APPENDIX 1: Additional background information

Understanding the virologic basis for breastmilk HIV transmission: additional detail

Cell-free vs. Cell-associated virus

Breastmilk can contain cell-free virus (measurable as a "viral load") and cell-associated virus. Cell-free viral load arises via passive leakage of the virus into breastmilk or viral replication in the mammary gland[1]. In contrast, cell-associated virus is latently integrated into the nuclear DNA of CD4 T-lymphocytes and macrophages in breastmilk[2].

Antiretroviral medications effectively eliminate cell-free virus in plasma but have no impact on latent cell-associated virus[3,4]. Limited data from small studies assessing HIV in breast milk from women on combination antiretroviral therapy (ART) in Africa demonstrate that the lack of effect of ART on cell-associated HIV DNA is also true for breastmilk[5].

Breast or Nipple inflammation (Mastitis)

Mastitis or local breast inflammation has been associated with increased breastmilk viral load[6]. An estimated 10%-33% of women experience mastitis, typically during the early breastfeeding period or during the mixed feeding and weaning stages[7]. Clinical and subclinical mastitis are associated with higher breast milk HIV viral load and breast-feeding–associated HIV transmission rates, demonstrated in infants of women who were not on combination ART[8–12]. More information about mastitis is available in the following "Maternal factors that can impact transmission risk" section.

Co-infection, reactivations, and gastrointestinal / oral thrush

Infections of the infant gastrointestinal tract could weaken barrier defenses, bring target cells to effected areas, and facilitate entry of HIV (see resources section for BHIVA "Safer Triangle" Resource)[13]. For example, infant exposure to *Candida* during vaginal delivery has been associated with increased risk for postnatal HIV transmission[8]. Oral thrush and gastrointestinal candidiasis can disrupt the integrity of the infant's oral and intestinal epithelial barrier, which may increase risk of HIV transmission through breastfeeding[14,15].

Reactivation of maternal latent viral infections, such as cytomegalovirus or Epstein-Barr virus, in breastmilk is associated with synergistically increasing HIV shedding[16].

Antiretroviral concentrations in breastmilk

Antiretrovirals are present in the breastmilk of mothers receiving ART[17]. The presence of these medications in breast milk may contribute to the reduction of HIV transmission through breastmilk by reducing both maternal plasma and breast milk HIV concentrations. The ingestion of antiretroviral medications in breast milk by the infant may also play a role in preventing transmission[17]. However, since the levels of antiretroviral medications in infant serum are likely sub-therapeutic, there is risk of antiretroviral medication resistance emergence if the child becomes infected[18,19]. The extent of antiretroviral transfer from the mother to the infant via breast milk is described in the "Infant - risk of resistance, antiretroviral toxicity from breast milk" section.

Colostrum and early milk

Colostrum and early milk, particularly in the first month after birth, have higher concentrations of antibodies and immune cells compared to mature milk[20]. Due to the high concentrations of immune cells that can also serve as reservoirs for HIV, concentration of cell-associated HIV is higher in colostrum and early milk compared to mature milk[21,22]. It has also been documented that higher median cell-free HIV viral load can occur in colostrum and early milk when compared to mature milk collected more than 14 days after delivery[23]. Adding to the complexity, cell-free HIV viral load can be below detection levels in milk from one breast but be detectable in milk from the other breast at the same time[24], regardless of noticeable breast inflammation.

Maternal factors that can impact transmission risk: additional information

Viral load

The ability to suppress plasma and potentially breastmilk viral load is the most important factor in reducing the risk of breastmilk transmission[25–27]. However, even if the viral load is fully suppressed transmission may still occur, probably because of transmission by cell associated virus.

Adherence to ART

Postpartum women living with HIV may find it challenging to remain engaged in HIV care and achieve optimal adherence to ART. In one systematic review, only 74% of postpartum women living with HIV achieve optimal adherence to ART[28]. A study performed in British Columbia showed ART adherence significantly decreased with each time interval (third trimester, and three, six, nine, and 12 months postpartum) of follow up postpartum between 1993-2006[29]. However, Canada-wide data from the Canadian HIV Women's Sexual Reproductive Cohort Study in 71 post-partum women, 89% remained undetectable within one year of delivery[30]**.** In British Columbia, not all women had undetectable viral loads at the time of their infant's birth[31]. In addition, there is a high prevalence (>85% in a

Zambian urban cohort) of depression, general distress, and other psychiatric symptoms among postpartum women living with HIV[32]. While there are no specific guidelines for perinatal depression in women living with HIV, assembling an appropriate care team and informative counselling[33] can improve engagement in HIV care and adherence to ART.

