Breastfeeding patterns and mothers'health in an HIV context in Sub Saharan Africa

Eric Nagaonlé Somé

Thesis for the Degree of Philosophiae Doctor (PhD)
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Scientific environment

This work is a planned ancillary study built on the PROMISE PEP clinical trial data. The PROMISE PEP trial (Promoting Infant health and Nutrition in Sub-Saharan Africa: Safety and Efficacy of prolonged anti-retroviral Peri Exposure Prophylaxis against HIV infection during breastfeeding) consortium included European and African Universities: the University of Bergen in Norway, the Universities of Montpellier and Paris V in France, the University of Uppsala in Sweden, the University of Ouagadougou in Burkina Faso, the University of Makerere in Uganda, the University of Western Cape in South Africa and the University of Zambia.

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Abstract in English

Introduction

It has been estimated that breastfeeding is a healthier alternative than replacement feeding because of its nutritional and immunological values. If optimally implemented it has the potential to save children's lives. Despite the HIV infection context, breastfeeding is recommended for infants born to HIV-infected women in low-income settings as the best feeding option when formula feeding is unsafe. However, both breastfeeding and HIV-infection are energy demanding. There are concerns about the effects of lactation on post-partum weight changes among HIV-infected women because low weight could increase the risk of HIV progression and worsen biological parameters, such as the CD4 cell count and HIV viral load of HIV-positive mothers.

Our objectives were i) to examine the feeding practices and the determinants of exclusive/ predominant or any breastfeeding among the trial's participants (paper 1); ii) to explore if exclusive or predominant breastfeeding is associated with a change of the body mass index (BMI) among breastfeeding HIV-1-positive women (paper 2); iii) to assess the mothers' HIV-1 disease progression in relation to the exclusive/predominant or any breastfeeding duration during the infant first 6 months of life and until week 50 post-partum (paper 3).

Methods

Our data were collected in the context of the ANRS 12174 phase 3 clinical trial (clinical trial no NCT0064026) between November 2009 and March 2013. The trial compared the efficacy of lamivudine with lopinavir/ritonavir in preventing postnatal HIV-1 transmission during lactation to infants born to HIV-1 positive mothers. Mothers infected with HIV-1 with CD4 cell count of >350 cell/µl and their uninfected offspring were followed from day 7 after birth for 50 weeks, keeping monthly records of their self-reported feeding patterns. Feeding was classified into 3 categories: 1) exclusive breastfeeding during the first 6 months, only breast-milk being given to infant for 6 months, 2) predominant breastfeeding, breast-milk with liquid-based items being given, and 3) mixed feeding, other non-breast milk or solid food being given in addition to breast milk with or without liquid-based items. The categories were merged into 2 groups: exclusive or predominant breastfeeding (EPBF) applying to infants aged <6 months and mixed feeding applying to infants of any age.

In paper 1 the feeding patterns are shown using Kaplan-Meier curves. A flexible parametric multiple regression model was used to identify the determinants of the mothers' feeding behaviour. In paper 2, we ran a linear mixed-effect model with BMI as the dependent variable and exclusive or predominant breastfeeding duration as the key explanatory variable. In paper 3 we ran a multiple logistic regression model with the HIV-1 disease progression as the dependent composite endpoint combining decrease in mothers' weight (<10% of initial weight), decrease in CD4 cell count (<350 cell/µl) and HIV-1 clinical stage, as per the world health organization (WHO) classification (above stage 2).

Results

In paper 1, a total of 1,225 mother-infant dyads provided feeding data from Burkina Faso (N = 204), South Africa (N = 213), Uganda (N = 274) and Zambia (N = 534). N=1 216 mother-infant dyads were included in paper 2 and 3 from Burkina Faso (203), South Africa (212), Uganda (272) and Zambia (529). The mean maternal age was 27.4 years, the mean BMI was 24.5 kg/m². Breastfeeding was initiated by 57.7% within the first hour and 93.9% within the first day. Overall, the median durations of any form of breastfeeding and EPBF were 40.6, and 20.9 weeks, respectively. No major change in mean BMI was seen in this cohort over a 50 weeks of lactation. The mean change between 26 and 50 weeks after birth was 0.7 kg/m². Baseline mean BMI (measured on day 7 post-partum) and CD4 cell count were positively associated with maternal BMI change, with a mean increase of 1.0 kg/m² (95% CI:0.9; 1.0) per each additional baseline-BMI kilogram and 0.3 kg/m² (95% CI:0.2; 0.5) for each additional CD4 cell/µl, respectively. In the adjusted model, there was no significant correlation between EPBF duration and HIV-1 disease progression.

Conclusion:

Better breastfeeding practice, as per WHO's recommendations, has been achieved in the ANRS 12174 clinical trial for the majority of the children. Breastfeeding did not affect the BMI or the HIV-1 disease progression in HIV-1 infected Sub-Saharan African mothers when their CD4 cell counts were >350 cells/µl. Considering the benefits of breast milk for infants, and the recurrent results from different studies 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 to 24 or more, provided that the right treatment or prophylaxis for the infection is given where formula feeding

is unsafe. Thus, there is a need to improve breastfeeding and complementary feeding practices of children, particularly those exposed to HIV and antiretroviral treatment, taking into account context and socio-demographic factors.

Abstract in French

Introduction

L'allaitement est perçu comme une alternative plus saine que le lait artificiel à cause de ses qualités nutritionnelles et immunologiques. En dépit du contexte marqué par l'infection à VIH, le lait maternel est recommandé comme la meilleure option alimentaire pour les enfants nés de mères séropositives dans les pays à faibles revenus, chaque fois que le lait artificiel présente des risques. Cependant, l'allaitement maternel comme l'infection à VIH entraîne des dépenses énergétiques plus importantes au niveau de l'organisme. Ces constats ont suscité des inquiétudes par rapport à l'influence de l'allaitement sur les variations de poids chez la femme séropositive, car une perte de poids pourrait être à l'origine d'un risque de progression plus rapide de l'infection à VIH dont une détérioration de paramètres biologiques tels que la numération CD4 et la charge virale.

Notre objectif était de i) décrire les pratiques d'allaitement et les déterminants de l'option alimentaire (allaitement maternel exclusif ou prédominant ou allaitement mixte) par les participants à l'essai clinique ANRS 12174 (publication 1) ; ii) définir la relation entre l'allaitement maternel exclusif ou prédominant et la variation de l'indice de masse corporelle parmi les femmes allaitantes et séropositives à l'infection à VIH-1 (publication 2) ; iii) identifier la relation entre la progression de l'infection à VIH-1 et la durée de l'allaitement par la mère en fonction du mode (exclusif, prédominant ou mixte) pendant les 6 premiers mois ou jusqu'à 50 semaines après la naissance de l'enfant (publication 3).

Méthodes

Nos données ont été collectées entre Novembre 2009 et Mars 2013 dans le contexte de l'essai clinique de phase 3 ANRS 12174 (numéro d'enregistrement NCT0064026). L'objectif de l'essai ANRS 12174 était de comparer l'efficacité de deux médicaments (lamivudine et lopinavir/ritonavir) à prévenir la transmission postnatale du VIH-1 aux enfants allaitant. Les mères séropositives au VIH-1 avec des taux de CD4>350 cell/µl et leurs nouveau-nés non infectés ont été suivis du septième jour à la 50ème semaine après la naissance avec une collecte mensuelle des données d'alimentation telles que rapportées par les mères. Le mode d'alimentation a été classé en trois catégories : 1) allaitement maternel exclusif pendant les six premiers mois ; uniquement le lait maternel est donné à l'enfant ; 2) l'allaitement maternel

prédominant ; le lait maternel est associé à des aliments essentiellement liquides ; 3) et l'alimentation mixte où autres laits non maternels ou aliments solides ont été servis à l'enfant en plus de lait maternel avec ou sans autre aliment liquide. Les deux premières catégories ont été fusionnées en un groupe pour obtenir deux grandes catégories : l'allaitement maternel exclusif ou prédominant pour les enfants âgés de moins de six mois et l'alimentation mixte quel que soit l'âge de l'enfant.

Dans la première publication, les modes d'alimentation ont été décrits en utilisant les courbes de survie de Kaplan-Meier. Un modèle de régression multiple paramétrique flexible a été utilisé pour identifier les déterminants des pratiques d'alimentation par les mères. Dans la deuxième publication, nous avons mis en œuvre un modèle linéaire mixte avec l'indice de masse corporelle comme variable dépendante et la durée de l'allaitement maternel exclusif ou prédominant comme principale variable indépendante. Dans la troisième publication un modèle de régression logistique a été utilisé avec la progression de l'infection à VIH-1 comme critère de jugement composite combinant la perte de poids maternel (au-dessous de 10% du poids initial), la baisse du taux de CD4 (<350 cell/µl) et le stade clinique de l'infection à VIH selon la classification de l'OMS (au-dessus du stade 2).

Resultats

Au total, les données de 1225 couples mères-enfants ont été utilisées du Burkina Faso (N=204), de l'Afrique du Sud (N=213), de l'Ouganda (N=274) et de la Zambie (N=534) pour la première publication. Dans les deuxième et troisième publications nous avons inclus 1216 couples mères-enfants dont 203, 212, 272 et 529 couples du Burkina Faso, de l'Afrique du Sud, de l'Ouganda et de la Zambie respectivement. L'âge moyen des mères était de 27.4 ans et l'indice de masse corporelle moyen de 24.5 kg/m² de surface corporelle. L'allaitement maternel a été initié par 57.7% des mères dans la première heure après la naissance et 93.9% dans la première journée. Au total les durées médianes de l'allaitement tout type confondu et de l'allaitement maternel exclusif ou prédominant étaient de 40.6 et 20.9 semaines respectivement. Aucune variation majeure n'a été constatée au niveau de l'indice de masse corporelle dans notre cohorte durant tout le suivi de 50 semaines. Une diminution moyenne de 0.7 kg/m² entre 26 et 50 semaines a été notée au niveau de l'indice de masse corporelle. L'indice de masse corporelle moyen initial et le taux de CD4 de base (mesuré à J7 postpartum) étaient positivement associés avec la variation de l'indice de masse corporelle maternel avec une augmentation moyenne de 1.0 kg/m² (IC à 95% : 0,9 ; 1,0) pour chaque kilogramme additionnel d'IMC de base et 0.3 kg/m²

(IC à 95% : 0.2; 0.5) pour chaque unité additionnelle de CD4 respectivement. Dans l'analyse ajustée, l'association entre la durée de l'allaitement maternel exclusif ou prédominant et la progression de l'infection à VIH-1 n'était pas significative.

Conclusion

Les meilleures pratiques d'allaitement maternel selon les recommandations de l'OMS ont été mises en œuvre au cours de l'essai clinique ANRS 12174 pour la plupart des enfants. L'allaitement maternel n'a influencé ni l'indice de masse corporelle, ni la progression de la maladie chez les mères séropositives au VIH-1 et vivant en Afrique au Sud du Sahara, lorsque leur taux de CD4 était >350 cells/µl. Considérant les multiples avantages du lait maternel pour les enfants et les résultats répétés de différentes études prouvant que l'allaitement maternel ne détériore pas la santé des mères infectées par le VIH-1, notre étude abonde dans le sens des recommandations 2016 de l'OMS sur l'alimentation de l'enfant. Ces recommandations soulignent que les mères vivant avec le VIH doivent allaiter pendant au moins 12 mois et jusqu'à 24 mois ou au-delà, à condition que le traitement ou la prophylaxie adaptée soit administré contre l'infection, et cela, chaque fois que la pratique de l'allaitement artificiel n'est pas sans risque. Par conséquent, il y a aussi un besoin d'améliorer la pratique de l'allaitement y compris l'introduction de l'alimentation complémentaire, particulièrement chez les enfants exposés au VIH et à une prophylaxie antirétrovirale, tout en prenant en compte le contexte et les facteurs socio-démographiques.

Abbreviations and acronyms

µl	Microliter
3TC	Lamivudine
AFASS	Acceptable, Feasible, Affordable, Sustainable and Safe
AIDS	Acquired Immuno-deficiency Syndrome
ANC	Ante Natal Care
ANRS	Agence Nationale pour la Recherche sur le SIDA et les hépatites virales (French agency for Research on AIDS and viral hepatitis)
AOR	Adjusted odd ratio
ART	Antiretroviral therapy
ARV	Antiretroviral (drug)
AZT	Zidovudine
BMI	Body Mass Index
CD4	Cluster of Differentiation 4
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
EBF	Exclusive Breastfeeding
eMTCT	Eliminating Mother-To-Child Transmission (of HIV)
EPBF	Exclusive or Predominant Breastfeeding
HAART	Highly Active Antiretroviral Treatment
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IQR	Interquartile Range
LMICs	Low and Middle Income Countries
LPV/r	Lopinavir/Ritonavir
MF	Mixed Feeding
MTCT	Mother To Child Transmission (of HIV)
PBF	Predominant Breastfeeding
PCR	Polymerase Chain Reaction
PMTCT	Prevention of Mother To Child Transmission (of HIV)
PreP	Pre exposure Prophylaxis
RNA	Ribonucleic Acid
sdNVP	Single-Dose Nevirapine
SES	Socio-Economic Status
SSA	Sub-Saharan Africa
UNICEF	United Nations Children Fund (original definition: United Nations International Children's Emergency Fund).

USA	United State of America
USAID	United States Agency for International Development
VCT	Voluntary Counselling and Testing
WHO	World Health Organization

Definitions

The definitions regarding the infant feeding patterns have been taken from WHO and UNICEF 2016 updates on HIV and infant feeding guidelines (1) and other WHO online tools.

Exclusive breastfeeding (EBF): only breast milk (including breast milk that has been expressed, or coming from a wet nurse) is given to the infant without any other kind of food or liquid, except medically prescribed drugs, mineral supplements or vitamins. WHO recommends that EBF starts within 1 h after birth and continues until the infant is 6 months of age.

Predominant breastfeeding (PBF): infant is fed with breast milk and incidentally with some liquid-based food such as juice, tea, sugar-water and salt-water including glucose without any kind of formula or animal milk.

Exclusive or predominant breastfeeding (EPBF): infant is either exclusively or predominantly fed with breast milk. This group is the result of the merging of both EBF and PBF groups in this thesis work.

Complementary feeding: is when breastmilk and other solid, semi-solid or liquid-based food is given to the infant because breastmilk alone is no longer sufficient to meet the infant's need. WHO recommends that complementary feeding starts from 6 months after birth and onward for 24 months or more.

Prelacteal feeding: any food given to the infant before the initiation of breastfeeding.

Formula or replacement feeding: breastmilk is completely stopped. An infant is given breastmilk substitutes in the form of commercial formula. Exclusive replacement feeding is when infant has never taken breastmilk.

Mixed feeding (MF): infant is fed with breast milk and other solid or liquid-based food, including other kinds of formula and non-human milks. This feeding mode is different from complementary feeding since it could start at any time in the infant feeding process regardless of WHO's breastfeeding recommendations.

Option A: a pregnant, HIV-positive and immunocompetent (CD4 cell count >350 cells/µl) woman is offered a prophylactic antiretroviral therapy (ART) regimen during antenatal and intrapartum period to prevent the vertical transmission of HIV to a child in-utero and during labor and delivery. An antiretroviral prophylaxis is also given to the mother postpartum ("tail" regimen) for a short period, to reduce the risk of drug resistance while an infant receives postpartum antiretroviral prophylaxis throughout the breastfeeding period (2).

Option B: a pregnant or breastfeeding, HIV-positive woman is offered ART to prevent the vertical transmission of HIV-infection to her child during pregnancy and postpartum throughout the breastfeeding period. After breastfeeding cessation, ART is stopped if the mother has CD4 cell count >350 cells/µl and will be resumed when the mother would need it for herself (2).

Option B+ or universal treatment program: a pregnant or breastfeeding woman is offered ART for life immediately after she is diagnosed as HIV-infected, regardless of her CD4 cell count. This ART aims to prevent the vertical transmission of HIV to her child, while benefitting the woman and protecting her sexual partner

List of publications

Eric N. Somé, Ingunn M. S. Engebretsen, Nicolas Nagot, Nicolas Meda, Roselyne Vallo, Marianne Peries, Chipepo Kankasa, James K. Tumwine, G. Justus Hofmeyr, Mandisa Singata, Kim Harper, Philippe Van De Perre, Thorkild Tylleskar for the ANRS 12174 Trial Group. **HIV-1 disease progression in immune-competent HIV1-infected and breastfeeding mothers participating in the ANRS 12174 clinical trial in Burkina Faso, South Africa, Uganda and Zambia: a cohort study.** BMJ Open 2018;8:e019239. doi:10.1136/bmjopen-2017-019239.

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Eric N. Somé, Ingunn M. S. Engebretsen, Nicolas Nagot, Nicolas Meda, Carl Lombard, Roselyne Vallo, Marianne Peries, Chipepo Kankasa, James K. Tumwine, G. Justus Hofmeyr, Mandisa Singata, Kim Harper, Philippe Van De Perre, Thorkild Tylleskar for the ANRS 12174 Trial Group. **Breastfeeding patterns among mothers living with HIV in Sub Saharan Africa: the ANRS 12174 trial.** International Breastfeeding Journal 2017; 12

All the articles were published in open access journals.

Introduction

The following germane questions were posed. How was breastfeeding practiced by participating women in the ANRS 12174 phase 3 clinical trial? What were the determinants that influenced the compliance of these women to the world health organization (WHO) breastfeeding recommendations for mothers infected with the human immunodeficiency virus (HIV) in resources constrained settings? Has breastfeeding a negative impact on HIV-infected mothers' nutritional status and on their HIV disease progression?

Throughout the introduction, I will present salient information on HIV and its modes of transmission. Thereafter I will discuss its association with women's and children's health relevant for placing the research findings in a biological and historical context. The prevention methods focusing on the prevention of mother to child transmission of HIV and the evolvement of the guidelines on feeding a child born to a HIV positive woman from early 2000 up to current challenges will be summarized. Further, I provide epidemiological patterns and current nutritional knowledge of breastfeeding. I will next elaborate on the ANRS 12174 clinical trial, its relevance for this thesis, and in the context of today's universal treatment program strategy and the possible use of the other options B, A or Pre-exposure prophylaxis (PreP). Lastly, the question of why it is important to provide scientific evidence that contribute to answer the above research questions will be explained.

1.1 HIV-1 transmission

The Human Immunodeficiency Virus type 1 (HIV-1, Figure 1) is an RNA virus that is transmitted through body fluids, including blood, semen, vaginal fluid, rectal fluid and breast milk. There is an increased risk of HIV-1 transmission when the body fluid of an HIV-1-infected person is in contact with vulnerable body parts of an HIV-uninfected person through, for instance, a rupture of the skin or a mucous membrane (3). In the case of an infection through, e.g., sexual contact, the virus has to pass through the different layers of cells of the mucous membrane (4). HIV-1 can cross these cell layers and enter the body on its own but some factors facilitate penetration. This is the case with sexually transmitted infections (herpes, syphilis; (5, 6), the micro-lesions occurring during sexual intercourse, the use of enemas, vaginal washing, brushing teeth, flossing, dental work and surgery. Other risk factors of HIV-1 infection include

the surface area of the mucosa in contact with the infectious fluid, the amount of virus (viral load), the infectiousness of the viruses in the fluid (more infectious during primary infection) or onsite inflammation attracting T-cells that are the main target for the virus (3, 7).

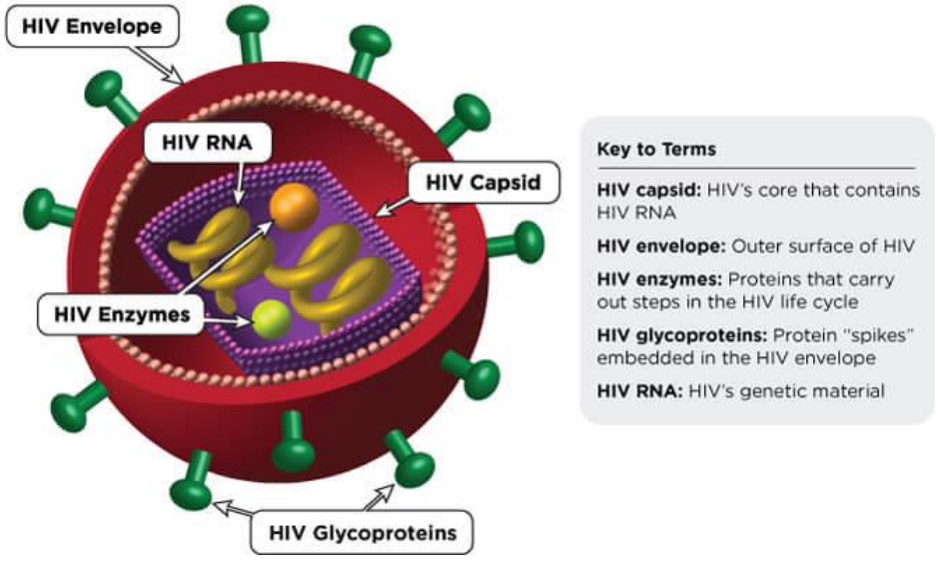


Figure 1: Human immunodeficiency virus (source: AIDSinfo; material in the public domain).

HIV-1 cannot multiply on its own. Typically it attacks a lymphocyte called T-helper cell or CD4 cell, fuses with it, takes control of it, replicates itself inside the cell, and then releases new HIV-1 virions into the blood. This life cycle include binding and fusion, reverse transcription and integration, replication, assembly, budding and maturation (8). These are all stages targeted by the anti-retroviral (ARV) drugs. Figure 2 is a reprint of the HIV life cycle and the corresponding classes of ARVs.

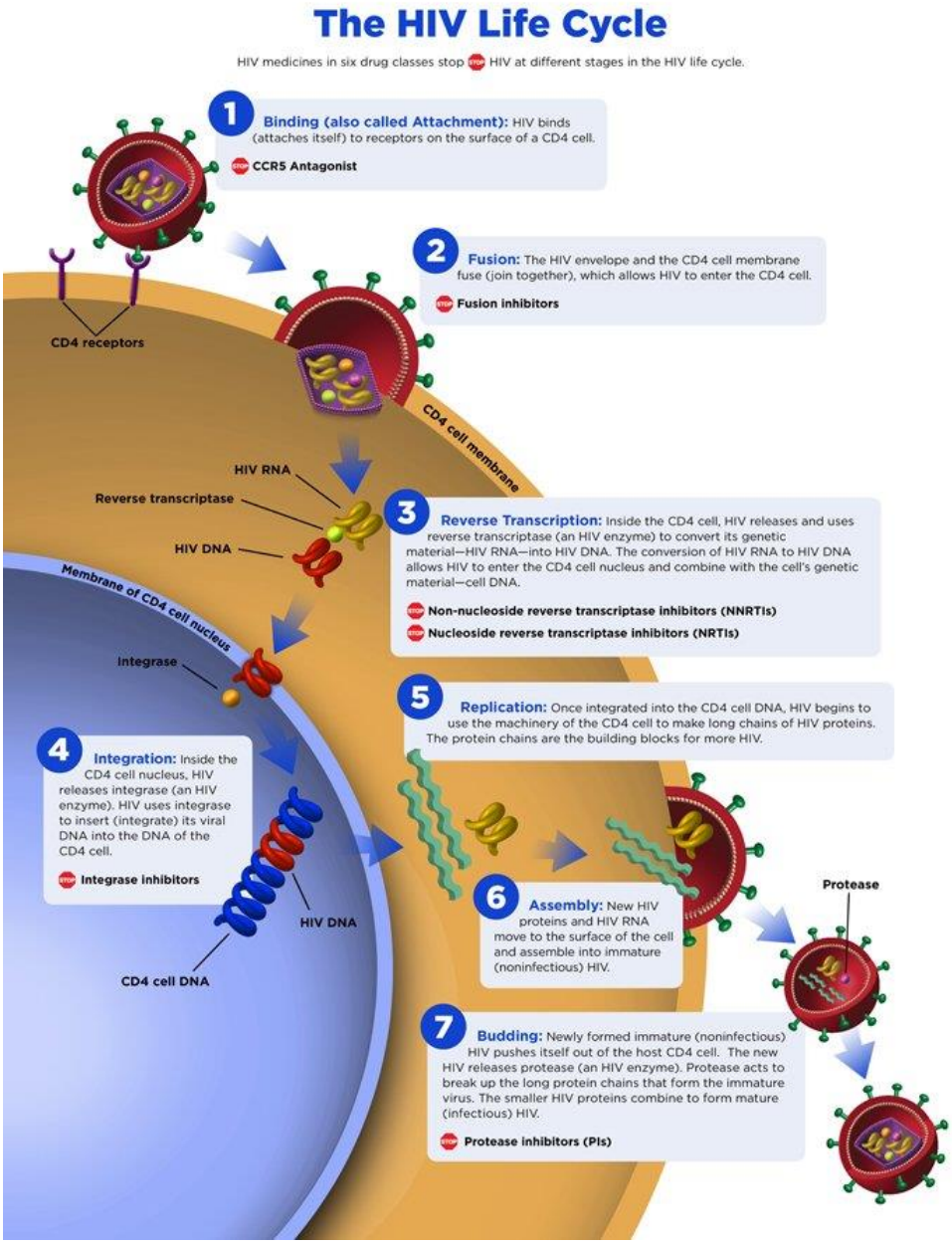


Figure 2: HIV life cycle (source: AIDSinfo; material in the public domain).

The rate of HIV transmission from male to female is 2-3 times higher than it is from female to male (6, 9). The specific risk factors for female acquisition of HIV infection include a higher affinity of some HIV serotypes for the Langerhans' cells of the cervix, vulval and vaginal inflammation or ulceration, and other sexually transmitted infections (10). In addition to these biological and clinical risk factors, socio-cultural factors also play a role in putting female partners more at risk for HIV infection. Gender inequality, poverty, less access to education, lack of employment opportunities, traditional practices and practices, such as vaginal washing, female circumcision, low use of female and male condoms, and the societal pressure to produce children, make women highly vulnerable to HIV acquisition (10).

1.1.1 HIV-1 during pregnancy, birth and lactation

During pregnancy, the woman's body experiences unique and complex changes that include the immune system. Both the humoral and cell-mediated immunity are involved in these changes (11, 12). Reports from resource-limited settings tend to show that HIV-1 disease is associated with poorer pregnancy outcomes, including complications both in early and late pregnancy (13-16). A pooled analysis on sub-Saharan African community data from 2013 found higher rates of spontaneous abortions, ectopic pregnancies, preterm labour, preterm rupture of membranes, placental abruption, low birth weight and stillbirths (16). In the same review, the postpartum period was also associated with more infectious morbidity in HIV-1-infected women. Women with more advanced HIV-1 disease had a higher risk of pregnancy loss and perinatal mortality. HIV-1 is the predominant underlying risk factors for maternal deaths, even when antiretroviral therapy (ART) is universally provided (17). Advanced maternal HIV-1 disease affects infant health and survival indirectly due to low birth weight and prematurity, and directly through the risk of HIV-1 transmission (18).

1.1.2 Mother-to-child transmission of HIV-1

Early HIV research on mother-to-child transmission (MTCT) of HIV-1 showed that, in the absence of any intervention, transmission ranged from 15 to 40%, with higher rates in Africa and Asia (19-21). The mode of delivery influenced the transmission rate; Caesarean section decreased the rate while vaginal delivery, prolonged rupture of membranes, intrapartum hemorrhage and invasive fetal monitoring increased the rates (20). More recent analyses showed that maternal immunological, nutritional and clinical status, maternal viral load and

resistance in plasma and breastmilk, viral phenotype and genotype, and breast conditions also affect the HIV-1 transmission to the fetus/child (22). Behavioural factors such as smoking, drug use and unprotected sexual intercourse during pregnancy increase the rate of HIV-1 transmission to the fetus (20, 21).

Untreated HIV-1 infection can be transmitted to the fetus in-utero during pregnancy (5-10%), labour and delivery (10-15%) and the breastfeeding period (5-20%) (21). During pregnancy, transmission can occur by direct contamination of maternal infected blood through the placenta to the fetal circulation. Such contamination increases with the age of the placenta, with in-utero infection more commonly occurring towards the end of pregnancy.

During labour and delivery, infection occurs through muco-cutaneous contact of the baby with maternal fluids, mainly blood and cervico-vaginal secretions. The transfer of fluids and viruses from the mother to the child is facilitated by uterine contractions during labour and delivery, the passage through the birth canal, or the ingestion and absorption of liquids through the fetal-neonatal digestive tract (23, 24). According to the classification of the timing of transmission based on the time of detection of HIV-1 in the infant, an infant is considered infected in-utero when virus is detectable within 48 h of birth. Later detection after an initial negative test indicates intrapartum or breast milk transmission. By definition, the infection occurred intrapartum if the tests are negative during the first week of life before turning positive between 7 and 90 days (10). When a woman is breastfeeding, it is impossible to find any difference between intrapartum and early postpartum transmission of HIV because the existence of a (window) period after HIV infection during which current technologies cannot detect the virus. However, late postnatal transmission can be documented and is defined as an infant HIV-infection 3-6 months after birth, where previous polymerase chain reaction (PCR) testing was negative before becoming positive between 2 and 6 months of an infant's age (25). This timing of HIV diagnosis in infants has been greatly reduced with the implementation of nucleic acid technologies (NAT) using HIV DNA or RNA PCR or even ultrasensitive P24 antigen detection. These technologies are used in PMTCT programmes to test HIV exposed infants at their sixth week of life. Earlier diagnosis of in-utero infection is possible, but is not yet recommended in routine practice (26).

Transmission of HIV-1 through breastfeeding was first reported by Philippe Van de Perre and colleagues (27). In the absence of maternal ART, it can occur at any time-point as long as the child is breastfed and the risk seems constant per day of breastfeeding. Three clinical factors,

including the rate of viral replication in the mother, the duration and mode of breastfeeding, and the inflammation of the mammary gland, increase the risk of MTCT of HIV (28). In mothers taking ART with a suppressed HIV viral load, the residual HIV transmission rate has been estimated at 0.2% per month of breastfeeding (29).

Some fetal and infant factors modulate the HIV-1 transmission from mother-to-child. Among them, prematurity, multiple pregnancy, mode of infant feeding (breast milk, formula or replacement feeding or mixed breast milk and formula/replacement feeding), child gastrointestinal tract factors (integrity of mucous membranes), immature immune system would be associated with higher risk of transmission (10, 30). Extensive research is focusing on whether the HIV transmission through lactation is cell-free and cell-associated; this has implications for pharmacological preventive strategies for the mother and child.

1.1.3 The dilemma of infant feeding and HIV transmission

With optimal viral suppression at delivery, postnatal infant prophylaxis and avoidance of breastfeeding, the risk of perinatal HIV-1 transmission is 0.1% (31). The global plan to eliminate MTCT (eMTCT) by 2020 set the motto that "children everywhere can be born free of HIV-1 and their mothers remain alive". The impact criteria required by WHO for validating eMTCT included 50 or fewer new pediatric HIV infections per 100,000 live births and a transmission rate of either 5% or less in breastfeeding populations, or 2% or less in non-breastfeeding populations (32). However, implementation of optimal prevention strategies were incomplete and still about 160,000 new HIV infections among children aged 0-14 years occurred in 2016. This was less than 300,000 in 2010 (33). In 2015, adolescent girls and young women accounted for 25% of new HIV-1 infections among adults, and women accounted for 56% of new infections in sub-Saharan Africa (34).

In many high income countries, including the United States, United Kingdom and Canada (35), the guidelines to prevent the MTCT of HIV have been very clear - HIV-infected mothers should not breastfeed. In resource-limited settings, avoidance of breastfeeding in HIV-1 contexts has remained a challenge. The feeding option relied primarily on the mothers' decision after nutrition counselling on the feeding context by a healthcare worker. The way a HIV positive mother decided to feed her baby affected the infant's risk of either becoming infected with HIV or dying from other infections (36). Other studies also found that replacement feeding is not safe in very poor contexts, even with maximum support (37). Before the 2010 guidelines, the

healthcare workers put emphasis on the WHO's main criteria for exclusive replacement feeding: "when replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding by HIV-infected mothers is recommended. Otherwise, exclusive breastfeeding is recommended during the first months of life" (38). These criteria have been heavily criticized as unethical and difficult, putting women in an impossible situation to make a decision (39-42).

The current guidelines have uniform feeding recommendations for all women in resource-limited settings, irrespective of HIV-1 or socio-economic status (1). Mothers living with HIV-1 should breastfeed for at least 12 months and continue breastfeeding for at least 24 months (similar to the general population), while being fully supported for ART adherence (43-45). This harmonisation has been made possible, thanks to ART.

A dichotomized approach remains since WHO includes HIV infection of mothers as an absolute contra-indication of breastfeeding, but only in high-resource countries. Supported breastfeeding is widely practiced in resource-limited settings by HIV-positive mothers and occasionally by individual women in high income countries where formula-feeding is expected. WHO 2016 guidelines seems then tailored for resource-limited settings, while at the same time the world is committed to zero tolerance for any infant HIV-1 acquisition (35, 46, 47).

1.2 Breastfeeding

1.2.1 Breastfeeding advantages

Breastfeeding has several advantages for both babies and breastfeeding mothers. However, an important community trial in Burkina Faso, South Africa and Uganda promoting exclusive breastfeeding for 6 months (the PROMISE-EBF trial) was unable to reconfirm these advantages of breastfeeding promotion on diarrhoeal diseases prevention (48), growth of children under-6 months old (49), and on neuropsychological outcomes in 5-8 year old children (50). This was probably due to the uniform breastfeeding practices in the study areas. Other studies have found that universal coverage with general nutritional interventions, including exclusive breastfeeding (EBF), could prevent 8% and 11.6% of under-3 and under-5-year old child mortality, respectively, as well as 10-15% of child stunting (51-54). Mortality risk for infants of <6 months old and not breastfed is 3.5 times higher than in any breastfed boys and 4.1 times higher in girls

(53). In children aged 6-23 months, 50, 36 and 58% reductions in deaths, sudden infant death and necrotizing enterocolitis were noted, respectively. For children, 823 000 (13.8%) annual deaths could have been averted in 75 high-mortality low-and-middle-income countries (LMICs) in 2015 if breastfeeding had been scaled up to near universal levels (53). About half of all diarrhoeal episodes and a third of respiratory infections could have been avoided by universal breastfeeding. Besides, breastfeeding could prevent 72 and 57% of admissions for diarrhoea and respiratory infections, respectively, and 68% reduction in malocclusions. Exclusive breastfeeding could prevent infant growth deficit (55, 56). Therefore, it is assumed globally that breastfed children are healthier than non-breastfed children, in which better health status results from reduced incidence and severity of communicable diseases, including diarrhoea, and improved feeding during illness. Other expected benefits are lower risk of auto-immune diseases and potential long-term effects on cardiovascular diseases and cognition (52).

Positive effects of breastfeeding on the mother include lactation amenorrhoea and birth spacing, protection against breast and ovarian cancer, type 2 diabetes and osteoporosis. Current global rates of breastfeeding seemingly avert ~19,500 annual breast cancer deaths compared with a scenario in which no woman breastfeeds (53).

1.2.2 Breastfeeding practices

Meta-analysis (53) showed that, worldwide over a 20-year period, exclusive breastfeeding rates increased from 24.9% in 1993 to 35.7% in 2013. In general, early initiation of and exclusive breastfeeding are still low in all settings. In children <6 months, 53, 61 and 63% are not exclusively breastfed in low-income, lower-middle-income and upper-middle-income countries, respectively. In children aged 6-23 months, 18, 34 and 55% were not receiving any breastmilk in low-income, lower-middle-income and upper-middle-income countries, respectively. The same meta-analysis indicated that breastfeeding prevalence was highest in Sub-Saharan Africa, South Asia and parts of Latin America. It was ~37% for children of <6 months. Continued breastfeeding was higher in West and Central Africa than in Eastern and Southern Africa. To the contrary, exclusive breastfeeding rate was higher in Eastern and Southern Africa, highest in Rwanda and lowest in Gabon. The feeling that breast milk is not enough to cover the child's needs was the main reason explaining why mothers stopped EBF early in Sub-Saharan Africa (53).

The main explanatory factors associated with increased EBF rates were mothers' of age 25-35 years, who had completed secondary school or above, had completed at least 4 antenatal care (ANC) visits, resided in rural area, belonged to the wealthiest quantile, delivered female or a singleton baby at a health facility or initiated breastfeeding early. Mothers' knowledge on EBF, mainly through counselling sessions during pregnancy and after delivery, was also an important factor in promoting EBF. At the country level, higher gross national income per capita was associated with lower EBF rate (57-59).

1.3 HIV and infant feeding recommendations

WHO recommends early initiation of breastfeeding, which means putting the baby on the breast within one hour of delivery. Early initiation of breastfeeding is associated with exclusive, as well as an extended duration, of breastfeeding. It helps keep the baby warm and helps mother-child bonding (59, 60). Early initiation of breastfeeding would reduce neonatal and early infant mortality through both EBF and other mechanisms (61, 62). EBF is recommended for the first 6 months.

