**Does U=U for breastfeeding mother-infant pairs? Breastfeeding for mothers on effective treatment for HIV infection in high-income settings**

Catriona Waitt 1,2,3, Nicola Low4, Philippe Van de Perre5, Fiona Lyons6, Mona Loutfy7,8, Karoline Aebi-Popp9

1 Department of Molecular and Clinical Pharmacology, 70 Pembroke Place, University of Liverpool, Liverpool, L69 3GF, UK

2 Infectious Diseases Institute, Makerere University College of Health Sciences, Kampala, Uganda

3 Royal Liverpool University Hospital, Prescot Street, Liverpool, L7 8XP, UK

4 Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

5 Pathogenesis and Control of Chronic Infections, INSERM, University of Montpellier, Etablissement français du Sang, CHU Montpellier, Montpellier, France

6 Department of Genitourinary Medicine and Infectious Diseases, St. James’s Hospital, Dublin, Ireland

7 Women’s College Research Institute, Women’s College Hospital, Toronto, Ontario, Canada

8 Department of Medicine, University of Toronto, Toronto, Ontario, Canada

9 Department of Infectious Diseases, University Hospital Bern, Bern, Switzerland

Corresponding author: Catriona Waitt, Department of HIV Pharmacology, 70 Pembroke Place, University of Liverpool, Liverpool, L69 3GF, UK.

Email [cwaitt@liverpool.ac.uk](mailto:cwaitt@liverpool.ac.uk)

Telephone 0151 794 5553

Synopsis

Can the campaign phrase, undetectable=untransmissible (U=U), established for the sexual transmission of HIV, be applied to the transmission of HIV through breastfeeding? European AIDS Clinical Society and, to some extent American guidelines, now state that HIV-infected mothers who wish to breastfeed should be supported, with ‘increased clinical and virological monitoring’. This paper summarises existing evidence about transmission of HIV through breastfeeding, differences in HIV dynamics and viral load between breastmilk and plasma, and the effects of antiretrovirals on infants. At present, there is insufficient evidence for clear recommendations about the frequency of clinical and virological monitoring required for mother and infant in a breastfeeding relationship or about the action to be taken in the event of viral rebound. We propose a roadmap for collaborative research to provide the missing evidence required to enable mothers who wish to breastfeed to make a fully informed choice.

*Background*

In October 2017, the European AIDS Clinical Society (EACS) stated that if ‘a woman insists upon breastfeeding, we recommend follow-up with increased clinical and virological monitoring of both the mother and the infant’, in an updated guideline.1 United States (US) guidelines were updated in March 2018 to describe how to counsel and support women who make this choice, whilst clearly recommending against breastfeeding in general.2 These recommendations recognise both the increasing numbers of women living with HIV who are virologically suppressed on combination antiretroviral therapy (cART) and wish to breastfeed their children and the framework of respect for human rights.

Globally, effective cART in pregnancy and postpartum has resulted in a dramatic reduction in rates of mother to child transmission of HIV (MTCT), such that elimination of MTCT is now embraced as a realistic goal.3 Since 2015, global guidelines have recommended that pregnant women with HIV start cART as soon as possible and remain on it for life.4 MTCT rates <1% have consistently been reported from high-income countries where most HIV-positive mothers do not breastfeed.5-7

The updated US guideline states that women ‘who desire to breastfeed should receive patient-centred, evidence-based counselling on infant feeding options.’2 But do we know enough to provide this evidence-based advice? That HIV-positive people who are virologically suppressed on cART cannot sexually transmit the virus to others is now well established. The Undetectable=Untransmissible (U=U) campaign, launched in early 2016,8 led to changes in HIV prevention advice given to sero-different sexual partners. The success of U=U for sexual transmission raises the question of its applicability to other contexts, such as in breastfeeding.

This viewpoint addresses major questions that need to be answered to produce evidence-based recommendations about breastfeeding and HIV in high-income settings. First, we summarise current recommendations and evidence about the risk of HIV transmission from breastfeeding mothers on cART. We then discuss biological factors which make transmission of HIV through breastfeeding different from other routes of exposure, the potential risks of infant exposure to maternal cART through breastfeeding, the clinical and virological monitoring required for a breastfeeding mother-infant pair and the action to be taken in the event of detectable maternal viral load (VL) while breastfeeding. Finally, we summarise research priorities in a roadmap for future collaborative research and knowledge exchange.