HIV disease stage

Advanced maternal HIV disease with low maternal CD4 count (<200 cells/uL) is associated with risk of breastmilk transmission in some studies, with a 5-fold increased risk of transmission compared to CD4 counts in the normal range (>500cells/uL)[34]. This is hypothesized to be due to the more advanced progression of HIV disease and lower immunological protective factors in breastmilk.

Timing of maternal infection

Acute HIV infection in breastfeeding women is associated with a significantly elevated risk of breastmilk transmission. For example, this was seen in case series of women who were acutely infected post-partum via blood transfusion[35]. Acute HIV infection is defined as the first few weeks to months where the virus disseminates into tissues and organs, establishing a pro-viral reservoir within days. After that, the viral load decreases and reaches a plateau set point that persists chronically[36].

Breast or Nipple inflammation (Mastitis)

Mastitis or local breast inflammation has been associated with increased breastmilk viral load[6]. Clinical and subclinical mastitis as indicated by elevated breastmilk sodium/potassium ratio or lactoferrin concentration is associated with higher breastmilk HIV viral load and breastfeeding–associated HIV transmission rates[37]. The ratio of extracellular ions (sodium) to intracellular ions (potassium) becomes elevated when cell membranes are disrupted, which may also facilitate greater leakage of HIV from plasma to breastmilk[38]. Clinical mastitis occurs in a few to 33% of women[39], however up to 50% of all breastfeeding associated transmission may be attributable to subclinical mastitis[40]. Breast related factors also play a role, as seen in a cohort of breastfeeding women living with HIV in Zimbabwe in which 30.8% had nipple disease (eczema 22.1%, cracked and sore nipples 10.6%)[41]. Nipple bleeding has been demonstrated as an independent risk factor as seen in one small observational study in Brazil where bleeding cracked nipples had a higher association with transmission among WLWH not on ART, not seen with a history of cracked nipples alone[42].

Nutritional status

Poor nutritional status of the mother is likely to increase the vertical transmission of HIV[43]. The potential effects include impaired systemic immune function; an increased rate of clinical, immunologic, and virologic disease progression; impaired epithelial integrity of the placenta and genital tract; increased viral shedding in breast milk from inflammation of breast tissue; increased risk of low birth weight and preterm birth; and impaired gastrointestinal immune function and integrity in fetuses and children[44]. Vitamin A is essential for maintaining immune function and maternal supplementation may reduce the risk of vertical HIV transmission in women not on ART[45]. All pregnant and breastfeeding women are recommended to take a prenatal vitamin.

REFERENCES

1. Van De Perre, P. *et al.* HIV-1 reservoirs in breast milk and challenges to elimination of breast-feeding transmission of HIV-1. *Science Translational Medicine* vol. 4 143sr3-143sr3 (2012).
2. Dutartre, H., Clavière, M., Journo, C. & Mahieux, R. Cell-Free versus Cell-to-Cell Infection by Human Immunodeficiency Virus Type 1 and Human T-Lymphotropic Virus Type 1: Exploring the Link among Viral Source, Viral Trafficking, and Viral Replication. *Journal of Virology* 90, 7607–7617 (2016).
3. Showa, S. P., Nyabadza, F. & Hove-Musekwa, S. D. On the efficiency of HIV transmission: Insights through discrete time HIV models. *PLoS ONE* 14, e0222574 (2019).
4. Milligan, C. & Overbaugh, J. The role of cell-Associated virus in motherto-child HIV transmission. *Journal of Infectious Diseases* 210, S631–S640 (2014).
5. Lehman, D. A. *et al.* HIV-1 persists in breast milk cells despite antiretroviral treatment to prevent mother-to-child transmission. *AIDS* 22, 1475–1485 (2008).
6. Semrau, K. *et al.* Dynamics of breast Milk HIV-1 RNA with unilateral mastitis or abscess. *Journal of Acquired Immune Deficiency Syndromes* 62, 348–355 (2013).
7. Boakes, E., Woods, A., Johnson, N. & Kadoglou, N. Breast Infection: A Review of Diagnosis and Management Practices. *European Journal of Breast Health* 14, 136 (2018).
8. Embree, J. E. *et al.* Risk factors for postnatal mother–child transmission of HIV-1 | Ovid. *AIDS* 14, 2535–2541 (2000).
9. Fawzi, W. *et al.* Transmission of HIV-1 through breastfeeding among women in Dar es Salaam, Tanzania. *Journal of Acquired Immune Deficiency Syndromes* 31, 331–338 (2002).
10. John, G. C. *et al.* Correlates of mother-to-child human immunodeficiency virus type 1 (HIV-1) transmission:

Association with maternal plasma HIV-1 RNA load, genital HIV-1 DNA shedding, and breast infections. *Journal of Infectious Diseases* 183, 206–212 (2001).
11. Semba, R. D. *et al.* Human immunodeficiency virus load in breast milk, mastitis, and mother- to-child transmission of human immunodeficiency virus type 1. *Journal of Infectious Diseases* 180, 93–98 (1999).
12. Kasonka, L. *et al.* Risk factors for subclinical mastitis among HIV-infected and uninfected women in Lusaka, Zambia. *Paediatric and Perinatal Epidemiology* 20, 379–391 (2006).
13. British HIV Association. *HIV and breastfeeding your baby*.
14. Hester, R. A. & Kennedy, S. B. Candida infection as a risk factor for HIV transmission. *Journal of Women's Health* 12, 487–494 (2003).
15. John-Stewart, G. *et al.* Breast-feeding and Transmission of HIV-1. *Journal of Acquired Immune Deficiency Syndromes* vol. 35 196–202 (2004).
16. Viljoen, J. *et al.* Cytomegalovirus, and possibly Epstein-Barr virus, shedding in breast milk is associated with HIV-1 transmission by breastfeeding. *AIDS* 29, 145–153 (2015).
17. Mirochnick, M. *et al.* Antiretroviral concentrations in breast-feeding infants of mothers receiving highly active antiretroviral therapy. *Antimicrobial Agents and Chemotherapy* 53, 1170–1176 (2009).
18. Zeh, C. *et al.* HIV-1 drug resistance emergence among breastfeeding infants born to HIV-infected mothers during a single-arm trial of triple-antiretroviral prophylaxis for prevention of mother-to-child transmission: A secondary analysis. *PLoS Medicine* 8, e1000430 (2011).
19. Fogel, J. *et al.* Initiation of antiretroviral treatment in women after delivery can induce multiclass drug resistance in breastfeeding HIV-infected infants. *Clinical Infectious Diseases* 52, 1069–1076 (2011).
20. Wheeler, T. T., Hodgkinson, A. J., Prosser, C. G. & Davis, S. R. Immune components of colostrum and milk - A historical perspective. *Journal of Mammary Gland Biology and Neoplasia* vol. 12 237–247 (2007).
21. Ruff, A. J. *et al.* Prevalence of HIV-1 DNA and p24 antigen in breast milk and correlation with maternal factors. *Journal of Acquired Immune Deficiency Syndromes* 7, 68–73 (1994).
22. Van de Perre, P. *et al.* Infective and anti-infective properties of breastmilk from HIV-1-infected women. *The Lancet* 341, 914–918 (1993).
23. Richardson, B. A. *et al.* Breast-milk infectivity in human immunodeficiency virus type 1-infected mothers. *Journal of Infectious Diseases* 187, 736–740 (2003).
24. Willumsen, J. F. *et al.* Variation in breastmilk HIV-1 viral load in left and right breasts during the first 3 months of lactation | Ovid. *AIDS* 15, 1896–1898 (2001).
25. Rousseau, C. M. *et al.* Longitudinal analysis of human immunodeficiency virus type 1 RNA in breast milk and of its relationship to infant infection and maternal disease. *Journal of Infectious Diseases* 187, 741–747 (2003).
26. Mmiro, F. A. *et al.* Predictors of early and late mother-to-child transmission of HIV in a breastfeeding population: HIV network for prevention trials 012 experience, Kampala, Uganda. *Journal of Acquired Immune Deficiency Syndromes* 52, 32–39 (2009).
27. Mofenson, L. M. *et al.* Risk Factors for Perinatal Transmission of Human Immunodeficiency Virus Type 1 in Women Treated with Zidovudine. *New England Journal of Medicine* 341, 385–393 (1999).
28. Nachega, J. B. *et al.* Adherence to antiretroviral therapy during and after pregnancy in low-income, middle-income, and high-income countries: A systematic review and meta-analysis. *AIDS* 26, 2039–2052 (2012).
29. Kaida A, Forrest J, Money D, Burdge D, Forbes J, F. K. Antiretroviral adherence during pregnancy and postpartum among HIV-positive women enrolled in the drug treatment program in British Columbia, Canada. in *Canadian Journal of Infectious Diseases and Medical Microbiology* 20(Suppl B):1B-112B (Abstract O046) (2009).
30. Boucoiran, Isabelle. No Title. (2021).
31. Boucoiran, I. *et al.* Human Immunodeficiency Virus Viral Load Rebound Near Delivery in Previously Suppressed, Combination Antiretroviral Therapy-Treated Pregnant Women. *Obstet Gynecol* 130, 497–501 (2017).
32. Kapetanovic, S., Dass-Brailsford, P., Nora, D. & Talisman, N. Mental health of HIV-seropositive women during pregnancy and postpartum period: A comprehensive literature review. *AIDS and Behavior* vol. 18 1152–1173 (2014).
33. World Health Organization. *Counselling for Maternal and Newborn Health Care: A Handbook for Building Skills.* . vol. 14 (World Health Organization, 2013).
34. Iliff, P. J. *et al.* Early exclusive breastfeeding reduces the risk of postnatal HIV-1 transmission and increases HIV-free survival. *AIDS* 19, 699–708 (2005).
35. Dunn, D. T., Newell, M. L., Ades, a. E. & Peckham, C. S. Risk of human immunodeficiency virus type 1 transmission through breastfeeding. *The Lancet* 340, 585–588 (1992).