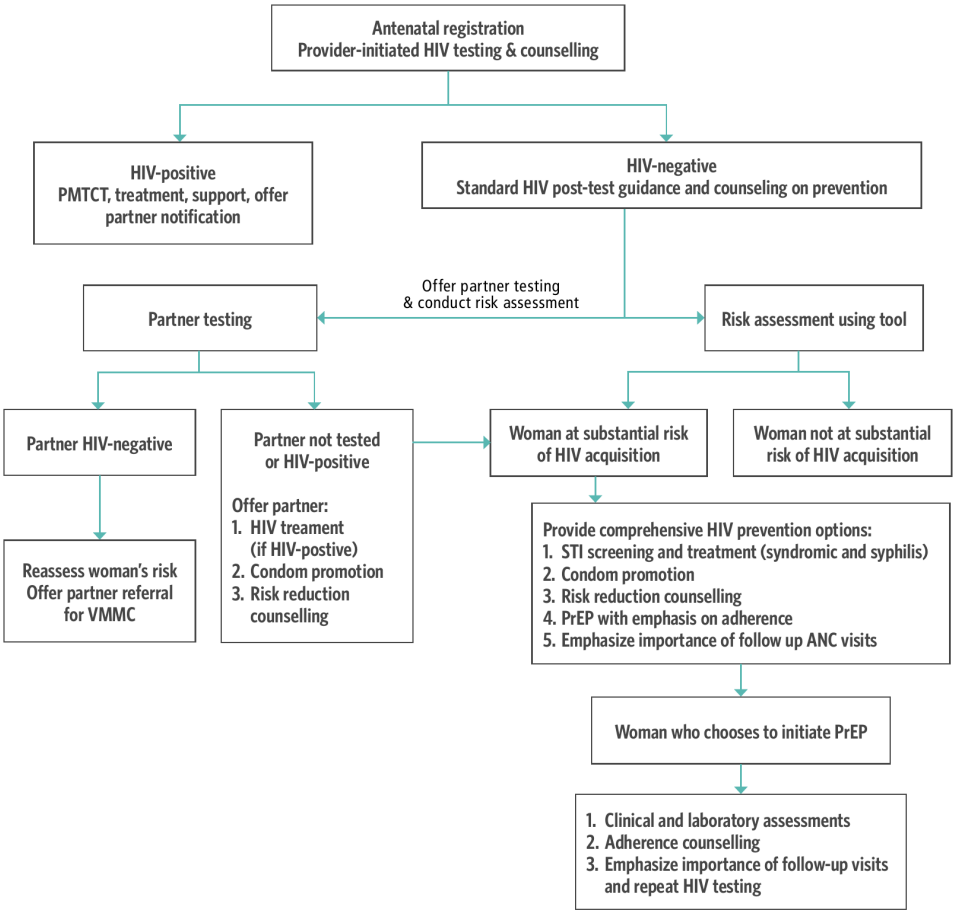
From 6 until 23 months of age, WHO recommends continued frequent on-demand breastfeeding, since breastfeeding benefits children older than 6 months. The 12-23-month aged breastfed child's energy intake comes 35-40% from breastmilk and the remainder from complementary foods (62). The importance of breastfeeding is emphasized during a child's illness, when appetite for other foods decreases but breast-milk intake is maintained. Complementary food usually starts at 6 months of age, which implies that the infant is receiving breast or formula milk in addition to solid or semi-solid food. According to WHO, it is when breast milk or infant formula alone is no longer sufficient to meet infants nutritional requirements. Breastfeeding protection and promotion is needed to achieve successful practices (48).

HIV-1 infected mothers must use highly active antiretroviral therapy (HAART) or antiretroviral (ARV) prophylaxis to reduce HIV transmission through breastfeeding. The main objective is to reduce the HIV viral load in the mother. In countries where health authorities have opted for breastfeeding and ARV prophylaxis as strategies to increase HIV-free survival among children born to HIV-infected mothers, WHO recommends EBF during the first 6 months, the

introduction of complementary food from 6 months and onwards, while going on with breastfeeding until 24+ months or until a safe breastmilk substitute can be provided (1).

1.3.1 Interventions to prevent mother-to-child transmission of HIV-1

WHO recommends HIV testing for all pregnant or breastfeeding women in every setting during antenatal and postnatal care visits to tailor care provisions (prevention or treatment) according to their HIV status. For HIV-positive women, the assisted partner notification service should be provided. ART should be started directly and continued for life regardless of CD4 count (universal treatment program) (45). HIV testing should also be offered to all sexual and drug-injecting partners. For HIV-negative pregnant or breastfeeding women, living in settings where HIV burden is high, or living within populations with HIV incidence greater than 3 per 100 person-years (substantial and increased risk of HIV acquisition during pregnancy and breastfeeding), pre-exposure prophylaxis (PreP) is recommended (63). However PreP is always optional and its initiation relies purely on the decision of the woman after being counselled by a qualified healthcare worker. Figure 4 gives an algorithm to assist in the decision making process.



VMMC: Voluntary medical male circumcision

STI: sexually transmitted infection

Figure 3: A suggested prioritization framework for offering PrEP to pregnant and breastfeeding women (63)WHO Technical brief: Preventing HIV during pregnancy and breastfeeding in the context of pre-exposure prophylaxis (PrEP). Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO)

1.3.2 Historical overview of HIV and infant feeding recommendations

In high income settings, formula feeding became the only feeding option offered to HIV-infected mothers once the transmission paths of HIV-1 (including the mother-to-child transmission) had been well understood (35, 64, 65). In low and middle-income countries, the history of infant feeding in the context of maternal HIV-1 infection started with the recommendation of formula feeding for all babies in 1985 (66, 67). However, the problem of increased mortality among HIV-1 exposed and formula-fed infants was identified. These infant deaths were associated with multiple factors, including lack of hygiene in preparation of the formula, non-sustainability of the formula by families, and infant malnutrition. These factors contributed to the increase in the prevalence of infectious diseases, including pneumonia and diarrhoea, leading to increased mortality among the infants (66). In 1998, WHO recommended EBF for 4 months, followed by abrupt weaning to address the morbidity and mortality issues. In 2007, it became evident that abrupt weaning was not a good solution to increase HIV-free survival of infants born to HIV-infected mothers (68-70). At this point, the WHO's recommendation was EBF during 6 months, then a progressive weaning over a 2-month period. Thereafter in 2010, mothers were allowed to breastfeed exclusively for 6 months, to add complementary food from 6 months and onwards, and to wean within 12 months of infant life. Further, WHO's 2016 guidelines for HIV-exposed children extended the period of continued breastfeeding to 2 years or beyond, as in the general population. Table 1 (summarized from Sinning, 66) explains the evolvement of feeding recommendations and ARV regimens since the early years after the discovery of HIV infection. While infants were being fed breast milk, prophylactic ARV was administered either to the mother or the child (1, 66).

Option A is the administration of ARV prophylaxis to an HIV-infected and immunocompetent pregnant or breastfeeding woman and to her HIV-exposed child in the postpartum period to prevent mother-to-child transmission. Option B is when an HIV infected mother is given ARV as mono- or dual-therapy or as a highly active antiretroviral therapy (HAART) that tries to achieve a level of viral suppression that reduces the transmission rate of HIV to the offspring either in-utero or during the lactation period. Option B+ or universal treatment program is the provision of HAART both as an HIV treatment for the mother and prevention to the child. This is lifelong therapy for the mother that starts with the diagnosis of HIV infection (2).

The provision of ART at higher CD4 counts is justifiable for medical, public health and human rights reasons. Therefore, the need to take a CD4 count as a condition to start ART was removed (71). The cost-effectiveness of this option includes reduced laboratory costs, reduced cost related to the management of ARV therapy by less trained health professionals because of a simplified protocol, and reduced mortality among women because they can access ARV therapy more quickly and earlier in disease progression (71). However, the MTCT rates remain much higher than expected under the B+ approach. The issues that need to be addressed include the acceptability of lifelong antiretroviral treatment, and adherence to and retention on treatment. Other biological explanations are discussed in the last chapter of the introduction.

In resource-limited settings, mostly in Sub-Saharan Africa, the national health guidelines including those on PMTCT used to be inspired by WHO guidelines. However, it is claimed that since the early 1990's, the WHO guidelines on PMTCT have slipped dangerously away from evidence-based to opinion-based recommendations of experts. The implementation of non-tested policies has exposed targeted populations to non-assessed risks, such as those associated with the abrupt weaning recommendation (72). Besides, the rapid succession of these guidelines is making their implementation in national programs very difficult.

Table 1: Evolution of the WHO guidelines, feeding recommendations, ART and ARV prophylaxis in the PMTCT context (summarized data from Sinning; 66)

Year	WHO guidelines	Main innovation	Treatment	PMTCT
1981	First reports of the condition that will become HIV/AIDS and WHO's enactment of the International Code of Marketing of Breast Milk Substitutes	Worldwide breastfeeding promotion and restrictions on formula feeding; breastfeeding becomes a public health priority. Transmission of HIV via breastmilk not yet established		
1985	First report on HIV transmission risk from breast milk	HIV-positive women are advised against breastfeeding. Transmission via breastfeeding suspected; not yet established		
1987	The first global recommendation on HIV and breastfeeding	HIV transmission risk via breastmilk is assessed by WHO as smaller than the risk in-utero and intrapartum; if the breastmilk alternative is not "safe" and "effective", women should continue breastfeeding.		
1992	Consensus statement from the WHO/UNICEF consultation on HIV transmission and breastfeeding	In setting where the primary cause of infant death is infectious disease and malnutrition, breastfeeding should continue regardless of HIV status. Otherwise, HIV-positive mothers should be advised not to breastfeed		
1998	UNICEF-USAID-WHO HIV and infant feeding: a review of HIV transmission through breastfeeding.	Risk of HIV transmission via breastmilk estimated at least 15%; mixed feeding could carry a greater risk of HIV transmission; identified barriers to replacement feeding: stigma, affordability, increased risk of infection, and increased risk of malnutrition; three feeding options for HIV-positive mothers: breastfeeding + early cessation, heat treatment of expressed breastmilk or wet-		

		nursing by a HIV-negative woman; ARV medicines could reduce the transmission risk.		
2000	New data on the prevention of mother-to-child transmission of HIV and their policy implications	Risk of HIV transmission via breast milk quantified at 10-20%; ARV prophylaxis recommended for pregnant women; When replacement feeding is acceptable, feasible, affordable, sustainable and safe (AFASS), avoidance of all breastfeeding by HIV-infected mothers	No recommendation	4 weeks azithromycin (AZT) during pregnancy; AZT+lamivudine (3TC) or single dose nevirapine (sdNVP) intrapartum
2004	HIV transmission through breastfeeding – a review of available evidence	EBF for 6 months + transition to replacement feeding as soon as it is AFASS	ART if CD4<200	AZT from 28 weeks + sdNVP
2007	HIV transmission through breastfeeding - a review of available evidence 2007 update.	Risk of postnatal HIV transmission estimated at 1% per month of breastfeeding; early cessation of breastfeeding no longer recommended; maternal ART as prophylaxis and post-exposure prophylaxis for the infant discussed	Art if CD4<200	AZT from 28 weeks + sdNVP + AZT/3TC
2010	Guidelines on HIV and infant feeding - principles and recommendations for infant feeding in the context of HIV and a summary of evidence	ART should be provided to either the breastfeeding mother or infant; options to either breastfeed while the mother or infant received ARVs or to avoid all breastfeeding	Art if CD4<350	Option A: AZT/sdNVP + infant NVP if breastfeeding; Option B: ART pregnancy/breastfeeding period
2012	WHO guidelines on HIV and infant feeding 2010 - an updated framework for priority action.	EBF with addition of ARVs for the mother or the infant would decrease the risk of transmission of HIV to ~2% for 6 months of exclusive breastfeeding and a risk of 4% for 12 months; options to either breastfeed while receiving ARVs (to the mother or infant) or to avoid breastfeeding		
2014	WHO consolidated guidelines on HIV prevention, diagnosis,	Triple ART for all pregnant or breastfeeding HIV+ women during the risk of MTCT or for life if mother is eligible for ART (option B); in	Art if CD4<500	Option B: ART pregnancy/breastfeeding period

	treatment and care for key populations.	generalized epidemics, ART for all pregnant or breastfeeding HIV+ women for life (option B+)		Option B+: Life-long ART
2015			Art regardless of CD4	Life-long ART for all Pregnant/breastfeeding women
2016	Update on HIV and infant feeding	HIV+ mothers should breastfeed as in the general population; ART for all pregnant or breastfeeding HIV+ women for life (option B+)	Art regardless of CD4	Life-long ART for all Pregnant/breastfeeding women

1.4 Current implementation and limitations of the B+ option

Despite the momentum and invested effort, the global pace towards the elimination of mother-to-child transmission (e-MTCT) of HIV and the 2020 target will be slowing, according to UNICEF. If the trend is maintained, ~100,000 instead of 20,000 new paediatric infections are expected in 2020. Using data from WHO (73), it is possible to estimate the overall mean mother-to-child transmission rate including breastfeeding period at 12.8% in 2016 for the African region of WHO spanning Eastern and southern Africa and Madagascar (mean transmission rate=11.4%) and Western and Central Africa (mean transmission rate 14.3%). The overall estimated mean percentage of ART coverage was 47.9%, including Eastern and southern Africa and Madagascar (56.2%) and Western and Central Africa (39.5%). The overall estimated mean percentage of HIV-positive women receiving ART for PMTCT was 70.1%, i.e. 71.6 and 68.5% for Eastern and southern Africa and Western and Central Africa, respectively.

1.4.1 Adherence

In a MITRA plus ancillary study published in 2015 (74), a sub-cohort of women with ≤200 CD4 cells/µl at enrolment was followed up for 24 months after delivery to determine virological and immunological responses, drug resistance and mortality. About 95% (64/68), 85% (56/66), 74% (39/53) and 65% (30/46) of participants reported drug adherence at 3, 6, 12 and 24 months, respectively. Response failures were more likely to occur among women who reported breach of adherence to ART 24 months postpartum (74). In a Cameroonian cohort, 88% of women were still in care at 6 months and 81.1% at 12 months. At 18 and 24 months, the retention rates were 74.2 and 73.3%, respectively. Women visiting small facilities with high staff turnover were more at risk of discontinuing Option B+ procedures [HR (95% CI) = 2.0 (1.2–3.4), P = 0.012] (75). In Malawi, a survey was conducted to explore the levels and determinants of the loss to follow-up under option B+ therapy. About 20% of women who tested HIV-positive in antenatal and postpartum clinics dropped out without initiating ART. Of the women who started ART under Option B+ (n=21 939), 17% appeared to be loss to follow-up 6 months after start. Most loss occurred in the first 3 months of therapy. Option B+ patients who started therapy during pregnancy were 5 times more likely than women who started ART at WHO HIV stage 3/4 or with a CD4 cell count ≤350 cells/µl, to never return after their initial clinic visit (odds ratio 5.0, 95% CI 4.2-6.1). Option B+ patients who started therapy while breastfeeding were

twice as likely to miss their first follow-up visit (odds ratio 2.2, 95% CI 1.8-2.8). Loss to follow-up and no follow-up were highest in pregnant Option B+ patients (29%), mostly among those who began ART at large clinics on the day they were diagnosed with HIV. Among non-pregnant women starting on ART for medical indication (low CD4 cell count or WHO clinical stage 3 or 4) in ART clinics, the no follow-up and loss to follow-up rate was 10% at 6 months. The higher retention rates among these women were explained by better adherence counselling including more time to prepare to start ART, longer experience and training of staff, and the motivation induced by a symptomatic HIV infection. In the same study, pregnant women reported being "scared" and "traumatized" by the seriousness of the commitment to lifelong ART. They felt pressured by their HIV-positive test, the need to disclose this new HIV status to their partner and initiate lifelong ART on the same day (76-78). In South Africa, 32% of women initiating ART during pregnancy were loss by 6 months postpartum. Later gestational age at initiation and being newly diagnosed with HIV were risk factors for disengagement from ART (79). Other factors associated with loss to follow-up among pregnant women include younger age (80), initiating treatment at higher CD4 counts (76, 81), timing of presentation to ANC (82), and receiving a new HIV diagnosis during pregnancy. The strongest predictors of loss to follow-up were primiparity, WHO clinical stage 1 or 2, and receiving only 1 to 4 weeks of ART before delivery (83, 84).

1.4.2 Coverage

The universal treatment program strategy was first initiated in Malawi. Its underlying rationale was to provide a highly effective ART intervention during pregnancy and the 24-months prolonged breastfeeding period, in a setting of high fertility with limited testing for CD4 cell count (71). The early results of this strategy rapidly achieved a 6-fold increase in the number of pregnant or breastfeeding HIV-infected women starting ART (77) and a PMTCT antiretroviral coverage of >60% nationwide. Following the experience of Malawi, WHO recommended in 2015 that all HIV-positive pregnant women be provided with lifelong ART. From 2010 to 2015, a progressive switch occurred from ARV prophylaxis to lifelong ART. By 2015, the global roll-out of the universal treatment program could cover 91% of 1.1 million women receiving ART to prevent MTCT (85). By mid-2016, the universal treatment program was the ARV regimen recommended nationally in almost all countries of the world.

1.5 Options A, B/B+ and pre-exposure prophylaxis are not exclusive to each other: findings from the ANRS 12174 trial

A good counselling or a worse health status can be significant determinants of strong adherence to the universal ART coverage. Although the universal treatment program is currently globally accepted and its implementation is being scaled up, it has been deemed "not good enough" (86). Therefore, option A improved with a PreP strategy can still have some indications mainly in eliminating mother-to-child transmission. It is well established that mothers antiretroviral therapy alone, though reducing tremendously the HIV transmission risks to the child, does not put the child at zero risk of being infected. The main reason in addition to low coverage and adherence is that the viral reservoirs in breast are hardly reached by mothers' ART (28). In this scenario, infant ARV prophylaxis as currently implemented (ARV drug to infants in B+ option for 6 weeks to cover the risk of HIV related to delivery), prolonged with a PreP scheme (meaning infants take ARV drug to cover the whole period of breastfeeding duration), can add to the child's protection by reducing HIV-1 residual transmission during maternal ART. When mothers are not ready to start lifelong ART or their viral load remains still detectable despite ART, when countries are unable to achieve full implementation of universal maternal ART, option A with PreP offers an efficacious and cost-effective alternative (87). Currently PreP is targeted only to uninfected pregnant or breastfeeding women in high incidence areas, to men who have sex with men, transgender women, injecting drug users, serodiscordant heterosexual couples, and commercial sex workers. HIV-exposed children are excluded, meaning they are not specifically targeted by these preventive health measures (86).

1.6 Breastfeeding, mothers' HIV infection and BMI

Weight loss and low BMI are markers of HIV disease progression. Body weight was previously used to make a clinical diagnosis of AIDS. A 10% weight loss without any other evident cause was a clinical criterion in the diagnosis of AIDS disease in remote areas without laboratories (88-91). Individuals with asymptomatic HIV-1 infection need to increase their energy intake by 10% to maintain their body weight; this increase is assessed to up to 20-30% among those who are already at a symptomatic HIV-1 stage (92, 93).

Besides, during pregnancy and breastfeeding, in a physiological context, women's bodies undergo important energy trade-off translated externally into weight variations. Put in a simple way, this is seen as a weight gain during pregnancy, followed by a return to the pre-pregnancy weight during the lactation period (94, 95). Studies have been inconsistent in their conclusion with respect to the effects of breastfeeding on the weight and health of HIV-1 infected mothers. For instance, the Vertical Transmission Study in South Africa comparing women practicing EBF to those opting for replacement or mixed feeding found that HIV-infected and uninfected mothers experienced similar weight loss over 24 months (92). The Kesho Bora study showed that 6 months of breastfeeding was not detrimental to the weight of well-nourished HIV-1 infected mothers (96). A study in Zambia randomized women into a short-duration (4 months EBF and abrupt weaning) and a long-duration (mean duration of 16 months including 6 months of EBF) breastfeeding period. It showed a net weight gain in HIV-1 infected women breastfeeding from 4 to 24 months postpartum. Effects of lactation in women with low CD4 counts were similar to those in women with higher CD4 counts (97). In Uganda, a report also concluded that food insecurity rather than breastfeeding is associated with changes in maternal body composition (98). In contrast, 2 Kenyan studies (99, 100) found weight loss among HIV-infected breastfeeding mothers compared to mothers using formula feeding. Additional studies, including our work, may help consolidate the body of knowledge and evidence on whether breastfeeding could be advised without any fear for the lactating mothers' health, mostly when they are infected with HIV.

2. RATIONALE

Exclusive Breastfeeding, including early initiation of breastfeeding, provides a better protection compared to mixed or unsafe formula feeding with regard to the mother-to-child transmission of HIV and the other common childhood infections in LMICS contexts (101). However, global breastfeeding prevalence is currently ~37%. Nevertheless, breastfeeding is traditional practice in Sub-Saharan Africa. It is implemented diversely by mothers and only a few women actually follow the WHO guidelines for breastfeeding, including EBF (57, 102). The breastfeeding modality (exclusive or not) is closely related to postnatal HIV-1 transmission (103-105). Because breastfeeding is often the only pragmatic way to feed infants born to HIV-positive mothers, the risk of mothers passing the virus on to their child is never nil in resources-limited settings. Strategies to lower the probability of HIV vertical transmission have proven efficient among the breastfeeding population. In this thesis, we will start with a description of breastfeeding patterns among the mother-infant pairs participating in the ANRS 12174 clinical trial, since the way mothers implement breastfeeding influence the risk of HIV-1 transmission.

The breastmilk quality depends on the mother's nutritional status; breastfeeding could be detrimental to the mother with a poor nutritional status (106). In the case of moderate malnutrition, the breast milk quality is a little or marginally affected. When it comes to severe malnutrition (deprivation of >1500 calories per day), breast production diminishes and the milk concentration in some nutrients may be seriously affected. These nutrients include vitamins A, B, C and D (107).

Food and nutrition are among the root causes of the epidemic of HIV and AIDS in Sub Saharan Africa. In a body infected with HIV, good nutritional status is paramount in maintaining the immune system, managing opportunistic infections, boosting the treatments and slowing the progression of the disease (108). Most Sub-Saharan African countries are areas of endemic chronic malnutrition mainly among women and children (109, 110). These factors combined with the HIV-1 infection-associated metabolic burden may contribute to a detrimental impact of breastfeeding on maternal nutritional status and HIV-1 disease progression. The ANRS 12174 clinical trial was to assess if infant prophylaxis to HIV-1 exposed uninfected infants during the whole breastfeeding period could prevent postnatal HIV-1 transmission through breastfeeding (111). In this phase III randomized trial, we nested an assessment of the correlation between breastfeeding and HIV-1 infected mothers' disease progression.

3. STUDY AIM AND OBJECTIVES

1. Aim of the study

The aim of this thesis was to assess infant feeding practices, nutritional status and HIV-1 disease progression of lactating HIV-1-positive mothers in relation to the feeding patterns of their infants.

2. Specific objectives

The specific objectives were:

- to describe the different feeding patterns implemented by the ANRS 12174 trial participants (paper 1)
- to assess the changes in BMI and hemoglobin concentration in breastfeeding women living with HIV-1 in relation to a CD4 count of >350 cells per microliter (paper 2)
- to assess the HIV-1 disease progression (measured by the change in weight, CD4 cell count and the HIV-1 viral load or disease stage) in relation to the breastfeeding patterns (paper 3).

4. METHODS

4.1 Study designs

The study design was a cohort of mother-infant pairs included in the ANRS 12174 phase 3 randomized controlled PMTCT clinical trial. This trial compared the efficacy of infant peri-exposure prophylaxis with lopinavir/ritonavir (LPV/r) versus lamivudine to prevent HIV-1 transmission during breastfeeding (trial registration number: www.clinicaltrials.gov registration # NCT00640263). The rationale behind the trial was to administer the prophylaxis to the infant and by doing so, to spare mothers from using HAART only to prevent child's infection, and thus avoid HAART side effects and selection of resistant strains later for the mothers. It was also to fill the gaps left by countries which are not ready to implement universal ART coverage. Infant PreP may be an efficacious route to prevent residual MTCT when mothers are already on ART with detectable viral load. The ANRS 12174 study protocol has been published (111).

The participants were recruited progressively in 2 steps, initially at the antenatal care visits and after the occurrence of births on the different sites. Screening was done before the child birth; the pregnant women were contacted while attending an ANC visit between 28th and 40th week of pregnancy. They were offered a voluntary counselling and testing for HIV with the possibility of receiving the result the same day. If positive for HIV-1 they were briefed on the ANRS 12174 trial and invited to participate during the post-test counselling session. They were also advised about the different feeding options for their child. Only women willing to breastfeed or those not yet clear about the feeding option they intend to implement were referred to the research clinic for further assessment regarding their HIV-1 stage, the level of CD4 count and the other inclusion/exclusion criteria listed below. Those who clearly did not want to breastfeed were excluded.

At the screening 2 or postnatal screening that took place within the first 6 days after delivery, the women who passed the screening 1 were re-assessed with their new-borns with regard to the inclusion/exclusion criteria (see below) before the final inclusion decision. Once included, the participants were distributed to the study arms (boosted lopinavir or lamivudine group) and followed up over 50 weeks. We described and analysed data extracted from this database, considering feeding modes as an exposure variable and different outcomes, including

mothers'BMI, Hemoglobin concentration, CD4 cell count, HIV-1 viral load and status, as well as HIV-1 disease progression in general. The overview of the different papers included in this study is summarized in Table 2.

Table 2: Summary of the methods for the papers included in the thesis

Paper title	**Objective**	**Exposure**	**Outcome**	**Analytical techniques**
1. Breastfeeding patterns and its determinants among mothers living with Human Immunodeficiency Virus-1 in 4 African countries participating in the ANRS 12174 trial	To study feeding practices and the determinants of exclusive and predominant breastfeeding practices of the participants in the ANRS 12174 trial	Exclusive or predominant breastfeeding	Time to mixed feeding	Survival analysis; non-proportional hazard flexible parametric multiple regression
2. Changes in BMI and hemoglobin concentration in breastfeeding women living with HIV-1 with a CD4 count >350: Results from 4 African countries (The ANRS 12174 trial)	To study the effect of breastfeeding on mothers' BMI and hemoglobin concentration. To determine other risk factors for weight changes in HIV-1-infected mothers during lactation	Exclusive or predominant breastfeeding	Body mass index Hemoglobin concentration	Linear mixed-effect model; Bonferroni-corrected paired t-tests for multiple comparisons
3. HIV-1 disease progression in non-immuno-compromised HIV-1 infected and breastfeeding mothers participating in the ANRS 12174 clinical trial in Burkina Faso, South Africa, Uganda and Zambia: a cohort design.	To assess mothers' HIV-1 disease progression (measured by the change in weight, CD4 cell count and HIV-1 disease stage as per WHO classification) in relation to exclusive breastfeeding or Duration of any	Exclusive or predominant breastfeeding	HIV-1 disease progression	Linear mixed-effect model ; multiple logistic regression

	breastfeeding during the infant's first 6 months of life and until week 50 post-partum.			

4.2 Study population

4.2.1 Study sites

The study sites were Ouagadougou (Burkina Faso), Mbale (Uganda), East London (South Africa) and Lusaka (Zambia). Ouagadougou is the capital of Burkina Faso in West Africa. The city is located in the central part of the country on flat land covering 274, 200 km^2. At the time of the ANRS 12174 trial, Ouagadougou had 1.2 million inhabitants (112). According to the region's health authority's statistics, the frequency of ANC visits and assisted delivery were 95 and 85%, respectively. The HIV prevalence among ANC-attendees was 5.9%. The participants were recruited from peripheral antenatal clinics and referred to the research clinic based at the paediatric university teaching hospital Charles De Gaulle in the eastern part of Ouagadougou. The data on 204 mother-infants pairs were included and analysed from Burkina Faso.

In South Africa, the ANRS 12174 study was conducted in East London, located on the southeast coast in Eastern Cape Province. The HIV prevalence among pregnant women was estimated at 23% and 213 participants were included from the East London site. The HIV voluntary counselling and testing (VCT) uptake among new antenatal visit attendees was 80%. About 50% of HIV-infected women would choose exclusive breastfeeding.

Mbale is located in eastern Uganda ~200 km from Kampala, the capital, with a total population of 720, 000, of which 274 participated in the study. The general HIV prevalence was 10% among pregnant women. HIV VCT was accepted by 70% of pregnant women, 80% of those accepting the test returned for results, and 80 to 90% intended to breastfeed..

Lusaka, the capital of Zambia, was the 4th study site. Its population was estimated at 2 million, of which 534 participants were included. HIV prevalence among females aged 15-49 years was estimated at 25%; 98% of pregnant women would attend at least one ANC visit and exclusive breastfeeding rate countrywide was 26% for children aged <4 months. The characteristics of the sites are presented in the Table 3.

Table 3: Characteristics of the ANRS 12174 trial sites

	Ouagadougou (Burkina Faso)	**East London (South Africa)**	**Mbale (Uganda)**	**Lusaka (Zambia)**
Population size of catchment area	1 200 000*	267000	720 000*	2 000 000*
Population density (persons per km^2)	51.8** (2006)	39.9 (113)	124 (114)	15 (115)
Percentage of HIV in the general population (%)	2*	9 (116)	6.2 (117)	27 (118)
Percentage of HIV among ANC attending women (%)	5.9*	23*	10*	25*
ANC attendance (%)	95*	92 (119)	97 (120)	98*
Number of included cases	204	213	274	534

* source: ANRS 12174 trial protocol

**source: INSD (http://www.insd.bf/n/contenu/Tableaux/T0316.htm [accessed on 12/06/2018]

4.2.2 Study participants

- ***Eligibility criteria***

HIV-1 positive, immune-competent women, delivering HIV-1-negative infants as per DNA PCR assessment on day 7 (+/- 2 days) postpartum and opting to exclusively breastfeed during 6 months after delivery were eligible to participate in the study. Further inclusion and exclusion criteria are provided in the Table 4.

Table 4: ANRS 12174 trial eligibility criteria (87)

	Inclusion criteria	**Exclusion criteria**
Baby	is a singleton	Presents clinical symptoms and/or biological abnormalities equal to or greater than grade II of the ANRS classification for adverse event on the day of enrolment, with exceptions for hemoglobin (baby excluded if Hb <12 g/dL) and absolute neutrophil count (baby excluded if neutrophils <1200 cells/µL)
	is breastfed at day 7 by her/his mother and her/his mother intends to continue breastfeeding for at least 6 months	presents with serious congenital malformation(s)
	has a post-partum blood sample with a negative HIV-1 DNA PCR test result on day 7 (+/- 2 days)	birth weight is <2.0 kg
	has received some antiretroviral prophylaxis at birth or during the first week	is participating in another clinical trial on the day of enrolment

Mother	has reached the local legal age for participating in medical research studies	has participated in the ANRS 12174 trial for a previous pregnancy
	is HIV-1 infected (with or without HIV-2 infection) and is not eligible for HAART	is participating in another clinical trial on the day of enrolment
	has received antiretroviral prophylaxis during pregnancy or delivery	
	has an antenatal lymphocyte CD4 count above the threshold for HAART initiation in pregnant women according to the national recommendation in each site	
	resides within the study area and is not intending to move out of the area in the next year	
	gives assent for the infant to participate and gives consent to participate	

- ***Sample size***

The ANRS 12174 clinical trial sample size has been calculated taking into account its primary objective, which was to compare the efficacy of infant LPV/r (80/20 mg twice a day) vs. lamivudine (12 mg twice daily if <6 kg, 24 mg per day if 6.0 to 9.0 kg, and 36 mg per day if ≥9.0 kg) from day 7 until one week after cessation of breastfeeding (maximum recommended duration of prophylaxis: 50 weeks for a maximum duration of breastfeeding of 49 weeks) to prevent postnatal HIV-1 acquisition between 7 days and 50 weeks of age. The derived primary outcome measures were acquisition of HIV-1 (as assessed by HIV-1 DNA PCR) between day 7 and 50 weeks of age. The trial planned to include 1500 infants to cover most scenarios under the study hypotheses. However, only 1267 infants were finally included due to field constraints including lower prevalence at the time of the protocol implementation which started in November 2009. Our cohort study was a planned ancillary study of the ANRS 12174 clinical trial.

4.3 Data management and analysis

Data were collected on paper case report form or directly entered online using the Electronic Data capture system, ®OpenClinica (https://www.openclinica.com). Twenty-four hours and one-week recalls of breastfeeding practice were collected during the enrolment visit on day 7 and the following visits on weeks 2, 6 and every 4 weeks (±2) until the end of the study at week 50. In all papers, we have defined the feeding categories: 1) exclusive breastfeeding (EBF - only breast milk is given to the infant without any other kind of food or liquid except medically prescribed drugs or vitamins); 2) predominant breastfeeding (PBF - breast milk 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 - breast milk with other solid or liquid-based food including other kind of milks). EBF and PBF were further combined into "Exclusive or predominant breastfeeding group" (EPBF) as PBF presented few cases and was assessed to present the same risk as EBF with regard to postnatal HIV transmission (104).

In paper 1, we analysed survival and presented curves describing the feeding behaviour of the participants. The outcome was to stop EPBF practice before the end of the follow-up period that was set at week 22, since after this time-point, stopping EPBF would be most likely induced

by the trial requirement. The outcome was reached when the mother gave any food item out of the list of liquid-based food allowed in EPBF practice. The censoring date was the date the woman stopped EPBF whatever the reason (child's death, discontinuation from the study, lost to follow-up) or at the end of the follow-up at 22 weeks. We also explored potentially modulating factors associated with early cessation of EPBF, as well as the early weaning before 50 weeks. To this purpose, we ran a non-proportional hazard flexible parametric multiple regression analysis with duration of EPBF, or any breastfeeding as continuous variables using the "stpm2" command in Stata (121, 122). Variables with $p \leq 0.20$ in the bivariate analysis were considered for the multivariate analysis in the pooled data analysis. Other covariates included the trial arm (lopinavir/ritonavir vs lamivudine), the trial country (Burkina Faso, South Africa, Uganda or Zambia) and the maternal age categorized into 3 age groups (<25 years, 25 to 30 or 30+). The rest of the covariates were used as dichotomised variables (single vs. married or co-habiting, primi- vs. multi-para, vaginal vs. caesarean section delivery, primary vs. secondary school level or higher and breastfeeding initiation in time within the first hour postpartum vs. later). We also presented a country-specific analysis catering for contextual variation in factors associated with the feeding behaviour of the neonates. Since South Africa has a different socio-economic and cultural context, we stratified the data by creating 2 models - South Africa alone, and Uganda and Zambia together. Burkina Faso was excluded from these analyses due to the very small numbers of the events (women initiating mixed feeding in the study period before week 26). The same list of variables was used in the country-specific analysis regardless of the p-value in the bivariate analysis.

In paper 2, we considered separately 2 dependent variables, the mothers' BMI and hemoglobin concentration. Both were used as continuous variables and the main exposure variables for both was the exclusive/predominant breastfeeding or any breastfeeding duration. First, we implemented a bivariate analysis, using the Bonferroni-corrected paired t-test for multiple comparisons, comparing the baseline mean BMI (measured at day 7) with mean BMI measured at weeks 14, 26, 38 and 50. Baseline mean hemoglobin concentration (week 14) was also compared with the mean hemoglobin concentration at week 38. Then, in order to take into account the country as well as the inter-subject variability and the confounders, we fitted a linear mixed-effect model with BMI as the dependent variable and EPBF or any breastfeeding duration as the key explanatory variables. One important variable that was also considered in the multiple regression model was the socio-economic status. To build this index, 16 asset-variables per country were used in the principal component analysis method.

Similarly in paper 3, we ran linear mixed-effect models where we considered separately HIV-1-positive mothers' weight, CD4 cell count and HIV-1 viral load change in relation to EPBF duration. When the inter-country variability was not significant, we simply ran linear or logistic regression models. Then we used a multiple logistic regression model with HIV-1 disease progression as a composite variable, combining mothers' weight, CD4 cell count and HIV-1 staging as per WHO classification. HIV-1 disease progression was accelerated when CD4 cell count decreased to <350 cells/µl or the HIV-1 infection was assessed by the study physician at stage 3 or above or the mothers lost at least 10% in weight. Otherwise, HIV-1 disease progression was absent or slow. Regarding mothers' weight loss, we generated a new variable called "weight loss", which was calculated as the mothers' weight at week 26 minus the baseline weight at day 7 post-partum which was compared to the baseline weight to assess if the loss reached 10%. In these risk factor analyses, participants were stratified into two strata: stratum 1 with South Africa alone because the South African context was specific enough and different from the contexts of the stratum 2, which included Burkina Faso, Uganda and Zambia together. The socio-economic, cultural and demographic environments of these latter countries were deemed sufficiently similar to form the second stratum together.

In papers 2 and 3, potential confounders and other covariates used to build the models included the mother's viral load, CD4 count, hemoglobin concentration, BMI, HIV stage, mode of delivery (vaginal vs. C-section), parity, age, education level, marital status and occupation. The child's characteristics included gender, birth weight, treatment group and breastfeeding initiation time.

The data was collected by trained physicians, pharmacists, biologists and counsellors. We analysed the data using STATA/SE 13.1 (4905, Lakeway Drive College Station, Texas 77845 USA) statistical software.

4.4 Methodological challenges

The challenges for the completion of this study were mainly statistical. In paper 1, the issue related to executing a survival analysis with a time-varying phenomenon. We had to determine what was happening at different time-points in addition to the simple time-to-end of EBF analysis; a recurrent event as a repeated occurrence of EPBF cessation; and interval censored data, since the exact EBF cessation time was unknown. Survival curves were plotted using the Kaplan-Meier method to describe the practice of exclusive or predominant breastfeeding from birth to the introduction of complementary food, loss to follow-up or the end of the study follow-up. To solve the issue of recurrent events with some mothers showing periods of relapses of EPBF, we considered only the first event in our analysis. A mother who stopped EPBF even for one day was censored and had no possibility to return to the EPBF group. The same principle was applied to breastfeeding as a whole. For example, after cessation of breastfeeding, 62 mothers (25, 20 and 17 in South Africa, Zambia and Uganda, respectively) resumed it. In total, 54 mothers resumed breastfeeding once and stopped breastfeeding permanently and 8 resumed twice. The mean duration of all periods with resumed breastfeeding was 17.2 days. In the calculation of this mean, 5 women who had resumed breastfeeding of long duration and had been characterized as outliers were disregarded (80, 93, 97, 134 and 137 days). The mean duration of the periods with resumed breastfeeding for those who resumed once was 18.2 days (once again without the 5 women), 14.9 and 13.2 days for the first and second period, respectively, for those who resumed breastfeeding twice. In the analysis, we could see that the phenomenon of reversal of breastfeeding was not significant. Presenting the mothers' breastfeeding practice at different time-points added no new information on the findings since in our data, the decline being almost steady.

In plus of the Kaplan-Meier graph, we had to fit a model that allows the inclusion of adjusting covariates in the assessment of the factors influencing EPBF and breastfeeding practice. Since the proportional hazard assumption was invalid, we reverted to a non-proportional hazard flexible parametric multiple regression model using "stpm2" command in Stata. This model allows proportional hazard and proportional odd to be fit, and can be extended to model a time-dependent effect, which we also had to deal with in our models.

