



# The correlation of rectal HIV-1 RNA shedding with plasma viral load is not affected by rectal STIs in MSM with access to cART

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## ABSTRACT

**Background:** Among persons using combination antiretroviral therapy (cART), the impact of rectal sexually transmitted infection (STI) on rectal HIV-1 shedding is not well understood. We examined the association of rectal HIV-1 RNA levels with blood viral loads in the presence and absence of rectal STIs among mostly cART-using men who have sex with men (MSM).

**Methods:** We used a convenience sample of 93 rectal swabs collected from 81 HIV-1-infected MSM enrolled in the CDC-sponsored SUN Study. The swabs were collected for *Neisseria gonorrhoea* (GC) and *Chlamydia trachomatis* (CT) screening by nucleic acid testing and were stored in Gen-Probe APTIMA® media, aliquots of which were centrifuged (400 x g) and filtered (0.45 µm pore size) before ultra-centrifugation (105 x g). Nucleic acids in the pelleted material were extracted and HIV-1 was quantified using an FDA-approved virus load kit. Preliminary virus spiking studies established that our protocol's sensitivity for quantification was 150 HIV-1 RNA copies/rectal swab. HSV antibody status and yearly anal cytology are collected on study participants. We determined the correlation between ordinal categories of rectal and plasma viral load with Kendall's tau. We used multinomial regression models with robust variances to examine factors associated with rectal viral load.

**Results:** Sixty-five (70%) swabs were from MSM on cART with a median plasma viral load of 2.18 log<sub>10</sub> copies/ml (IQR <1.7-4.34) and CD4 count 457 cells/mm<sup>3</sup> (IQR 312-554). Fifty-five (68%) men were HSV-2 seropositive at baseline and 40 (49%) had abnormal rectal cytology. Thirty-two (34%) swabs were positive for rectal GC or CT. Rectal and plasma HIV-1 RNA levels were highly correlated (Kendall's tau 0.68 p<.0001). The presence of rectal GC or CT did not enhance detection of rectal HIV-1 shedding when plasma viral loads were <2.0 log<sub>10</sub> copies/ml (p=0.94). Only plasma viral load, but not rectal GC or CT infection, CD4 cell count, abnormal anal cytology, or HSV-2 serostatus, was significantly associated with rectal HIV RNA levels in multinomial regression.

**Conclusions:** In this small sample of HIV-infected MSM with a high prevalence of STI and access to cART, plasma HIV-1 viral load was the only independent correlate of rectal HIV-1 shedding. Rectal GC or CT infection did not appreciably alter this relationship. Suppressing plasma HIV-1 viral load with cART is a priority in order to reduce HIV transmission related to exposure to rectal secretions.