36. Krebs, S. J. & Ananworanich, J. Immune activation during acute HIV infection and the impact of early antiretroviral therapy. *Curr Opin HIV AIDS* 163–172 (2015) doi:10.1097/COH.0000000000000228.
37. Semba, R. D. *et al.* Mastitis and immunological factors in breast milk of lactating women in Malawi. *Clinical and Diagnostic Laboratory Immunology* 6, 671–674 (1999).
38. Lunney, K. M. *et al.* Associations between Breast Milk Viral Load, Mastitis, Exclusive Breast-Feeding, and Postnatal Transmission of HIV. *Clinical Infectious Diseases* 50, 100201120631034–000 (2010).
39. World Health Organization. *Mastitis Causes and Management*. (2000).
40. Kantarci, S., Koulinska, I. N., Aboud, S., Fawzi, W. W. & Villamor, E. Subclinical mastitis, cell-associated HIV-1 shedding in breast milk, and breast-feeding transmission of HIV-1. *J Acquir Immune Defic Syndr* 46, 651–4 (2007).
41. Kambarami, R. The Prevalence of Nipple Disease Among Breast Feeding Mothers of HIV Seropositive Infants. *Cent Afr J Med.* 43, 20–22 (1997).
42. Tess, Beatriz H, Rodrigues, Laura C, Newell, Marie-Louise, Dunn, David T, Lago, Tania DG, and the S. P. C. S. for V. T. of H.-1. Infant Feeding and Risk of Mother-to-Child Transmission of HIV-1 in Sao Paulo State, Brazil. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* 19, 189–194 (1998).
43. Villamor, E. *et al.* Wasting during pregnancy increases the risk of mother-to-child HIV-1 transmission. *Journal of Acquired Immune Deficiency Syndromes* 38, 622–626 (2005).
44. Dreyfuss, M. L. & Fawzi, W. W. Micronutrients and vertical transmission of HIV-1. *American Journal of Clinical Nutrition* vol. 75 959–970 (2002).
45. Wiysonge, C. S., Ndze, V. N., Kongnyuy, E. J. & Shey, M. S. Vitamin A supplements for reducing mother-to-child HIV transmission. *Cochrane Database of Systematic Reviews* vol. 2017 (2017).