The analysis of risk factors stratified by site suffered from small sample size. Sparse cells explained some of the large discrepancies between adjusted and unadjusted hazard ratios in the

tables. To cope with the small cells issue and also to make the data more heterogeneous, we stratified the countries into 2 groups (South Africa vs. Uganda and Zambia). The data from Burkina Faso was dropped from the analysis because it was considered too homogenous with regard to every few events of interest.

In paper 2, the introduction of the socio-economic status variable implied an important challenge in the calculation of the SES index. Sixteen asset-variables were considered, including owning a bike, a fridge, a phone, a radio, a scooter, a television, or a washing machine; the material used for the dwelling roof, floor and wall, the source of drinking water, of heat used for cooking and having electricity or not, and the type of toilet. Two new assets (means of communication and house-keeping tools) resulting from the combination of owning a phone, a radio and a television, and owning a fridge and a washing machine, were created and added to these items. Since the countries involved in the study presented diverse socio-economic contexts, we stratified our SES index by country. The asset-variables were categorized and included in different ways in each analysis of a country, and were excluded each time when their distribution among the participants was too homogenous. The first component (explaining 33, 39, 29 and 34% of the variation for Burkina Faso, South Africa, Uganda and Zambia, respectively) was used to reflect the socio-economic status, and thus it was divided into tertiles.

To assess the impact of breastfeeding on mothers' BMI, we were unable to consider the mothers' calories (food) intake because of the lack of data on this variable. This was indeed an unresolved challenge that we mitigated as the consequences of our results by using the SES index in the country-stratified models. The linear mixed effect model implemented in paper 2 contributed also to mitigating the consequences of the lack of data on food intake.

This linear mixed effect model allows the inclusion of a maximum of subjects, regardless of the missing data; the differences in measurement times (although the study visits were well planned for all participants), women were counselled not to start the weaning process before eight months post-partum until a maximum of 50 weeks follow-up period); regardless of correlations resulting from different measurements in the same subject; regardless of major variabilities in the study incurred by different countries with relevant differences in socio-economic contexts; regardless of the between-subject and within-subject variabilities. All these variabilities were controlled in the random-effect part of the model.

In paper 3, our outcome was a composite endpoint, which has a lot of advantages, including a higher frequency of the study events that allows a more precise grasp of an eventual intervention effect and a reduction in the necessary sample size. However, it has also some pitfalls, the main one being how to accurately include the component endpoints so that they are of more or less of equivalent importance from a clinical point of view, that they are of equal frequency, and finally that they are similarly impacted by the study intervention. Otherwise, the interpretation of the findings may be misleading. For example, the conclusion may be that the intervention is highly efficacious, when it has only greatly reduced a less important, but highly frequent, event while the main important one was less influenced or completely uninfluenced. An example of such a misleading interpretation may be, for example, a composite endpoint including death or chest pain as the component endpoint. The intervention may be deemed efficacious when it has only reduced the occurrence of chest pain.

Our composite endpoint was set up to assess the participant progression from HIV-1 infection to AIDS disease. This disease is a syndrome and there is no test to diagnose a patient with AIDS as for malaria, meningitis or cancer. The clinician relies on a certain number of symptoms and biological markers to make a diagnosis. Therefore, our composite endpoint needed to include relevant AIDS-defining criteria. We chose as component endpoints a weight loss of >10% of the initial weight, a CD4 count <350 cells/µl, or a WHO-clinical stage of HIV infection >2. The component endpoints we used to build up our composite endpoint were coherent and sufficiently equivalent.

4.5 Ethics

For the ANRS 12174 trial, ethical clearances were obtained from the different sites, including the approval from the health research national ethic committee in Burkina Faso (n° 2008-039 on 29 may 2008), the institutional review board of Stellenbosch University in South Africa (M09/11/043), Uganda (ref: HS470), Zambia (ref: 008-02-08) and Norway (200802523-5/KST017/400). To obtain the informed consent, the participants were informed that the data may be used beyond the need of the clinical trial for further studies, and they agreed upon signed consent forms allowing the use of the data for ancillary studies.

5. Results

5.1 Study flow chart

The trial considered 3,199 pregnant HIV-1 infected women for the antenatal screening. Some 2,238 women were eligible for the post-natal screening, 1,274 eligible for the enrolment visit and 1,273 enrolled and randomized to the 2 arms of the trial. Of these, 6 randomizations were excluded afterwards because of protocol violations. Further, 42 participants were excluded from the analysis of our first paper due to a lack of breastfeeding data after the randomization. We therefore analysed a sample of 1,225 participants in paper 1, 204 from Burkina Faso, 213 from South Africa, 274 from Uganda and 534 from Zambia.

In papers 2 and 3, 9 more participants were dropped because of missing data for the initial mother's weight variable, leaving 1,216 participants' data (Burkina Faso 203, South Africa: 212, Uganda: 272 and Zambia: 529) for the analysis. The eligibility criteria were the same for all papers.

5.2 Baseline characteristics

The median (interquartile range) age, body mass index and gestational age of the mothers were 27.0 (23.3; 31.0) years, 23.7 (21.3; 27.0) kg/m² and 38.0 (38.0; 40.0) weeks, respectively. The medians CD4+ cells count, HIV-1 viral load and hemoglobin concentration were 530 (432.5; 668.5) cells/µl, 3.0 (0.8; 13.7)*10^3 copies/µl, and 12.2 (11.3; 13.1) g/dl respectively (Table 5a). The number (and percentage) of participants having completed secondary school or further was 626 (51.1%) for a total literacy rate of 1,062 (86.7%). Employed, married or cohabiting, primiparous and vaginal-delivered mothers represented 296 (24.2%), 966 (78.9%), 276 (22.5%) and 1100 (89.8%), respectively. The infant median birth weight was 3.0 (2.8; 3.3) kg. Female infants represented 594 (48.5%) and 616 (50.3%) of the children were allocated to the lamivudine arm (Table 5b).

Table 5

Table 5a: Baseline characteristics, continuous variables

Paper 2 & 3					
	Burkina Faso	**South Africa**	**Uganda**	**Zambia**	**All sites**
	N=203	**N=212**	**N=272**	**N=529**	**N=1216**
	Median (IQR[a])	**Median (IQR[a])**	**Median (IQR[a])**	**Median (IQR[a])**	**Median (IQR[a])**
Maternal age (years)	28.0 (24.3; 32.0)	27.3(23.5; 32.8)	26.9 (23.0; 29.9)	26.8 (23.3; 31.0)	27.0 (23.3; 31.0)
BMI (kg/m^2)	23.0 (21.2; 25.4)	27.8 (24.2; 31.8)	22.8 (20.8; 24.6)	23.2 (21.2; 26.5)	23.7 (21.3; 27.0)
CD4+ (cells/µL)	526 (428; 653)	505 (426; 648.5)	524.5 (429.5; 624)	548 (445; 705)	530 (432.5; 668.5)
Gestational age (weeks)	39.0 (37.0; 40.0)	38.0 (38.0; 38.0)	40.0 (39.0; 40.0)	38.0 (37.0; 40.0)	38.0 (38.0; 40.0)
HIV-1 viral load[b] (x10^3 copies/mL)	1.8 (0.7; 7.2)	2.1 (0.8; 7.9)	4.1 (0.6; 17.5)	3.8 (1.2; 20.6)	3.0 (0.8; 13.7)
Hemoglobin[c] (g/dL)	11.2 (10.5; 12.1)	12.0 (11.3; 12.9)	12.4(11.7; 13.2)	12.5 (11.6; 13.3)	12.2 (11.3; 13.1)
Birth weight (kg)	2.9 (2.7; 3.2)	3.2 (2.8; 3.5)	3.0 (2.8; 3.3)	3.0 (2.8; 3.3)	3.0 (2.8; 3.3)

[a]Interquartile range

[b]N= 173, 167, 180, 477 and 997 for Burkina Faso, South Africa, Uganda, Zambia and overall, respectively

[c] N=175, 161, 218, 454 and 1008 for Burkina Faso, South Africa, Uganda, Zambia and overall, respectively

Table 5b: Baseline characteristics: categorical variables

Paper 2 & 3					
	Burkina Faso	**South Africa**	**Uganda**	**Zambia**	**All sites**
	N=203	**N=212**	**N=272**	**N=529**	**N=1216**
	% (95% CI[a])	**% (95% CI[a])**	**% (95% CI[a])**	**% (95% CI[a])**	**% (95% CI[a])**
CD4 cell count >500	57.1 (50.2; 63.8)	51.4 (44.7; 58.1)	57.3 (51.4; 63.1)	59.9 (55.7; 64.0)	57.4 (54.6; 60.2)
Education level					
Non-completed primary	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; 38.8)
Completed primary	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 and more	24.1 (18.7; 30.5)	91.0 (86.4; 94.2)	35.7 (30.2; 41.5)	53.3 (49.0; 57.5)	51.1 (48.2; 53.9)
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)
Married/co-habiting	90.6 (85.8; 93.0)	39.1 (32.8; 45.9)	82.0 (76.9; 86.1)	88.7 (85.7; 91.1)	78.9 (76.5; 81.1)
Mode of delivery (vaginal)	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)
Parity (primiparous)	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)

Trial arm (lamivudine)	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)
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)
Child sex (male)	41.9 (35.2; 48.8)	49.1 (42.4; 55.8)	52.9 (47.0; 58.8)	48.4 (44.1; 52.7)	48.4 (45.6; 51.2)
ART prophylaxis postpartum	100.0	7.1 (4.3; 11.4)	71.0 (65.3; 76.1)	100.0	77.3 (74.9; 79.6)
Breastfeeding initiation time					
Within 1st 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)
After 1st hour and within 1st day	64.0 (57.2; 70.4)	45.7 (39.1; 52.5)	41.2 (35.5; 47.1)	18.9 (15.8; 22.5)	36.1 (33.4; 38.8)
After 1st day	29.1 (23.2; 35.7)	2.8 (1.3; 6.2)	2.9 (1.5; 5.8)	0.4 (0.1; 1.5)	6.2 (4.9; 7.7)
EPBF[b] 1st 3 days	93.1 (88.7; 95.9)	94.3 (90.3; 96.8)	97.8 (95.2; 99.0)	99.0 (97.7; 99.6)	97.0 (95.8; 97.8)
EPBF[b] last 4 days	94.1 (89.8; 96.6)	95.7 (92.1; 97.8)	97.4 (94.7; 98.8)	99.6 (98.5; 99.9)	97.5 (96.5; 98.3)
Any breastfeeding 1st week	100.0	97.6 (94.4; 99.0)	100.0	100.0	99.6 (99.0; 99.8)
EPBF[b] 1st week	96.5 (92.9; 98.3)	97.2 (93.8; 98.7)	98.9 (96.6; 99.6)	99.8 (98.7;100.0)	98.6 (97.8; 99.1)
SES[c] tertiles					
Highest tertile	31.5 (30.3; 32.8)	19.8 (18.8; 20.9)	38.2 (37.1; 39.3)	42.3 (41.5; 43.1)	
Middle tertile	39.4 (38.1; 40.7)	73.6 (72.4; 74.7)	27.9 (26.9; 29.0)	17.4 (16.8; 18.0)	
Lowest tertile	29.1 (27.9; 30.3)	6.6 (6.0; 7.3)	33.8 (32.7; 34.9)	40.3 (39.5; 41.1)	

[a]Confidence Interval

[b]Exclusive/predominant breastfeeding

[c]Socio-economic status

During pregnancy antiretroviral prophylaxis was given 100% of women in all 4 countries.

5.3 Breastfeeding patterns and its determinants among HIV-1 infected mothers (paper 1)

5.3.1 Breastfeeding patterns

In all 4 countries, 57.7% of mothers initiated breastfeeding within the first hour after delivery; 25.1% between 2 and 5 h post-delivery. In total 93.9% of the women initiated breastfeeding within the first day after birth. During the first week post-delivery, 99.0 and 95.9% of the mothers initiated any or exclusive breastfeeding, respectively (Table 5b). The food items provided as prelacteal food to the infants in the first week included water and water-based items, such as water plus sugar or glucose, water plus salt, juice, infant formula, cow's milk and liquids as part of traditional practices.

The median duration of any breastfeeding and EBF was 40.6 (IQR; 32.3; 45.4) weeks and 20.9 (IQR: 19.1; 21.1) weeks, respectively. PBF was reported only during 59 monthly visits (4/1,000 person-year). At week 22, 185 (90.7%), 104 (48.8%), 200 (73.5%) and 434 (83.1%) were exclusively or predominantly breastfeeding in Burkina Faso, South Africa, Uganda and Zambia, respectively (Figure 2, paper 1). At week 50, 24 (11.8%), 10 (4.7%), 7 (2.5%) and 17 (3.2%) were continuing to breastfeed in the same countries, respectively (Figure 3, paper 1). There were 457 (37.3%) and 456 (37.2%) infants still on EPBF at week 22 in the lamivudine and lopinavir/ritonavir arms, respectively, whereas at week 50, there were 5.1 and 4.4% of breastfed children in the lamivudine and lopinavir/ritonavir arms, respectively (Figure 4). Figures 5 and 6 present the Kaplan Meier curves by country, and trial arms for any breastfeeding and EPBF practices. In these curves, South African women showed the fastest decline among all the breastfeeding and EPBF infants.

Porridge or cereals were introduced at week 10 and 18 in Zambia and Uganda, respectively whereas in Burkina Faso they were used from week 26. Soup, meat, fish or eggs were introduced as early as week 18 in South Africa, Uganda and Zambia whereas these items were first seen in infant's food only at week 26 (0.5% for meat, fish or egg) in Burkina Faso.

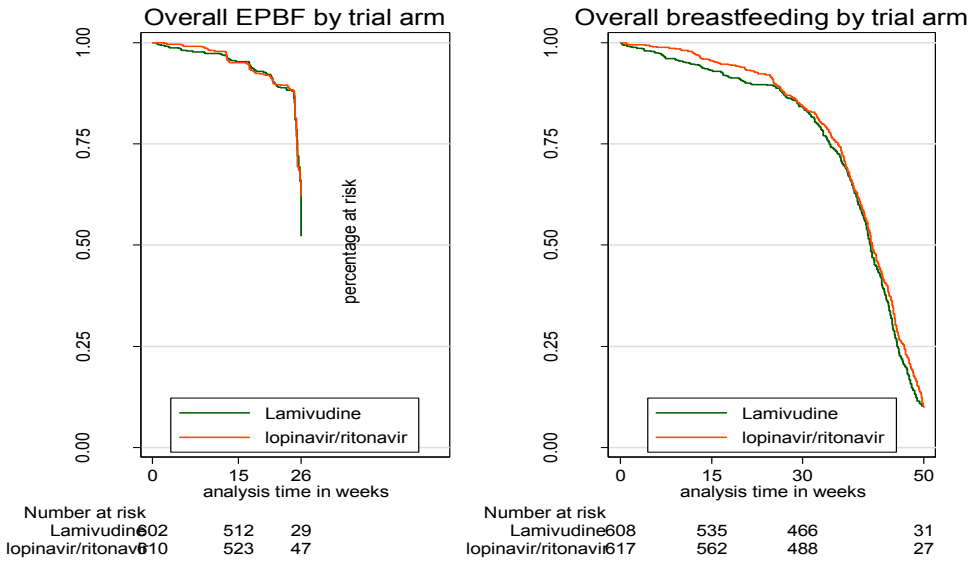


Figure 4: Infant's feeding option by trial arm

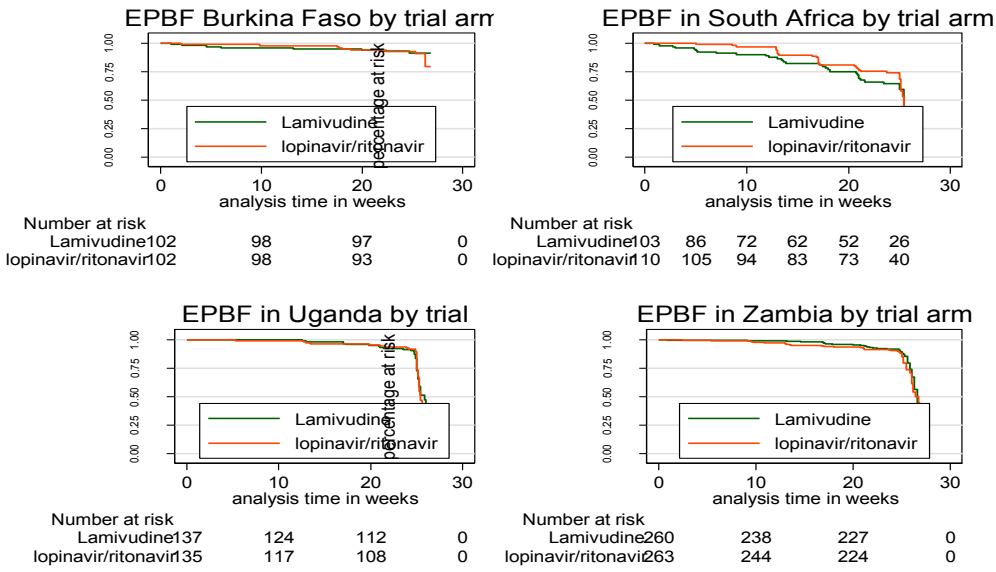


Figure 5: Exclusive or predominant breastfeeding (EPBF) by country and trial arm.

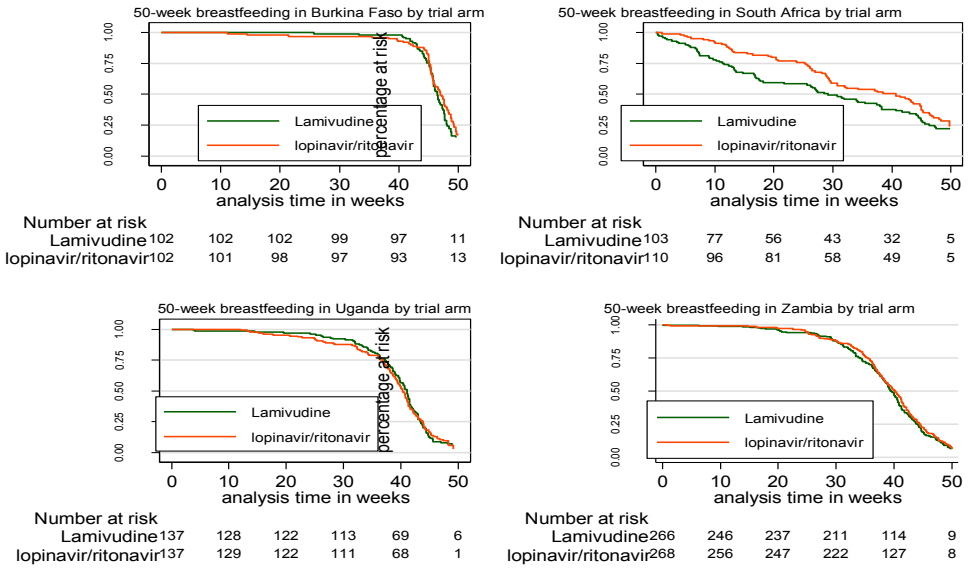


Figure 6: Any form of breastfeeding by country and trial arm

5.3.2 The determinants of breastfeeding patterns

Risk factor analysis was restricted to South Africa, Uganda and Zambia. Burkina Faso was excluded because the feeding behaviour of the women from this country was homogeneous. Table 6 summarizes the objectives, main findings and risk factors of each sub-topic of our study.

5.4 BMI changes and hemoglobin concentration (paper 2)

Overall, the BMI of breastfeeding mothers decreased from baseline to week 26 before plateauing through to week 50 (Table 2, paper 2). With the Bonferroni-corrected paired t-test for multiple comparisons regarding BMI of day 7 post-partum, overall we found a significant mean BMI (95% CI) decrease of -0.5 (-0.7; -0.4), -0.7 (-1.0; -0.3), -0.7 (-0.9; -0.4) and -0.7 (-1.0; -0.3) kg/m² at weeks 14, 26, 38 and 50, respectively. The same linear trend in the

association between breastfeeding duration and BMI decrease was found in the country-specific analysis, except in Uganda, where there was no significant decrease. The largest mean BMI decrease was found in Burkina Faso, reaching 1.1 kg/m² at week 26. In South Africa and Zambia, the maximum mean BMI decreases were -0.9 (-1.8; -0.0) and-0.6 (-0.9; -0.2) kg/m², respectively at week 38. Regarding hemoglobin concentration, no significant change was found both in overall and country-specific analysis.

Categorizing women into 3 BMI groups, we found that slimmer women (BMI <18.5 kg/m²) showed no statistically significant change in their BMI postpartum (p>0.05), whereas the other groups (BMI between 18.5 and 24.9 kg/m² and BMI≥25 kg/m²) showed a significant decrease over the breastfeeding period (Table 4, paper 2). The thinnest group of women had the steepest drop in hemoglobin concentration at week 38,but the difference was not significant -0.3 (-1.5; 0.9) g/dl; Table 5, paper 2). Overall, the multivariate analysis found no association between EPBF duration and mothers' BMI (Table 6, paper 2).

5.5 HIV-1 disease progression among HIV-1-infected mothers in relation to their feeding patterns (paper 3)

In all models, including mothers' weights, CD4 count, HIV-1 viral load or HIV-1 disease progression as outcome variables, no association was found between these outcomes and EPBF duration (Table 6). However, an increase in HIV-1 viral load was associated with any breastfeeding duration in South Africa (7.7 (95% CI: 3.4; 12.1) $*10^3$ copies/µl) and at the pooled analysis (6.1 (95%CI: 1.0; 11.2) $*10^3$ copies/µl) (Table 6, paper 3).

Table 6: study objectives, main findings and associated factors

Study objectives	Main finding	Positive determinants	Negative determinants
To describe the feeding patterns implemented by the ANRS 12174 trial participants, (paper 1)	Median duration (p25;p75) of any breastfeeding: 40.6 (32.3; 45.4) weeks		Factors prompting an early stopping of any breastfeeding: • Infant allocated to lopinavir/ritonavir arm • Participant from Uganda or Zambia • Mothers aged between 25-30 years • Mothers of secondary school level or more • Employed mothers • Delivery by c-section
	Median duration (p25;p75) of exclusive breastfeeding: 20.9 (19.1; 21.1) weeks		
	Median duration (p25;p75) of exclusive and predominant breastfeeding: 20.9 (19.7; 21.1) weeks	Factors prompting a longer duration of EPBF • Mothers from Uganda or Zambia	Factors prompting an early stopping of EPBF: • Infant allocated to lopinavir/ritonavir arm • Mothers aged between 25-30 years • Married mothers • Employed mothers • Multiparous mothers

To assess the changes in BMI in breastfeeding women living with HIV-1 with a CD4 cell count >350 cells per microliter (paper 2)	Mean decrease (95% CI) in mothers's BMI at paired t-test at: • Week 14 post-partum: -0.5 (-0.7; -0.4) kg/m² • Week 26 post-partum: -0.7 (-1.0; -0.3) kg/m² • Week 38 post-partum : -0.7 (-0.9; -0.4) kg/m² • Week 50 post-partum: -0.7 (-1.0; -0.3) kg/m²		
	Mean decrease (95% CI) in mothers's BMI per month of EPBF duration at the multivariate analysis: -0 (0; 0) kg/m²	Factors increasing the mothers' BMI during EPBF period: • Baseline BMI • CD4 count ≥500 cells/µl	Factors decreasing the mothers' BMI during EPBF period: • Mothers who completed primary school level • Married/cohabiting mothers
To assess the changes in haemoglobin concentration in breastfeeding women living with HIV-1 with a CD4 count > 350 cells per microliter (paper 2);	Mean change (95% CI) in mothers's hemoglobin concentration between weeks 14 and 38 post-partum at paired t-test: 0.1 (-0.1; 0.3) g/dl		
To assess the changes in CD4 cell count in relation to EPBF duration (paper 3)	Mean change (95% CI) in CD4 cell count per month of EPBF duration: 4.5 (−6.2 to 15.1) CD4 cells/ µl	Factors increasing the mean CD4 count at multivariate analysis:	Factors decreasing the mean CD4 count at multivariate analysis: • Mother's age

		• Mothers' baseline BMI • Mothers' hemoglobin concentration	• Breastfeeding initiation after 1 hour • HIV stage >1
To assess the change in HIV-1 viral load in relation to EPBF duration (paper 3)	Mean change (95% CI) in HIV-1 viral load per month of EPBF duration: 1.7 (−7.3 to 10.8)*10^3 copies/µl	Factors decreasing the mean viral load at multivariate analysis: • Baseline BMI • Mother's age • Delivering a female baby • Mothers of Secondary school level or more	Factors Increasing the mean viral load at multivariate analysis: • Delivery by c-section • Multiparous mothers • Infants allocated to lopinavir/ritonavir arm
To assess the HIV-1 disease progression (measured by the change in weight, CD4 cell count and the WHO's HIV-1 disease stage) in relation to EPBF duration (paper 3)	EPBF duration does not speed up HIV-1 disease progression: Adjusted OR 1.1 (95% CI: 1.0; 1.2):		Factors accelerating HIV-1 disease progression at multivariate analysis: • Single mothers • Infants allocated to lopinavir/ritonavir arm
To assess the change in CD4 cell count in relation to any breastfeeding duration (paper 3)	Mean change (95% CI) in CD4 cell count per month of any breastfeeding duration: 5.7 (0.4 to 10.9) CD4 cells/µl	Factors increasing the mean CD4 count at multivariate analysis: • Mothers' baseline BMI	Factors decreasing the mean CD4 count at multivariate analysis: • Mother's age • Mothers' HIV stage >1 • Single mothers

		• Mothers' haemoglobin concentration	
To assess the change in HIV-1 viral load in relation to any breastfeeding duration (paper 3)	Mean change (95% CI) in HIV-1 viral load per month of any breastfeeding duration: 6.1(1.0 to 11.2)*10^3 copies/µl	Factors decreasing the mean viral load at multivariate analysis: • Mothers' baseline BMI • Mother's age • Delivery of female babies • Mothers of secondary school level or further	Factors Increasing the mean viral load at multivariate analysis: • C-section delivery • Multiparous mothers • Infants allocated to lopinavir/ritonavir arm
To assess the HIV-1 disease progression (measured by the change in weight, CD4 cell count and the WHO's HIV-1 disease stage) in relation to any breastfeeding duration (paper 3)	Any breastfeeding duration does not speed HIV-1 disease progression: Adjusted OR (95% CI): 1.0 (0.9 to 1.0)	Factors slowing down HIV-1 disease progression at multivariate analysis: • Mothers of secondary school level or further	Factors accelerating HIV-1 disease progression at multivariate analysis: • Mothers' HIV stage >1 • Infants allocated to lopinavir/ritonavir arm

6. Discussion

6.1 The main findings

In general, the mothers in the ANRS 12174 phase 3 clinical trial adhered to the breastfeeding recommendations. Considered separately, no variations in the mothers' weight, CD4 cell count or HIV-1 viral load were related to EPBF or "any breastfeeding". When these outcomes are combined in a composite endpoint representing HIV-1 disease progression, there was still no variation in relation to EPBF or "any breastfeeding". Not surprisingly, the mothers' baseline BMI was consistently associated with an increase in the mothers' weight, in CD4 cell count, and a lower mean HIV-1 viral load for both EPBF, and any breastfeeding groups. At a bivariate analysis, there was a significant mean reduction of 0.7 kg/m² in mothers' BMI from 26 to 50 weeks after delivery. This was also the case for changes that occurred mainly after 26 weeks in hemoglobin concentration. Associated with the study outcomes, but in an opposite direction, was also the allocation of the babies to the lopinavir/ritonavir arm, which seemingly was associated with an acceleration of the mother's HIV-1 disease progression (AOR : 1.3 (95% CI: 1.0; 1.6)), a higher HIV-1 viral load (31.1 (95% CI: 7.1; 55.0)*10^3 copies/µl), and, though not significantly, a lower CD4 cell count (-19.2 (95% CI: -41.9; 3.6) cells/µl) and lower mothers' weight (AOR: -0.1 (95% CI: -0.7; 0.4) kg).

6.2 Discussion of findings

6.2.1 The breastfeeding pattern and its determinants among HIV-1 positive women

In spite of clear breastfeeding recommendations, only 40-43% of infants were exclusively breastfed worldwide in 2017 and 2018, and ~36% over from 2007 to 2014 (123, 124). The global estimate of EBF ranges from 22 to 37% in Sub-Saharan Africa where breastfeeding is a traditional practice, although EBF is not (52, 58, 125-128).

Recent studies conducted in the general population and among mothers living with HIV found that the proportion of women initiating breastfeeding within the first hour postpartum varied

from 40 to 70% (129-132). In our study, the country having the lowest percentage of women initiating breastfeeding within the first hour was Burkina Faso (only 6.9%).

In our trial, <10% of the participants across all sites gave prelacteal feeds compared to 22 to 57% in other studies (125, 130-132). The Burkina Faso site in our trial had the highest proportion of women giving prelacteal feeds (>16%). The same feeding patterns were found in the PROMISE-EBF trial promoting breastfeeding by peer-counsellors in the Burkina Faso rural site, with <4 and 11% of participants initiating breastfeeding within the first hour and giving prelacteal feeding, respectively, in the intervention clusters (133). In Burkina Faso, however, women breastfed longer compared to the other countries, for which we have no clear explanation. Our hypothesis is that the tradition through a cultural influence, better counselling sessions and a poorer economic context that could be accompanied with difficulties in affording quality complementary food for children, may have been important factors. More mothers in South Africa, and to some extent in Zambia, had to resume formal work after a few months, preventing them from continuing breastfeeding.

The Kesho Bora study was another PMTCT trial in overlapping countries preceding the ANRS 12174 trial, where the intervention assessed the efficacy and safety of a 3-drug ARV regimen compared with zidovudine plus single-dose nevirapine for PMTCT of HIV-1 during pregnancy, delivery and breastfeeding for women with CD4 counts of 200 and 500 cells per cubic millimetre (134). The proportion of women initiating breastfeeding in the first week and the median duration of any breastfeeding were higher in our study than that in Kesho Bora (99% and 41 weeks vs. 70% and 20 weeks, respectively). In the Kesho Bora trial, there was a lower proportion of EBF (22% at 5 months) compared to our trial (75.3% at 5 months). Even comparing the countries that participated in both the Kesho Bora study and our own trial (Burkina Faso and South Africa), the exclusive breastfeeding rate was higher in our trial. In other breastfeeding studies among HIV-infected women, from contexts similar to this trial, the median duration of EBF varied from 1.8 (135) to 5 months (129, 135, 136) and the proportion of EBF at 6 months ranged from 0 to 84% (125, 132, 134, 135, 137). Therefore one has to acknowledge the high variability of infant feeding practices in sub-Saharan Africa among HIV-positive women.

In general, the ANRS12174 participants adhered to a large extent to the WHO infant feeding recommendations. The trial setup and the frequent counselling sessions are the most likely explanation for this. Thus it is possible that frequent quality counselling sessions are required

for the promotion of safe infant feeding practices among HIV-1 positive women. However, we have some methodological concerns that will be discussed below.

Interestingly, the trial allocation was a factor associated with breastfeeding behaviour, but in South Africa alone, the lopinavir/ritonavir group had a 3 times higher risk of shorter EPBF compared to the lamivudine group. It is possible that the poor palatability of lopinavir/ritonavir influenced South African participants to stop EPBF earlier than recommended. Why this happened only in South Africa is unclear, but formula-feeding is more common in South Africa, where it has a central role in infant feeding as it was provided to HIV-infected women by the health system for almost 10 years (138). It is also possible that, due to bad taste, women in South Africa mixed the drug with other foods than milk, unlike women in Uganda and Zambia who mixed it with breastmilk. Other risk factors limiting EBF practices included age between 25 and 30 years, living as a couple, being employed, multiparity or being from South Africa. Other authors have found that older age (35-49 years) (57) favoured EBF. With regards to any breastfeeding, better educated women (i.e. having completed secondary school or beyond) were more at risk to stop breastfeeding earlier.

6.2.2 Breastfeeding and HIV-1 positive mothers' health

Mothers' nutritional status and hemoglobin concentration

The mean BMI decrease over time compared with baseline BMI at day 7 postpartum in our study (Table 2, paper 2) was generally small, and therefore is probably of limited clinical significance. The largest decrease (-1.1 kg/m²) was seen in Burkina Faso at week 26. The median BMI for this country was 23.0 (interquartile range: 21.2; 25.4) kg/m² and the mean BMI was 23.8 (95% CI: 23.2; 24.3) kg/m². Therefore, a mean decrease of 1.1 kg/m² did not push the participants into the underweight category. This finding was supported in a country-pooled analysis where the greatest decrease was seen after 50 weeks and women who were underweight (<18.5 kg/m²) had a maximum weight loss of 0.5 kg/m² (95% CI: -2.1; 1.1), a statistically insignificant estimate with low precision (Table 4, paper 2).

Our study tested a dose-response relationship by looking at the duration of EPBF and any breastfeeding, respectively, in relation to BMI change of HIV1-infected mothers. In addition, we also investigated this relationship where HIV-1 disease progression including maternal weight loss was the main outcome variable. These analyses showed no relationship between exposure and the nutritional outcomes in the women. This was also seen in a Tanzanian study

(139), which, despite its loss to follow-up, concluded that neither the modality of breastfeeding (exclusive or any), nor the duration of breastfeeding had any influence on the weight of mothers with HIV infection.

Analysis of Zambian data (97) covering the first 2 years after birth, controlling for confounding variables, such as socio-economic status, obstetric history, season and food shortage, reported a net weight gain rather than a weight loss. This has also been found in undernourished lactating mothers (140-142). Hartmann (142) explained this weight gain by a homeorhetic theory of metabolic adjustment in favour of a dominant physiological state, such as lactation. This could explain why the thinnest mothers lost no weight during the EPBF period in our cohort (Table 4, paper 2).

Some studies have compared breastfeeding and formula-feeding (99, 100), or HIV-positive with HIV-negative breastfeeding mothers (143). Unlike our findings, studies in Kenya (99, 100) and South Africa (143) found that breastfeeding was a risk factor to a faster decrease in mothers' BMI, CD4 cell count and more frequent deaths (99, 100) among HIV-positive mothers.

The literature is extensive regarding breastfeeding influence on mothers' body weight or BMI in the general population. It is assumed that when there is a decrease in lactating mothers' body weight, this is caused by an imbalance between the energy needs of the lactating mothers (energy needs of lactation plus normal energy needs) and their energy intake. Breastfeeding mothers combine different adaptive strategies, including increased food intake, mobilization of tissue stores or reducing energy expenditure by reducing physical activity (144). Breastfeeding women undergo a mild progressive weight loss in the first 6 months postpartum. The mean rates of the weight loss in those first 6 months (ranging from 0.1 kg/m²/month in resource-limited settings to 0.8 kg/ m^2/month in high income countries) is comparable to our findings as significant in the bivariate pooled analysis of the HIV-positive women (-0.7 kg/m²/month). Weight gains have also been observed (145). Considering the mothers' energy intake, usually breastfeeding mothers consume more than women who formula feed. Therefore, breastfeeding may play a role both in postpartum weight loss and even in weight gain if there is a high energy intake (145, 146).

Except in extreme cases of malnutrition, milk production in quantity and quality is scarcely affected by the mother's nutritional status (107). The priority of the biological functions goes

towards the baby's need. Therefore, even if the mother is facing deficiencies, most nutrients, such as iron, zinc, calcium and copper, continue to be adequately supplied by the breastmilk at the expense of the mother's stores. Duration and intensity of breastfeeding have a significant impact on maternal nutritional requirements, as has age, the baseline postpartum weight, and the level of activity (her metabolism). Some nutrients such as the water-soluble vitamin concentrations in breastmilk come from maternal intake, whereas the fat-soluble ones depend on maternal stores. The nutritional requirements in women through the reproductive cycle is highest during the breastfeeding period. Milk produced during the first 4 months of lactation constitutes an energy level that is equivalent to the total energy expenditure of gestation, since during the same period an infant will double the weight gained during the whole pregnancy period (107).

During the first 6 months post-delivery, 750 ml of breastmilk would be produced every day, providing a daily energy of 525 kcal to the infant. This is equivalent to 700 kcal/litre of breastmilk produced by the mother. The women's food requirement should not be mathematically balanced since a third of the energy needed to produce breastmilk comes from the mother's stores, physiologically often gained during pregnancy. When a diet provides <1800 kcal/day, these stores may be depleted beyond a pre-pregnancy state. Furthermore, low energy intake of <1500 kcal/day may lead to decreased milk supply. The total daily energy intake recommended for breastfeeding mothers ranges from 2300 to 2500 kcal to feed a single child, and 2600 to 3000 kcal for twins, on average (107). Another study estimated that the total energy cost of breastmilk production over the first 6 month period would be 117000 kcal. This energy would represent the mobilization of 13 kg of the mother's body fat if nothing changes in the energy metabolism, but in reality this is seldom observed. In the absence of other energy saving methods than physical activity, increased energy (food) intake remains the main explanation why lactating women do not lose a lot of body weight (147). Weight loss can occur in breastfeeding women when the energy intake is below the minimum requirement. This may be what we observed in Burkina Faso, probably the highest weight loss in our study. The Burkina Faso mothers breastfed more intensively than in the other countries, and their food intake may have probably been insufficient. Weight loss can be rapid when it is combined with excessive physical activity.

A consistent weakness of the studies on weight change and lactation (148) is that the mean change in the mothers' weight does not in fact convey the changes in individual participants. It has been suggested that differences in mean weight changes may be due to differences in

gestational weight gains, cultural practices, physical activity levels, seasonal food availability and desired weight loss. Other confounding factors associated with postpartum weight change include pre-pregnancy weight, age, parity, race, smoking and return to work outside the home. Gestational weight gain was by far the most consistent and strongest associated factor across these studies, whereas lactation was a minor contributor (144, 149). In a systematic review, whereas the majority of studies showed little or no association between lactation and weight change, 5 studies considered higher methodological quality reported that breastfeeding was positively associated with weight change (148). To sum up, our findings accord with the existing literature on weight change in the general population, confirming physiological weight change from lactation. There is limited evidence for other interpretations of relevance to HIV-positive women.