## Introduction

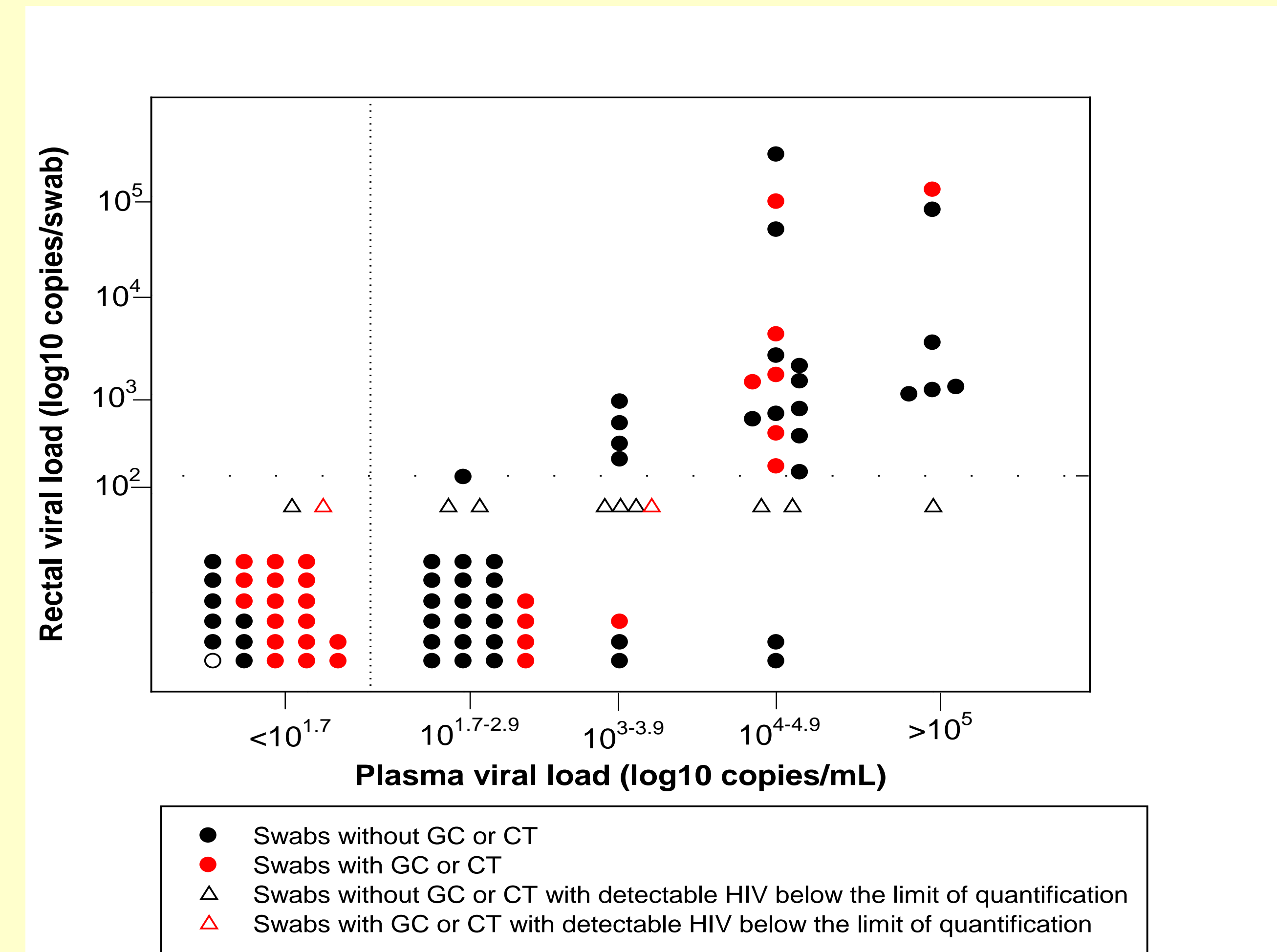
- ❖ While the rectum is an important site of HIV transmission in men who have sex with men, very little is known about HIV in rectal secretions in the era of effective cART.
- ❖ All published studies to date have collected rectal secretions by anoscopy, a procedure not practiced uniformly by HIV providers.
- ❖ Previous studies have associated rectal viral loads with plasma viral loads, but there are mixed results on the effect of local inflammation on rectal viral load.
- ❖ While the presence of sexually transmitted infections in the male and female genital tract is associated with increased shedding of HIV, it is not known if the same is true for rectal secretions, where the vast majority of infections are asymptomatic.

