Association between Genital Tract HIV-1 RNA Shedding and Mucosal Inflammation among Women on Effective Antiretroviral Therapy with Undetectable Plasma Viral Load

Chia-ching J. Wang[1], Tao Liu[2], Brenna Anderson[3], Allison DeLong[2], Stacey Chapman[1], Jaclynn Kurpewski[1], Jessica Ingersoll[4], Angela Caliendo[4], Susan Cu-Uvin[1,3]

[1]*The Miriam Hospital*, [2]*Center for Statistical Sciences - Brown University*, [3]*Women and Infants Hospital*, [4]*Emory University*

Contact Information:
Chia-ching (Jackie) Wang
cwang1@ lifespan.org
164 Summit Ave, Providence, RI 02906
Phone (401) 793-4317 Fax (401) 793-4779

Background:
• Some women on effective antiretroviral therapy (ART) with undetectable plasma viral load (PVL) shed HIV in the genital tract.
• This discordance is not wholly explained by genital tract infections.

Methods:
• The study enrolled women on effective ART with PVL ≤ 80 copies/mL for at least 6 months.
• Serial paired plasma and genital tract HIV-1 RNA were measured every 4 weeks for 12 months.
• HIV-1 RNA was measured by nucleic acid sequence-based amplification assay (NucliSens).
• Genital tract secretions were collected by cervicovaginal lavage (CVL) and Sno-Strips, and lower genital tract infections were tested (see Table 1)
• GT-WBC was measured by hemacytometry.
•Generalized estimating equations with robust standard errors were used to estimate the prevalence and odds of detectable GT HIV-1 RNA when GT-WBC was present.

Results:
52 women contributed 364 visits over 12 months. Among all 364 visits with PVL ≤ 80 copies/mL, there were 8% (30/364) of visits with detectable HIV-1 RNA in genital tract, and 65% (238/364) of visits with detectable white blood cells in CVL. mucosal inflammation as measured by GT-WBC is associated with genital tract HIV-1 RNA shedding, both in the absence (p=0.008) and presence (p=0.012) of diagnosed genital tract infections.

Conclusion:
In HIV-infected women on effective ART with undetectable PVL, the prevalence of genital tract HIV-1 RNA shedding is low. In this study, mucosal inflammation as measured by genital tract white blood cells is associated with genital tract HIV-1 RNA shedding.

Background:

• Virologic and immunologic co-factors, such as plasma HIV-1 RNA concentration and CD4 cell count, influence genital tract shedding.
• In addition, pregnancy, hormonal contraceptives and phases of menstrual cycle also impact HIV-1 viral shedding.
• Both ulcerative and non-ulcerative genital tract infections have been shown to increase HIV-1 shedding.
• Some women on effective antiretroviral therapy (ART) with undetectable plasma viral load (PVL) shed HIV in the genital tract. This discordance is not wholly explained by genital tract infections.

Objective:

We aimed to determine the association between mucosal inflammation (as measured by genital tract white blood cells [GT WBC]) and genital tract shedding of HIV-1 RNA.

Methods:

• The study enrolled 52 women on effective ART with PVL ≤ 80 copies/mL for at least 6 months. Only visits with PVL ≤ 80 copies/mL were included.
• Serial paired plasma and genital tract HIV-1 RNA were measured every 4 weeks for 12 months.
• Genital tract secretions were collected by cervicovaginal lavage (CVL) and Sno-Strips.
• GT-WBC was measured by hemacytometry.
• HIV-1 RNA was measured by nucleic acid sequence-based amplification assay (NucliSens). Limit of detection: plasma ≤ 80 copies/mL, Sno-strips ≤ 3,300 copies/mL.

(Continued)

Methods (continued):

• Gonorrhea (GC), chlamydia (CT) by urine NAAT and syphilis by RPR were tested at baseline and if clinically indicated.
• Bacterial vaginosis (BV), *Candida* vaginitis, and trichomoniasis were diagnosed by wet mount at baseline and at every 4 weeks.
• HSV-2 DNA PCR on CVL was performed at all study visits on women who were HSV-2 antibody (Ab) positive.