We searched PubMed (U.S. National Library of Medicine) using the search terms ‘HIV transmission OR mother to child transmission AND breastfeeding’, and ‘antiretroviral AND breast milk’. We selected publications that appeared to be relevant and supplemented these with references that were cited in identified papers.

*Breastfeeding guidelines differ between high- and low-income regions*

Clinical guidelines from high-income countries all recommend against breastfeeding, even though recent updates acknowledge that women who choose to breastfeed should be supported.2,9,10 In low-income settings, the World Health Organization (WHO) recommends breastfeeding for at least six months and continuing up to 12 or 24 months.11 The recommendations are based on the same sources of data but the balance of benefits and harms of breastfeeding differs. In low-income settings, the morbidity and mortality from infection in infants receiving formula milk outweighs the risks of HIV transmission through breast milk, because of unclean water and lost protection from maternal antibodies in breast milk.11

*Evidence about the risk of HIV transmission through breastfeeding*

The risk of HIV transmission through breastfeeding in high-income countries remains unknown because randomised controlled trials of prevention of MTCT (PMTCT) using cART are not feasible. A 2017 meta-analysis of six studies in low-income settings in which mothers started cART before or during their current pregnancy, estimated a postnatal HIV transmission rate of 1.08% (95% CI 0.32-1.85) at six months, with higher rates from mothers who commenced ART in later pregnancy.12 The Promoting Maternal Infant Survival Everywhere (PROMISE) trial in southern Africa,13 comparing maternal cART with prolonged infant nevirapine, reported MTCT of 0.3% (95% CI 0.1-0.8) and 0.7% (95% CI 0.3-1.4), at six and 12 months, respectively in the maternal cART arm.13

The estimates from low- and middle-income countries are likely to overestimate HIV transmission risk in high-income settings. First, early trials included women who started cART at any time up to, and including, the third trimester and discontinued cART at six months postpartum.14-18 Given the time taken to attain virological suppression, the increased risk of postnatal transmission from mothers who started therapy in the third trimester is not surprising.14,16,17,19 Second, most studies reported transmission events after discontinuation of maternal cART and reported cessation of breastfeeding, so the findings might not be generalisable to mothers who remain on cART lifelong.

*Association between transmission and maternal plasma and breast milk HIV viral load*

Elevated maternal plasma and breast milk HIV-RNA are associated with an increased risk of transmission to the infant, but a ‘safe’ threshold has not been defined. Earlier studies using assays with detectability thresholds of 1000 15,17 or 400 16 copies per mL might have missed clinically important viraemia.

In the Malawian Breastfeeding, Antiretrovirals and Nutrition (BAN) study, amongst mothers receiving 28 weeks of postpartum cART, detectable HIV-RNA in breast milk was associated with increased transmission (hazard ratio 3.8, 95% CI 1.2-12.1) in a secondary analysis.20 All transmitting mothers had at least one plasma HIV-RNA sample of >100 copies/mL; 73% had detectable HIV-RNA in breast milk at any time and 53% had so at the nearest time point to the transmission.20 Two mothers had detectable HIV-RNA in breast milk with undetectable HIV-RNA in plasma. In a large case-control study nested into the Vertical Transmission Study in KwaZulu Natal, South Africa, the estimated total breast milk exposure to HIV-RNA was strongly associated with postnatal transmission.21 Transmission of HIV during breastfeeding has occurred despite undetectable HIV-RNA in breast milk20,22 and postnatal HIV transmission was documented from a woman who had both plasma and breast milk HIV-RNA <37 copies/mL at the time point closest to transmission.18 Whilst these transmission events might represent elevations in HIV-RNA between sampling intervals or assay limitations, the presence of cell-associated HIV-DNA is also a possible explanation.

*The question of breast milk cells in relation to MTCT*

Both cell-associated DNA and cell-free RNA in breast milk have been associated with HIV transmission from mother to child.23,24 A study comparing the infecting virus (in the infant) with viruses characterised by their C2 to C5 *env* fragment sequences in the cellular and acellular fractions of breast milk demonstrated that before nine months postpartum, HIV-1 is mainly transmitted by cells containing HIV-1 provirus while cell-free virus is frequently involved later.25 It has been suggested that the breast is seeded with a long-lived lineage of latently infecting resting T-cells.26 Decline of HIV-RNA, but not HIV-DNA in breast milk was seen with cART, although mothers had been on treatment for a median of 98 days;27 this may not directly inform the situation where a woman has been on long term suppressive cART.