Regarding hemoglobin concentration, lactation is protective against iron deficiency, as breastfeeding mothers usually are in amenorrhea during the first 6 months of exclusive breastfeeding. Therefore, a net decrease up to 50% in mothers' daily iron requirement can be expected (147). In addition, iron secretion in breastmilk is very limited (147). These combined factors may be the reason why we found no decrease in hemoglobin concentration in our research.

HIV disease progression

Regarding HIV-1 disease progression in Durban, South Africa, a study found no deleterious effect of breastfeeding in HIV-1-infected mothers, in agreement with our own results (150). This study had as outcome variables CD4 and CD8 cells counts, the mothers' illness and mortality, and the hemoglobin levels. Other studies in Malawi and South Africa also found that breastfeeding, regardless of the modality, exclusive or not, was not associated with higher risk of maternal morbidity or mortality (150, 151). A study in Zambia concluded much the same direction that at 12 months after delivery there was no difference in mortality between groups that were recommended to breastfeed short (4 months) versus prolonged (68). An individual patient data meta-analysis on mortality among HIV-infected women according to the infant's feeding modality confirmed that the risk of dying within 18 months postpartum was not significantly different regarding infant's feeding modality (breastfed vs. never breastfed) (152). In a randomized trial on prolonged breastfeeding and maternal mortality in Zambia (153), there seemed to be no harmful effect of breastfeeding.

Regarding the change in CD4 cell count, the above-mentioned South African study concluded that CD4 cell count did not differ significantly between women who breastfed and those who did not (150), which is contrary to the Kenyan where the rate of CD4 cell count decline was higher in breast-feeding mothers than in mothers who never breast-fed. However, in that Kenyan study, HIV-1 RNA levels did not differ significantly between breast-feeding mothers and women feeding their babies with formula (100). Even if the Kenyan study generated many questions, it also discussed issues related to the study design and implementation, including higher baseline HIV viral load (though statistically non-significant) in the breastfeeding group, the differential implementation of follow-up of the study groups (the formula feeding group having had more interaction with the clinical team), the smaller number of events of interest, and a higher rate of loss to follow-up of up to 18% (99, 100). Given our comparable results to other HIV studies, we likewise have to discuss the specific methodological challenges of this thesis.

6.3 Discussion of the research methods

The design of the 3 papers in this thesis was a cohort-design; however, the cohort was established as a phase 3 randomized control trial (RCT), namely the ANRS 12174 trial. An RCT is in nature a cohort study with an allocation of an intervention randomly assigned to the study population. Thus, using an RCT as a cohort population requires consideration as to the success of the trial criteria specific for trials and criteria common for trials and cohort studies.

The aim of the trial has been to prevent mother-to-child transmission of HIV, thus an HIV-1 positive population of pregnant women were included. This makes any comparison of practices or non-HIV related health outcome, such as BMI, with a HIV-negative population of women impossible. HIV-positive women also had a CD4 cell count of >350 cells/µl due to the health policies of the countries at the time of intervention. The intervention was not given when the mothers were on ART for their own health, which was provided below a threshold of 350 cells/µl. Therefore the study population was a selected group of HIV-positive women enrolled into the trial cohort from antenatal care screening. Other constraints were a willingness to breastfeed, physical characteristics of the baby and informed consent to trial participation. One could argue that, given a classical cohort design including HIV-positive pregnant women, there would have been a larger variation in population characteristics, such

as health status. In that respect, the cohort study of this thesis must acknowledge its restrictive selection of participants in its final interpretation.

On the other hand, the clinical RCT design facilitated rigorous follow-up of the trial cohort, given its frequently scheduled events and tracing system in place, which are often not affordable in cohort studies. The loss of follow-up was 3.3% in the first paper and 4% in the last 2 papers over a period of 50 weeks, which makes the differential loss to follow-up bias between participation and non-participation no major concern for interpretation of the outcomes. In addition, we used statistical methods (survival analyses, non-proportional hazard flexible parametric multiple regression and linear mixed effect models) that coped with the issues of missing data, as well as the loss to follow-up.

Even if the trial intervention was not the exposure of interest in the cohort analysis, a classical RCT presentation is interested in whether the intervention is randomly allocated to the study population. In the ANRS 12174 trial, randomisation was considered successful, meaning that baseline characteristics, such as biomarkers and socio-economic characteristics, were evenly distributed between the arms. Therefore, it was unexpected that the lopinavir/ritonavir infant prophylaxis was associated with a faster HIV-1 disease progression and increased HIV-1 viral load in mothers compared to lamivudine given to the babies. We have no clear explanation for this finding at this time.

The sample size was estimated based on the trial's outcome of interest, and not for secondary outcomes or any cohort study. For the descriptive paper on breastfeeding practices, we are confident that the sample size was sufficient as we could estimate the population characteristics with high precision. Even if this was the case for the 2 analytical papers where we looked at feeding practices as exposure, and nutritional status and elements of HIV progression as an outcome, there are concerns over suboptimal sample size as to drawing any confident conclusion. There is the possibility of a type 2 error being present. In epidemiology, this is the failure to reject the H_0 hypothesis when H_0 is not true, where H_0 indicates no association between exposure and outcome. The reason for this concern was that there was limited variation in the exposure variable; most women were practicing EPBF and any breastfeeding with limited variation, and furthermore the outcome variable also showed limited variation. Regression coefficients generally indicated no association. In paper 2, no association was seen between feeding practices and women's BMI, which may be due to non-variability. In order to be more confident on the lack of any relationship between feeding

practices and mothers' health, the composite variable as an endpoint was constructed in paper 3, which reconfirmed the findings in paper 2. Thus, one can be confident from this trial cohort that no association existed between feeding practices and the investigated mothers' health outcome; however, type 2 error cannot be ruled out, as mentioned on a more important research question on the relationship between the feeding practices and HIV-1 positive mothers' health.

In addition to design issues, any study has to consider information bias. In this trial cohort, in some sites such as Burkina Faso, the nutritional counsellors were also collecting feeding data. Therefore, there was a risk of a "Hawthorne effect" or social desirability bias, which is a bias occurring when the mothers under-report non-recommended practices while emphasizing the recommended ones mainly because they know they are being "observed" (147). However, the counsellors were senior staff with considerable experience in research and knew the techniques needed to probe participants in obtaining accurate data. Furthermore, the Burkina Faso participants had the highest proportion of prelacteal feeding and delayed initiation of breastfeeding, which suggests that information bias was limited. Moreover, our findings regarding prelacteal feeding practices and breastfeeding initiation time in Burkina Faso are comparable with the PROMISE-EBF findings (48), where peer counsellors were not the same as the data collectors.

It is recommended that one considers postpartum weight change, the energy intake, the physical activity level and the intentional restriction of energy intake when studying breastfeeding and mothers' health (147). The ideal design for reliable assessment of the outcome variable would be a longitudinal study considering a baseline weight measured within the first few weeks postpartum, i.e. not too early to allow the evacuation of fluids usually retained during the early postpartum period and not too late to catch up the early changes associated with the feeding method. Subsequent weights should be measured during a follow-up period of at least one year. Our trial cohort considered weight separately and BMI only, and did not consider energy intake and physical activity. Our baseline BMI was measured on day 7 postpartum, which may not have been sufficient time for the women to stabilize their weight. The next measurement at 14 weeks was considered too late to provide baseline information. The anthropometric data were collected by trained personnel using validated equipment which has minimized information and classification bias for nutritional status. The validity of the biochemical outcome was very high as the lab procedure were certified.

As mentioned in discussing the design, there are restrictions to generalisation of the study findings. Another challenge with the trial cohort was the fixed time of 50 weeks for provision of the infant prophylaxis. This limited the generalisation of the data in particular on 'any breastfeeding'.

The international multicentre design included participants from countries in West Africa (Burkina Faso), East Africa (Uganda) and Southern Africa (Zambia and South Africa). Though the study sites inside the participating countries were mostly urban or semi-urban, the participants were deemed representative of the target population in Sub-Saharan Africa. One could argue that largely rural areas, with a poor infrastructure dominating a large part of Africa, are under-represented in this trial cohort. We introduced a socio-economic status (SES) variable that was challenging because the study environments in the 4 countries were diverse. For this reason we built a country-specific SES variable, since a pooled 4-country index was not meaningful. The clinical trial context may have also distorted the SES effect on BMI change. However, these issues were less likely to influence the association between EPBF duration and BMI change. To deal with the large differences between South Africa and the other countries, we stratified the participants into 2 strata, viz. South Africa vs. Burkina Faso, Uganda and Zambia. This stratification reduced the sample size in South Africa. Some models for South Africa could not converge well enough and the findings regarding the risk factors in this country may not reflect the reality. Furthermore, the nutritional, financial (transport costs) and technical support provided to all participants in the trial, combined with the relative homogeneity in our study population, tended to minimize the effect of disparities in general, as also those that were eventually related to food intake and physical activities. The participants generally had a good nutritional status (only 50 participants had a BMI of <18.5 kg/m²), making it representative for that group of women. Therefore we suggest that our results can be generalized to all breastfeeding HIV-1 infected and immune-competent mothers in similar settings.

6.4 Implications and recommendations

Even if the incidence of HIV transmission is reduced, MTCT continues to constitute the major reason (90% of all pediatric HIV infection) for the 1.8 million pediatric infections seen globally (154). One of the main contributing factors to the HIV incidence decline has been the roll-out of the universal treatment program (2, 155). However, as already mentioned,

suboptimal coverage and adherence remain challenges to the successful elimination of MTCT (76-78). Postnatal transmission still constitutes a large proportion of MTCT, disproportionally high in LMICs. Even if postnatal transmission is a major problem, breastfeeding promotion is held as one of the most important strategies to increase neonatal and infant survival (136, 156, 157).

This thesis supports the interpretation that EBF for 6 months and breastfeeding for a year does not harm HIV-1 positive women's nutritional status or accelerate their HIV-1 disease progression. In addition, recommended feeding practices are achievable given sufficient support and motivation. One important finding of a systematic review on the association between breastfeeding and postpartum weight change has been that the difficulty in assessing such a relationship using observational research. "Although the available evidence challenges the widely held belief that breastfeeding promotes weight loss", the authors recommended that "more robust studies are needed to reliably assess the impact of breastfeeding on postpartum weight management" (148). It is not clear what type of design that can be, because one cannot randomize for ethical reasons pregnant women into feeding options not including breastfeeding, given today's knowledge about its benefits.

Even if there is a progress, there is still a clinical and implementation research needs. The ultimate aim is viral suppression in women. The universal treatment program was implemented for its programmatic efficiency; however, other strategies may still lead towards e-MTCT, but have not yet been operationally tested. One such example is the integration of the infant PrEP within existing public health program. This could mitigate the gap in infant protection where coverage or adherence to universal treatment program is weak. Other opportunities exist with new drug regimens, administration and indication methods, and preventive options, all of which would need clinical and implementation research (28, 86, 158). Even if opportunities exist in HIV research, there is still a need to identify and tackle existing bottlenecks in the universal treatment program particularly related to adherence to ART regimen and current feeding recommendations. For instance, working women may quit breastfeeding too early as they do not manage EBF in the initial phase and fear transmission due to earlier messages (159). Other groups that are in need of specific attention are women with comorbidities, hard-to-reach groups and child and adolescent parents (160-162). Multi-disciplinary research is needed to understand and tackle the bottlenecks.

Nutritional counselling and monitoring has been on the global agenda since the Alma Ata declaration (163). Key strategies have been the baby-friendly hospital initiative in resource-limited settings. This initiative with its correlated 10 steps to successful breastfeeding is a very useful tool to achieve good breastfeeding practices (124). Promotion of breastfeeding and improved complementary feeding are 2 main nutritional specific interventions that need full implementation. Strategies to successfully deliver these interventions need contextual scientific implementation. For example, it is not clear why late initiation of breastfeeding and use of prelacteal feeding in Burkina Faso is more frequent both in rural and urban sites (134, 164). Even if evidence exists that EBF is safe, questions remain about breast milk production and composition. This is of particular relevance in situations of undernutrition such as in mothers with a BMI < 18.5. To improve breastfeeding practices and achieve the 2025 goal of 50% of EBF prevalence (165), there remains a need for clinical and implementation science in the field of infant feeding.

High-level policy commitment and broad societal support, national law and social security systems need to improve for successful achievement of safe infant feeding practices. WHO recommends 6 months of mandatory paid maternity leave as well as policies that encourage women to breastfeed in the workplace and in public (166). This could be equally recommended as policies focusing solely on behavioural aspects in the women.

Finally, a holistic approach is needed in policy adjustment in favour of breastfeeding, to embrace the nutritional issue as a whole. As stated by Webb in his publication entitled "Why nutrition must feature prominently in the post-2015 Sustainable Development Goals" (167): "Poor nutrition doesn't just represent a lack of nutritious and safe food; it derives from a host of interacting processes that link healthcare, education, sanitation and hygiene, access to resources, women's empowerment and more. We know that good nutrition leads to higher individual earnings and mental acuity, which in turn support macroeconomic and societal growth… good nutrition needs to be understood and positioned as both an input to, and an outcome of the success of the SDGs as a whole." This thesis looked at the breastfeeding situation and mothers' health in the context of HIV in Sub-Saharan Africa; it recommends continued breastfeeding support to HIV-positive women.

7. Conclusion

Improved breastfeeding practices as per WHO's recommendations have been achieved in the ANRS12174 clinical trial for the majority of the children. However, late initiation of breastfeeding and prelacteal feeding remains important issues that need to be addressed specifically during the nutritional counselling to the mothers. In our study, these practices were more pronounced in Burkina Faso.

Breastfeeding did not affect the BMI in HIV-1 infected Sub-Saharan African mothers when their CD4 counts were >350 cells/µl. However, a higher baseline BMI and a CD4 cell count >500 cells/µl led to an increase in BMI during the EPBF period. Our study showed that breastfeeding (exclusive or not) was not a risk factor for the CD4 cell count or HIV-1 viral load change, nor for the HIV-1 disease progression. Higher weight and hemoglobin concentration at baseline were important factors consistently associated with an improvement of the outcome variables at stake including the changes in the mothers' weight, CD4 cell count, HIV-1 viral load or HIV-1 disease progression. A higher education level (secondary school or further) was also a factor associated with a better HIV-1 infection status (higher weight, lower HIV-1 viral load and slower HIV-1 disease progression).

There is a need to improve breastfeeding and complementary feeding practices of children, particularly those exposed to HIV and anti-retrovirals, taking into account context and socio-demographic factors. Considering the benefits of breast milk for infants, and the recurrent 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 behave like mothers in the general population with regard to breastfeeding practices, provided that the right treatment or prophylaxis for the infection is provided.

References

1. World Health Organization, UNICEF. Guideline: updates on HIV and infant feeding: the duration of breastfeeding and support from health services to improve feeding practices among mothers living with HIV. Geneva: World Health Organization. 2016.
2. UNICEF. Options B and B+: key considerations for countries to implement an equity-focused approach; eliminating new HIV infections among children and keeping mothers living with HIV alive and well. UNICEF. 2012.
3. Wilton J. From exposure to infection: The biology of HIV transmission. http://www.catie.ca/en/pif/fall-2011/exposure-infection-biology-hiv-transmission [Acessed on 2nd December 2017]. From prevention in focus. 2011.
4. Real F, Sennepin A, Ganor Y, Schmitt A, Bomsel M. Live imaging of HIV-1 transfer across T cell virological synapse to epithelial cells that promotes stromal macrophage infection. Cell Reports 2018;23:1794–805.
5. Peterman TA, Newman DR, Maddox L, Schmitt K, Shiver S. Risk for HIV following a diagnosis of syphilis, gonorrhoea or chlamydia: 328,456 women in Florida, 2000-2011. International journal of STD & AIDS. 2015;26(2):113-9.
6. Boily M, Baggaley R, Wang L, Masse B, White R, Hayes R, et al. Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies. Lancet Infect Dis. 2009;9:118–29.
7. Sagar M. HIV-1 transmission biology: selection and characteristics of infecting viruses. J Infect Dis. 2010;202 Suppl 2:S289-96.
8. Greene W, Peterlin B. Molecular insights into HIV biology. http://hivinsite.ucsf.edu/InSite?page=kb020101 [accessed on 2nd December 2017]. HIV In Site. 2006.
9. European Study Group on Heterosexual Transmission of HIV. Comparison of female to male and male to female transmission of HIV in 563 stable couples. BMJ. 1992;304:809-13.
10. World Health Organization, UNAIDS. HIV in pregnancy: a review. WHO. 1998:63.
11. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. American journal of reproductive immunology. 2010;63(6):425-33.
12. Weinberg E. Pregnancy-associated immune suppression: risks and mechanisms. Microbial Pathogenesis. 1987;3:393-7.
13. Moran NF, Moodley J. The effect of HIV infection on maternal health and mortality. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2012;119 Suppl 1:S26-9.
14. Gorman SE. A new approach to maternal mortality: the role of HIV in pregnancy. International journal of women's health. 2013;5:271-4.
15. Calvert C, Ronsmans C. The contribution of HIV to pregnancy-related mortality: a systematic review and meta-analysis. AIDS. 2013;27(10):1631-9.
16. Zaba B, Calvert C, Marston M, Isingo R, Nakiyingi-Miiro J, Lutalo T, et al. Effect of HIV infection on pregnancy-related mortality in sub-Saharan Africa: secondary analyses of pooled community-based data from the network for Analysing

Longitudinal Population-based HIV/AIDS data on Africa (ALPHA). The Lancet. 2013;381(9879):1763-71.
17. Buchmann EJ, Mnyani CN, Frank KA, Chersich MF, McIntyre JA. Declining maternal mortality in the face of persistently high HIV prevalence in a middle-income country. BJOG : an international journal of obstetrics and gynaecology. 2015;122(2):220-7.
18. Kim HY, Kasonde P, Mwiya M, Thea DM, Kankasa C, Sinkala M, et al. Pregnancy loss and role of infant HIV status on perinatal mortality among HIV-infected women. BMC pediatrics. 2012;12:138.
19. The Working Group on Mother-To-Child Transmission of HIV. Rates of mother-to-child transmission of HIV-1 in Africa, America, and Europe: results from 13 perinatal studies. Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology. 1995;8(5):506-10.
20. Newell ML. Vertical transmission of HIV-1 infection. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000;94:1-2.
21. Kaizad R, Parikshit T, Mamatha L. Recent trends in mother to child transmission of HIV in pregnancy. J Obstet Gynecol India. 2010;60(5):395-402.
22. Bucagu M, Bizimana J, Muganda J, Humblet C. Socio-economic, clinical and biological risk factors for mother - to – child transmission of HIV-1 in Muhima health centre (Rwanda): a prospective cohort study. Archives of Public Health. 2013;71:4.
23. Kourtis AP, Bulterys M. Mother-to-child transmission of HIV: pathogenesis, mechanisms and pathways. Clinics in perinatology. 2010;37(4):721-37.
24. Newell ML. Mechanisms and timing of mother-to-child transmission of HIV-1. AIDS. 1998;12:831–7.
25. UNICEF, UNAIDS, WHO. A review of HIV transmission through breastfeeding. World Health Organization. 1998.
26. World Health Organization. WHO recommendations on the diagnosis of HIV infection in infants and children. Geneva, World Health Organization. 2010.
27. Van de Perre P, Simonon A, Msellati P, Hitimana D, Vaira D, Bazubagira A, et al. Postnatal transmission of human immunodeficiency virus type 1 from mother to infant. N Engl J Med. 1991;325(9):593-8.
28. Van de Perre P, Rubbo P, Viljoen J, Nagot N, Tylleskär T, And Lepage P. HIV-1 reservoirs in breast milk and challenges to elimination of breast-feeding transmission of HIV-1. Sci Transl Med. 2012;4(143):143sr3.
29. Rollins N, Mahy M, Becquet R, Kuhn L, Creek T, Mofenson L. Estimates of peripartum and postnatal mother-to-child transmission probabilities of HIV for use in Spectrum and other population-based models. Sex Transm Infect. 2012;88 Suppl 2:i44-51.
30. UNICEF, UNAIDS, WHO, UNFPA. HIV transmission through breastfeeding A review of available evidence. World Health Organization. 2004:32.
31. Townsend C, Cortina-Borja M, Peckham C, De Ruiter A, Lyall H, Tookey P. Low rates of mother-to-child transmission of HIV following effective pregnancy interventions in the United Kingdom and Ireland, 2000–2006. AIDS. 2008;22:973–81.
32. Idele P, Hayashi C, Porth T, Mamahit A, Mahy M. Prevention of mother-to-child transmission of HIV and paediatric HIV care and treatment monitoring: from

measuring process to impact and elimination of mother-to-child transmission of HIV. AIDS Behav. 2017;21(suppl 1):23–33.
33. UNICEF. Children and AIDS: statistical update. https://data.unicef.org/wp-content/uploads/2017/11/HIVAIDS-Statistical-Update-2017.pdf [Accessed on 13th June 2018]. 2017.
34. UNAIDS. Global AIDS update 2016. http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016 [Accessed on 17th October 2016]. UNAIDS 2016.
35. Levison J, Weber S, Cohan D. Breastfeeding and HIV-infected women in the United States: harm reduction counseling strategies. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2014;59(2):304-9.
36. Moland KM, de Paoli MM, Sellen DW, van Esterik P, Leshabari SC, Blystad A. Breastfeeding and HIV: experiences from a decade of prevention of postnatal HIV transmission in Sub-Saharan Africa. International breastfeeding journal. 2010;5:10.
37. Kagaayi J, Gray R, Brahmbhatt H, Kigozi G, Nalugoda F, Wabwire-Mangen F, et al. Survival of infants born to HIV-positive mothers, by feeding modality, in Rakai, Uganda. PloS one. 2008;3(12):e3877.
38. World Health Organization. HIV and infant feeding: framework for priority action. World Health Organization, Geneva. 2003.
39. Amibor P, Ade N. A review of infant feeding policy in Botswana within the context of best practices for the prevention of mother-to-child transmission (PMTCT) of human immunodeficiency virus (HIV): 1998 to 2012. Journal of AIDS and HIV Research. 2013;5(8):275-83.
40. Doherty T, Sanders D, Goga A, Jackson D. Implications of the new WHO guidelines on HIV and infant feeding for child survival in South Africa. Bull World Health Organ 2011;89:62–7.
41. Adegbehingbe S, Paul-Ebhohimhen V, Marais D. Development of an AFASS assessment and screening tool towards the prevention of mother-to-child HIV transmission (PMTCT) in Sub-Saharan Africa - A Delphi survey. BMC Public Health. 2012;12:402.
42. Woldegiyorgis B. Replacement feeding experiences of HIV-positive mothers in Ethiopia. Journal of Community Practice. 2012;20:69-88.
43. World Health Organization. Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: recommendations for a public health approach. World Health Organization Geneva. 2010.
44. Becquet R, Ekouevi DK, Arrive E, Stringer JS, Meda N, Chaix ML, et al. Universal antiretroviral therapy for pregnant and breast-feeding HIV-1-infected women: towards the elimination of mother-to-child transmission of HIV-1 in resource-limited settings. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2009;49(12):1936-45.
45. World Health Organization. Consolidated guidelines on HIV prevention, diagnosis, treatment and care for key populations; 2016 update World Health Organization. 2016;Geneva.
46. Saloojee H, Gray G, McIntyre J. HIV and infant feeding – one step forward, two steps back. Southern African Journal of HIV Medicine. 2011;12(4):1-11.

47. World Health Organization, UNICEF. Acceptable medical reasons for use of breast-milk substitutes. World Health Organization, Geneva. 2009.
48. Tylleskär T, Jackson D, Meda N, Engebretsen IMS, Chopra M, Diallo AH, et al. Exclusive breastfeeding promotion by peer counsellors in sub-Saharan Africa (PROMISE-EBF): a cluster-randomised trial. The Lancet. 2011;378(9789):420-7.
49. Engebretsen I, Jackson D, Fadnes L, Nankabirwa V, Diallo A, Doherty T, et al. Growth effects of exclusive breastfeeding promotion by peer counsellors in sub-Saharan Africa: the cluster-randomised PROMISE EBF trial. BMC Public Health. 2014;14:633.
50. Tumwine JK, Nankabirwa V, Diallo HA, Engebretsen IMS, Ndeezi G, Bangirana P, et al. Exclusive breastfeeding promotion and neuropsychological outcomes in 5-8 year old children from Uganda and Burkina Faso: Results from the PROMISE EBF cluster randomized trial. PloS one. 2018;13(2):e0191001.
51. Black R, Victora C, Walker S, Bhutta W, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet. 2013;382(9890):26.
52. Black R, Allen L, Bhutta Z, Caulfield L, de Onis M, Ezzati M, et al. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet. 2008;371:243–60.
53. Victora C, Bahl R, Barros A, França G, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet. 2016;387:475–90.
54. Sankar MJ, Sinha B, Chowdhury R, Bhandari N, Taneja S, Martines J, et al. Optimal breastfeeding practices and infant and child mortality: a systematic review and meta-analysis. Acta Paediatr. 2015;104(467):3-13.
55. Kramer M, Guo T, Platt R, Sevkovskaya Z, Dzikovich I, Collet J, et al. Infant growth and health outcomes associated with 3 compared with 6 mo of exclusive breastfeeding. Am J Clin Nutr. 2003;78:291–5.
56. Dewey K, Cohen R, Brown K, Rivera L. Effects of Exclusive Breastfeeding for Four versus Six Months on Maternal Nutritional Status and Infant Motor Development: Results of Two Randomized Trials in Honduras. J Nutr 2001;131:262–7.
57. Maonga A, Mahande M, Damian D, Msuya S. Factors affecting exclusive breastfeeding among women in Muheza district Tanga Northeastern Tanzania: A mixed method community based study. Matern Child Health J 2016;20:77–87.
58. Chola L, Fadnes L, Engebretsen I, Nkonki L, Nankabirwa V, Sommerfelt H, et al. Cost-effectiveness of peer counselling for the promotion of exclusive breastfeeding in Uganda. PloS one. 2015;10(11):e0142718.
59. Yalcin SS, Berde AS, Yalcin S. Determinants of exclusive breast feeding in Sub-Saharan Africa: a multilevel approach. Paediatr Perinat Epidemiol. 2016;30(5):439-49.
60. Moore ER, Anderson GC, Bergman N, Dowswell T. Early skin-to-skin contact for mothers and their healthy newborn infants. The Cochrane database of systematic reviews. 2012;5:CD003519.

61. NEOVITA Study Group. Timing of initiation, patterns of breastfeeding, and infant survival: prospective analysis of pooled data from three randomised trials. Lancet Glob Health 2016;4:e266–75.
62. World Health Organization. Essential Nutrition Actions: improving maternal, newborn, infant and young child health and nutrition. World Health Organization. 2013;Geneva.
63. World Health Organization. Preventing HIV during pregnancy and breastfeeding in the context of PreP. World Health Organization technical brief. 2017;WHO/HIV/2017.09.
64. American Academy of Pediatrics. Policy statement. Breastfeeding and the use of human milk. http://pediatrics.aappublications.org/content/129/3/e827.full.pdf+html. [Accessed 29 October 2018]. Pediatrics. 2012;129(3):e827-41.
65. American Academy of Pediatrics. Policy statement. Infant feeding and transmission of human immunodeficiency virus in the United States. Pediatrics. 2013;131(2):391-6.
66. Sinning K. The evolution of the intersection of HIV and breastfeeding in global policy. Wright State University; Dayton, Ohio. 2016.
67. Centers for Disease Control and Prevention (CDC). Current trends recommendations for assisting in the prevention of perinatal transmission of Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus and Acquired Immunodeficiency Syndrome. Morbidity and Mortality Weekly Report. 1985;34(48):721-6.
68. Kuhn L, Aldrovandi G, Sinkala M, Kankasa C, Semrau K, Mwiya M, et al. Effect of early, abrupt weaning on HIV-free survival of children in Zambia. N Engl J Med. 2008;359:130-41.
69. Fawzy A, Arpadi S, Kankasa C, Sinkala M, Mwiya M, Thea DM, et al. Early weaning increases diarrhea morbidity and mortality among uninfected children born to HIV-infected mothers in Zambia. J Infect Dis. 2011;203(9):1222-30.
70. Kuhn L, Aldrovandi GM, Sinkala M, Kankasa C, Semrau K, Kasonde P, et al. Differential effects of early weaning for HIV-free survival of children born to HIV-infected mothers by severity of maternal disease. PloS one. 2009;4(6):e6059.
71. van den Akker T, de Vroome S, Mwagomba B, Ford N, van Roosmalen J. Peripartum infections and associated maternal mortality in rural Malawi. Obstetrics and gynecology. 2011;118:266-72.
72. Van de Perre P, Tylleskar T, Delfraissy JF, Nagot N. How evidence based are public health policies for prevention of mother to child transmission of HIV? BMJ. 2013;346:f3763.
73. World Health Organization. HIV country profiles. http://www.who.int/hiv/data/profiles/en/ [Accessed on 14 October 2018]. World Health Organization, Geneva. 2017.
74. Ngarina M, Kilewo C, Karlsson K, Aboud S, Karlsson A, Marrone G, et al. Virologic and immunologic failure, drug resistance and mortality during the first 24 months postpartum among HIV-infected women initiated on antiretroviral therapy for life in the Mitra plus Study, Dar es Salaam, Tanzania. 2015;15:175.

75. Atanga P, Ndetan H, Achidi E, Meriki H, Hoelscher M, Kroidl A. Retention in care and reasons for discontinuation of lifelong antiretroviral therapy in a cohort of Cameroonian pregnant and breastfeeding HIV-positive women initiating 'Option B+' in the South West Region. Tropical Medicine and International Health. 2017;22(2):161–70.
76. Tenthani L, Haas A, Tweya H, Jahn A, van Oosterhoutf J, Chimbwandira F, et al. Retention in care under universal antiretroviral therapy for HIV-infected pregnant and breastfeeding women ('Option B+') in Malawi. AIDS. 2014;28:589–98.
77. Shaffer N, Abrams E, and Becquet R. Option B+ for prevention of mother-to-child transmission of HIV in resource-constrained settings: great promise but some early caution. AIDS. 2014;28:599–601.
78. Kieffer M, Mattingly M, Giphart A, van de Ven R, Chouraya C, Walakira M, et al. Lessons learned from early implementation of option B+: The Elizabeth Glaser Pediatric AIDS Foundation experience in 11 African countries. J Acquir Immune Defic Syndr. 2014;67:S188–S94.
79. Phillips T, Thebus E, Bekker LG, McIntyre J, Abrams EJ, Myer L. Disengagement of HIV-positive pregnant and postpartum women from antiretroviral therapy services: a cohort study. Journal of the International AIDS Society. 2014;17:19242.
80. Boyles TH, Wilkinson LS, Leisegang R, Maartens G. Factors influencing retention in care after starting antiretroviral therapy in a rural South African programme. PloS one. 2011;6(5):e19201.
81. Westreich D, Evans D, Firnhaber C, Majuba P, Maskew M. Prevalent pregnancy, biological sex, and virologic response to antiretroviral therapy. J Acquir Immune Defic Syndr. 2012;60(5):489-94.
82. Panditrao M, Darak S, Kulkarni V, Kulkarni S, Parchure R. Socio-demographic factors associated with loss to follow-up of HIV-infected women attending a private sector PMTCT program in Maharashtra, India. AIDS Care. 2011;23(5):593-600.
83. Kaplan R, Orrell C, Zwane E, Bekker LG, Wood R. Loss to follow-up and mortality among pregnant women referred to a community clinic for antiretroviral treatment. AIDS. 2008;22(13):1679-81.
84. Bengtson A, Chibwesha C, Westreich D, Mubiana-Mbewe M, Chi B, Miller W, et al. A risk score to identify HIV-infected women most likely to become lost to follow-up in the postpartum period. AIDS Care. 2016;28(8):1035–45.
85. UNAIDS. Prevention gap report. UNAIDS, Geneva. 2016.
86. Van de Perre P, Kankasa C, Nagot N, Meda N, Tumwine J, Coutsoudis A, et al. Pre-exposure prophylaxis for infants exposed to HIV through breast feeding. BMJ. 2017;356:j1053
87. Nagot N, Kankasa C, Tumwine J, Meda N, Hofmeyr G, Vallo R, et al. Extended pre-exposure prophylaxis with lopinavir–ritonavir versus lamivudine to prevent HIV-1 transmission through breastfeeding up to 50 weeks in infants in Africa (ANRS 12174): a randomised controlled trial. The Lancet. 2015;387(10018):566–73.
88. World Health Organization. Workshop on AIDS in Central Africa: Bangui, Central African Republic; 22 to 25 October 1985. WHO/CDS/AIDS. 1985;85(1).

89. World Health Organization. WHO case definitions for AIDS surveillance in adults and adolescents. Wkly epidem rec. 1994;69(37):279 - 80.
90. Organization WH. Acquired Immuno Deficiency Syndrome WHO/CDC case definition for AIDS. Wkly epidem rec. 1986;61:69 -76.
91. World Health Organization. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. World Health Organization 2007. 2007.
92. Chetty T, Carter RJ, Bland RM, Newell ML. HIV status, breastfeeding modality at 5 months and postpartum maternal weight changes over 24 months in rural South Africa. Tropical medicine & international health : TM & IH. 2014;19(7):852-62.
93. Kosmiski L. Energy expenditure in HIV infection. Am J Clin Nutr. 2011;94(6):1677S-82S.
94. Ministry of Health. Food and Nutrition Guidelines for Healthy Pregnant and Breastfeeding Women: A background paper. Wellington: Ministry of Health. 2006.
95. Kuhn L, Stein Z, Susser M. Preventing mother-to-child HIV transmission in the new millennium: the challenge of breast feeding. Paediatr Perinat Epidemiol. 2004;18:10–6.
96. Cames C, Cournil A, de Vincenzi I, Gaillard P, Meda N, Luchters S, et al. Postpartum weight change among HIV-infected mothers by antiretroviral prophylaxis and infant feeding modality in a research setting. AIDS. 2014;28(1):85-94.
97. Murnane PM, Arpadi SM, Sinkala M, Kankasa C, Mwiya M, Kasonde P, et al. Lactation-associated postpartum weight changes among HIV-infected women in Zambia. International journal of epidemiology. 2010;39(5):1299-310.
98. Widen E, Collins S, Khan H, Biribawa C, Acidri D, Achoko W, et al. Food insecurity, but not HIV-infection status, is associated with adverse changes in body composition during lactation in Ugandan women of mixed HIV status. Am J Clin Nutr. 2017:doi: 10.3945/ajcn.116.142513.
99. Nduati R, Richardson B, John G, Mbori-Ngacha D, Mwatha A, Ndinya-Achola J, et al. Effect of breastfeeding on mortality among HIV-1 infected women: a randomised trial. Lancet 2001;357:1651–55.
100. Otieno PA, Brown ER, Mbori-Ngacha DA, Nduati RW, Farquhar C, Obimbo EM, et al. HIV-1 disease progression in breast-feeding and formula-feeding mothers: a prospective 2-year comparison of T cell subsets, HIV-1 RNA levels, and mortality. J Infect Dis. 2007;195(2):220-9.
101. Berde A, and Yalcin S. Determinants of early initiation of breastfeeding in Nigeria: a population-based study using the 2013 demograhic and health survey data. BMC Pregnancy and Childbirth 2016;16:32.
102. Aishat U, David D, Olufunmilayo F. Exclusive breastfeeding and HIV/AIDS: a crossectional survey of mothers attending prevention of mother-to-child transmission of HIV clinics in southwestern Nigeria. Pan Afr Med J. 2015;21:309.
103. Tournoud M, Ecochard R, Kuhn L, and Coutsoudis A. Diversity of risk of mother-to-child HIV-1 transmission according to feeding practices, CD4 cell count, and haemoglobin concentration in a South African cohort. Tropical medicine & international health : TM & IH. 2008;13(3):310–8.

104. Becquet R, Bland R, Leroy V, Rollins N, Ekouevi D, Coutsoudis A, et al. Duration, pattern of breastfeeding and postnatal transmission of HIV: pooled analysis of individual data from West and South African cohorts. PloS one. 2009;4(10):e7397.
105. Becquet R, Ekouevi D, Menan H, Amani-Bosse C, Bequet L, Viho I, et al. Early mixed feeding and breastfeeding beyond 6 months increase the risk of postnatal HIV transmission: ANRS 1201/1202 Ditrame Plus, Abidjan, Côte d'Ivoire. Preventive medicine. 2008;47 27–33.
106. World Health Organization. The quantity and quality of breast milk: report on the WHO collaborative study on breast milk. World Health Organization, Geneva. 1985.
107. Segura S, Ansótegui J, Díaz-Gómez N, en representación del Comité de Lactancia Materna de la Asociación Espa˜nola de Pediatría. The importance of maternal nutrition during breastfeeding: Do breastfeeding mothers need nutritional supplements? An Pediatr (Barc). 2016;84:347.e1-.e7.
108. Fanzo J. The nutrition challenge in Sub-Saharan Africa. UNDP; Regional Bureau for Africa. 2012.
109. Beiersmann C, Bountogo M, Tiendrebeogo J, Louis VR, Gabrysch S, Ye M, et al. Malnutrition in young children of rural Burkina Faso: comparison of survey data from 1999 with 2009. Tropical medicine & international health : TM & IH. 2012;17(6):715-21.
110. Müller O, Garenne M, Kouyate B, and Becher H. The association between protein–energy malnutrition, malaria morbidity and all-cause mortality in West African children. TMIH. 2003;8(6):507–11.
111. Nagot N, Kankasa C, Meda N, Hofmeyr J, Nikodem C, Tumwine J, et al. Lopinavir/Ritonavir versus Lamivudine peri-exposure prophylaxis to prevent HIV-1 transmission by breastfeeding: the PROMISE-PEP trial Protocol ANRS 12174. BMC Infect Dis. 2012;12:246.
112. Ministère de l'économie et des finances Burkina Faso. Recensement général de la population et de l'habitation de 2006: Résultats définitifs. Ouagadougou. 2008.
113. United Nations Economic Commission for Africa, UNFPA. The Demographic Profile of African Countries. Economic Commission for Africa, Addis Ababa, Ethiopia. 2016.
114. Uganda Bureau of Statistics, Macro International Inc. Uganda: Demographic and Health Survey 2006. Kampala, Uganda. 2007.
115. FAO. Irrigation in Africa in figures – AQUASTAT Survey 2005. Aquastat. 2005.
116. Phaswana-Mafuya N, Seager J, Peltzer K, Jooste S, Mkhonto S. Social Determinants of HIV in the Eastern Cape [Report]. HSRC, SAHARA. 2010:66.
117. The Revised National Strategic Framework for HIV/AIDS Activities in Uganda: 2003/04 –2005/06. The Revised National Strategic Framework for HIV/AIDS Activities in Uganda: 2003/04 – 2005/06. Republic of Uganda. 2004.
118. U.S. Census Bureau. HIV/AIDS profile, Zambia https://www.census.gov/population/international/files/hiv/zambia08.pdf [Accessed on 25 October 2017]. 2006.
119. Department of Health, Medical Research Council, OrcMacro. South Africa Demographic and Health Survey 2003. Pretoria, Department of Health. 2007.