Statistical Analysis:

Generalized estimating equations with robust standard errors were used to estimate the prevalence and odds of detectable GT HIV-1 RNA when GT-WBC was present.

Results:

52 women contributed 364 visits over 12 months.

Note: This study is still ongoing, and only partial data are presented here.

Table 1. Demographic Characteristics, N = 52 women, n (%)

Median age (years)	44 (range: 31 - 63)
Race/ethnicity: White	17 (33)
Black	24 (46)
Hispanic	8 (15)
Other	3 (6)
Median CD4 count (cells/uL)	445 (range: 120 - 1346)
Median GT-WBC (cell/cmm)	10 (range: 0 - 4,100)
On PI-containing ART regimen	24 (46)
On NNRTI-containing ART regimen	27 (52)
Status-post hysterectomy	10 (19)

Table 2. Prevalence of Lower Genital Tract Infections, N = 364 visits

Sexually Transmitted Infections	Number of visits	Prevalence (% of visits)
Gonorrhea	0	0
Chlamydia	0	0
Syphilis	0	0
Trichomoniasis	3	0.8
Candida vaginitis	30	8.3
Bacterial vaginosis	73	20.0
HSV-2 serum Ab (+)	364	100.0
Asymptomatic HSV-2 shedding	26	7.1
Symptomatic herpetic episodes	5	1.4

• Among all 364 visits with PVL ≤ 80 copies/mL, there were **8% (30/364)** of visits with detectable HIV-1 RNA in genital tract, and **65% (238/364)** of visits with detectable white blood cells in CVL.

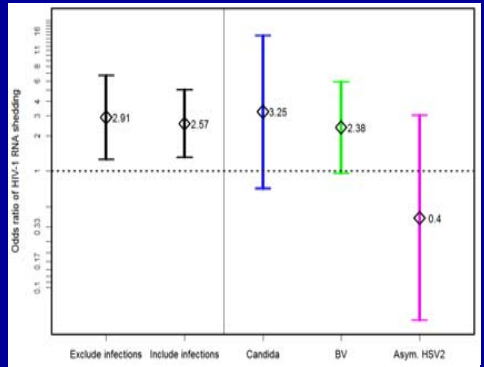

Figure 1. The Association between GT-WBC and HIV-1 RNA Genital Tract Shedding; in the absence and presence of genital tract infections

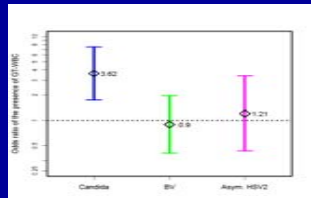

Figure 2. The association between various genital tract infections and GT-WBC

Figure 2:
Only Candida was found to be associated with presence of GT-WBC.

Figure 1:
• Presence of GT-WBC was associated with increased odds of HIV-1 RNA shedding in genital tract, both in the absence and presence of genital tract infections. .
• None of the diagnosed genital tract infections were associated with HIV-1 RNA shedding.

Conclusions:

• In HIV-infected women on effective ART with undetectable PVL, the prevalence of genital tract HIV-1 RNA shedding is low.
• In this study, mucosal inflammation as measured by GT-WBC is associated with genital tract HIV-1 RNA shedding, both in the absence (p=0.008) and presence (p=0.012) of diagnosed genital tract infections.
• Our data suggest that it may not be the infections themselves, but rather the inflammation caused by infections that drives the viral shedding. The increase in HIV shedding could be due to cytokines and chemokines produced by neutrophils of certain patients.
• There are several limitations of this study:
  ❑ We only tested for GC, CT, and syphilis at baseline and when clinically indicated.
  ❑ BV, *Candida* vaginitis, and trichomoniasis were included or excluded from the analysis based on test with imperfect sensitivity and specificity (wet mount).
  ❑ Cytokine measurements were also not done.
• Future studies need to explore the role of mucosal inflammation, mucosal immunology, and vaginal ecology in genital tract HIV-1 viral shedding.

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