Van de Perre and colleagues suggest postnatal transmission from a mother receiving suppressive cART can result from cell-associated viral transmission, due to several differences between blood and breast milk cells.28 Breast milk immune cells are frequently activated and express homing markers signalling their mucosal origin (maternal gut, respiratory mucosae).33 Latently HIV-infected, resting CD4+ T lymphocytes harbour HIV-1 pro-viral DNA. These cells have a half-life of about 44 months,29 are not affected by current cART regimens,30 and constitute an inducible reservoir of HIV-producing cells, which can transcribe HIV-DNA generating infectious viral particles.38 Activated CD4 cells spontaneously secreting HIV-1 antigen detected by enzyme-linked immunospot assay (ELISPOT) are found in both breast milk and blood, irrespective of cART.31 Even when HIV-DNA is comparable in blood and breast milk, polyclonal activation resulted in ten times more HIV antigen-secreting cells in breast milk than blood, making the breast milk CD4 cells 17-times more effective than their blood counterparts in producing HIV antigens.32

Furthermore, breast milk contains other cell types susceptible to HIV infection, such as macrophages, dendritic cells and CD4+ progenitor T cells that may also be involved in transmission.28 The transfer and persistent nesting of maternal cells into infant tissues (maternal microchimerism) involving breast milk cells is reported in many mammalian species and has recently been suggested in humans.33

The role of mastitis is uncertain. Activation of breast milk cells and leakage of HIV from plasma into the breast could promote HIV shedding,34 and data from the pre-ART era indicate an increased risk of MTCT in this situation.35 Reactivation of latent viral infections such as cytomegalovirus or Epstein-Barr virus in breast milk may similarly and synergistically favour HIV shedding.36 There are no data about the effects of mastitis on HIV-RNA in breast milk or cell-associated HIV-DNA among women on cART who have suppressed plasma HIV-RNA.

*What about drug exposure to breastfed infant?*

The two main concerns about transmammary exposure to maternal cART are the development of HIV-resistance in infants should transmission occur and toxicities resulting from long-term low-dose exposure to drug. Almost all data on drug exposure to the breastfed infant are derived from breastfeeding populations in LMIC. Exclusively breastfed infants receive up to 10% of the weight-adjusted infant dose of nucleoside reverse transcriptase inhibitors (NRTIs) and non-NRTIs (NNRTIs), whereas transfer of protease inhibitors (PIs) to the infant is low.37 Genetic differences, such as CYP2B6 polymorphisms in the case of efavirenz, result in higher infant drug exposure through pregnancy and breastfeeding.38 Individual patient data indicate transfer of dolutegravir to the breastfed infant,39 with ongoing studies exploring this question (DolPHIN-1, NCT02245022). Reduced infant clearance of dolutegravir likely relates to the immature metabolism of the neonates.40 No breast milk pharmacokinetic data yet exist for drugs including tenofovir alafenamide, which are increasingly used in high-income countries. Infant ingestion of low concentrations of ART through breast milk can promote emergence of drug-resistant mutants in the infant. Two large PMTCT studies demonstrated that infants who acquired HIV during breastfeeding despite maternal cART had high rates of multi-class drug resistance, with demonstration of different resistance patterns to those seen in their mothers.41,42

Pharmacovigilance of infant exposure to drugs through breastfeeding is poor, with under-reporting of adverse drug reactions and a likely skew towards the most serious events.43 Whilst the Antiretroviral Pregnancy Registry is well established, no parallel system exists to systematically collect data relating to clinical outcomes, growth and development in the breastfed infant. Furthermore, the clinical tools needed to assess for subtle toxicities alongside clinical care have not been established. Few large PMTCT trials conducted in low-income countries reported on infant safety data. The recent PROMISE study described similar rates of ≥ grade 3 adverse events between infants given prolonged treatment with nevirapine compared to having transmammary exposure to maternal cART.13

*What is optimal infant prophylaxis?*

Given the uncertainty about the absolute risk of HIV transmission to the breastfed infant, prophylaxis might be an option, particularly as a ‘rescue strategy’ in the event that the mother experiences viral rebound. EACS guidelines make no reference to infant prophylaxis.1 Consistent with most European guidelines,44 British HIV Association guidelines recommend nevirapine monotherapy for four weeks in the infant whose mother has a plasma HIV-RNA level <50 copies/mL; maternal cART is seen to provide sufficient prophylaxis for the breastfed infant of a virologically suppressed mother.10 The Swiss national guidelines do not recommend infant post-exposure prophylaxis (PEP) in mothers with undetectable HIV-RNA at delivery.45 The US guidelines recommend at least 6 weeks of infant zidovudine and/or nevirapine.2 A recent systematic review of the evidence to support the optimal infant ART prophylactic regimen in the ‘high risk’ infant included European data.46 However, ‘high risk’ included cases in which a pregnant woman has never received ART, has received ‘insufficient’ ART or had a detectable VL around the time of delivery, but did not mention breastfeeding as a risk factor.47 From such data, the virologically suppressed mother who chooses to breastfeed would not be considered high risk.