120. Karamagi CA, Tumwine JK, Tylleskar T, Heggenhougen K. Antenatal HIV testing in rural eastern Uganda in 2003: incomplete rollout of the prevention of mother-to-child transmission of HIV programme? BMC international health and human rights. 2006;6:6.
121. Lambert PC, Royston P. Further development of flexible parametric models for survival analysis. Stata J. 2009;9(2):265–90.
122. Royston P. Flexible parametric alternatives to the Cox model: update. Stata J. 2004;4(1):98–101.
123. World Health Organization. Exclusive breastfeeding rate, by country, 2018. https://data.unicef.org/topic/nutrition/infant-and-young-child-feeding/ [Accessed on 25 October 2018]. World Health Organization, Geneva. 2018.
124. World Health Organization. National implementation of the baby-friendly hospital initiative 2017. World Health Organization, Geneva. 2017.
125. Engebretsen IM, Wamani H, Karamagi C, Semiyaga N, Tumwine J, Tylleskar T. Low adherence to exclusive breastfeeding in Eastern Uganda: a community-based cross-sectional study comparing dietary recall since birth with 24-hour recall. BMC pediatrics. 2007;7:10.
126. Nor B, Ahlberg B, Doherty T, Zembe Y, Jackson D, Ekström E, et al. for the PROMISE-EBF Study Group. Mother's perceptions and experiences of infant feeding within a community-based peer counselling intervention in South Africa. Maternal & child nutrition. 2012;8:448–58.
127. Buskens I, Jaffe A, Mkhatshwa H. Infant feeding practices: realities and mindsets of mothers in Southern Africa. AIDS Care. 2007;19(9):1101 - 9.
128. Doherty T, Sanders D, Jackson D, Swanevelder S, Lombard C, Zembe W, et al. Early cessation of breastfeeding amongst women in South Africa: an area needing urgent attention to improve child health. BMC pediatrics. 2012;12:105.
129. Njom Nlend A, and Ekani B. Preliminary assessment of breastfeeding practices in HIV 1-infected mothers (prior to weaning) under the Djoungolo programme on the prevention of mother-to-child transmission of HIV. J Trop Pediatr. 2010;56(6):436-9.
130. Kamau-Mbuthia E, Elmadfa I, Mwonya R. The impact of maternal HIV status on infant feeding patterns in Nakuru, Kenya. J Hum Lact. 2008;24(1):34-41.
131. Fadnes LT, Engebretsen IM, Wamani H, Semiyaga NB, Tylleskar T, Tumwine JK. Infant feeding among HIV-positive mothers and the general population mothers: comparison of two cross-sectional surveys in Eastern Uganda. BMC Public Health. 2009;9:124.
132. Gewa C, Oguttu M, and Savaglio L. Determinants of early child-feeding practices among HIV-infected and non-infected mothers in rural Kenya. J Hum Lact. 2011;27(3):239 –49.
133. Engebretsen I, Nankabirwa V, Doherty T, Diallo A, Nankunda N, Fadnes L, et al. Early infant feeding practices in three African countries: the PROMISE-EBF trial promoting exclusive breastfeeding by peer counsellors. Int Breastfeed J. 2014;9:19.
134. Bork K, Cames C, Cournil A, Musyoka F, Ayassou K, Naidu K, et al. Infant Feeding Modes and Determinants Among HIV-1– Infected African Women in the Kesho Bora Study. J Acquir Immune Defic Syndr. 2013;62:109–18.

135. Young SL, Israel-Ballard KA, Dantzer EA, Ngonyani MM, Nyambo MT, Ash DM, et al. Infant feeding practices among HIV-positive women in Dar es Salaam, Tanzania, indicate a need for more intensive infant feeding counselling. Public Health Nutr. 2010;13(12): 2027–33.
136. Coovadia H, Rollins N, Bland R, Little K, Coutsoudis A, Bennish M, et al. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. Lancet. 2007;369:1107-16.
137. Kuhn L, Sinkala M, Kankasa C, Semrau K, Kasonde P, Scott N, et al. High uptake of exclusive breastfeeding and reduced early post-natal HIV transmission. PloS one. 2007;2(12):e1363.
138. Kuhn L, Kroon M. Breastfeeding and the 2015 South African guidelines for prevention of mother-to-child transmission of HIV. South Afr J HIV Med. 2015;16(1):Art. #377.
139. Sedgh G, Spiegelman D, Larsen U, Msamanga G, and Fawzi W. Breastfeeding and maternal HIV-1 disease progression and mortality. AIDS 2004;18:1043–9.
140. Winkvist A, Rasmussen K, and Lissner L. Associations between reproduction and maternal body weight: examining the component parts of a full reproductive cycle. Eur J Clin Nutr. 2003;57:114–27.
141. Ladner J, Castetbon K, Leroy V, Nyiraziraje M, Chauliac M, Karita E, et al. Pregnancy, body weight and human immunodeficiency virus infection in African women: a prospective cohort study in Kigali (Rwanda), 1992-1994. International journal of epidemiology. 1998;27:1072-7.
142. Hartmann P, Sherriff J, and Mitoulas L. Homeostatic mechanisms that regulate lactation during energetic stress. J Nutr. 1998;128:394S–9S.
143. Papathakis P, Van Loan M, Rollins N, Chantry C, Bennish M, and Brown K. Body composition changes during lactation in HIV-Infected and HIV-uninfected South African Women. J Acquir Immune Defic Syndr. 2006;43:467–74.
144. Butte N, Hopkinson J. Body composition changes during lactation are highly variable among women. J Nutr. 1998;128:381S– 5S.
145. Manning-Dalton C. The effects on lactation on energy and protein consumption , postpartum weight change and body composition of well nourished north american women. Nurtrition research. 1983;3:293-308.
146. Brewer M, Bates M, Vannoy L. Postpartum changes in maternal weight and body fat depots in lactating vs nonlactating women. Am J Gun Nutr 1989;49:259 - 65.
147. Dewey KG. Impact of breastfeeding on maternal nutritional status Department of Nutrition, One Shields Avenue, . 2004;University of Califomia, Davis, CA 95616-8669.
148. Neville CE, McKinley MC, Holmes VA, Spence D, Woodside JV. The relationship between breastfeeding and postpartum weight change; a systematic review and critical evaluation. Int J Obes (Lond). 2014;38(4):577-90.
149. Haiek L, Kramer M, Ciampi A, Tirado R. Postpartum weight loss and infant feeding. J Am Board Fam Pract 2001;14:85–94.
150. Coutsoudis A, Coovadia H, Pillay K, Kuhn L. Are HIV-infected women who breastfeed at increased risk of mortality? AIDS. 2001;15(5):653 -5.

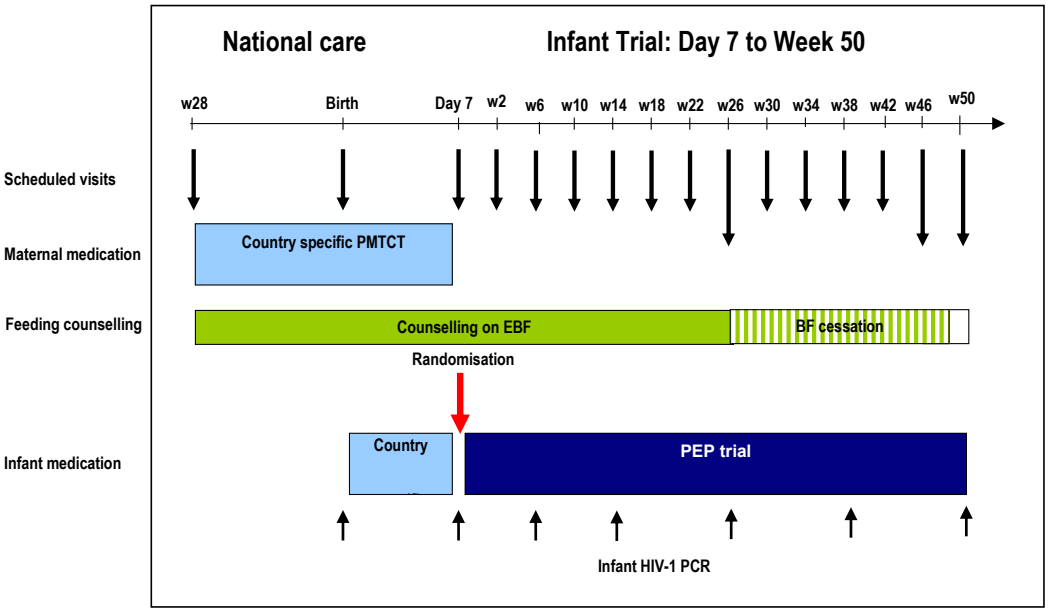
151. Taha T, Kumwenda N, Hoover D, Kafulafula G, Fiscus S, Nkhoma C, et al. The impact of breastfeeding on the health of HIV-positive mothers and their children in sub-Saharan Africa. Bull World Health Organ. 2006;84:546-54.
152. Breastfeeding and HIV International Transmission Study Group. Mortality among HIV-1–infected women according to children's feeding modality; an individual patient data meta-analysis. J Acquir Immune Defic Syndr. 2005;39:430–8.
153. Kuhn L, Kasonde P, Sinkala M, Kankasa C, Semrau K, Vwalika C, et al. Prolonged breast-feeding and mortality up to two years post-partum among HIV-positive women in Zambia. AIDS 2005;19:1677–81.
154. Lindegren M, Steinberg S, Byers R. Epidemiology of HIV/AIDS in children. Pediatric clinics of North America. 2000;47(1):1-20.
155. Etoori D, Kerschberger B, Staderini N, Ndlangamandla M, Nhlabatsi B, Jobanputra K, et al. Challenges and successes in the implementation of option B+ to prevent mother-to-child transmission of HIV in southern Swaziland. BMC Public Health. 2018;18(1):374.
156. Cournil A, De Vincenzi I, Gaillard P, Cames C, Fao P, Luchters S, et al. Relationship between mortality and feeding modality among children born to HIV-infected mothers in a research setting: the Kesho Bora study. AIDS. 2013;27(10):1621-30.
157. Cournil A, Van de Perre P, Cames C, de Vincenzi I, Read JS, Luchters S, et al. Early infant feeding patterns and HIV-free survival: findings from the Kesho-Bora trial (Burkina Faso, Kenya, South Africa). The Pediatric infectious disease journal. 2015;34(2):168-74.
158. Coovadia H, Moodley D. Improving HIV pre-exposure prophylaxis for infants. http://dx.doi.org/10.1016/S0140-6736(15)00983-6 [Accessed 27 January 2016]. The Lancet. 2015.
159. Murtagh L, and Moulton A. Governement, politics and law: strategies to protect vulnerable populations; working mothers, breastfeeding, and the law. Am J Public Health. 2011;101:217– 23.
160. Smith P, Coley S, Labbok M, Cupito S, Nwokah E. Early breastfeeding experiences of adolescent mothers: a qualitative prospective study. International breastfeeding journal. 2012;7:13.
161. Hunter L, Magill-Cuerden J. Young mothers' decisions to initiate and continue breastfeeding in the UK: tensions inherent in the paradox between being, but not being able to be seen to be, a good mother. Evidence Based Midwifery 2014;12(2):46-51.
162. Kendall-Tackett K. Mental health and the breastfeeding mother. https://womenshealthtoday.blog/2017/04/07/mental-health-and-the-breastfeeding-mother/ [Accessed on 27 October 2018]. Women's Health Today. 2018.
163. The International Conference on Primary Health Care. Declaration of Alma-Ata. http://www.who.int/publications/almaata_declaration_en.pdf [Accessed on 27 October 2018]. Alma-Ata, USSR. 1978.
164. Somé E, Engebretsen I, Nagot N, Meda N, Lombard C, Vallo R, et al. Breastfeeding patterns and its determinants among mothers living with Human Immuno-deficiency Virus -1 in four African countries participating in the ANRS 12174 trial. International breastfeeding journal. 2017;12:22.

165. World Health Organization. Comprehensive implementation plan on maternal, infant and young child nutrition. World Health Organization, Geneva. 2014.
166. World Health Organization, UNICEF. Global nutrition targets 2025 breastfeeding policy brief. World Health Organization, Geneva. 2014.
167. Webb P. Why nutrition must feature prominently in the post2015 Sustainable Development Goals. [Blog] https://wwwglopanorg/news/why-nutrition-must-feature-prominently-post-2015-sustainable-development-goals [Accessed on 14 October 2018]. 2018.

Appendices:

7.1 Appendix 1. ANRS 12174 schematic trial design

Multicentre randomised controlled clinical trial



7.2 Appendix 2. Flow chart of the study outlining follow-up visits and assessments at each visit

	Screening 1 (ante-natal)	**Screening 2 (post-delivery)**	**D 7**	**W 2**	**W 6**	**W 10**	**W 14**	**W 18**	**W 22**	**W 26**	**W 30**	**W 34**	**W 38**	**W 42**	**W 46**	**W 50**
Acceptable time shift (days)		1-6	± 2	± 4	± 7	± 7	± 7	± 7	± 7	± 7	± 7	± 4	± 4	± 7	± 7	± 7
Mother visits																
- Informed consent	X	X	X													
- Eligibility of mother	X	X	X													
- Follow up visits Interview (mother)				X	X	X	X	X	X	X	X	X	X	X	X	X
- Clinical assessment	X		X				X						X			X
- Infant feeding counselling	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lab tests on mother:																
- HIV-1 serology	X															
FBC							X						X			
- CD4	X						X						X			
- Plasma HIV-1 RNA			X				X						X			
- Stored plasma (HIV-1 genotyping)			X		X		X			X			X			
- Breast milk (storage)			X		X		X			X			X			
Infant																
- Eligibility of infant		X	X													
- Randomization			X													
- Clinical assessment, AE, SAE & anthropometry			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lab tests on infant:																
- HIV-1 DNA PCR and stored DBS		X	*		X		X			X			X			X
- ALT, FBC**, creat, natremia, kalemia, stored blood		X			X					X			X		**	**
- Cholesterol, HDL cholesterol, LDL cholesterol, triglycerides					X								X			
- Plasma 3TC and LPV/r		X			X								X			

* Collect 5 spots on DBS card for storage. Only perform HIV DNA PCR if HIV DNA PCR is positive at W6

FBC = Full blood count

** Only if the baby is still on study treatment or if alteration of ALT, FBC, creat, natremia, kalemia at last sample

Paper I

Eric N. Somé, Ingunn M. S. Engebretsen, Nicolas Nagot, Nicolas Meda, Carl Lombard, Roselyne Vallo, Marianne Peries, Chipepo Kankasa, James K. Tumwine, G. Justus Hofmeyr, Mandisa Singata, Kim Harper, Philippe Van De Perre, Thorkild Tylleskar for the ANRS 12174 Trial Group. **Breastfeeding patterns among mothers living with HIV in Sub Saharan Africa: the ANRS 12174 trial.** International Breastfeeding Journal 2017; 12

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RESEARCH Open Access

Breastfeeding patterns and its determinants among mothers living with Human Immuno-deficiency Virus -1 in four African countries participating in the ANRS 12174 trial

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Abstract

Background: HIV-1 transmission rates have been reduced over the last decade, an estimated 2 million new infections per year arise, including 220,000 paediatric cases. The main post-natal HIV exposure is through breastfeeding, where both its duration and modality (exclusive or not) are associated with postnatal transmission. The ANRS 12174 trial compared HIV-1 postnatal transmission of 2 prophylaxis drugs for infants during lactation (lamivudine and lopinavir-ritonavir). Our objective has been to examine the feeding practices and the determinants of exclusive/ predominant (EPBF) or any breastfeeding among the participants of this trial in Burkina Faso, South Africa, Uganda and Zambia.

Methods: Mothers infected with HIV-1 and their uninfected offspring were followed from day 7 after birth for 50 weeks, keeping monthly records of their feeding patterns. Feeding was classified into 3 categories: 1) exclusive breastfeeding during the first six months, only breast-milk being given to infant for 6 months, 2) predominant breastfeeding, breast-milk with liquid-based items being given, and 3) mixed feeding, other non-breast milk or solid food being given in addition to breast milk with or without liquid-based items. The categories were merged into 2 groups: EPBF applying to infants aged <6 months and mixed feeding applying to infants of any age. The feeding patterns have been given as Kaplan-Meier curves. A flexible parametric multiple regression model was used to identify the determinants of the mothers' feeding behaviour.

(Continued on next page)

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Results: A total of 1,225 mother-infant pairs provided feeding data from Burkina Faso ($N = 204$), South Africa ($N = 213$), Uganda ($N = 274$) and Zambia ($N = 534$) between November 2009 and March 2013. The mean maternal age was 27.4 years and the mean BMI was 24.5. 57.7 and 93.9% of mothers initiated breastfeeding within the first hour and first day, respectively. Overall, the median durations of any form of breastfeeding and EPBF were 40.6, and 20.9 weeks, respectively. Babies randomized to the lopinavir/ritonavir group in South Africa tended to do less EPBF than those in the lamivudine group.
Overall the group of mothers aged between 25 and 30 years, those married, employed or multiparous tended to stop early EPBF. Mothers living in Uganda or Zambia, those aged between 25 -30 years, better educated (at least secondary school level), employed or having undergone C-section stopped any breastfeeding early.

Conclusions: There is a need to improve breastfeeding and complementary feeding practices of children, particularly those exposed to HIV and anti-retrovirals, taking into account context and socio-demographic factors.

Trial registration: Clinical trial registration: NCT00640263.

Keywords: HIV infection, Exclusive breastfeeding, Vertical transmission, Prevention, Sub Saharan Africa, Risk factors, Cohort study

Background

Worldwide, there are 2.6 million children <15 years old living with the Human Immuno-deficiency Virus (HIV). Mother-to-child transmission of HIV-1 (MTCT) through pregnancy, childbirth and breastfeeding are the main routes of transmission according to estimates made in 2015 through the United Nations programme on HIV/AIDS (UNAIDS) [1]. Even if transmission rates have been reduced over the last decade, an estimated 2 million new HIV-1 infections occur per year including 220,000 paediatric cases [1]. Improved prevention of mother-to-child transmission of HIV-1 (PMTCT) strategies and programme implementation strengthening are therefore needed. Postnatal HIV-1 exposure can be avoided by replacement feeding, but this has been detrimental in settings with a high child mortality [2–4]. Non-breastfed children will also be deprived of the many known benefits of breastfeeding - better survival rates, and immunological and nutritional status [5–9]. Developmental [10] benefits include bonding of the mother-infant dyad, natural spacing of pregnancies [11] and cultural acceptability [12]. Mixed feeding seems to be the most risky option during the first 6 months of life regarding HIV transmission for infants born to mothers living with HIV-1 where there is no antiretroviral therapy (ART) [13–15]. Reasons for mixed feeding include social, cultural, tradition and individual factors of the mother [16–19]. Breastfeeding is traditional in Sub-Saharan Africa, but exclusive breastfeeding (EBF) is not [16, 19–24]. The prevalence of EBF ranges from 22 to 37% in this region, whereas it is estimated globally to be 35% [25, 26]. Factors known to negatively influence the practice of EBF are a) late initiation of breastfeeding (after one h postnatally), b) not giving colostrum, and c) provision of pre-lacteal feeds [25, 27, 28]. By giving ART to pregnant or breastfeeding women, postnatal HIV-transmission can be substantially reduced [29].

For these reasons, the World Health Organization (WHO) currently recommends HIV-infected pregnant women to breastfeed when formula is unsafe, i.e. as in most of Sub-Saharan Africa. At the time of the trial, exclusive breastfeeding for 6 months was recommended and thereafter, to introduce complementary food while continuing breastfeeding up to a year or until safe replacement feeding could be provided [30]. Potential strategies included the infant's protection during the breastfeeding period, either by ART to the mother (option B or B+) or peri-exposure prophylaxis to the child (option A) [29–31]. However, maternal ART does not entirely eliminate postnatal MTCT, probably because of the persistence of a residual stable CD4+ T cell-associated reservoir of HIV-1 in breast milk [32]. Thus prolonged infant prophylaxis covering the entire recommended breastfeeding period was tested in the ANRS 12174 trial [33]. The trial aimed to compare the efficacy of lopinavir/ritonavir versus lamivudine to prevent the mother to child transmission of HIV-1 during breastfeeding period. The postnatal transmission rates were 1.4% (95% confidence interval [CI]; 0.4;2.5) and 1.5% (CI 0.7;2.5), respectively, in the lopinavir/ritonavir and lamivudine arms [34]. However, as breastfeeding is the main post-natal HIV exposure, and because its duration and modality (exclusive or not) are associated with postnatal transmission, we examined feeding practices and the determinants of exclusive and predominant breastfeeding practices of all participants in this trial.

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 [33, 34]. Briefly, HIV-1 infected pregnant women at the time ineligible for highly active antiretroviral therapy (HAART) because their CD4 count was >350 cells/mm3, aged 18 or above, planning to breastfeed and with between 28 and 40 weeks of amenorrhea, were identified from antenatal clinics. They received a pre-test counselling session before being tested for HIV infection. As part of the post-test session, they were informed of the different feeding options available for their babies. Only women wanting 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 site-stratified randomisation at day 7 postpartum. From 28 weeks of pregnancy to day 7 after birth, programmatic mother to child transmission prophylaxis was implemented according to national guidelines, but mainly with antenatal zidovudine, an intrapartum single dose nevirapine and zidovudine-lamivudine for mothers and nevirapine for infants for 7 days postpartum. We excluded twins and triplets, infants positive in the 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 DAIDS classification for adverse events) on the day of enrolment [35].

The intervention was infant prophylaxis in the breastfeeding period plus one week from day 7 to 50 weeks of age with either lopinavir/ritonavir or lamivudine. Lamivudine is generally well tolerated and accepted; it has been widely used in research and programs. Lopinavir/ritonavir paediatric formulation has been a promising prophylactic drug with low risks for resistance, high antiviral potency and a good safety profile [33]. However, it has poor palatability, which mattered less when introduced very early. Breastfeeding recalls of 24-h and one week were collected during the enrolment visit at day 7 ± 2 days after birth, and the 13 monthly scheduled follow-up visits starting at week 2. Prelacteal feeding data (which we defined as any food items except mothers' milk given to infants before mothers initiated breastfeeding) were also collected at enrolment.

Data management and analysis

Data were collected on case report forms or directly entered online using the Electronic Data capture system, viz. OpenClinica® (https://www.openclinica.com). Based on the data collected at each visit, we categorized mothers into one of the following groups: 1) exclusive breastfeeding (EBF - only breast milk being given to the infant without any other kind of food or liquid except medically prescribed drugs or vitamins); 2) predominant breastfeeding (PBF - breast milk 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 - breast milk with other solid or other kind of milks with or without liquid-based food). We thereafter combined EBF and PBF into one group called "exclusive and predominant breastfeeding" (EPBF) as PBF presented few cases and was assessed to present the same risk than EBF at least with regard to postnatal HIV transmission [15]. The entire cohort was in this EPBF group at the beginning of the study and were followed up to detect any change to mixed feeding or non-breastfeeding by week 26 post-partum, the time when it is recommended to change from exclusive breastfeeding to complementary feeding. Any breastfeeding was defined as women breastfeeding whatever the pattern (exclusive, predominant or mixed feeding). Any breastfeeding applied during the whole duration of the study from day 7 to week 50 and was opposed to non-breastfeeding. We have also included women who stopped breastfeeding and eventually resumed it. We determined the number and mean duration of the periods with resumed breastfeeding between day 7 and week 50 postpartum.

For continuous variables, the median with interquartile range (IQR) were reported, and percentages for categorical variables. Comparison across categorical variables was done using the Chi-squared test. We described the participants' feeding patterns using Kaplan-Meier survival curves for each country. The censoring date was the date of the child's death, discontinuation, the last EPBF date or 22 weeks for those who completed the study without reporting any mixed feeding. The exit date for survival analysis was set at week 22, since prior to this time-point any stopping of EPBF was assumed to be participant-driven whereas at week 26 or later, it was induced by the trial requirements.

We also explored potentially modulating factors associated with early cessation of EPBF, as well as the early weaning before 50 weeks in a non-proportional hazard flexible parametric multiple regression analysis with duration of EPBF, or any breastfeeding as continuous variables using the "stpm2" command in Stata [36, 37]. Variables with a $p \leq 0.20$ in the bivariate analysis were considered for the multivariate analysis in the pooled-data analysis of all the countries. Other covariates included the trial arm, trial country and the maternal age in 3 age groups (<25 years, 25 to 30 and 30+). The rest of the covariates were used as dichotomised variables (single vs. married or co-habiting, primi- vs. multipara, vaginal vs. caesarean section delivery, primary vs. secondary school or higher, breastfeeding initiation in

time within the first hour postpartum vs. later. We also presented a country-specific analysis catering for contextual variation in factors associated with the feeding behaviour of the neonates. Since South Africa has a different socio-economic and cultural context, we stratified the data by creating 2 models - South Africa alone, and Uganda and Zambia together. Burkina Faso was excluded from these analyses due to the very small numbers of observations (women initiating mixed feeding in the study period before week 26). The same list of variables was used in the country-specific analysis regardless of the p-value in the bivariate analysis. Statistical analysis was done using STATA/SE 13.1 (4905 Lakeway Drive College Station, Texas 77845 USA).

Ethics consent and permissions

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 was approved by the relevant ethics committees in the four participating countries and the Medicines Control Council in South Africa.

Results

Baseline characteristics and feeding patterns

In the ANRS 12174 trial, 1,273 mother-infant pairs were randomized, of which 6 were excluded due to protocol violation. Of the remaining 1,267 participants, 204 were from Ouagadougou, 222 from East London, 278 from Mbale and 563 from Lusaka. Another 42 were excluded from analysis due to lack of breastfeeding data after inclusion (Fig. 1). Trial profile.

The mothers excluded from analysis tended to be younger (mean age 25 vs. 27 years), have better education and fewer children. Regarding baseline characteristics, South African mothers had the highest body mass index (BMI), and Ugandan mothers the highest number of children (Table 1).

Breastfeeding was initiated within 1 h and on the first day postpartum by 57.7% and 93.9% of mothers, respectively (Table 2), and 99% had started any breastfeeding within the first week (Table 3). The main reason for delayed initiation of breastfeeding was reported as a lack of breast milk. EBF, PBF and MF were practiced by 95.9, 1.6 and 1.5%, respectively, in the first week (Table 3). Water-based liquids were the most common prelacteal items (6.2% of the participants) during this week (Table 4).

The median duration of any breastfeeding was 40.6 weeks (interquartile range (IQR; 32.3; 45.4; Table 5). Burkina Faso had the longest median duration and South Africa the shortest. The median duration of EBF was 20.9 (IQR: 19.1; 21.1) weeks. PBF was reported only during 59 monthly visits (4/1000 person-year). When PBF was combined with EBF, the overall median duration remained the same (20.9; IQR: 19.7; 21.1).

At week 22, EPBF was practiced by 90.7, 48.8, 79.6 and 83.1% in Burkina Faso, South Africa, Uganda and Zambia, respectively (Fig. 2 and Additional file 1). Nevertheless at week 22 there was no significant difference between the trial arms ($p = 0.05$, log rank test; in the lopinavir/ritonavir arm, 70.3% practiced EBF vs. 69.1% in the lamivudine arm).

At week 50, 11.8, 4.7, 2.5 and 3.2% of the mothers were continuing to breastfeed in Burkina Faso, South Africa, Uganda and Zambia, respectively (Fig. 3).

The details of the food items that the infants received are set out in detail in Table 4 (first week) and in the Additional files 2, 3 (first year). There were major country differences in prelacteal feeds (Table 4). In the first 3 days of life, water-based items (including water, water and sugar or glucose, water and salt, cow's milk, infant formula and traditional beverages) were given to 24, 12.5, 4.7 and 1% of the children in Burkina Faso, Uganda, South Africa and Zambia, respectively. After the first week of life (Additional file 2, 3), milk items were mainly used in South Africa, Uganda and Zambia. Porridge or cereals were were introduced at week 10 and 18 in Zambia and Uganda, respectively while in Burkina Faso they were used from week 26. Soup, meat, fish or egg were introduced as early as week 18 in South Africa, Uganda and Zambia while these items were first seen in infant's food only at week 26 (0.5% for meat, fish or egg) in Burkina Faso (additional file 2c). After cessation of breastfeeding, 62 (5.1%) of 1225 mothers (25 (11.7%), 20 (7.3%) and 17 (3.2%) in South Africa, Zambia and Uganda, respectively) resumed breastfeeding. In total, 54 (4.4%) mothers resumed breastfeeding once and stopped breastfeeding permanently, and 8 (0.6%) resumed it twice. The mean duration of all periods with resumed breastfeeding was 17.2 days, and in calculating this mean, we disregarded 5 women who had resumed breastfeeding of long duration (mean duration of 108.2 days) and were characterized as outliers. The mean duration of these periods with resumed breastfeeding for mothers who resumed once was 18.2 days (i.e. without these 5 women), 14.9 and 13.2 days for the first and second period, respectively, for those who resumed twice. We did not find any difference between the women who stopped breastfeeding earlier (before 26 weeks) and resumed it and those who stopped it later (after 26 weeks).

Risk factors analysis

Almost all the Burkina Faso mothers continued EPBF beyond 22 weeks after birth. We therefore removed them from the pooled multivariable analysis on risk factors for stopping EPBF (Additional file 2: Table S6). The groups of mothers significantly more at risk to stop EPBF before

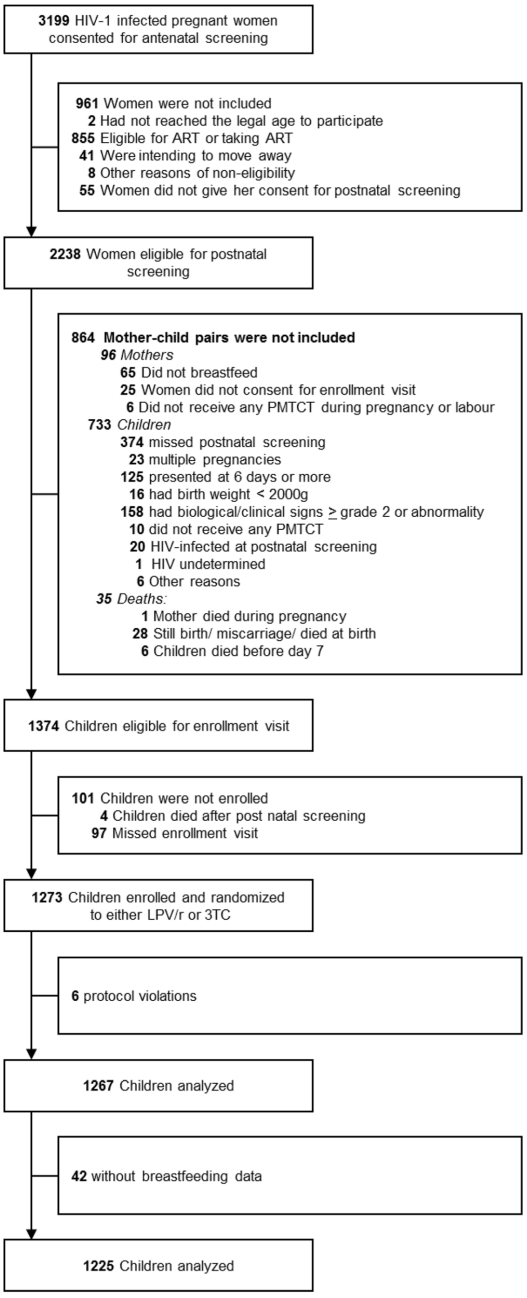

Fig. 1 Study flow-chart

Table 1 Study participants' baseline characteristics

	Burkina Faso	South Africa	Uganda	Zambia	All sites
	N = 204	*N* = 213	*N* = 274	*N* = 534	*N* = 1225
	n (%)	n (%)	n (%)	n (%)	n (%)
Age group					
<25 years	54 (26.5)	73 (34.3)	107 (39.0)	200 (37.8)	434 (35.6)
25 – 30 years	75 (36.8)	67 (31.5)	97 (36.1)	175 (33.1)	414 (34.1)
30 and above	75 (36.8)	73 (34.3)	68 (24.8)	154 (29.0)	370 (30.3)
Literacy rate	103 (50.5)	211 (99.1)	261 (95.3)	487 (91.2)	1062 (86.7)
Education level (%)					
Primary school	155 (76.0)	19 (0.5)	176 (15.7)	249 (18.7)	599 (13.0)
Secondary school and higher	49 (24.0)	194 (91.1)	98 (35.7)	285 (53.4)	626 (51.1)
Occupation: employed	18 (8.8)	89 (41.8)	97 (35.4)	92 (17.2)	296 (24.2)
Married/co-habiting	185 (90.7)	83 (38.0)	225 (82.1)	473 (88.6)	966 (78.9)
Primiparous	44 (21.6)	71 (33.3)	49 (17.9)	112 (21.0)	276 (22.5)
Surgical breast history	8 (3.9)	1 (0.5)	8 (2.9)	1 (0.2)	18 (1.5)
Mother's HIV stage 1	190 (93.1)	210 (98.6)	253 (92.3)	533 (99.8)	1184 (96.6)
Facility delivery	200 (98.0)	211 (99.1)	212 (77.4)	518 (97.0)	1141 (93.1)
Vaginal delivery	191 (93.6)	139 (65.3)	256 (93.4)	514 (96.2)	1100 (89.8)
Female infant	86 (42.2)	105 (49.3)	144 (52.5)	259 (48.5)	594 (48.5)
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
Mean BMI	23.8 (23.2; 24.3)	28.3 (27.5; 29.0)	23.0 (22.6; 23.4)	24.1 (23.7; 24.4)	24.5 (24.3; 24.8)
Mean number of children	2.8 (2.6; 3.0)	2.0 (1.9; 2.2)	3.5 (3.3; 3.7)	2.6 (2.5; 2.7)	2.7 (2.7; 2.8)

26 weeks in the remaining 3 countries were the 25-30 year-old married, employed, and multiparous mothers, or those in the lopinavir/ritonavir arm. On the other hand, Ugandan and Zambian women were more likely to continue EPBF until 26 weeks than South African women. The behaviour of South African women to stop EPBF early affected the overall analysis, showing that the lopinavir/ritonavir arm was a risk factor in this situation.

With regard to any breastfeeding, Burkina Faso was once again dropped from the multivariate analysis of the determinants. The survival curve (Fig. 3) of any breastfeeding by country shows a dramatic drop of the South African curve from the beginning until around week 40. From week 40, the remaining South African mothers tended to breastfeed longer than those mothers in the other countries. Having completed secondary school or beyond was an independent risk factor for early cessation of any breastfeeding (Additional file 2: Table S6).

In the country-specific analyses (Additional file 2: Table S7a), there were in general no large differences in the infant feeding behavior, except for South Africa where there was an interaction with trial allocation. Children in the lopinavir/ritonavir group were 3 times more likely to stop EPBF early than children from the lamivudine group. In Uganda and Zambia, the trial drug had no significant influence on infant feeding behavior.

Table 2 Time to initiation of breastfeeding after birth

Hours	Burkina Faso	South Africa	Uganda	Zambia	All sites
	N = 204	*N* = 213	*N* = 274	*N* = 534	*N* = 1225
	n (%)	n (%)	n (%)	n (%)	n (%)
0-1	14 (6.9)	109 (51.2)	152 (55.5)	432 (80.9)	707 (57.7)
2-5	73 (35.8)	85 (39.9)	76 (27.7)	74 (13.9)	308 (25.1)
6-12	54 (26.5)	10 (4.7)	32 (11.7)	22 (4.1)	118 (9.6)
12-24	4 (2.0)	3 (1.4)	6 (2.2)	4 (0.7)	17 (1.4)
Total initiated 1st day	145 (71.1)	207 (97.2)	266 (97.1)	532 (99.6)	1150 (93.9)

Table 3 Feeding pattern during the first week of life by country

Breast feeding pattern	Burkina Faso	South Africa	Uganda	Zambia	All sites
	N = 204	*N* = 213	*N* = 274	*N* = 534	*N* = 1225
	n (%)	n (%)	n (%)	n (%)	n (%)
Any breastfeeding first 3 days	200 (98.0)	206 (96.7)	272 (99.3)	532 (99.6)	1210 (98.8)
Any breastfeeding day 4-7	202 (99.0)	205(96.2)	273 (99.6)	533 (99.8)	1213 (99.0)
Exclusive breastfeeding throughout first 3 days	156 (76.5)	199 (93.4)	250 (91.2)	528 (98.9)	1133 (92.5)
Exclusive breastfeeding throughout days 4-7	177 (86.8)	200 (93.9)	266 (97.1)	532 (99.6)	1175 (95.9)
Predominant breastfeeding throughout first 3 days	34 (16.7)	2 (0.9)	18 (6.6)	1 (0.2)	55 (4.5)
Predominant breastfeeding throughout days 4-7	15 (7.3)	4 (1.9)	1 (0.4)	0 (0.0)	20 (1.6)
Mixed feeding first 3 days	10 (4.9)	5 (2.3)	4 (1.5)	3 (0.6)	22 (1.8)
Mixed feeding day 4-7	10 (4.9)	1 (0.5)	6 (2.2)	1 (0.2)	18 (1.5)

Contrary to any breastfeeding, having a secondary school or higher education level was beneficial for EPBF practice, allowing up to 40, 60 and 40 of babies to benefit from EPBF in South Africa, Uganda and Zambia, respectively. However, this association was significant only for Zambian children. Being married or living as a couple was a risk factor for shorter EPBF, with a significant adjusted hazard ratio (AHR) of 1.6 (95% CI: 1.2; 2.1) and 2.6 (95% CI: 1.9; 3.6) in South Africa and Zambia, respectively. A similar pattern was observed with employed mothers and multiparous mothers. Late initiation of breastfeeding (after 1 h) was associated with shorter EPBF in Uganda and Zambia.