## Methods

- ❖ Rectal swabs were collected twice yearly for GC/CT NAAT testing in GenProbe Aptima media and stored.
- ❖ HIV RNA was extracted from rectal swabs in a convenience sample of 61 MSM without and 32 MSM with rectal GC or CT detected (sample dates 2006-2008) who had a wide spectrum of plasma viral loads.
- ❖ A 1 ml aliquot of the Aptima media was cleared of flocculent material by low speed centrifugation, then filtered through a 0.45µm filter (pre-coated with FBS) to remove PCR inhibitors and cellular material. The sample was then centrifuged (10<sup>5</sup>g x 30 min) and the RNA pellet was extracted using the Omega Bio-tek E.Z.N.A. spin column procedure
- ❖ The Roche Amplicor HIV-1 Monitor Ultra-sensitive assay was used for amplification and detection.
- ❖ The lower limit of HIV-1 RNA quantification was 2.18 log<sub>10</sub> copies/swab as determined by virus spiking experiments. Swabs that had detectable HIV-1 RNA that was below the limit of quantification were given arbitrary values of 1.87 log<sub>10</sub> for statistical analysis.
- ❖ Plasma and rectal viral loads were categorized into an ordinal variable to account for many observations that were below the limit of detection.
- ❖ Kendall's tau correlation coefficient was calculated for higher ordinal category of rectal viral load.
- ❖ Multinomial regression with robust variance was used to determine correlates of higher ordinal category of rectal viral load.

## Results

### Plasma viral load is correlated with rectal viral load for MSM with and without rectal GC or CT



### Plasma viral load is the only significant correlate of rectal viral load in a multivariable regression model

Clinical Characteristic	Univariate Odds Ratio	P value	Multivariable Odds Ratio	P value
<b>Plasma viral load category in log<sub>10</sub> copies/ml</b>				
>5.0	827.7	<.0001	639.2	<0.0001
4.0-4.99	268.9	<.0001	162.7	0.0002
3.0-3.99	27.3	<.0003	21.1	0.008
1.7-2.99	1.8	0.55	2.59	0.4
<1.7 (referent)	--	--	--	--
CD4 count in cells/mm <sup>3</sup> (sqrt)	0.82	.05	.90	0.07
On combination ART	0.07	<.0001	3.42	0.09
Presence of rectal GC or CT	0.52	0.13	1.7	0.4
HSV-2+ antibody status	1.2	0.74	0.64	0.5
Abnormal anal cytology	1.1	0.79	1.0	1.0

- ❖ Kendall's tau correlation coefficient for all MSM=0.68. For MSM without STI r=0.66. For MSM with STI r=0.73. P<.0001 for all correlations.
- ❖ The strength of the association between plasma viral load and rectal viral load was greater for MSM with STI (p=.05).
- ❖ The plasma viral load that distinguished a detectable rectal viral low from undetectable (i.e. threshold) was 3.15 log<sub>10</sub> copies/ml (95% CI: 2.7-3.6).

### The presence of GC or CT does not increase detection of HIV in rectal secretions when plasma VL<2.0log<sub>10</sub> copies/ml

Plasma viral load in log <sub>10</sub> copies/ml	Detectable HIV in rectal swab % (n)		P-value
	STI negative	STI positive	
<2.0	5 (1/19)*	5 (1/21)*	1.0
2.0-4.0	45 (10/22)	73 (8/11)**	NA
>4.0	90 (18/20)		

\* One swab PCR inhibited in this group.  
\*\* Categories 2.0-4.0 and >4.0 combined for STI positive swabs due to small sample size.

## CONCLUSIONS

- ❖ Blind, clinician sampling of rectal secretions by swab is a simple and useful methodology for sampling rectal secretions for HIV in MSM.
- ❖ Plasma HIV viral load is the only significant correlate of rectal viral load in this sample of MSM with access to cART.
- ❖ Rectal GC or CT, HSV-2 antibody status or abnormal anal cytology were not associated with a higher rectal viral load.
- ❖ We were not powered to detect a difference at higher plasma viral loads, but at plasma viral loads <2.0 log<sub>10</sub> copies/ml, the presence of rectal GC or CT did not increase the detection of HIV in the rectal swabs.
- ❖ The presence of inflammation due to STI may have a greater effect on HIV shedding in the male and female genital tract or rectal CT and GC may cause minimal inflammation.
- ❖ Suppressing plasma viral load with combination ART is a priority to reduce transmission from rectal secretions in US MSM.