*Postpartum adherence to cART and virological suppression*

Adherence to cART in the postpartum period can be more challenging than either the mother or healthcare provider predict. In one systematic review of 51 studies involving 20 153 HIV-infected pregnant women from the US, Kenya, South Africa and Zambia, 76% were found to have adequate adherence (defined as >80%) antepartum, but only 53% postpartum.48 Several studies in high-income countries indicate that problems for adherence amongst postpartum women. The UK Collaborative HIV Cohort compared data from 623 parturient women who with 1225 women who did not deliver between 2006 and 2011. 10.7% of postpartum women experienced viral rebound (HIV-RNA > 200 copies/mL), compared to 7.4% of controls.49 Among the 363 women had been diagnosed with HIV and commenced on ART during the recent pregnancy, 27% had evidence of viral rebound within six months postpartum.49 The Swiss cohort study found, that 12% of 695 women were lost to follow up in the first year postpartum.50 In the US, a retrospective cohort found that only 39% of 695 mothers were retained in care and 31% virologically suppressed at one year postpartum.51

*What virological monitoring should be undertaken for women on cART who are breastfeeding?*

There are no specific data to guide the required frequency of “increased virological monitoring”,1 whether VL should be monitored in plasma alone or also in breast milk, or the action to be taken in the event of an unexpected result. The British HIV Association recommends monthly testing of both mother and infant if breastfeeding takes place, with grading 1C (a strong recommendation, but where some of the supporting evidence is of low quality).10 Swiss guidelines suggest testing the mother and the infant after one month, then 2-3 monthly until weaning.45 US guidelines recommend maternal viral load 1-2 monthly, and to ‘consult an expert’ in the event of detectable viraemia.2

Future guidelines should consider whether increased contact with healthcare providers for monitoring might improve patient-clinician relationship and adherence or might overburden the postpartum woman. Qualitative research might inform the best models of care.

*When and how to stop breastfeeding in the event of virological rebound?*

Firm data defining a ‘rescue strategy’, if a breastfeeding mother has detectable HIV-RNA in plasma or breast milk, are lacking. Clinical judgement might suggest immediate cessation of exposure to breast milk and infant antiretroviral prophylaxis as both pre- or post-exposure prophylaxis.52 It is, however, difficult to stop breastfeeding abruptly and mammary tissue remains partially functional for a long period, which may lead to intermittent breastfeeding against medical advice. Professional support from an expert in lactation is recommended when cessation of breastfeeding for other medical reasons is needed.53 As adherence to cART is crucial, HIV-positive women need continued emotional and social support. Anecdote suggests that women may feel pressurised to inform clinicians that they are no longer breastfeeding, when this is not the case. As summarised above, most studies indicate MTCT occurred in women who stated that breastfeeding has ceased,12 suggesting that this is a real phenomenon.

*Research Priorities*

Our synthesis of the salient issues shows that there are important gaps in the evidence-base needed to define the ‘optimal’ clinical and virological monitoring of a breastfeeding HIV-positive woman and her infant in a well-resourced setting. Principles may be of relevance to low-income settings as access to resources improves.

If women with HIV wish to breastfeed, both clinicians and patients need accurate, up-to-date information about the risks and benefits to enable an informed decision. Detailed information about the necessary additional tests and their frequency for breastfeeding mother-infant pairs is needed. Should viral rebound occur, statements about the need for cessation of breastfeeding, together with advice on how to support the mother in this process, or how to choose the optimal PrEP/PEP for the infant must be clear. New research studies would help to understand the motivations for breastfeeding and issues related to cART adherence support for the diverse populations of women with HIV infection, including women who have migrated from low-income countries for whom breastfeeding is the first choice and those born in high-income countries who wish to breastfeed and have high levels of motivation to adhere to cART.