Discussion

In general, the mothers in this trial adhered to the breastfeeding recommendations: 58% initiated breastfeeding within the first hour and 94% within the first day. EPBF was practiced at a high rate in all countries throughout the 22-week period. This performance can probably be

Table 4 Proportion of infants receiving different food items during the first week of life by country

Nutrient or other intake	Burkina Faso	South Africa	Uganda	Zambia	All sites
	N = 204	*N* = 213	*N* = 274	*N* = 534	*N* = 1225
	n (%)	n (%)	n (%)	n (%)	n (%)
Days 1 to 3					
Breast milk	200 (98.0)	206 (96.7)	272 (99.3)	532 (99.6)	1210 (98.8)
Water	34 (16.7)	5 (2.3)	12 (4.4)	0 (0.0)	51 (4.2)
Water + sugar or glucose	6 (2.9)	0 (0.0)	15 (5.5)	0 (0.0)	21 (1.7)
Water + salt	0 (0.0)	0 (0.0)	4 (1.5)	0 (0.0)	4 (0.3)
Juice	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.2)	2 (0.2)
Cow's milk	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.1)
Infant formula	2 (1.0)	4 (1.9)	0 (0.0)	3 (0.6)	9 (0.7)
Liquid as part of traditional practice	6 (2.9)	1 (0.5)	3 (1.1)	0 (0.0)	10 (0.8)
Other	2 (1.0)	0 (0.0)	2 (0.4)	0 (0.0)	3 (0.2)
Days 4 to 7					
Breast milk	20 2(99.0)	205 (96.2)	273 (99.6)	533 (99.8)	1213 (99.0)
Water	19 (9.3)	4 (1.9)	1 (0.4)	0(0.0)	24 (2.0)
Water + sugar or glucose	1 (0.5)	1 (0.5)	1 (0.4)	0 (0.0)	3 (0.2)
Tea	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Juice	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)
Infant formula	1 (0.5)	0 (0.0)	1 (0.4)	1 (0.2)	3 (0.2)
Powdered milk	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)
Liquid as part of traditional practice	6 (2.9)	1 (0.5)	3 (1.1)	0 (0.0)	10 (0.8)
Other	3 (1.5)	0 (0.0)	1 (0.4)	0 (0.0)	4 (0.3)

Table 5 Median duration of "any breastfeeding", "exclusive breastfeeding" and "exclusive and predominant breastfeeding" in weeks

Feeding patterns	Burkina Faso	South Africa	Uganda	Zambia	All sites
	N = 204	*N* = 213	*N* = 274	*N* = 534	*N* = 1225
	Median (p25; p75)	Median (p25; p75)	Median (p25; p75)	Median (p25; p75)	Median (p25; p75)
Any breastfeeding	46.5 (45.0; 48.7)	29.1 (13.0; 46.3)	39.9 (34.1; 43.0)	39.0 (33.0; 43.6)	40.6 (32.3; 45.4)
Exclusive breastfeeding	20.7 (18.9; 21.3)	19.5 (12.4; 21.0)	20.9 (19.6; 21.0)	21.0 (20.4; 21.1)	20.9 (19.1; 21.1)
Exclusive and predominant breastfeeding	20.9 (20.0; 21.5)	19.8 (12.9; 21.0)	20.9 (19.9; 21.0)	21.0 (20.6; 21.1)	20.9 (19.7; 21.1)

attributed to the study design, including close tracking of the mothers during the antenatal period and frequent follow-up visits with infant feeding counselling. The median durations of any and exclusive breastfeeding were 40.6 and 20.9 weeks, respectively. At week 22, >75% of the children were on EPBF. Only 4.7% were breastfed at week 50.

In other recent studies of both the general population and mothers living with HIV, findings showed that the proportion of women initiating breastfeeding within the first hour postpartum was between less than half and 76% [18, 38–40]. However, with respect to our study, the country having the lowest percentage of women initiating breastfeeding within the first hour was Burkina Faso (only 6.9%).

Under 10% of the participants in this trial gave prelacteal feeds compared to 22 to 57% in other studies [18, 20, 39, 40]. Burkina Faso had the highest proportion of women giving prelacteal feeds (>16%). The same feeding patterns were found in the PROMISE-EBF study in Burkina Faso site with <4 and 11% of participants initiating breastfeeding within the first hour and giving prelacteal feeding, respectively [41]. In Burkina Faso, however, women breastfed longer compared to the other countries, for which we have no clear explanation. Our hypotheses are that the tradition through a cultural influence, better counselling sessions and a poorer economic context that could be accompanied with difficulties in affording quality complementary food for children, may have been important factors. More mothers in South Africa (and to some extent in Zambia) had to resume formal work after a few months, preventing them from continuing breastfeeding.

In the Kesho Bora study [42], the participants from Burkina Faso had a considerably lower level of education, than in our study. In contrast to our study, South Africa had the longest median duration of any breastfeeding compared with Burkina Faso and Kenya. The proportion of women initiating breastfeeding in the first week and the median duration of any breastfeeding were higher in our study (99% and 41 weeks vs. 70% and 20 weeks, respectively). Likewise, the Kesho Bora study had a lower proportion of EBF (22% at 5 months) compared to our study (75.3% at 5 months). Even comparing the countries that participated in both the Kesho Bora and our trial (Burkina Faso and South Africa), the exclusive breastfeeding rate was higher in our study. In other EBF studies with contexts similar to this trial, the

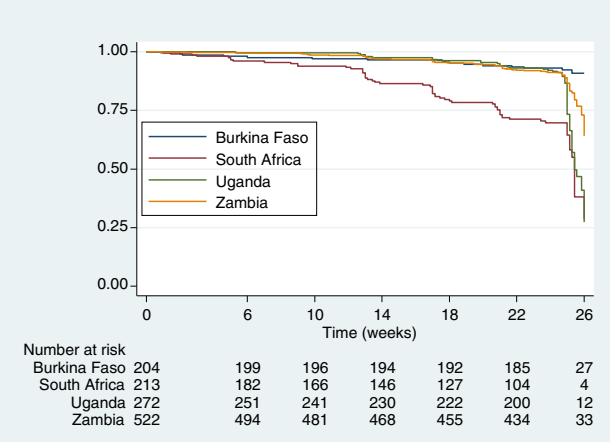

Fig. 2 Kaplan-Meier survival curve by country for exclusive and predominant breastfeeding until week 26 post-partum

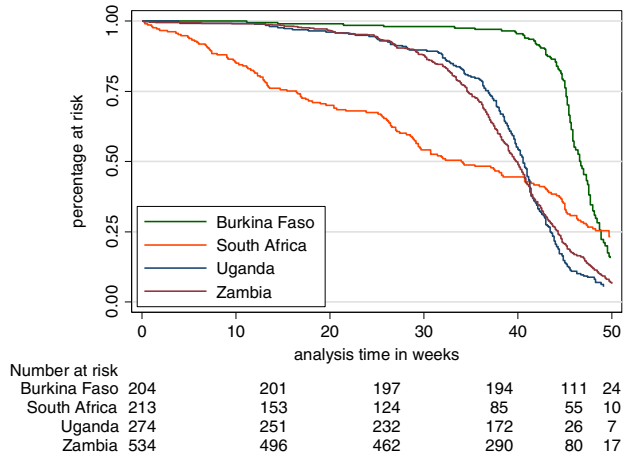

Fig. 3 Kaplan-Meier survival curve by country for any breastfeeding until week 50 post-partum

median durations of EBF varied from 1.8 [43] to 5 months [38, 43, 44] and the proportion of EBF at 6 months ranged from zero percent to 84% [18, 20, 42, 43, 45, 46]. Therefore one has to acknowledge the high variability of infant feeding practices in sub-Saharan Africa among HIV-positive women. In general, the ANRS12174 participants adhered to a large extent to the WHO HIV and infant feeding recommendations. The trial setup and the frequent counselling is the most likely explanation for that; thus, it is possible that frequent quality counselling is required for the promotion of the safe infant feeding practices among HIV-1 positive women.

Interestingly, the trial allocation was a factor associated with breastfeeding behaviour, but only in South Africa where the lopinavir/ritonavir group had a 3 times higher risk of shorter EPBF compared to the lamivudine group. It is possible that the poor palatability of lopinavir/ritonavir influenced the South African participants to stop EPBF earlier than recommended. Why this happened only in South Africa is unclear, but formula-feeding is more common in South Africa, which may be one explanation. It is also possible that, due to the bad taste, women in South Africa mixed the drug with other foods than milk, unlike women in Uganda and Zambia where they mixed it with breastmilk.

Our findings together with the ANRS 12174 trial results [34] demonstrated enough that breastfeeding in HIV-1 infected women could be almost as safer as in HIV-1 uninfected mothers for the infants, provided it is practised according to the international recommendations. However, it is currently commonly accepted to breastfeed babies born to HIV-1 infected mothers while the peri-exposure prophylaxis is assured by the mothers taking lifelong anti-retroviral medicines (option B+). Nonetheless, the efficacy demonstrated for the option A in our studies required some reflections on how to combine all these options for an improved PMTCT strategy as we know that some HIV reservoirs (including in breastmilk) [32] are not fully controlled by the mother's HAART. In the other hands, some mothers may not be able to afford or to take HAART.

There are certain limitations to our study. In some sites, including Burkina Faso, the nutritional counsellors were also collecting feeding data; therefore, there was a risk for the mothers to under-report non-recommended practices while emphasizing the recommended ones. However, the counsellors were senior staff with considerable experience in research and knew the techniques needed to probe participants to obtain accurate data. Furthermore, the Burkina Faso participants had the highest proportion of prelacteal feeding, delayed initiation of breastfeeding, among other items, which suggests that information bias was limited. Moreover, our findings regarding prelacteal feeding practices and breastfeeding initiation time in Burkina Faso are comparable with PROMISE-EBF findings [41], where peer counsellors were not the same as the investigators who collected the data.

Other potential limitations are the trial context with a fixed time of 50 weeks for provision of the infant prophylaxis, which limits the generalisability of our data particularly on 'any breastfeeding', and the selection criteria that may not allow any extrapolation of the results to non-research situations. However, our large

sample size, the long duration of our follow-up, the international multicentre design, as well as the stringent randomised clinical trial context and the cohort design, added value and accuracy to our findings.

Conclusion

ANRS 12174 trial participants were relatively more successful in practicing EPBF than has been seen in several previous studies. However, in Burkina Faso, late initiation of breastfeeding postpartum and the extensive use of prelacteal feeds remain prevalent. Why women in the lopinavir/ritonavir arm were more likely to stop EPBF in South Africa is a question requiring further investigation. There is a need to improve breastfeeding and complementary feeding practices of children, particularly those exposed to HIV and anti-retrovirals, taking into account context and socio-demographic factors.

Additional files

Additional file 1: Figure S4. Non-exclusive breastfeeding survival curves by country until week 50. This figure shows survival curves by country presenting the women nonexclusively breastfeeding their children during the 50-week follow-up period. (PDF 115 kb)

Additional file 2: Table S6. Non-proportional hazard models of early cessation of 'exclusive or predominant breastfeeding' and 'any breastfeeding' [As a result table]. This table presents overall factors determining early cessation of exclusive or predominant and any breastfeeding. **Table S7a**. Flexible parametric non-proportional hazard models of shorter duration of 'exclusive or predominant breastfeeding' by country. [As a result table]. This table gives factors determining early cessation of exclusive or predominant breastfeeding stratified by country. **Table S7b**. Flexible parametric non-proportional hazard models of shorter duration of 'exclusive or predominant breastfeeding' isolating South-Africa. [As a result table]. This table details factors determining early cessation of exclusive or predominant and any breastfeeding stratified by country in 2 different models, including South African model alone and another model for Uganda and Zambia together. (DOCX 47.1 kb)

Additional file 3: Table S8a. Infant feeding practices in detail: liquid-based items given during the study period. This additional file is a table describing the different liquid-based food items other than breastmilk given to the child during the study follow-up period. **Table S8b**. Infant feeding practices in detail: milk-based items given during the study period. This additional file is a table describing the different milk-based food items other than breastmilk given to the child during the follow-up period. **Table S8c**. Infant feeding practices in detail: solids items given during the study period. This additional file is a table describing the different solid food items other than breastmilk given to the child during the study follow-up period. (DOCX 52.9 kb)

Abbreviations

ART: Antiretroviral therapy; BMI: Body mass index; EBF: Exclusive breastfeeding; EPBF: Exclusive or predominant breastfeeding; HAART: Highly active antiretroviral therapy; HIV: Human Immuno-deficiency Virus; IQR: Interquartile range; MF: Mixed feeding; MTCT: Mother-to-child transmission of HIV-1; PBF: Predominant breastfeeding; PMTCT: Prevention of mother-to-child transmission of HIV-1; UNAIDS: United Nations programme on HIV/AIDS; WHO: World Health Organization

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Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NES, IMSE, NN, NM and TT designed the study. NES, RV and MP proceeded to the data management and NES, IMSE, TT, MP, RV and CL analysed the data. NES, IMSE NN, NM and TT wrote the first draft of the manuscript. NM, CK, JKT, GJH were the site principal investigators of the ANRS 12174 trial and reviewed the draft of the manuscript. MS and KH were the site leads in South Africa. PVP and TT were the protocol chairs for the whole trial. All authors approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

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 ethic committees in the four participating countries and the Medicines Control Council in South Africa. The ethics committees approval references are : i) Burkina Faso health research ethics committee : N° 2008-039; ii) South Africa Medicines Control Council: 20090938; iii) Uganda National Council for Science and Technology: HS470; iv) Zambia : 008-02-08.

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References

1. UNAIDS: Core epidemiology. *UNAIDS* July 2015, http://www.unaids.org/en/resources/documents/2015/20150714_coreepidemiologyslides_ppt; [Accessed 11 Nov 2015].
2. Kagaayi J, Gray R, Brahmbhatt H, Kigozi G, Nalugoda F, et al. Survival of infants born to HIV-positive mothers, by feeding modality, in Rakai, Uganda. PloS One. 2008;3(12):e3877.
3. Kuhn L, Stein Z, Susser M. Preventing mother-to-child HIV transmission in the new millennium: the challenge of breast feeding. Paediatr Perinat Epidemiol. 2004;18:10–6.
4. Taha T, Kumwenda N, Hoover D, Kafulafula G, Fiscus S, Nkhoma C, et al. The impact of breastfeeding on the health of HIV-positive mothers and their children in sub-Saharan Africa. Bull World Health Organ. 2006;84:546–54.
5. Bhutta Z, Das J, Rizvi A, Gaffey M, Walker N, Horton N. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet. 2013;382:452–77.
6. Black R, Victora C, Walker S, Bhutta W, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet. 2013;382:427–51.
7. Doherty T, Sanders D, Goga A, Jackson D. Implications of the new WHO guidelines on HIV and infant feeding for child survival in South Africa. Bull World Health Organ. 2011;89:62–7.
8. Kusunoki T, Morimoto T, Nishikomori R, Yasumi T, Heike T, Mukaida K, et al. Breastfeeding and the prevalence of allergic diseases in schoolchildren: Does reverse causation matter? Pediatr Allergy Immunol. 2010;21:60–6.
9. Jones G, Steketee RW, Black RE, Bhutta ZA, Morris SS. How many child deaths can we prevent this year? Lancet. 2003;362:65–71.
10. Victora C, Horta B, de Mola C, Quevedo L, Pinheiro R, Gigante D, et al. Association between breastfeeding and intelligence, educational attainment and income at 30 years of age: a prospective birth cohort study from Brazil. Lancet. 2015;3(4):e199–205.
11. Prieto C, Cardenas H, Croxatto H. Variability of breast sucking, associated milk transfer and the duration of lactational amenorrhoea. J Reprod Fertil. 1999;115(2):193–200.
12. Adejuyigbe N, Orji E, Onayade A, Makinde N, Anyabolu H. Infant feeding intentions and practices of HIV-positive mothers in Southwestern Nigeria. J Hum Lact. 2008;24(3):303–10.
13. Tournoud M, Ecochard R, Kuhn L, Coutsoudis A. Diversity of risk of mother-to-child HIV-1 transmission according to feeding practices, CD4 cell count and haemoglobin concentration in a South African cohort. Trop Med Int Health. 2008;13(3):310–8.
14. Becquet R, Ekouevi D, Menan H, Amani-Bosse C, Bequet L, Viho I, et al. Early mixed feeding and breastfeeding beyond 6 months increase the risk of postnatal HIV transmission: ANRS 1201/1202 Ditrame Plus, Abidjan, Côte d'Ivoire. Prev Med. 2008;47:27–33.
15. Becquet R, Bland R, Leroy V, Rollins N, Ekouevi D, Coutsoudis A, et al. Duration, pattern of breastfeeding and postnatal transmission of HIV: pooled analysis of individual data from West and South African cohorts. PloS One. 2009;4(10):e7397.
16. Buskens I, Jaffe A, Mkhatshwa H. Infant feeding practices: realities and mindsets of mothers in Southern Africa. AIDS Care. 2007;19(9):1101–9.
17. Doherty T, Chopra M, Nkonki L, Jackson D, Persson L. A longitudinal qualitative study of infant-feeding decision making and practices among HIV-positive women in South Africa. J Nutr. 2006;136:2421–6.
18. Gewa C, Oguttu M, Savaglio L. Determinants of early child-feeding practices among HIV-infected and noninfected mothers in rural Kenya. J Hum Lact. 2011;27:239–49.
19. Bland R, Rollins N, Coutsoudis A, Coovadia H, for the Child Health Group. Breastfeeding practices in an area of high HIV prevalence in rural South Africa. Acta Paediatr. 2002;91:704–11.
20. Engebretsen IM, Wamani H, Karamagi C, Semiyaga N, Tumwine J, Tylleskar T. Low adherence to exclusive breastfeeding in Eastern Uganda: a community-based cross-sectional study comparing dietary recall since birth with 24-h recall. BMC Pediatr. 2007;7:10.
21. Shapiro R, Lockman S, Thior I, Stocking L, Kebaabetswe P, Wester C, et al. Low adherence to recommended infant feeding strategies among HIV-infected women: results from the pilot phase of a randomized trial to prevent mother to child transmission in Botswana. AIDS Educ Prev. 2003;15(3):221–30.
22. Nor B, Ahlberg B, Doherty T, Zembe Y, Jackson D, Ekström E for the PROMISE-EBF Study Group. Mother's perceptions and experiences of infant feeding within a community-based peer counselling intervention in South Africa. Matern Child Nutr. 2012;8:448–58.
23. Doherty T, Sanders D, Jackson D, Swanevelder S, Lombard C, Zembe W, For the PROMISE EBF study group, et al. Early cessation of breastfeeding amongst women in South Africa: an area needing urgent attention to improve child health. BMC Pediatr. 2012;12:105.
24. Black R, Allen L, Bhutta Z, Caulfield L, de Onis M, Ezzati M, for the Maternal and Child Undernutrition Study Group. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet. 2008;371:243–60.
25. Maonga A, Mahande M, Damian D, Msuya S. Factors affecting exclusive breastfeeding among women in Muheza district Tanga Northeastern Tanzania: A mixed method community based study. Matern Child Health J. 2016;20:77–87.
26. Aishat U, David D, Olufunmilayo F. Exclusive breastfeeding and HIV/AIDS: a crossectional survey of mothers attending prevention of mother-to-child transmission of HIV clinics in southwestern Nigeria. Pan Afr Med J. 2015;21:309.
27. Hailemariam T, Adeba A, Sufa A. Predictors of early breastfeeding initiation among mothers of children under 24 months of age in rural part of West Ethiopia. BMC Public Health. 2015;15:1076.
28. UNICEF. State of the world's children 2013: children with disabilities. New York: UNICEF, 2013. New York: UNICEF; 2013 [https://www.unicef.org/sowc2013/files/Table_2_Stat_Tables_SWCR2013_ENGLISH.pdf] [accessed 01 Mar 2017].
29. Mofenson L. Antiretroviral prophylaxis to reduce breast milk transmission of HIV type 1: new data but still questions. J Acquir Immune Defic Syndr. 2008;48(3):237–40.
30. WHO, UNAIDS, UNFPA, UNICEF. Guidelines on HIV and infant feeding: principles and recommendations for infant feeding in the context of HIV and a summary of evidence. Geneva: World Health Organization; 2010. p. 58.
31. WHO. Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: recommendations for a public health approach. Geneva: World Health Organization; 2010. p. 117.
32. Van de Perre P, Rubbo P, Viljoen J, Nagot N, Tylleskär T, Lepage P. HIV-1 reservoirs in breast milk and challenges to elimination of breast-feeding transmission of HIV-1. Sci Transl Med. 2012;4(143):143sr3.
33. Nagot N, Kankasa C, Meda N, Hofmeyr J, Nikodem C, Tumwine J, et al. Lopinavir/Ritonavir versus Lamivudine peri-exposure prophylaxis to prevent HIV-1 transmission by breastfeeding: the PROMISE-PEP trial Protocol ANRS 12174. BMC Infect Dis. 2012;12:246.
34. Nagot N, Kankasa C, Tumwine J, Meda N, Hofmeyr G, Vallo R, et al. Extended pre-exposure prophylaxis with lopinavir–ritonavir versus lamivudine to prevent HIV-1 transmission through breastfeeding up to 50 weeks in infants in Africa (ANRS 12174): a randomised controlled trial. Lancet. 2015;387(10018):566–73.
35. ANRS: ANRS scale to grade the severity of adverse events in adults; version n° 1.0 4 November 2008. file:///C:/Users/install/Downloads/ANRS-GradeEI-V1-En-2008pdf; [Accessed 14 Jan 2016 2008].
36. Lambert PC, Royston P. Further development of flexible parametric models for survival analysis. Stata J. 2009;9(2):265–90.
37. Royston P. Flexible parametric alternatives to the Cox model: update. Stata J. 2004;4(1):98–101.
38. Njom Nlend A, Ekani B. Preliminary assessment of breastfeeding practices in HIV 1-infected mothers (prior to weaning) under the Djoungolo programme on the prevention of mother-to-child transmission of HIV. J Trop Pediatr. 2010;56(6):436–9.
39. Kamau-Mbuthia E, Elmadfa I, Mwonya R. The impact of maternal HIV status on infant feeding patterns in Nakuru, Kenya. J Hum Lact. 2008;24:34–41.
40. Fadnes LT, Engebretsen IM, Wamani H, Semiyaga NB, Tylleskar T, Tumwine JK. Infant feeding among HIV-positive mothers and the general population mothers: comparison of two cross-sectional surveys in Eastern Uganda. BMC Public Health. 2009;9:124.

41. Engebretsen I, Nankabirwa V, Doherty T, Diallo A, Nankunda N, Fadnes L, et al. Early infant feeding practices in three African countries: the PROMISE-EBF trial promoting exclusive breastfeeding by peer counsellors. Int Breastfeed J. 2014;9:19.
42. Bork K, Cames C, Cournil A, Musyoka F, Ayassou K, Naidu K, for the Kesho Bora Study Group, et al. Infant feeding modes and determinants among HIV-1–infected African women in the Kesho Bora Study. J Acquir Immune Defic Syndr. 2013;62:109–18.
43. Young SL, Israel-Ballard KA, Dantzer EA, Ngonyani MM, Nyambo MT, Ash DM, et al. Infant feeding practices among HIV-positive women in Dar es Salaam, Tanzania, indicate a need for more intensive infant feeding counselling. Public Health Nutr. 2010;13(12):2027–33.
44. Coovadia H, Rollins N, Bland R, Little K, Coutsoudis A, Bennish M, et al. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: an intervention cohort study. Lancet. 2007;369:1107–16.
45. Kramer MS, Chalmers B, Hodnett ED, Sevkovskaya Z, Dzikovich I, Shapiro S, et al. Promotion of breastfeeding intervention trial (PROBIT) a randomized trial in the Republic of Belarus. JAMA. 2001;285:413–20.
46. Kuhn L, Sinkala M, Kankasa C, Semrau K, Kasonde P, Scott N, et al. High uptake of exclusive breastfeeding and reduced early post-natal HIV transmission. PloS One. 2007;2(12):e1363.

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Paper II

Eric N. Somé, Ingunn M. S. Engebretsen, Nicolas Nagot, Nicolas Meda, Roselyne Vallo, Marianne Peries, Chipepo Kankasa, James K. Tumwine, G. Justus Hofmeyr, Mandisa Singata, Kim Harper, Philippe Van De Perre, Thorkild Tylleskar for the ANRS 12174 Trial Group. **Changes in body mass index and hemoglobin concentration in breastfeeding women living with HIV with a CD4 count over 350: Results from 4 African countries (The ANRS 12174 trial).** PLoS ONE, May 2017; 12(5):e0177259

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RESEARCH ARTICLE

Changes in body mass index and hemoglobin concentration in breastfeeding women living with HIV with a CD4 count over 350: Results from 4 African countries (The ANRS 12174 trial)

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Abstract

Introduction

Breastfeeding is recommended for infants born to HIV-infected women in low-income settings. Both breastfeeding and HIV-infection are energy demanding. Our objective was to explore how exclusive and predominant breastfeeding changes body mass index (BMI) among breastfeeding HIV1-positive women participating in the ANRS12174 trial (clinical trial no NCT0064026).

Methods

HIV-positive women (n = 1 267) with CD4 count >350, intending to breastfeed HIV-negative infants were enrolled from Burkina Faso, South Africa, Uganda and Zambia and counselled on breastfeeding. N = 1 216 were included in the analysis. The trial compared Lamivudine and Lopinavir/Ritonavir as a peri-exposure prophylaxis. We ran a linear mixed-effect model with BMI as the dependent variable and exclusive or predominant breastfeeding duration as the key explanatory variable.

Research – National Agency for Research on AIDS and Viral Hepatitis (Inserm–ANRS), the European Developing Countries Clinical Trials Partnership (EDCTP; grant number CT.2006.33020.004), the Research Council of Norway (GlobVac grant number 183600) and the Total Foundation. NES benefited from a PhD grant from the Norwegian Quota Scheme. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Results

Any breastfeeding or exclusive/predominant) breastfeeding was initiated by 99.6% and 98.6% of the mothers respectively in the first week after birth. The median (interquartile range: IQR) duration of the group that did any breastfeeding or the group that did exclusive /predominant breastfeeding were 9.5 (7.5; 10.6) and 5.8 (5.6; 5.9)) months, respectively. The median (IQR) age, BMI, CD4 count, and HIV viral load at baseline (day 7) were 27 (23.3; 31) years, 23.7 (21.3; 27.0) kg/m^2, 530 (432.5; 668.5) cells/μl and 0.1 (0.8; 13.7)1000 copies/mL, respectively. No major change in mean BMI was seen in this cohort over a 50-week period during lactation. The mean change between 26 and 50 weeks after birth was 0.7 kg/m^2. Baseline mean BMI (measured on day 7 postpartum) and CD4 count were positively associated with maternal BMI change, with a mean increase of 1.0 kg/m^2 (0.9; 1.0) per each additional baseline-BMI kilogram and 0.3 kg/m^2 (0.2; 0.5) for each additional CD4 cell/μl, respectively.

Conclusion

Breastfeeding was not negatively correlated with the BMI of HIV-1 infected Sub-Saharan African mothers. However, a higher baseline BMI and a CD4 count >500 cells/μl were associated with maternal BMI during the exclusive/ predominant breastfeeding period. Considering the benefits of breast milk for the infants and the recurrent results from different studies that breastfeeding is not harmful to the HIV-1-infected mothers, this study also supports the WHO 2016 guidelines on infant feeding that mothers living with HIV should breastfeed where formula is not safe for at least 12 months and up to 24 months, given that the right treatment or prophylaxis for the infection is administered. These findings and conclusions cannot be extrapolated to women who are immune-compromised or have AIDS.

Introduction

HIV infection is among the leading causes of mortality among women of childbearing age [1]. It will be associated with an increased risk of infections from non-obstetric and direct obstetric causes, making HIV-infected mothers more vulnerable than uninfected mothers [2,3]. HIV in pregnancy contributed to high maternal mortality rates with a 9% contribution in Sub-Saharan Africa (SSA) between 1990 and 2008. It is the leading cause of death during pregnancy and the postpartum period in countries with a high prevalence of HIV [4,5]. A recent meta-analysis pointed out clearly the significance of HIV infection in global maternal mortality rates, with an estimated 5% pregnancy-related deaths worldwide and 25% in SSA [6]. The reasons of this high mortality in HIV-infected women are unclear. The risk of obstetric complications may be increased in HIV-infected women or pregnancy might accelerate HIV progression [6–8].

Weight loss and low body mass index (BMI) can serve as markers of HIV disease progression. Weight has been used to diagnose clinical AIDS disease; a 10% weight loss in the absence of any other evident cause was one of the early WHO clinical criteria in areas without laboratories [9–13]. Individuals with asymptomatic HIV need an extra 10% energy intake to maintain body weight, which increases to 20–30% among those who have symptomatic HIV [14,15]. Moreover during pregnancy and lactation, the woman's body in a normal physiological state undergoes massive energy trade-off seen through weight changes, typically an increase during pregnancy and a loss to pre-pregnancy weight during lactation [16,17].

There has been a dilemma during the last 20 years in choosing between exclusive breastfeeding (EBF) and replacement feeding [18], which was only considered on the basis of the best nutritional and survival outcome for the infant. Specifically, in the pre-ART period the focus was on ensuring the HIV-free survival of infants exposed to HIV.However, in socio-economically deprived settings, breastfeeding has proven to be a key survival strategy for infants born to women living with HIV because increased morbidity and mortality have been associated with replacement feeding [19]. After 2010, availability of, and accessibility to, antiretroviral drugs during pregnancy and lactation have increased. The programs of prevention of mother-to-child transmission (PMTCT) of HIV yielded 2 antiretroviral (ARV) prophylaxis options, including regimen A (prophylactic ARV drugs are given to the mothers and children during the risk period); and regimen B (antiretroviral therapy is given to the mothers during the risk period), or regimen B+ (extending regimen B to lifelong treatment) [20]. In the WHO's 2010 recommendations, women living with HIV who opted to breastfeed were recommended to practice exclusive breastfeeding (EBF) until the infant was 6 months old, introduce appropriate complementary foods thereafter, and continue breastfeeding for the first 12 months of the infant's life. The 2016 recommendations recommend mothers living with HIV to prolong the breastfeeding period to up to 24 months, as advised for the general population (i.e. HIV-uninfected women). The importance of having a nutritionally adequate and safe diet for the child, a lifelong ARV therapy, with adherence counselling and support for breastfeeding for the mothers, is stressed as a prerequisite before breastfeeding can be gradually stopped. Thus, option B+ has made it easier for countries to opt for exclusive breastfeeding for 6 months as the preferred early child feeding option [21–23].

Regarding the mother's health, studies have reached inconsistent conclusions as to the effect of breastfeeding on the weight of those infected with HIV [14,24,25]. The South African Vertical Transmission Study that compared women practicing EBF compared to those opting for replacement feeding or mixed feeding concluded that a) HIV-infected and uninfected mothers experienced similar weight loss over 24 months; and b) postpartum weight change was not associated with feeding practices for the first 5 months of the baby's life. [14]. In this study, the mothers received a single-dose of nevirapine as PMTCT treatment. The Kesho Bora study, a combination of a randomized clinical trial and a prospective cohort study, comprised 3 parts: part IA included women with CD4 count <200 cells/mm^3, receiving Zidovudine (AZT), lamivudine and nevirapine (NVP) twice daily; part IB included women with CD4 count >500 cells/mm^3, receiving AZT 300 mg taken by the mother twice daily starting from 34 to 36 weeks of pregnancy until the onset of labor, plus one 600 mg dose of AZT and one 200 mg dose of NVP at the onset of labor; and part II or the RCT part, included women with a CD4 count between 200 and 500 cells/mm^3, randomized to receive the same prophylaxis as part IB or triple-ARV prophylaxis (AZT [300 mg], 3TC [150 mg] and lopinavir/ritonavir [LPV/r, 400 mg/100 mg]) twice daily from 34 to 36 weeks of pregnancy, through delivery and during breastfeeding to a maximum of 6 months postpartum [26]. It showed that 6 months of breastfeeding was not detrimental to the weight of well-nourished HIV-infected mothers [24]. A study in Zambia randomized women into a short-duration (4 months EBF and abrupt weaning) and a long-duration (mean duration of 16 months including 6 months EBF) breastfeeding period; it showed a net weight gain in HIV-infected women breastfeeding from 4 up to 24 months postpartum [27]. All mothers in the Zambian study were categorized into low ($\leq$350 cells/μl) and high ($>$350 cells/ μl) CD4 count groups. The subjects had received a single-dose nevirapine as MTCT prophylaxis. When antiretroviral treatment became available in May 2004, 26 women were started on the first-line regimen. CD4 count was associated with lower weight. Effects of lactation in women with low CD4 counts were similar to the effects in women with higher CD4 counts. In contrast, two Kenyan studies [28,29] found weight loss

among HIV-infected breastfeeding mothers compared to mothers using formula feeding. Though the design of these studies made a comparison difficult with the precedent studies. they were worse in showing adverse outcomes due to breastfeeding, which included more maternal deaths. These studies in Nairobi, representing two different analyses of the same data, compared mothers who breastfed from delivery until 2 years of age to formula-feeding in a cohort study where all the pregnant women received a short course of zidovudine prophylaxis. When antiretroviral treatment became available, women were referred to highly active antiretroviral treatment programs.

HIV infection also seems to be detrimental to maternal hemogloblin concentration [30,31], which could be worsened by the use of some antiretroviral substances [32–34]. Our aim was therefore to explore within the ANRS12174 trial population how breastfeeding might change BMI and hemoglobin concentration. We also assessed other factors that might have influenced weight changes of HIV-infected mothers during lactation.

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 primary analysis having been published [35,36]. Briefly, pregnant women who tested positive for HIV-1 infection in the context of routine antenatal clinic service making them ineligible for highly active antiretroviral therapy because their CD4+ count was >350 cells/ml, who had to be at least 18 years old and who were planning to breastfeed, were identified at antenatal clinics between 28 and 40 weeks of pregnancy. They received a pre-test counselling session before testing for HIV infection. As part of the post-test session, they were informed of 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 enrolment and randomisation at day 7 postpartum. From 28 weeks of pregnancy to day 7 after birth, programmatic mother-to-child transmission prophylaxis was followed with antepartum zidovudine, intrapartum single dose nevirapine, and zidovudine-lamivudine for mothers and nevirapine for infants for 7 days postpartum. The intervention implemented during the trial was infant prophylaxis in the breastfeeding period starting from 7 days to 50 weeks of age with either lopinavir/ritonavir or lamivudine. Twins and triplets, infants with a positive HIV-1 DNA PCR test result at day 7 (+/- 2) postpartum, and low birth-weight or ill babies (ranked grade II or above of the ANRS classification for adverse events) on the day of enrolment were excluded [37].

Lamivudine, generally well tolerated and accepted, has been widely used in research and clinical trials. The lopinavir/ritonavir paediatric formulation has been a very promising prophylactic combination with low risks for resistance, high antiviral potency and a good safety profile [35]. However, it is known to be distasteful (https://www.medicines.org.uk/emc/medicine/4602; https://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf), which was thought to matter less when introduced very early.

Data management and analysis

Data were collected on a paper case-report form or directly entered online using the Electronic Data capture system OpenClinica™ (www.openclinica.com). Twenty-four hours and one week breastfeeding recalls were collected during the enrolment visit on day 7 (±2) after birth and during the 13 monthly-scheduled follow-up visits that started at week 2. During these visits, mothers were particularly asked if they gave their infants other foods/liquids in addition to

breastmilk. Pre-lacteal feeding data which was defined as any food item except mothers' milk given to infants before initial breastfeeding) were also collected at the enrolment visit. 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 categorized mothers at each visit into the following groups: 1) exclusive breastfeeding, EBF (only breastmilk being given to the infant without any other kind of 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 and predominant breastfeeding" (EPBF) because the number of women who practised PBF was too small and the practice was occasional. The entire cohort was in this latter group at the beginning of the study, which was followed up to detect any change in EPBF status, i.e. change to mixed feeding by week 26 post-partum, which is the time when exclusive breastfeeding is supposed to be changed to complementary feeding. Data on maternal dietary intake were not collected

In a bivariate analysis, we compared the mean BMI at weeks 14, 26, 38 and 50 with the mean BMI at day 7 (baseline) post-delivery using Bonferroni-corrected paired t-tests for multiple comparisons. Data were presented for all 4 sites, by country and baseline BMI groups. Group 1 included women with BMI$<$18.5 kg/m^2 (underweight), Group 2 included those with a BMI between 18.5 and 24.9 kg/m^2 (normal range), and Group 3 were those with a BMI $\geq$25 kg/m^2 (overweight) according to the WHO classification system (http://apps.who.int/bmi/index.jsp?introPage=intro_3.html) [38]. We also compared the mean hemoglobin concentration at week 38 with week 14 (because this data was available only for these visits) in the same way. To take into account the inter-country and inter-subject variability, we ran a further linear mixed-effect model with BMI as the dependent variable and EPBF duration as the key explanatory variable adjusted for baseline BMI and other covariates, including the mother's viral load, CD4 count, hemoglobin concentration, HIV stage, mode of delivery (vaginal versus C-section), parity, age, education level, marital status and occupation. The child's characteristics included its gender, birth weight, treatment group and the breastfeeding initiation time. We checked that the total variance due to country effect was always $>$10% by running a variance component analysis and a likelihood ratio test to confirm that the differences related to countries were significant. Variables with p$\leq$0.20 in the bivariate analysis were considered for the multivariate analysis in the pooled-data analysis for all the countries. We also stratified by country and ran the same multivariate analysis, introducing a country-specific socio-economic status (SES) index.

The principal component methods was used to construct the SES index, [39]. Sixteen asset variables were variously included in the principal component analysis, considering country specificities. The first components (explaining 33, 39, 29 and 34% of the variation for Burkina Faso, South Africa, Uganda and Zambia, respectively) were retained to weigh the variables and calculate the index at the household level. This was the sum of the different variables' weight/score per subject, which was divided into tertiles.

For continuous variables, the mean values with 95% confidence interval (CI) were estimated, and for categorical variables, percentages were used. Medians (IQR) were also reported. Associations between variables were tested using the Chi-square test for categorical variables. STATA/SE 13.1 (4905 Lakeway Drive College Station, Texas 77845 USA) was the statistical software used.

Ethics

Prior to enrolment, the mothers signed 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, the Biomedical Research Ethics Committee in Zambia, the Uganda National Council for Science and Technology, the Stellenbosch University ethical committees, the Medicines Control Council in South Africa and the Regional Committee for Medical Research Ethics of Norway.

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. Thus 1,216 subjects were included in the analysis.

At baseline (Table 1), South Africa had the largest mean BMI, and the highest frequency of single women and C-section delivery. Burkina Faso participants had the lowest HIV viral load, the lowest hemoglobin concentration, and the lowest literacy and formal occupation frequency. Breastfeeding was initiated later in Burkina Faso where EPBF frequency was also the lowest the first week.

During pregnancy antiretroviral prophylaxis was given 100% of women in all 4 countries

Breastfeeding duration and BMI changes

The median (Interquartile Range (IQR)) durations of EPBF and any breastfeeding were 5.8 (5.6; 5.9) and 9.5 (7.5; 10.6) months, respectively. The median (IQR) durations of EPBF were 20.9 (20.0; 21.5), 19.8 (12.9; 21.0), 20.9 (19.9; 21.0), 21.0 (20.6; 21.1) for Burkina Faso, South Africa, Uganda and Zambia, respectively

The BMI of breastfeeding mothers decreased from baseline to week 26 before plateauing until week 50 (Table 2). The same linear trend in the association between breastfeeding duration and BMI decrease was found in the country-specific analysis except in Uganda where we did not find any significant decrease. The maximum weight loss was at week 26 ($p<0.001$) in Burkina Faso and Zambia, whereas it was at week 38 for South Africa and Uganda.

Comparing the hemoglobin concentration at weeks 14 and 38, there was no overall change, as also in the country-stratified analysis ($p>0.05$; see Table 3).

Categorizing women into 3 BMI groups, we found that slimmer women (BMI <18.5 kg/m2) had no statistically significant change in their BMI postpartum ($p>0.05$), while the others had significant decrease over the breastfeeding period (Table 4). The thinnest group of women (BMI <18.5) had the steepest drop in hemoglobin at week 38; however, the difference was not significant (-0.3 (-0.8; 0–3); see Table 5).

Factors associated with BMI changes in mothers

Overall, the univariate analysis showed that EPBF duration and all other controlled variables were significantly associated with the BMI change, but not with the CD4 count, possibly because the most vulnerable group, i.e. those with CD4 counts <350, were excluded from the analysis. However, this variable was kept in the final model because of the known association between CD4 count and HIV disease progression, usually leading to weight loss (Table 6). In

Table 1. Baseline characteristics.

A	Burkina Faso	South Africa	Uganda	Zambia	All sites
	N = 203	N = 212	N = 272	N = 529	N = 1216
	% (95% CI[a])	% (95% CI[a])	% (95% CI[a])	% (95% CI[a])	% (95% CI[a])
CD4>500	57.1 (50.2; 63.8)	51.4 (44.7; 58.1)	57.3 (51.4; 63.1)	59.9 (55.7; 64.0)	57.4 (54.6; 60.2)
Education level					
Incomplete primary	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; 38.8)
Completed primary	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 and more	24.1 (18.7; 30.5)	91.0 (86.4; 94.2)	35.7 (30.2; 41.5)	53.3 (49.0; 57.5)	51.1 (48.2; 53.9)
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)
Married/co-habiting	90.6 (85.8; 93.0)	39.1 (32.8; 45.9)	82.0 (76.9; 86.1)	88.7 (85.7; 91.1)	78.9 (76.5; 81.1)
Mode of delivery (vaginal)	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)
Parity (primiparous)	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)
Trial arm (lamivudine)	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)
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)
Child sex (male)	41.9 (35.2; 48.8)	49.1 (42.4; 55.8)	52.9 (47.0; 58.8)	48.4 (44.1; 52.7)	48.4 (45.6; 51.2)
ART prophylaxis postpartum	100.0	7.1 (4.3; 11.4)	71.0 (65.3; 76.1)	100.0	77.3 (74.9; 79.6)
Breastfeeding initiation time					
Within 1st 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)
After 1st hour and within 1st day	64.0 (57.2; 70.4)	45.7 (39.1; 52.5)	41.2 (35.5; 47.1)	18.9 (15.8; 22.5)	36.1 (33.4; 38.8)
After 1st day	29.1 (23.2; 35.7)	2.8 (1.3; 6.2)	2.9 (1.5; 5.8)	0.4 (0.1; 1.5)	6.2 (4.9; 7.7)
EPBF[b] 1st 3 days	93.1 (88.7; 95.9)	94.3 (90.3; 96.8)	97.8 (95.2; 99.0)	99.0 (97.7; 99.6)	97.0 (95.8; 97.8)
EPBF[b] last 4 days	94.1 (89.8; 96.6)	95.7 (92.1; 97.8)	97.4 (94.7; 98.8)	99.6 (98.5; 99.9)	97.5 (96.5; 98.3)
Any BF 1st week	100.0	97.6 (94.4; 99.0)	100.0	100.0	99.6 (99.0; 99.8)
EPBF[b] 1st week	96.5 (92.9; 98.3)	97.2 (93.8; 98.7)	98.9 (96.6; 99.6)	99.8 (98.7;100.0)	98.6 (97.8; 99.1)
SES[c] tertiles					
Highest tertile	31.5 (30.3 32.8)	19.8 (18.8 20.9)	38.2 (37.1 39.3)	42.3 (41.5 43.1)	
Middle tertile	39.4 (38.1 40.7)	73.6 (72.4 74.7)	27.9 (26.9 29.0)	17.4 (16.8 18.0)	
Lowest tertile	29.1 (27.9 30.3)	6.6 (6.0 7.3)	33.8 (32.7 34.9)	40.3 (39.5 41.1)	
B					
	N = 203	N = 212	N = 272	N = 529	N = 1216
	Median (IQR[d])	Median (IQR[d])	Median (IQR[d])	Median (IQR[d])	Median (IQR[d])
Maternal age (years)	28.0 (24.3; 32.0)	27.3 (23.5; 32.8)	26.9 (23.0; 29.9)	26.8 (23.3; 31.0)	27.0 (23.3; 31.0)
BMI (kg/m^2)	23.0 (21.2; 25.4)	27.8 (24.2; 31.8)	22.8 (20.8; 24.6)	23.2 (21.2; 26.5)	23.7 (21.3; 27.0)
CD4+ cells/μL	526 (428; 653)	505 (426; 648.5)	524.5 (429.5; 624)	548 (445; 705)	530 (432.5; 668.5)
Gestational age (weeks)	39.0 (37.0; 40.0)	38.0 (38.0; 38.0)	40.0 (39.0; 40.0)	38.0 (37.0; 40.0)	38.0 (38.0; 40.0)
HIV viral load[e] (x1000 /mL)	1.8 (0.7; 7.2)	2.1 (0.8; 7.9)	4.1 (0.6; 17.5)	3.8 (1.2; 20.6)	3.0 (0.8; 13.7)
Haemoglobin (g/dL)	11.2 (10.5; 12.1)	12.0 (11.3; 12.9)	12.4(11.7; 13.2)	12.5 (11.6; 13.3)	12.2 (11.3; 13.1)
Birth weight (kg)	2.9 (2.7; 3.2)	3.2 (2.8; 3.5)	3.0 (2.8; 3.3)	3.0 (2.8; 3.3)	3.0 (2.8; 3.3)

[a] Confidence Interval

[b] Exclusive and predominant breastfeeding

[c] Socio-economic status

[d] Interquartile range

[e] N = 173, 167, 180, 477 and 997 for Burkina Faso, South Africa, Uganda, Zambia and overall, respectively

[f] N = 175, 161, 218, 454 and 1008 for Burkina Faso, South Africa, Uganda, Zambia and overall, respectively

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the multivariate analysis shown in Table 6, no association between EPBF duration and mothers' BMI was found. Only baseline BMI, CD4 counts, education level, marital status and child

Table 2. Country-stratified paired t-test comparing mean BMI at different endpoints with baseline mean BMI.

	Burkina Faso	South Africa	Uganda*	Zambia	All sites
	Mean difference (kg/m^2) (95% CI[a])	Mean difference (kg/m^2)(95% CI[a])	Mean difference (kg/m^2) (95% CI[a])	Mean difference (kg/m^2)(95% CI[a])	Mean difference (kg/m^2)(95% CI[a])
BMI at week 14 minus BMI at baseline (D7)	-0.8 (-1.1; -0.4) §	-0.8 (-1.4; -0.3) §	-0.2 (-0.5; 0.1)	-0.5(-0.8; -0.2) §	-0.5(-0.7; -0.4)§
BMI at week 26 minus BMI at baseline (D7)	-1.1 (-2.4; 0.2)	-0.8 (-1.5; -0.1) §		-0.6(-0.9; -0.2) §	-0.7 (-1.0; -0.3)§
BMI at week 38 minus BMI at baseline (D7)	-0.9 (-1.5; -0.4) §	-0.9 (-1.8; -0.0) §	-0.4 (-0.9; 0.1)	-0.6(-1.0; -0.2) §	-0.7 (-0.9; -0.4)§
BMI at week 50 minus BMI at baseline (D7)	-1.0 (-1.7; -0.2) §	-0.8 (-1.7; 0.1)		-0.6(-1.0; -0.2) §	-0.7 (-1.0; -0.3)§

* data on mothers' weight were missing on weeks 26 and 50 in Uganda

§ Significant results

https://doi.org/10.1371/journal.pone.0177259.t002

birth weight remained significant risk factors for BMI changes. Each additional kilogram of mother's baseline weight increased BMI by 1.0 kg/m^2 during the lactation period.

Compared to mothers with a CD4 count of 350–500 cells/μl, the group with the higher count had an increase in their BMI of 0.3 kg/m^2. Regarding education level, the group that completed primary and secondary or further education had a reduced BMI of 0.3 and 0.1 kg/m^2, respectively, over the lactation period. Eighty per cent of women in the last 2 categories were employed compared to 18.8% among those who had zero or some years of primary schooling. Living with a partner also conveyed a mean decrease in BMI of 0.3 kg/m^2 compared to being single. Finally, mothers of babies born with a birth weight of >3,500 g had a mean BMI increase of 0.2 kg/m^2 compared to mothers of babies with <2,500 g.

The multivariate analysis stratified by country (Table 7) shows EPBF had a significantly decreased BMI of 0.1 kg/m^2/month for Zambian participants. South Africa had exactly opposite (of borderline significance) outcome of EPBF on mothers' BMI. No effect from EPBF was seen in the other countries. This relationship may be partially explained by the median duration of EPBF and the proportion of women still on EPBF at week 26 at the different study sites. The median duration of EPBF was highest in Burkina Faso, Uganda and Zambia (5.8 months) and lowest in South Africa (4.9 months). At weeks 26, 27 (13.2%), 4 (0.02%), 12 (0.04%) and 33 (0.06%) were still practicing EPBF in Burkina Faso, South Africa, Uganda and Zambia, respectively. Conversely, baseline BMI was consistently and significantly associated with an increase in mothers' subsequent BMI measures in all countries throughout the lactation period. A higher CD4 count increased BMI in Burkina Faso and Zambia, whereas an HIV stage greater than one decreased BMI in Zambia. In the 4 countries, delivering by C-section or delivering a girl was associated with mean BMI changes in different ways. In Burkina Faso, single mothers had lowered BMI, whereas in South Africa it increased, these participants being in the highest socio-economic tertile. The trial arm was not associated with any BMI change.

Table 3. Country-stratified paired t-test comparing mean hemoglobin at week 14 and week 38 (hemoglobin at week 14 > hemoglobin at week 38).

	Burkina Faso	South Africa	Uganda	Zambia	All sites
Mean hemoglobin (g/dl) at W14 (95% CI)	11.2 (10.8; 11.7)	12.0 (11.5; 12.5)	12.4 (12.1; 12.8)	12.4 (12.1; 12.7)	12.1 (11.9; 12.3)
Mean hemoglobin (g/dl) at W38 (95% CI)	11.2 (10.7; 11.6)	12.1 (11.6; 12.6)	12.5 (12.2; 12.9)	12.6 (12.3; 12.8)	12.3 (12.1; 12.5)
Mean difference (g/dl) (95% CI)	-0.1 (-0.5; 0.3)	0.1 (-0.3; 0.5)	0.1 (-0.2; 0.4)	0.2 (-0.1; 0.5)	0.1 (-0.1; 0.3)

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Table 4. Women stratified in 3 BMI-categories: Paired t-test comparing mean BMI at different endpoints with baseline mean BMI.

	BMI<18.5 N = 50	BMI between 18.5 and 24.9 N = 715	BMI≥25 kg N = 451	All categories N = 1,216
	Mean difference (kg/m^2) (95% CI[a])	Mean difference (kg/m^2) (95% CI[a])	Mean difference (kg/m^2) (95% CI[a])	Mean difference (kg/m^2) (95% CI[a])
BMI at week 14<BMI at baseline (D7)	-0.0 (-1.1; 1.0)	-0.4 (-0.6; -0.2)§	-0.8 (-1.1; -0.4)§	-0.5 (-0.7; -0.4)§
BMI at week 26<BMI at baseline (D7)	-0.2 (-1.6; 1.2)	-0.6 (-1.0; -0.2)§	-0.8 (-1.3, -0.2)§	-0.7 (-1.0; -0.3)§
BMI at week 38<BMI at baseline (D7)	-0.1 (-1.2; 0.9)	-0.7 (-0.9; -0.4)§	-0.8 (-1.3; -0.2)§	-0.7 (-0.9; -0.4)§
BMI at week 50<BMI at baseline (D7)	-0.5 (-2.1; 1.1)	-0.7 (-1.2; -0.3)§	-0.6 (-1.2; -0.0)§	-0.7 (-1.0; -0.3)§

§ Significant results

https://doi.org/10.1371/journal.pone.0177259.t004

Discussion

Considering our results, no marked change in BMI was seen in this cohort over a 26-week period of lactation covering the EPBF period neither in the pooled analysis (Table 6) nor in the country-stratified analysis.The mean change from 26 to 50 weeks after birth was 0.7 kg/m^2 at the paired t-test. This was also the case for changes in hemoglobin concentration. However, the thinnest mothers had the largest change, although not statistically significant. The major factor contributing to the biggest change in BMI was the mother's own BMI after birth. The heaviest mothers gained most weight during lactation. Higher birth weights and CD4 counts correlated with some increase in BMI. Education level showed the opposite pattern, with a higher educational status being associated with a slight reduction in BMI. In the country-stratified analysis, socio-economic status was not a risk factor for BMI change, except in South Africa where high SES was associated with an increase in BMI. However, a HIV stage above 1 correlated with a small decrease in BMI in Zambia.

The magnitude of the mean BMI decrease observed in Table 2 does not seem enough important to have a clinical significance. The most important decrease (minus 1.1 kg/m^2) was seen in Burkina Faso at week 26. The mean BMI for this country was 23.8 (95% CI: 23.2; 24.3) kg/m^2 [data not shown]. Therefore, a decrease of 1.1 kg/m^2 did not put a participant of this site in an underweight category. This analysis was confirmed by the findings in Table 4A where the underweight women group (<18.5 kg/m^2) had a maximum non statistically-significant weight loss of 0.5 kg/m^2 smaller than the overall maximum of 1.1 kg/m^2.

To the best of our knowledge, only one study [40] has tested the dose-response relationship between breastfeeding and HIV disease progression including maternal weight loss. We also tested whether the duration of EPBF was detrimental to the BMI of HIV-infected mothers.

Table 5. BMI-categories-stratified paired t-test comparing mean hemoglobin at weeks 14 and 38 (hemoglobin at week 14> hemoglobin at week 38).

	Number of observations	Mean hemoglobin (g/dl) at W14 (95% CI)	Mean hemoglobin (g/dl) at W38 (95% CI)	Mean difference (g/dl) (95% CI)
BMI<18.5	36	12.1 (10.9; 13.3)	11.8 (10.7; 13.0)	-0.3(-1.5; 0.9)
BMI between 18.5 and 24.9	502	12.2 (11.9; 12.5)	12.3 (12.0; 12.6)	0.1 (-0.2; 0.3)
BMI≥25 kg	295	12.1 (11.8; 12.42)	12.3 (12.0; 12.6)	0.2 (-0.1; 0.5)

§ Significant results

https://doi.org/10.1371/journal.pone.0177259.t005

Table 6. Unadjusted and adjusted mixed-effect model analysis testing BMI change over duration of extended EBF.

	Unadjusted Coefficient (95% CI)	Adjusted Coefficient (95% CI)
EPBF duration	-0.1 (-0.1; -0.0)	-0 (0; 0)
Baseline BMI	1.0 (0.9; 1.0) §	1.0 (0.9; 1.0) §
Mother's age	0.2 (0.1; 0.2) §	0 (0; 0)
CD4 count		
<500	1	1
> = 500	0 (0; 0)	0.3 (0.2; 0.5) §
Number of children	0.4 (0.3; 0.5) §	-
Number of child death	-1.0 (-1.5; -0.5) §	-
BF initiation time	0 (0; 0)	
Within 1 hour	1	-
After 1 hour and within 1st day	-0.1 (-0.4; 0.1)	-
After 1st day	0.9 (0.3; 1.5) §	-
Child gender		
Male	1	-
Female	0.2 (0.0; 0.5)	-
HIV stage		
1	1	1
>1	-1.1 (-1.8; -0.3) §	-0.1 (-0.4; 0.2)
Education level	0.3 (0.1; 0.5) §	
No education or some primary	1	1
Complete primary	1.0 (0.6; 1.5) §	-0.3 (-0.4; -0.1) §
Secondary and more	0.6 (0.3; 1.0) §	-0.1 (-0.3; -0.0)
Marital status		
Single	1	1
Married/cohabiting	-0.9 (-1.2; -0.6) §	-0.3 (-0.4; -0.2) §
Mode of delivery		
Vaginal	1	-
C-section	1.8 (1.4; 2.1) §	-
Parity		
Primiparous	1	-
Multiparous	1.8 (1.5; 2.0) §	-
Trial arm		
lamivudine	1	1
lopinavir/ritonavir	0.2 (0.0; 0.4)	-0.0 (-0.1; 0.0)
Child birth weight	0 (0; 0)	
<2.5 kg	1	1
2.5 to 3.4 kg	1.0 (0.5; 1.4) §	-0.0 (-0.2; 0.2)
> = 3.5 kg	3.2 (2.8; 3.7) §	0.2 (0.0; 0.4)
Gestational age	0.5 (0.4; 0.6) §	-

§ Significant results

https://doi.org/10.1371/journal.pone.0177259.t006

Other studies [28,29] compared any breastfeeding group with a formula-feeding group. A Tanzanian study [40], despite its loss to follow-up, concluded that neither the modality of breastfeeding (exclusive or any), nor the duration of breastfeeding had an influence on the of mothers with HIV infection. However, this research was focused on HIV disease progression, and considered parameters that included maternal HIV viral load, CD4 count and weight loss.

Table 7. Multivariate country-stratified analysis testing BMI change in breastfeeding mothers over the duration of extended EBF.

	Adjusted Coefficient (95% CI)			
	Burkina Faso	**South Africa**	**Uganda**	**Zambia**
EPBF duration	0.0 (-0.1; 0.2)	0.1 (-0.0; 0.1)	-0.0 (-0.1; 0.1)	-0.1 (-0.1; -0.0)
Baseline BMI	0.9 (0.9; 1.0) §	1.0 (0.9; 1.0) §	1.0 (0.9; 1.0) §	1.0 (0.9; 1.0) §
Mothers' age	-0.0 (-0.0; 0.0)	0.0 (-0.0; 0.1)	-0.00 (-0.0; 0.0)	-0.0 (-0.0; 0.0)
CD4 count				
<500	1	1	1	1
> = 500	0.5 (0.2; 0.8) §	0.2 (-0.1; 0.4)	0.1 (-0.1; 0.4)	0.2 (0.0; 0.4)
Child sex				
Male	1	1	1	1
Female	-0.4(-0.6;-0.1) §	0.5 (0.1; 0.9) §	-0.5 (-0.8; -0.2) §	-0.8 (-1.8; 0.1)
HIV stage				
1	1	1	1	1
>1	0.4 (-0.1; 0.8)	0.6 (-0.4; 1.6)	-0.0 (-0.1; 0.1)	-0.1 (-0.1; -0.0)
Marital status				
Single	1	1	1	1
Married/cohabiting	-1.0 (-1.5; -0.5) §	-	-	-
Mode of delivery				
Vaginal	1	1	1	1
C-section		-0.5 (-1.0; -0.1) §		0.6 (0.0; 1.2)
Trial arm				
lamivudine	1	1	1	1
lopinavir/ritonavir	-0.0 (-0.3; 0.3)	0.2 (-0.2; 0.6)	-0.16 (-0.3; 0.0)	-0.2 (-0.4; 0.0)
Child birth weight				
<2.5 kg	1	1		
> = 2.5 to 3.4 kg	0.1 (-0.4; 0.6)	-0.5 (-1.2; 0.2)	-0.1 (-0.6; 0.3)	0.3 (-0.1; 0.8)
> = 3.5 kg	0.3 (-0.3; 0.9)	0.0 (-0.7; 0.8)	0.2 (-0.7; 0.2)	0.4 (-0.2; 0.9)
SES (tertiles)				
Highest tertile	1			
Middle tertile	-0.3 (-0.6; 0.0)	0.0 (-0.5; 0.5)	0.2 (-0.4; 0.1)	-0.1 (-0.4; 0.1)
Lowest tertile	0.0 (-0.3; 0.4)	0.6 (0.1; 1.1) §	0.2 (-0.0; 0.5)	0.2 (-0.1; 0.6)

§ Significant results

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It recorded relative risks as association measures, making it difficult to compare with our study in terms of absolute numbers. In a randomized trial on prolonged breastfeeding and maternal mortality in Zambia [41], there seemed to be no harmful effect of breastfeeding. The study was well designed, but its main defect lies in the failure toadhere fully to random assignment by the participants, although the investigators deemed this had no significant effect on the findings. Another analysis of the same Zambian data [27] over 2 years, in which there was control for confounding, such as SES, obstetric history, season and food shortage, reported net weight gain rather than weight loss, which has also been found in undernourished lactating mothers [25,42,43]. Hartmann [43] explained this weight gain by a "homeorhetic theory of metabolic adjustment" in favor of a dominant physiological state such as lactation. This may also explain why the thinnest mothers did not lose weight during EPBF period in our cohort. However, we do not have sufficient data to address issues such as food shortages, or other practical or mental issues, that could have led to the underweight status in the first place.

Our study did not have a non-breastfeding group for comparison, and therefore cannot confirm or refute the findings from Kenya [28,29], where breastfeeding women were found that lose more weight than non-breastfeeding ones. Another study in South Africa [44] showed that breastfeeding HIV-positive mothers lost more weight than breastfeeding HIV-negative mothers. In addition to the limitations of the study (including the lack of power of the study), the design did not truly allow attribution of the weight change to the breastfeeding factor, since it did not compare breastfeeding and formula groups, nor assess a dose-response by considering the duration of breastfeeding; instead, the study compared cross-sectional data at different time points.

The different studies referred to above were not directly comparable, mainly because of differences in follow-up time. It was 24 months in the Vertical Transmission Study [14]; 20 months in the Zambian study with follow-up starting at 4 months post-partum [27]; and 6 months in the Kesho Bora trial [24]. The Kenyan studies had a number of methodological issues [45].

In an analysis of 2 sets of data from Honduras [46], EBF did not affect maternal weight loss in one study regarding 119 full-term low birth-weight infants. In the second study of 141 infants of low-income primiparous women, the EBF group lost more weight than mothers feeding their children with solid foods between 4 and 6 months of age. Being of a low income group could contribute to poor dietary intake, thereby contributing to the weight loss. The study design may not have controlled properly all potential confounders.

Validity of our findings

This analysis was done on data collected from a large sample of HIV-positive mothers from 4 different countries in a clinical trial setting. However, the rigorous selection criteria may have resulted in a recruited a study population that was not strictly representative of the general population. Hence, the association between the HIV stage and BMI change may not reflect that in the general population of HIV-positive mothers, since only patients with CD4 counts above 350 and at stage 1 or 2 were included, and almost all of them remained at the same asymptomatic stage until the end of the study. The SES index calculation proved challenging because the study environments in the 4 countries were diverse; for this reason we built a country-specific SES since a pooled 4-country index was not meaningful. Moreover, the clinical trial context was not the routine or standard care environments for most women, which may have distorted the SES effect on BMI change. However, these issues were less likely to influence the association between EPBF duration and BMI change.

It is also possible that some reporting bias regarding our variable of interest was involved, since in some sites (e.g. Burkina Faso), the study team in charge of nutritional counseling also collected the breastfeeding data. No data was collected to validate the mothers' reports of their own feeding practices. We dropped all subjects without breastfeeding data or with obvious inaccurate data to partially mitigate this issue.

We also failed to consider the mothers' daily food intake and physical activities as important factors in our assessment of BMI change. Nevertheless, we believe that the nutritional, financial (transport costs) and technical support provided to all participants in the trial, combined with the relative homogeneity of our study population tended to minimize the effect of disparities in general and also those that were eventually related to food intake and physical activities.

Conclusion

Breastfeeding did not affect the BMI in HIV-1 infected Sub-Saharan African mothers when their CD4 counts were >350 cells/μl. However, a higher baseline BMI and a CD4 count >500

cells/μl led to an increase in BMI during the EPBF period. Considering the benefits of breast milk for infants, and the recurrent 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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References

1. WHO/IER/MHI/STM.09.1 (2009) Women and health: today evidence, tomorrow agenda. Geneva: 10.
2. van den Akker T, de Vroome S, Mwagomba B, Ford N, van Roosmalen J (2011) Peripartum infections and associated maternal mortality in rural Malawi. Obstet Gynecol 118: 266–272. https://doi.org/10.1097/AOG.0b013e3182254d03 PMID: 21775841
3. Moran NF, Moodley J (2012) The effect of HIV infection on maternal health and mortality. Int J Gynaecol Obstet 119 Suppl 1: S26–29.
4. Gorman SE (2013) A new approach to maternal mortality: the role of HIV in pregnancy. Int J Womens Health 5: 271–274. https://doi.org/10.2147/IJWH.S46872 PMID: 23766661
5. WHO, UNICEF, UNFPA, Bank aTW (2010) Trends inMaternal Mortality: 1990 to 2008. World Health Organization: 52.
6. Calvert C, Ronsmans C (2013) The contribution of HIV to pregnancy-related mortality: a systematic review and meta-analysis. AIDS 27: 1631–1639. https://doi.org/10.1097/QAD.0b013e32835fd940 PMID: 23435296
7. Van der Paal L, Shafer L, Mayanja B, Whitworth J, and Grosskurth H (2007) Effect of pregnancy on HIV disease progression and survival among women in rural Uganda. Tropical Medicine and International Health 12: 9.
8. Ross A, Van der Paal L, Lubega R, Mayanja B, Shafer L, et al. (2004) HIV-1 disease progression and fertility: the incidence of recognized pregnancy and pregnancy outcome in Uganda. AIDS 18: 6.
9. WHO (1985) Workshop on AIDS in Central Africa: Bangui, Central African Republic; 22 to 25 October 1985. WHO/CDS/AIDS 85: 16.
10. WHO (1994) WHO case definitions for AIDS surveillance in adults and adolescents. Wkly epidem rec 69: 3.
11. WHO (1986) Acquired Immuno Deficiency Syndrome WHO/CDC case definition for AIDS. Wkly epidem rec 61: 5.
12. Organization PAH (1989) Working group on AIDS case definition. Epidemiological Bulletin 10: 4.

13. World Health Organization (2007) WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. World Health Organization 2007: 52.
14. Chetty T, Carter RJ, Bland RM, Newell ML (2014) HIV status, breastfeeding modality at 5 months and postpartum maternal weight changes over 24 months in rural South Africa. Trop Med Int Health 19: 852–862. https://doi.org/10.1111/tmi.12320 PMID: 24720779
15. Kosmiski L (2011) Energy expenditure in HIV infection. Am J Clin Nutr 94: 1677S–1682S. https://doi.org/10.3945/ajcn.111.012625 PMID: 22089443
16. Stuebe AM, Rich-Edwards JW (2009) The reset hypothesis: lactation and maternal metabolism. Am J Perinatol 26: 81–88. https://doi.org/10.1055/s-0028-1103034 PMID: 19031350
17. Ministry of Health (2006) Food and Nutrition Guidelines for Healthy Pregnant and Breastfeeding Women: A background paper. Wellington: Ministry of Health: 212.
18. Kuhn L, Stein Z, and Susser M (2004) Preventing mother-to-child HIV transmission in the new millennium: the challenge of breast feeding. Paediatr Perinat Epidemiol 18: 7.
19. Cournil A, De Vincenzi I, Gaillard P, Cames C, Fao P, et al. (2013) Relationship between mortality and feeding modality among children born to HIV-infected mothers in a research setting: the Kesho Bora study. AIDS 27: 1621–1630. https://doi.org/10.1097/QAD.0b013e32835d5226 PMID: 23262499
20. Kieffer M, Mattingly M, Giphart A, van de Ven R, Chouraya C, et al. (2014) Lessons Learned From Early Implementation of Option B+: The Elizabeth Glaser Pediatric AIDS Foundation Experience in 11 African Countries. J Acquir Immune Defic Syndr 67: 7.
21. WHO (2016) Consolidated guidelines on HIV prevention, diagnosis, treatment and care for key populations. WHO Guidelines: 180.
22. World Health Organization, United Nations Children's Fund (2016) Guideline: updates on HIV and infant feeding: the duration of breastfeeding, and support from health services to improve feeding practices among mothers living with HIV. Geneva: World Health Organization.
23. Kayira D, Bentley ME, Wiener J, Mkhomawanthu C, King CC, et al. (2012) A lipid-based nutrient supplement mitigates weight loss among HIV-infected women in a factorial randomized trial to prevent mother-to-child transmission during exclusive breastfeeding. Am J Clin Nutr 95: 759–765. https://doi.org/10.3945/ajcn.111.018812 PMID: 22258269
24. Cames C, Cournil A, de Vincenzi I, Gaillard P, Meda N, et al. (2014) Postpartum weight change among HIV-infected mothers by antiretroviral prophylaxis and infant feeding modality in a research setting. AIDS 28: 85–94. https://doi.org/10.1097/01.aids.0000433243.24481.c3 PMID: 24413262
25. Winkvist A, Rasmussen K, and Lissner L (2003) Associations between reproduction and maternal body weight: examining the component parts of a full reproductive cycle. Eur J Clin Nutr 57: 14.
26. Kesho Bora Study G (2011) Safety and effectiveness of antiretroviral drugs during pregnancy, delivery and breastfeeding for prevention of mother-to-child transmission of HIV-1: the Kesho Bora Multicentre Collaborative Study rationale, design, and implementation challenges. Contemp Clin Trials 32: 74–85. https://doi.org/10.1016/j.cct.2010.09.008 PMID: 20854932
27. Murnane PM, Arpadi SM, Sinkala M, Kankasa C, Mwiya M, et al. (2010) Lactation-associated postpartum weight changes among HIV-infected women in Zambia. Int J Epidemiol 39: 1299–1310. https://doi.org/10.1093/ije/dyq065 PMID: 20484334
28. Nduati R, Richardson B, John G, Mbori-Ngacha D, Mwatha A, et al. (2001) Effect of breastfeeding on mortality among HIV-1 infected women: a randomised trial. Lancet 357: 1651–1655. https://doi.org/10.1016/S0140-6736(00)04820-0 PMID: 11425369
29. Otieno PA, Brown ER, Mbori-Ngacha DA, Nduati RW, Farquhar C, et al. (2007) HIV-1 disease progression in breast-feeding and formula-feeding mothers: a prospective 2-year comparison of T cell subsets, HIV-1 RNA levels, and mortality. J Infect Dis 195: 220–229. https://doi.org/10.1086/510245 PMID: 17191167
30. Laar A, Grant F, Addo Y, Soyiri I, Nkansah B, et al. (2013) Predictors of fetal anemia and cord blood malaria parasitemia among newborns of HIV-positive mothers. BMC research notes 6: 9.
31. Orish VN, Onyeabor OS, Boampong JN, Acquah S, Sanyaolu AO, et al. (2013) The effects of malaria and HIV co-infection on hemoglobin levels among pregnant women in Sekondi-Takoradi, Ghana. Int J Gynaecol Obstet 120: 236–239. https://doi.org/10.1016/j.ijgo.2012.09.021 PMID: 23219288
32. Ziske J, Kunz A, Sewangi J, Lau I, Dugange F, et al. (2013) Hematological changes in women and infants exposed to an AZT-containing regimen for prevention of mother-to-child-transmission of HIV in Tanzania. PLoS One 8: e55633. https://doi.org/10.1371/journal.pone.0055633 PMID: 23405185
33. Parkes-Ratanshi R, Katende D, Levin J, Wakeham K, Heiner G, et al. (2015) Development of Severe Anemia and Changes in Hemoglobin in a Cohort of HIV-Infected Ugandan Adults Receiving

Zidovudine-, Stavudine-, and Tenofovir-Containing Antiretroviral Regimens. J Int Assoc Provid AIDS Care 14: 455–462. https://doi.org/10.1177/2325957414557264 PMID: 25425638

34. Odhiambo C, Zeh C, Angira F, Opollo V, Akinyi B, et al. (2016) Anaemia in HIV-infected pregnant women receiving triple antiretroviral combination therapy for prevention of mother-to-child transmission: a secondary analysis of the Kisumu breastfeeding study (KiBS). Trop Med Int Health 21: 373–384. https://doi.org/10.1111/tmi.12662 PMID: 26799167

35. Nagot N, Kankasa C, Meda N, Hofmeyr J, Nikodem C, et al. (2012) Lopinavir/Ritonavir versus Lamivudine peri-exposure prophylaxis to prevent HIV-1 transmission by breastfeeding: the PROMISE-PEP trial Protocol ANRS 12174. BMC Infect Dis 12: 246. https://doi.org/10.1186/1471-2334-12-246 PMID: 23039034

36. Nagot N, Kankasa C, Tumwine J, Meda N, Hofmeyr G, et al. (2015) Extended pre-exposure prophylaxis with lopinavir–ritonavir versus lamivudine to prevent HIV-1 transmission through breastfeeding up to 50 weeks in infants in Africa (ANRS 12174): a randomised controlled trial. The Lancet 14: 8.

37. ANRS (2008) ANRS scale to grade the severity of adverse events in adults; version n° 1.0 4 November 2008. file:///C:/Users/install/Downloads/ANRS-GradeEI-V1-En-2008pdf; Accessed 14 Jan 2016: 10.

38. WHO (1995) Physical status: the use and interpretation of anthropometry. WHO technical report series 854: 452.

39. Filmer D, And Pritchet L (2001) Estimating wealth effects without expenditure data- or tears: an application to educational enrollment in States of India. Demography 38: 19.

40. Sedgh G, Spiegelman D, Larsen U, Msamanga G, and Fawzi W (2004) Breastfeeding and maternal HIV-1 disease progression and mortality. AIDS 18: 1043–1049. PMID: 15096808

41. Kuhn L, Kasonde P, Sinkala M, Kankasa C, Semrau K, et al. (2005) Prolonged breast-feeding and mortality up to two years post-partum among HIV-positive women in Zambia. AIDS 19: 1677–1681. PMID: 16184038

42. Ladner J, Castetbon K, Leroy V, Nyiraziraje M, Chauliac M, et al. (1998) Pregnancy, body weight and human immunodeficiency virus infection in African women: a prospective cohort study in Kigali (Rwanda), 1992–1994. Int J Epidemiol 27: 6.

43. Hartmann P, Sherriff J, and Mitoulas L (1998) Homeostatic Mechanisms that Regulate Lactation during Energetic Stress. J Nutr 128: 6.

44. Papathakis P, Van Loan M, Rollins N, Chantry C, Bennish M, et al. (2006) Body Composition Changes During Lactation in HIV-Infected and HIV-Uninfected South African Women. J Acquir Immune Defic Syndr 43: 8.

45. Kuhn L, Thea DM, Aldrovandi GM (2007) Re: HIV-1 disease progression in breast-feeding and formula-feeding mothers: a prospective 2-year comparison of T cell subsets, HIV-1 RNA levels, and mortality. J Infect Dis 195: 2.

46. Dewey K, Cohen R, Brown K, LL aR (2001) Effects of Exclusive Breastfeeding for Four versus Six Months on Maternal Nutritional Status and Infant Motor Development: Results of Two Randomized Trials in Honduras. J Nutr 131: 6.

Paper III

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BMJ Open

HIV-1 disease progression in immune-competent HIV-1-infected and breastfeeding mothers participating in the ANRS 12174 clinical trial in Burkina Faso, South Africa, Uganda and Zambia: a cohort study

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ABSTRACT

Objective We have assessed HIV-1 disease progression among HIV-1-positive mothers in relation to duration of any or exclusive breast feeding in the context of ANRS 12174 trial.