There is currently insufficient evidence to state with certainty that U=U in the context of breastfeeding. To address gaps in the specific evidence applicable to clinical guidance in high-income countries, we would propose an Expert Networking Group to exchange knowledge based on cases in real-time. Anecdote suggests that each clinical centre is likely to have only a small number of patients who choose to breastfeed whilst on suppressive cART, and therefore multi-centre collaboration and shared research protocols will be necessary to maximise the knowledge gained.

Current networks, including the Paediatric European Network for Treatment of AIDS (PENTA) and Women Against Viruses in Europe (WAVE) within EACS and the North American Perinatal HIV Clinicians Network, which is linked through the HIVE54 ReproID HIV listserv, could provide the logistical support for a collaboration which should involve patient groups. At the first level, this would be simple documentation of the mother-infant pair data, including the country of origin, timing and type of maternal cART, VL at delivery and subsequent (possibly monthly) time-points, and the final infant HIV status after cessation of breastfeeding.

The Table highlights the key unanswered questions and research priorities. Alongside these efforts, collaborators should develop protocols for pharmacokinetic, virological and pharmacovigilance studies and social science research that includes evaluation of maternal quality of life. Most of all, a coalition of researchers and mothers living with HIV would generate the research to allow a consensus of the best available evidence for clinicians and mothers about the prevention of HIV transmission through breastfeeding.

Declaration of Interests

ML has received funding for research, travel and presentations from Gilead Science, ViiV Healthcare and Merck Frosst Canada.

Acknowledgements

CW is funded by a Wellcome Postdoctoral Training Fellowship for Clinicians WT104422MA

We thank Claire Thorne for critical review of the manuscript.

Author Contributions

CW and KA-P: initial concept for the paper, literature searches, data interpretation and writing

NL, FL, ML: critical input into the selection and interpretation of material reviewed and synthesis of conclusions presented

PVP: major contribution to sections relating to cell-associated virus, and critical input throughout

**Table:** Unanswered questions and research priorities related to breastfeeding and HIV

|  |  |  |
| --- | --- | --- |
| Question | What is known | Research priorities |
| What is the significance of cell-associated virus | May be associated with transmissions in women with or without suppressed viral load | Does this still hold for women on long term ART?  Do any newer drugs influence cell-associated virus? |
| What is the genuine rate of transmission? Is it truly ‘zero’? | Very low rates are reported in the context of suppressive ART, and most transmissions can be explained through detectable virus or poor adherence | Establishment of a registry of mother-infant pairs to capture any transmissions |
| What are the pharmacokinetics of newer ARVs in mother-infant pairs | Data exist surrounding NNRTI, NRTI and older PIs, with emerging data on DTG | Sparse PK sampling from mother-infant pairs in Europe where the mother has elected to breastfeed |
| How do we monitor infants for toxicities? | Little data exist for breastfeeding exposure to newer ART | Establishment of a registry linked to clinical care for longer term follow up of exposed infants |
| Are any regimens better suited for use in breastfeeding? | Almost all data exist for NNRTI/2 NRTI regimens in low resource settings | Clinical monitoring and PK among mother-infant pairs on individualised regimens |
| What is the optimal frequency of virological monitoring? | No evidence base on which to form a guideline. | Establishment of a cohort to describe experience |
| What steps should be taken in the event of detectable viraemia? | No evidence base on which to form a guideline. | Cohort data correlating viral rebounds with adverse events  Qualitative research on maternal attitudes and practice if abrupt weaning advised |
| What is the significance of clinical or subclinical mastitis | In pre-ART era, mastitis was associated with increased breast milk HIV RNA, and risk of MTCT | Evaluation of subclinical mastitis and breast milk HIV viral load among breastfeeding mothers on ART |
| Should infant prophylaxis be given during breastfeeding?  If so, which is the optimal regimen and duration? | Existing evidence largely from sub-Saharan Africa | Definition of optimal prophylaxis for the breastfed infant whose mother has a plasma HIV VL of <50 copies/ mL  To define optimal infant prophylaxis to be given in the event of detectable maternal HIV RNA |
| What clinical/ psychosocial support would benefit these mothers? | Existing qualitative work on postnatal ART intake and adherence stems from LMIC  Do different subpopulations of women exist who require different models of care? | Qualitative research among mothers who choose to breastfeed in well-resourced settings |

Abbreviations: ART antiretroviral therapy; NRTI nucleoside reverse transcriptase inhibitor; NNRTI non-nucleoside reverse transcriptase inhibitor; PI protease inhibitor; ARVs, antiretrovirals; DTG dolutegravir; MTCT, mother-to-child transmission; VL, viral load.

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