Methods The analysis was completed on 203, 212, 272 and 529 HIV-1-positive and lactating mothers with CD4 count >350 cells/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 endpoint called HIV-1 disease progression where HIV-1 viral load was replaced by the HIV-1 clinical stage. Exclusive or predominant breast feeding (EPBF) 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 to 7.7) CD4 cells/µL per each additional kilogram per square metre of BMI) and haemoglobin concentration (19.4 (95% CI 11.4 to 27.4) CD4 cells/µL per each additional gram per decilitre of haemoglobin 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 Breast feeding 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.

Trial registration number NCT0064026; Post-results.

Strengths and limitations of this study

- Our study has been implemented in four 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 minimised the loss 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 endpoints (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 was 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 recognised as major criteria of the diagnosis of AIDS.[8] This weight loss is also associated

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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, breast feeding prolongs the increased metabolic demands. Despite this, WHO still recommends HIV-1-infected women to breast feed 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 breast feeding in HIV-1-infected mothers. Some studies found that breast feeding was harmful to HIV-positive mothers by either accelerating HIV disease progression as assessed by the mother's weight loss, a decrease in CD4 cell count or even an increased risk of maternal mortality, suggesting that metabolic, immunological or hormonal changes associated with breast feeding 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 breast feeding protective, allowing weight gain in HIV-1-infected breastfeeding mothers.[15 18–22]

In the ANRS 12174 trial, we assessed mothers' HIV-1 disease progression (measured by the change in weight, CD4 cell count and HIV-1 disease stage as per WHO classification) in relation to exclusive breast feeding or duration of any breast feeding 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 and planning to breast feed were identified from antenatal clinics between 28 and 40 weeks of amenorrhoea. As part of the HIV post-test counselling session, they were informed on the different feeding options for their babies. Only women intending to breast feed 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 post partum. 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) post partum, low birthweight 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 1 week from day 7 to 50 weeks of age with either lopinavir/ritonavir or lamivudine.

Data management and analysis

Data were collected on a paper case-report form or directly entered online using the Electronic Data capture system: OpenClinica (http://www.openclinica.com). Twenty-four-hour and 1-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 breast milk. Prelacteal feeding data—

defined as any food item except mothers' milk given to infants before initial breast feeding—were also collected at the enrolment visit.

The mothers at each visit were categorised into the following groups: (1) exclusive breast feeding, EBF (only breast milk being given to the infant without any other food or liquid, except medically prescribed drugs or vitamins); (2) predominant breastfeeding, PBF (breast milk 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 (breast milk with other solid-based 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 dependent 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 post partum, which was compared with 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

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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 g 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 dependent variables, and EPBF or any breast feeding as key independent variables. The loss 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 (body mass index (BMI), education level, marital status, haemoglobin concentration) or on day 7 post partum (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 two strata comprising South African mothers (stratum 1) and Burkina Faso, Uganda and Zambia together (stratum 2) because South Africa presented important socioeconomic, cultural and demographic differences compared with the other countries. For continuous variables, the mean values with 95% CI were estimated, and for categorical variables, percentages were used. Associations between variables were tested using the χ^2 test for categorical variables. STATA/SE V.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.

RESULTS

In the ANRS 12174 trial, 1273 mother–infant pairs were randomised and six were excluded due to protocol violations. Of the remaining 1267 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 1216 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

Table 1a Baseline characteristics collected at screening one or on day 7 post partum 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 zidovudineregimen post-delivery (days)*	6.6 (6.5 to 6.8)	7 (7.0 to 7.0)	6.8 (6.7 to 6.9)	7.0 (6.9 to 7.0)	6.9 (6.8 to 7.0)
Mean duration of lamivudine regimen post-delivery (days)*	6.6 (6.5 to 6.8)	Data not available	6.7 (6.6 to 6.8)	7.0 (6.9 to 7.0)	6.8 (6.8 to 6.9)
Mean baseline CD4 count×10^2 cells/µL	5.6 (5.4 to 5.8)	5.5 (5.3 to5.7)	5.6 (5.4 to 5.8)	6.0 (5.8 to 6.2)	5.8 (5.7 to 5.9)
Mean baseline viral load×10^3 copies/µL	23.0 (7.3 to 38.7)	13.5 (7.5 to 19.6)	34.9 (19.7 to 50.0)	29.1 (21.5 to 36.6)	26.4 (21.1 to 31.8)
Baseline mothers' weight (kg)	62.9 (61.4 to 64.5)	72.1 (70.0 to 74.1)	58.1 (57.0 to 59.2)	62.0 (61.0 to 62.9)	63.0 (62.3 to 63.7)
Mean EPBF duration (months)	6.3 (6.2 to 6.4)	4.8 (4.7 to 4.9)	5.6 (5.5 to 5.7)	6.0 (5.9 to 6.1)	5.8 (5.7 to 5.9)
Mean breastfeeding duration (months)	10.5 (10.4 to 10.6)	6.7 (6.6 to 6.8)	8.4 (8.3 to 8.5)	8.4 (8.3 to 8.5)	8.4 (8.3 to 8.5)

*AZT and 3TC (zidovudine and lamivudine) 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.
EPBF, exclusive or predominant breast feeding.

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Table 1b Baseline characteristics collected at screening one or on day 7 post partum 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 to 32.6)	34.4 (28.3 to 41.1)	39.3 (33.7 to 45.3)	37.8 (33.8 to 42.0)	35.6 (33.0 to 38.3)
25–30	36.9 (30.6 to 43.8)	31.2 (25.2 to 37.7)	35.7 (30.2 to 41.5)	33.1 (29.2 to 37.2)	34.0 (31.3 to 36.7)
30 and above	36.9 (30.6 to 43.8)	34.4 (28.3 to 41.1)	25.0 (20.2 to 30.5)	29.1 (25.4 to 33.1)	30.4 (27.9 to 33.1)
HIV stage 1	93.1 (88.7 to 95.9)	98.6 (95.7 to 99.5)	92.3 (88.4 to 94.9)	99.8 (98.7 to 100.0)	96.8 (95.6 to 97.6)
Education					
Did not complete primary school	68.5 (61.7 to 74.5)	8.5 (5.4 to 13.1)	48.5 (42.6 to 54.5)	28.2 (24.5 to 32.2)	36.0 (33.4 to 0.38.8)
Completed primary school	7.4 (4.5 to 11.9)	0.5 (0.1 to 3.3)	15.8 (11.9 to 20.6)	18.5 (15.4 to 22.1)	12.9 (11.1 to 14.9)
Secondary school and more	24.1 (18.7 to 30.5)	91.0 (86.4 to 94.2)	35.7 (0.30.2 to 41.5)	53.3 (49.0 to 57.5)	51.1 (48.2 to 53.9)
Marital status (married)	90.6 (85.8 to 94.0)	39.1 (32.8 to 45.9)	82.0 (76.9 to 86.1)	88.7 (85.7 to 91.1)	78.9 (76.5 to 81.1)
Occupation (employed)	8.9 (5.6 to 13.6)	41.5 (35.0 to 48.3)	35.3 (29.8 to 41.2)	17.0 (14.0 to 20.5)	24.0 (21.7 to 26.5)
Primipara	21.7 (16.5 to 27.9)	33.5 (27.4 to 40.1)	18.0 (13.9 to 23.0)	20.6 (17.4 to 24.3)	22.4 (20.2 to 24.9)
Vaginal delivery	93.6 (89.3 to 96.2)	65.1 (58.4 to 71.2)	93.4 (89.7 to 95.8)	96.2 (94.2 to 97.5)	89.7 (87.9 to 91.3)
Breastfeeding initiation time (within 1 hour)	6.9 (4.1 to 11.3)	51.4 (44.7 to 58.1)	55.9 (49.9 to 61.7)	80.7 (77.1 to 83.9)	57.7 (54.9 to 60.5)
Lamivudine arm	49.7 (42.9 to 56.6)	51.9 (45.1 to 58.6)	49.6 (43.7 to 55.6)	50.3 (46.0 to 54.5)	50.3 (47.5 to 53.1)
Female baby	41.9 (35.2 to 48.8)	49.1 (42.4 to 55.8)	52.9 (46.0 to 58.8)	48.4 (44.1 to 52.7)	48.4 (45.6 to 51.2)

and any breastfeeding durations shortest in South Africa where the HIV-1 viral load was also the lowest (table 1a and b).

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 with those who did not complete primary school (table 2a).

The association between CD4 cell count and EPBF duration was non-significant (5.4 (95% CI −0.1 to 10.9) and 4.5 (95% CI −6.2 to 15.1) CD4 cells/µL increase per month of EPBF duration at univariate and multivariate analyses, respectively). The association was significantly positive between the mothers' baseline BMI, haemoglobin concentration and CD4 cell count yielding a mean increase of 4.9 (95% CI 2.1 to 7.7) CD4 cells/µL per additional BMI unit and 19.4 (95% CI 11.4 to 27.4) CD4 cells/µL per additional unit of haemoglobin 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, randomisation to the lopinavir/ritonavir arm or being a single mother led to a significantly adjusted OR of 1.3 (95% CI 1.0 to 1.6; P=0.04) and 1.6 (95% CI 1.3 to 2.1), respectively (table 2d).

Considering any breastfeeding duration, there was no weight change at univariate and multivariate analyses overall (table 3a). Still regarding any breast feeding, overall, there was a significant mean increase of 5.7 (95% CI 0.4 to 10.9) CD4 cells/µL per month of any breast feeding. We found also that being a single mother was associated with a mean decrease of −43.3 (95% CI −72.8 to −13.7) CD4 cells/µL as compared with 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).

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Table 2a Mothers' 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)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)
Dependent variable=mother's weight						
EPBF duration (months)	0.1 (−0.7 to 0.9)	−0.2 (−0.6 to 0.1)	−0.1 (−0.5 to 0.3)	0.1 (−0.0 to 0.3)	−0.2 (−0.5 to 0.2)	−0.1 (−0.2 to 0.1)
Baseline BMI (kg/m^2)	2.5 (2.4 to 2.7)	2.4 (2.3 to 2.6)	2.5 (2.4 to 2.6)	2.4 (2.3 to 2.5)	2.5 (2.4 to 2.6)	2.4 (2.3 to 2.5)
Mother's age (years)	0.8 (0.5 to 1.2)	0.1 (0.0 to 0.3)	0.5 (0.4 to 0.6)	0.1 (0.1 to 0.2)	0.5 (0.4 to 0.7)	0.1 (0.1 to 0.2)
HIV disease stage						
HIV stage 1						
HIV stage >1	12.4 (−4.5 to 29.4)	6.7 (0.4 to 13.1)	−2.8 (−6.4 to 0.8)			
Education						
Did not complete primary school			1	1	1	1
Completed primary school			3.9 (2.0 to 5.9)	0.2 (−0.7 to 1.1)	4.4 (2.2 to 6.5)	0.6 (−0.3 to 1.5)
Secondary school and more			2.7 (1.2 to 4.1)	0.7 (0.1 to 1.4)	3.1 (1.5 to 4.6)	1.1 (0.4 to 1.8)
Marital status						
Married/ cohabiting mothers	1				1	
Single mothers	−3.1 (−7.2 to 0.9)				−1.2 (−3.0 to 0.5)	
Delivery						
Vaginal delivery	1		1		1	1
C-section delivery	3.7 (−0.4 to 7.9)		4.5 (1.5 to 7.6)		4.4 (2.1 to 6.7)	−1.1 (−2.1 to −0.1)
Parity						
Primipara	1		1		1	–
Multipara	6.1 (1.9 to 10.3)		3.1 (1.4 to 4.7)		3.9 (2.3 to 5.4)	–
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ ritonavir arm	2.0 (−2.0 to 6.1)	0.8 (−0.7 to 2.3)	−0.1 (−1.4 to 1.3)	−0.3 (−0.9 to 0.2)	0.3 (−1.0 to 1.6)	−0.1 (−0.7 to 0.4)

BMI, body mass index; EPBF, exclusive or predominant breast feeding.

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, b, c and d). 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 breast feeding 1 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 breast feeding and the mothers' weight, CD4 cell count and HIV-1 disease progression in any of the strata (table 3a, b and d). 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

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Table 2b Mothers' CD4 cell 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)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)
Dependent variable=CD4 cell count						
EPBF duration (months)	−1.0 (−8.9 to 7.0)	−6.4 (−18.6 to 5.8)	9.3 (2.3 to 16.3)	7.9 (−4.2 to 20.1)	5.4 (−0.1 to 10.9)	4.5 (−6.2 to 15.1)
Baseline BMI (kg/m^2)			4.9 (2.4 to 7.3)	5.9 (2.5 to 9.2)	3.3 (1.3 to 5.3)	4.9 (2.1 to 7.7)
Mother's age (years)	−3.1 (−7.5 to 1.2)		−4.9 (−7.3 to −2.5)	−6.2 (−8.6 to −3.8)	−4.7 (−6.9 to −2.6)	−6.2 (−8.4 to −4.1)
Haemoglobin concentration (g/dL)	33.3 (12.7 to 53.8)	34.8 (14.4 to 55.1)	15.2 (7.8 to 22.6)	12.9 (4.6 to 21.2)	19.3 (12.3 to 26.4)	19.4 (11.4 to 27.4)
Breastfeeding initiation time						
Breastfeeding initiation within 1 hour	1	1	1	1	1	–
Breastfeeding initiation after 1 hour	−56.2 (−94.9 to −17.4)	−39.9 (−90.3 to 10.6)	−40.5 (−60.1 to −20.9)		−42.5 (−61.1 to −23.9)	–
Child's gender						
Male babies	1	1	1			
Female babies	−53.1 (−104.7 to −1.6)	−52.9 (−103.0 to −2.9)	21.8 (−3.9 to 47.4)			
HIV disease stage						
HIV stage 1			1	1	1	1
HIV stage >1			−85.8 (−131.5 to −40.2)	−86.5 (−147.1 to −26.0)	−70.2 (−115.5 to −25.0)	−83.7 (−144.1 to −23.4)
Education						
Did not complete primary school			1		1	1
Completed primary school			29.2 (−8.6 to 67.1)		25.1 (−12.6 to 62.9)	24.4 (−12.9 to 61.6)
Secondary school and more			1.0 (−26.8 to 28.9)		−7.8 (−34.9 to 19.3)	−9.3 (−36.8 to 18.2)
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			−34.5 (−73.2 to 4.2)	−44.6 (−83.1 to −6.03)	−24.6 (−55.9 to 6.6)	−29.7 (−61.0 to 1.6)
Delivery						
Vaginal delivery			1	1		
C-section delivery			71.6 (11.7 to 131.4)	71.1 (11.1 to 131.2)		
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	−33.4 (−69.2 to 2.3)	−65.3 (−116.4 to −14.1)	−12.8 (−31.6 to 6.1)	−12.9 (−38.2 to 12.4)	−15.8 (−32.6 to 1.0)	−19.2 (−41.9 to 3.6)

BMI, body mass index; EPBF, exclusive or predominant breast feeding.

load related to EPBF or any breast feeding. The same conclusion applied to these outcomes was 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 breast feeding or generally between the mode

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Table 2c Mothers' 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)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)
Dependent variable=viral load (coefficient×10^3)						
EPBF duration (months)	4.5 (–3.4 to 12.4)	–3.6 (–11.5 to 4.4)	5.4 (–7.1 18.0)	2.0 (–11.3 15.4)	6.2 (–2.5 to 14.9)	1.7 (–7.3 to 10.8)
Baseline BMI (kg/m^2)	–7.7 (–10.9 to –4.6)	–14.5 (–17.9 to –11.0)	–4.7 (–8.5 to –1.0)	–5.7 (–9.8 to –1.6)	–6.5 (–9.2 to –3.8)	–8.0 (–11.0 to –4.9)
Mother's age (years)	–2.7 (–5.3 to –0.1)	–2.7 (–5.5 to 0.1)	–1.9 (–4.6 to 0.8)	–4.5 (–7.7 to –1.4)	–2.1 (–4.3 to 0.1)	–4.5 (–7.0 to –2.0)
Breastfeeding initiation time						
Breastfeeding initiation <1 hour	1	1				
Breastfeeding initiation >1 hour	70.5 (41.3 to 99.7)	45.1 (13.5 to 76.7)				
Child's gender						
Male babies	1	1	1	1	1	1
Female babies	–49.1 (–79.4 to 18.7)		–19.5 (–48.5 to 9.4)	–36.5 (–66.0 to 7.2)	–25.3 (–49.2 to –1.4)	–35.2 (–59.2 to –11.1)
Education						
Did not complete primary school			1	1	1	1
Completed primary school			–10.2 (–53.9 to 33.5)	2.9 (–41.4 to 47.2)	–5.0 (–45.1 to 35.2)	13.4 (–26.9 to 53.8)
Secondary school and more			–76.7 (–108.1 to –45.3)	–73.4 (–105.9 to –41.0)	–72.7 (–98.7 to –46.7)	–62.0 (–89.7 to –34.3)
Marital status						
Married/cohabiting mothers	1	1				
Single mothers	55.6 (21.6 to 89.5)	127.9 (92.8 163.0)				
Delivery						
Vaginal delivery	1	1	1	1	1	1
C-section delivery	118.5 (86.4 to 150.5)	143.2 (108.8 to 177.5)	72.6 (6.8 to 138.4)	84.2 (17.6 to150.7)	90.8 (49.8 to 131.8)	105.5 (65.2 to 145.7)
Parity						
Primipara	1	1	1	1	1	1
Multipara	66.5 (34.8 to 98.2)	125.9 (90.5 to 161.2)	47.7 (12.1 to 83.2)	56.7 (15.1 to 98.2)	54.8 (26.7 to 83.0)	65.1 (32.8 to 97.4)
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	–48.4 (–77.6 to –19.2)	–37.6 (–67.5 to –7.6)	39.9 (12.4 to 67.4)	47.0 (17.9 to 76.1)	22.6 (–0.0 to 45.2)	31.1 (7.1 to 55.0)
Birth weight (g)	0.0 (0.0 to 0.1)	0.1 (0.0 to 0.1)	0.0 (0.0 to 0.1)	0.1 (0.0 to 0.1)		

BMI, body mass index; EPBF, exclusive or predominant breast feeding.

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 year 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 newborn.[29 30] The lower rates of vaginal deliveries in South Africa were likely due to the country policies (influenced by the scientific

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Table 2d Mothers' 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 OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
EPBF duration (months)	1.0 (0.9 to 1.1)		1.1 (1.0 1.2)	1.0 (0.9 to1.1)	1.1 (1.0 to 1.1)	1.1 (1.0 to 1.2)
Mother's age (years)			1.0 (1.0 to 1.1)	1.0 (1.0 to 1.1)		1.0 (1.0 to 1.0)
Child's gender						
Male babies			1		1	1
Female babies			0.8 (0.6 to 1.0)		0.8 (0.6 to 1.0)	0.8 (0.6 to 1.0)
HIV disease stage						
HIV stage 1			1	1		
HIV stage >1			4.0 (2.5 to 6.2)	4.2 (2.6 to 6.5)		
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			1.6 (1.1 to 2.2)	1.8 (1.3 to 2.6)	1.5 (1.2 to 1.9)	1.6 (1.3 to 2.1)
Trial arm						
Lamivudine arm			1	1	1	1
Lopinavir/ritonavir arm			1.3 (1.0 to 1.6)	1.3 (1.0 to 1.7)	1.3 (1.0 to 1.6)	1.3 (1.0 to 1.6)
Birth weight (g)			0.9 (0.8 to 1.0)			

EPBF, exclusive or predominant breast feeding.

evidence) that supported HIV-infected women towards 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 practice 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 categorisation of the education variable. In our study, less educated participants included only women with primary school level, meaning around 6 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 body weight, 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 breast feeding.[15]

Regarding the change in CD4 cell count, the South African data support the conclusion that CD4 cell count did not differ significantly between women who breast fed 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 breastfeeding than in non-breastfeeding mothers.[13] However, in that Kenyan study, HIV-1 RNA levels did not differ significantly between breastfeeding and formula-feeding mothers.

Regarding HIV-1 disease progression, the same data showed no deleterious effect of breast feeding 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 haemoglobin levels.[32] Another study from Malawi reached the

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Table 3a Mothers' 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)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
		Weight				
Any breastfeeding duration (months)	0.3 (–0.2 to 0.8)	–0.1 (–0.3 to 0.0)	–0.0 (–0.3 to 0.2)	0.1 (0.0 to 0.3)	–0.0 (–0.3 to 0.2)	–0.0 (–0.2 to 0.1)
Baseline BMI (kg/m^2)	2.5 (2.4 to 2.7)	2.5 (2.3 to 2.6)	2.5 (2.4 to 2.6)	2.4 (2.3 to 2.5)	2.5 (2.4 to 2.6)	2.4 (2.3 to 2.5)
Mothers' age (years)	0.8 (0.5 to 1.2)	0.2 (0.0 to 0.3)	0.5 (0.4 to 0.6)	0.1 (0.1 to 0.2)	0.5 (0.4 0.7)	0.1 (0.1 to 0.2)
HIV disease stage						
HIV stage 1	1	1	1			
HIV stage >1	12.4 (–4.5 to 29.4)	6.4 (0.0 to 12.7)	–2.8 (–6.4 to 0.8)			
Education						
Did not complete primary school			1	1	1	1
Completed primary school			3.9 (2.0 to 5.9)	0.3 (–0.6 to 1.1)	4.4 (2.2 to 6.5)	0.6 (–0.3 to 1.5)
Secondary school and further			2.7 (1.2 to 4.1)	0.9 (0.2 to 1.5)	3.1 (1.5 to 4.6)	1.0 (0.4 to 1.7)
Marital status						
Married/cohabiting mothers	1				1	
Single mothers	–3.1 (–7.2 to 0.9)				–1.2 (–3.0 to 0.5)	
Delivery						
Vaginal delivery			1		1	
C-section delivery	3.7 (–0.4 to 7.9)	–1.6 (–3.2 to 0.0)	4.5 (1.5 to 7.6)		4.4 (2.1 to 6.7)	–1.2 (–2.1 to –0.2)
Parity						
Primipara	1	1	1	1	1	
Multipara	6.1 (1.9 to 10.3)		3.1 (1.4 to 4.7)		3.9 (2.3 to 5.4)	
Trial arm						
Lamivudine arm	1	1	1	1	1	
Lopinavir/ritonavir arm	2.0 (–2.0 to 6.1)	0.7 (–0.8 to 2.2)	–0.1 (–1.4 to 1.3)	–0.3 (–0.9 to 0.3)	0.3 (–1.0 to 1.6)	0.1 (–0.7 to 0.4)

BMI, body mass index.

same conclusion that breast feeding 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 breast fed for a short duration (4 months) versus those who breast fed 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 post partum was not significantly affected by the infants' feeding modality (ie, ever vs never breast fed).[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

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Table 3b Mothers' 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)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
		CD4 cell count				
Any breastfeeding duration (months)	0.4 (–6.8 to 7.6)	–2.4 (–9.5 to 4.7)	1.2 (–5.8 to 8.3)	9.8 (–2.1 to 21.8)	1.5 (–3.9 to 7.0)	5.7 (0.4 to 10.9)
Baseline BMI (kg/m^2)			4.9 (2.4 to 7.3)	5.7 (2.4 to 9.1)	3.3 (1.3 to 5.3)	4.2 (1.5 to 6.9)
Mother's age (years)	–3.1 (–7.5 to 1.2)		–4.9 (–7.3 to –2.5)	–6.5 (–8.9 to –4.1)	–4.7 (–6.9 to –2.6)	–6.2 (–8.4 to –4.1)
Haemoglobin concentration (g/dL)	33.3 (12.7 to 53.8)	33.9 (13.5 to 54.3)	15.2 (7.8 to 22.6)	15.7 (7.2 to 24.3)	19.3 (12.3 to 26.4)	16.7 (9.0 to 24.4)
Breastfeeding initiation time						
Within 1 hour			1		1	
After 1 hour	–56.2 (–94.9 to –17.4)		–40.5 (–60.1 to –20.9)		–42.5 (–61.1 to –23.9)	
Child's gender						
Male babies	1	1	1			
Female babies	–53.1 (–104.7 to –1.6)	–54.1 (–104.3 to –3.6)	21.7 (–3.9 to 47.4)			
HIV stage						
HIV stage 1			1	1	1	1
HIV stage >1			–85.8 (–131.5 to –40.2)	–91.5 (–152.9 to –30.1)	–70.2 (–115.5 to –25.0)	–88.8 (–148.3 to –29.3)
Education						
Did not complete primary school			1		1	1
Completed primary school			29.2 (–8.6 to 67.1)		25.1 (–12.6 to 62.9)	19.9 (–16.7 to 56.5)
Secondary school and further			1.0 (–26.8 to 28.9)		–7.8 (–34.9 to 19.3)	–17.4 (–43.7 to 9.5)
Marital status						
Married/cohabiting mothers			1	1	1	1
Single mothers			–34.5 (–73.2 to 4.2)	–43.1 (–81.5 to –4.6)	–24.6 (–55.9 to 6.6)	–43.3 (–72.8 to –13.7)
Delivery						
Vaginal delivery			1	1		
C-section delivery			71.6 (11.7 to 131.4)	71.8 (11.9 to 131.7)		
Parity						
Primipara			1			
Multipara						
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	–33.4 (–69 to 2.3)	–58.7 (–109.6 to –7.8)	–12.8 (–31.6 to 6.1)	–13.4 (–38.6 to 11.8)	–15.8 (–32.6 to 1.0)	–19.2 (–41.9 to 3.6)

BMI, body mass index.

requirement and thus the metabolic stress related to breast feeding 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 breast feeding. This evidence inspires the idea that option A peri-exposure antiretroviral prophylaxis might still have pertinent indications since breast feeding remained the most frequent feeding

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Table 3c Mothers' 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)	Adjusted coefficient (95% CI)	Unadjusted coefficient (95% CI)	Adjusted coefficient (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
		HIV-1 viral load (coefficient×10^3) copies/µL				
Any breastfeeding duration (months)	11.2 (6.8 to 15.6)	7.7 (3.4 to 12.1)	5.9 (−1.5 to 13.2)	2.5 (−5.2 to 10.2)	9.8 (4.9 to 14.7)	6.1 (1.0 to 11.2)
Baseline BMI (kg/m^2)	−7.7 (−10.9 to −4.6)	−14.0 (−17.4 to −10.6)	−4.7 (−8.5 to −1.0)	−5.7 (−9.8 to −1.5)	−6.5 (−9.2 to −3.8)	−7.6 (−10.7 to −4.5)
Mother's age (years)	−2.7 (−5.3 to −0.1)	−3.4 (−6.2 to −0.6)	−1.9 (−4.6 to 0.8)	−4.6 (−7.8 to −1.4)	−2.1 (−4.3 to 0.1)	−4.8 (−7.3 to −2.3)
Breastfeeding initiation time						
Within 1 hour	1	1				
After 1 hour	70.5 (41.3 to 99.7)	34.2 (2.7 to 65.7)				
Child's gender						
Male babies	1		1	1	1	1
Female babies	−49.1 (−79.4 to −18.7)		−19.5 (−48.5 to 9.4)	−37.2 (−66.6 to −7.9)	−25.3 (−49.2 to −1.4)	−36.2 (−60.2 to −12.2)
Education						
Did not complete primary school			1	1	1	1
Completed primary school			−10.2 (−53.9 to 33.5)	4.7 (−39.8 to 49.3)	−4.9 (−45.1 to 35.2)	16.6 (−23.8 to 57.0)
Secondary school and further			−76.7 (−108.1 to −45.3)	−70.7 (−104.4 to −37.1)	−72.7 (−98.7 to −46.7)	−54.5 (−82.8 to −26.1)
Marital status						
Married/cohabiting mothers	1	1			1	
Single mothers	55.6 (21.6 to 89.5)	124.9 (89.9 to 160.0)				
Delivery						
Vaginal delivery	1	1	1	1	1	1
C-section delivery	118.5 (86.4 to 150.5)	137.0 (102.7 to 171.2)	72.6 (6.8 to 138.4)	84.4 (18.0 to 150.7)	90.8 (49.8 to 131.8)	104.7 (64.5 to 144.9)
Parity						
Primipara	1	1	1	1	1	1
Multipara	66.5 (34.8 to 98.3)	125.0 (89.8 to 160.3)	47.7 (12.1 to 83.2)	57.2 (15.6 to 98.8)	54.8 (26.7 to 83.0)	65.0 (32.7 to 97.3)
Trial arm						
Lamivudine arm	1	1	1	1	1	1
Lopinavir/ritonavir arm	−48.4 (−77.6 to −19.2)	−35.0 (−64.8 to −5.3)	39.9 (12.4 to 67.4)	47.6 (18.6 to 76.7)	22.6 (−60.2 to 45.2)	31.9 (8.0 to 55.8)

BMI, body mass index.

option in sub-Saharan Africa and since breast milk might still host HIV-1 reservoirs that mothers' 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 minimised the loss 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, that is, South Africa versus Burkina Faso, Uganda and Zambia. This stratification reduced the sample size in South Africa. Thus, some

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Table 3d Mothers' 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 OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
		HIV disease progress				
Any breastfeeding duration (months)			1.1 (1.0 to 1.2)	1.0 (0.9 to 1.1)	1.0 (0.9 to 1.0)	1.0 (0.9 to 1.0)
Baseline BMI (kg/m^2)			1.0 (1.0 to 1.1)			
Mother's age (years)				1.0 (1.0 to 1.1)	1.0 (0.9 to 1.0)	
Breastfeeding initiation time						
Within 1 hour	1					
After 1 hour						
Child's gender						
Male babies			1		1	
Female babies			0.8 (0.6 to 1.0)		0.8 (0.6 to 1.0)	
HIV stage						
HIV stage 1			1	1	1	1
HIV stage >1			4.0 (2.5 to 6.2)	4.2 (2.6 to 6.6)	4.4 (2.8 to 6.7)	4.6 (2.9 to 7.3)
Education						
Did not complete primary school			1			1
Completed primary school						1.4 (0.9 to 2.0)
Secondary school and further						0.7 (0.5 to 0.9)
Marital status						
Married/cohabiting mothers			1	1		
Single mothers			1.6 (1.1 to 2.2)	1.8 (1.2 to 2.6)	1.5 (1.2 to 1.9)	
Trial arm						
Lamivudine arm			1	1	1	1
Lopinavir/ritonavir arm			1.3 (1.0 to 1.6)	1.3 (1.0 to 1.7)	1.3 (1.0 to 1.6)	1.3 (1.0 to 1.6)

BMI, body mass index.

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

Breast feeding, 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/µL. The mothers' baseline high weight and high haemoglobin 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 breast feeding does not harm HIV-1-infected mothers, this study also supports the WHO 2016 guidelines on infant feeding, which indicate that mothers living with HIV should breast feed 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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REFERENCES

1. UNAIDS. Core epidemiology. 2015 http://www.unaids.org/en/resources/documents/2015/20150714_coreepidemiologyslides_ppt; (accessed 11 Nov 2015).
2. UNAIDS. Global AIDS update 2016. http://www.unaids.org/en/resources/documents/2016/Global-AIDS-update-2016 (accessed 17 Oct 2016).
3. Eaton JW, Rehle TM, Jooste S, *et al*. Recent HIV prevalence trends among pregnant women and all women in sub-Saharan Africa: implications for HIV estimates. *AIDS* 2014;28(suppl 4):8.
4. Younas M, Psomas C, Reynes J, *et al*. Immune activation in the course of HIV-1 infection: causes, phenotypes and persistence under therapy. *HIV Med* 2016;17:89–105.
5. Jacob JA. Men with HIV age faster according to DNA methylation study. *JAMA* 2016;316:135.
6. Zevin AS, McKinnon L, Burgener A, *et al*. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. *Curr Opin HIV AIDS* 2016;11:182–90.
7. DeVaughn S, Müller-Oehring EM, Markey B, *et al*. Aging with HIV-1 Infection: motor functions, cognition, and attention—a comparison with Parkinson's disease. *Neuropsychol Rev* 2015;25:424–38.
8. World Health Organization. *Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach [2006 revision]*134: World Health Organization, 2006.
9. Koyanagi A, Humphrey JH, Moulton LH, *et al*. Predictive value of weight loss on mortality of HIV-positive mothers in a prolonged breastfeeding setting. *AIDS Res Hum Retroviruses* 2011;27:1141–8.
10. Zaba B, Calvert C, Marston M, *et al*. Effect of HIV infection on pregnancy-related mortality in sub-Saharan Africa: secondary analyses of pooled community-based data from the network for Analysing Longitudinal Population-based HIV/AIDS data on Africa (ALPHA). *Lancet* 2013;381:1763–71.
11. Guttmacher Institute. HIV linked to many pregnancy-related deaths in sub-Saharan Africa. *International perspective on sexual and reproductive health* 2013;39:4.
12. World Health Organization, United Nations Children's Fund. *Guideline: updates on HIV and infant feeding: the duration of breastfeeding, and support from health services to improve feeding practices among mothers living with HIV*. Geneva: World Health Organization, 2016.
13. Otieno PA, Brown ER, Mbori-Ngacha DA, *et al*. HIV-1 disease progression in breast-feeding and formula-feeding mothers: a prospective 2-year comparison of T cell subsets, HIV-1 RNA levels, and mortality. *J Infect Dis* 2007;195:220–9.
14. Nduati R, Richardson BA, John G, *et al*. Effect of breastfeeding on mortality among HIV-1 infected women: a randomised trial. *Lancet* 2001;357:1651–5.
15. Papathakis PC, Van Loan MD, Rollins NC, *et al*. Body composition changes during lactation in HIV-infected and HIV-uninfected South African women. *J Acquir Immune Defic Syndr* 2006;43:467–74.
16. Sedgh G, Spiegelman D, Larsen U, *et al*. Breastfeeding and maternal HIV-1 disease progression and mortality. *AIDS* 2004;18:1043–9.
17. Kuhn L, Kasonde P, Sinkala M, *et al*. Prolonged breast-feeding and mortality up to two years post-partum among HIV-positive women in Zambia. *AIDS* 2005;19:1677–81.
18. Winkvist A, Rasmussen KM, Lissner L. Associations between reproduction and maternal body weight: examining the component parts of a full reproductive cycle. *Eur J Clin Nutr* 2003;57:114–27.
19. Murnane PM, Arpadi SM, Sinkala M, *et al*. Lactation-associated postpartum weight changes among HIV-infected women in Zambia. *Int J Epidemiol* 2010;39:1299–310.
20. Ladner J, Castetbon K, Leroy V, *et al*. Pregnancy, body weight and human immunodeficiency virus infection in African women: a prospective cohort study in Kigali (Rwanda), 1992–1994. Pregnancy and HIV Study Group (EGE). *Int J Epidemiol* 1998;27:1072–7.
21. Hartmann PE, Sherriff JL, Mitoulas LR. Homeostatic mechanisms that regulate lactation during energetic stress. *J Nutr* 1998;128:394S–9.
22. Somé E, Engebretsen I, Nagot N, *et al*. R_BreastfeedingandBMI_Main_ENSOME_WithoutTrackChange.pdf. *PloS One* 2017.
23. Nagot N, Kankasa C, Meda N, *et al*. Lopinavir/ritonavir versus Lamivudine peri-exposure prophylaxis to prevent HIV-1 transmission by breastfeeding: the PROMISE-PEP trial Protocol ANRS 12174. *BMC Infect Dis* 2012;12:246.
24. Nagot N, Kankasa C, Tumwine JK, *et al*. Extended pre-exposure prophylaxis with lopinavir-ritonavir versus lamivudine to prevent HIV-1 transmission through breastfeeding up to 50 weeks in infants in Africa (ANRS 12174): a randomised controlled trial. *Lancet* 2016;387:566–73.
25. ANRS. ANRS scale to grade the severity of adverse events in adults; version n° 1.0 4 November 2008. file:///C:/Users/install/Downloads/ANRS-GradeEI-V1-En-2008.pdf (accessed 14 Jan 2016).
26. Becquet R, Bland R, Leroy V, *et al*. Duration, pattern of breastfeeding and postnatal transmission of HIV: pooled analysis of individual data from West and South African cohorts. *PLoS One* 2009;4:e7397.
27. Somé EN, Engebretsen IMS, Nagot N, *et al*. Breastfeeding patterns and its determinants among mothers living with Human Immunodeficiency Virus-1 in four African countries participating in the ANRS 12174 trial. *Int Breastfeed J* 2016;12.
28. Crowell DT. Weight change in the postpartum period. A review of the literature. *J Nurse Midwifery* 1995;40:418–23.
29. Maguire A, Sánchez E, Fortuny C, *et al*. Potential risk factors for vertical HIV-1 transmission in Catalonia, Spain: the protective role of

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cesarean section. The Working Group on HIV-1 Vertical Transmission in Catalonia. *AIDS* 1997;11:1851–7.
30. Kind C, Rudin C, Siegrist CA, *et al*. Prevention of vertical HIV transmission: additive protective effect of elective Cesarean section and zidovudine prophylaxis. Swiss Neonatal HIV Study Group. *AIDS* 1998;12:205–10.
31. Rogers IS, Golding J, Emmett PM. The effects of lactation on the mother. *Early Hum Dev* 1997;49:S191–S203.
32. Coutsoudis A, Coovadia H, Pillay K, *et al*. Are HIV-infected women who breastfeed at increased risk of mortality? *AIDS* 2001;15:653–5.
33. Taha TE, Kumwenda NI, Hoover DR, *et al*. The impact of breastfeeding on the health of HIV-positive mothers and their children in sub-Saharan Africa. *Bull World Health Organ* 2006;84:546–54.
34. Breastfeeding and HIV International Transmission Study Group. Mortality among HIV-1-infected women according to children's feeding modality: an individual patient data meta-analysis. *J Acquir Immune Defic Syndr* 2005;39:9.
35. Trumbo P, Schlicker S, Yates AA, *et al*. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc* 2002;102:1621–30.
36. Lovelady C. Balancing exercise and food intake with lactation to promote post-partum weight loss. *Proc Nutr Soc* 2011;70:181–4.
37. Van de Perre P, Rubbo PA, 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;4:143sr3.

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HIV-1 disease progression in immune-competent HIV-1-infected and breastfeeding mothers participating in the ANRS 12174 clinical trial in Burkina Faso, South Africa, Uganda and Zambia: a cohort study

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