# HSV-2 Serostatus is Not Associated with Inflammatory or Metabolic Markers in ART-Treated HIV

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## Background

- Systemic inflammation and immune activation may persist in HIV-infected persons despite suppressive antiretroviral therapy (ART)
- This inflammation may contribute to adverse health outcomes
- In a recent randomized trial, we observed no impact of valacyclovir on inflammatory markers in this setting (CID 2013;57:1331-8) but the explanation for these negative findings is unclear
- We compared markers of immune activation, inflammation and glucose / lipid metabolism according to HSV-2 serostatus in ART-treated adults

## Methods

- **Setting:**
  - Two academic hospital-based HIV clinics in Toronto, Canada
- **Key eligibility criteria:**
  - HIV-infected adult aged ≥18 years
  - Viral load <50 copies/mL on ART ≥12 months
  - No chronic anti-HSV therapy nor need for it
  - No active opportunistic infection ≥12 months, active HBV or HCV co-infection, prior cardiovascular event
  - No immunomodulatory medications
  - Life expectancy > 24 months
- **Study visits:** baseline, 3 months, 6 months
- **Primary predictor variable:** HSV-2 serostatus
  - HerpeSelect ELISA (Focus Technologies) using cutoff of 1.1
  - Sensitivity analyses considered cutoff of 3.5
- **Primary outcome**: Percentage of activated (CD38+HLADR+) CD8 T-cells
- **Secondary outcomes:**
  - Immune: activated CD4, regulatory T-cells
  - Inflammatory: hsCRP, D-dimer, IL-1b, IL-6, MCP-1, TNF, sICAM-1, sVCAM-1, Ang1/Ang2 ratio
  - Metabolic:
    - Impaired fasting glucose (IFG), impaired glucose tolerance (IGT), diabetes (DM) defined by
    - Insulin sensitivity (Matsuda index) & resistance (HOMA-IR)
    - Fasting lipids
- **Analysis:** Impact of HSV-2 seropositivity estimated using GEE regression models
- **Sample size:** Target enrollment 90 allowing for 10% dropout
  - Analyses on 80 participants → minimum detectable difference of 1.9% in activated CD8+ T-cells between HSV-2 positive & negative participants
- **Ethical approval**: obtained from Research Ethics Boards of the University Health Network and St. Michael's Hospital

## Results

**Figure 1. Participant enrollment flowchart**

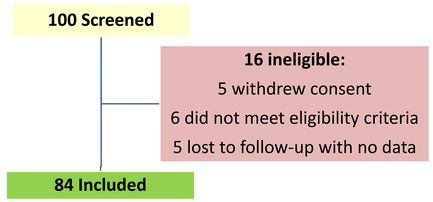


**Table 1. Participant Characteristics [a]**

| Characteristic | HSV2 Negative (n=46) | HSV2 Positive (n=38) |
|---|---|---|
| Age | 49 (40-53) | 49 (45-56) |
| Male | 45 (98%) | 28 (74%) |
| Race | | |
| Caucasian | 33 (72%) | 19 (50%) |
| Black | 4 (9%) | 12 (32%) |
| Asian | 6 (13%) | 3 (8%) |
| Other | 3 (7%) | 4 (11%) |
| Smoking Status | | |
| Never | 17 (37%) | 15 (41%) |
| Former | 10 (22%) | 13 (35%) |
| Current | 19 (41%) | 9 (24%) |
| HIV Risk Factor | | |
| MSM | 33 (72%) | 25 (66%) |
| Heterosexual | 15 (33%) | 17 (45%) |
| Other | 9 (20%) | 3 (8%) |
| Active Comorbidities | | |
| Hypertension | 7 (15%) | 7 (19%) |
| Hyperlipidemia | 12 (26%) | 10 (27%) |
| Diabetes | 1 (2%) | 3 (8%) |
| Asthma | 4 (9%) | 2 (5%) |
| Other[b] | 4 (9%) | 1 (3%) |
| HSV-1 Seropositive | 39 (85%) | 31 (89%) |
| History of HSV symptoms | 25 (54%) | 17 (50%) |
| Years since HIV diagnosis | 10 (7-18) | 12 (7-19) |
| Years since 1st ART | 9 (5-13) | 10 (3-15) |
| Years on current regimen | 3 (1-4) | 2 (1-3) |
| Antiretroviral regimen | | |
| 2 NRTIs + NNRTI | 28 (61%) | 16 (43%) |
| 2 NRTIs + PI | 8 (17%) | 8 (22%) |
| 2 NRTIs + Raltegravir | 0 (0%) | 1 (3%) |
| Other | 10 (22%) | 12 (32%) |
| Baseline CD4 | | |
| Cell/mm$^3$ | 466 (329-640) | 478 (342-621) |
| Percentage | 29 (26-34) | 32 (22-37) |

[a] Values shown are median (interquartile range) and frequency (percentage)
[b] Other comorbidities: Prior acute coronary syndrome, inflammatory bowel disease, chronic kidney disease & atrial fibrillation for HSV-2 negatives; Hashimoto's thyroiditis on thyroid replacement therapy for HSV-2 positive

- **HSV signs & symptoms were uncommon during the study**
  - 11 (13%) reported symptoms between baseline & month 3
  - 12 (14%) reported symptoms between month 3 & month 6
  - 3 (4%) had oral lesions on exam consistent with HSV at BL
  - 1 (1%) had oral lesions on exam consistent with HSV at mo. 6
  - No genital or perianal lesions seen on exam during study

**Table 2. No significant associations between HSV-2 serostatus and immune / inflammatory markers**

| Outcome | Univariate HSV2 | | Multivariable HSV2 [a] | |
|---|---|---|---|---|
| | Estimate (95% CI) | p | Estimate (95% CI) | p |
| **Immune Outcomes** | | | | |
| CD38+ CD8+ | -0.02 (-1.33,1.29) | 0.98 | 0.26 (-1.20,1.72) | 0.72 |
| CD38+ CD4+ | 0.00 (-0.47,0.47) | 1.00 | 0.05 (-0.45,0.54) | 0.85 |
| CD25+ | 0.09 (-0.41,0.58) | 0.73 | 0.05 (-0.45,0.56) | 0.84 |
| **Biomarker Outcomes** | | | | |
| Ang1/Ang2 Log10 | 0.13 (-0.12,0.37) | 0.30 | 0.15 (-0.11,0.40) | 0.26 |
| CRP Log10 | 0.11 (-0.14,0.36) | 0.40 | 0.06 (-0.17,0.30) | 0.59 |
| D-Dimer Log10 | 0.09 (-0.00,0.19) | 0.05 | 0.08 (-0.01,0.18) | 0.09 |
| sICAM Log10 | 0.04 (-0.04,0.12) | 0.31 | 0.06 (-0.01,0.13) | 0.09 |
| IL-1β [b] | 1.05 (0.34,3.27) | 0.94 | 0.99 (0.31,3.09) | 0.98 |
| IL-6 [b] | 1.19 (0.42,3.31) | 0.75 | 1.21 (0.40,3.61) | 0.74 |
| MCP-1 [b] | 1.80 (0.82,3.95) | 0.14 | 1.81 (0.79,4.13) | 0.16 |
| TNF [b] | 1.04 (0.37,2.93) | 0.94 | 1.18 (0.38,3.64) | 0.77 |
| sVCAM Log10 | 0.05 (-0.03,0.12) | 0.23 | 0.06 (-0.01,0.13) | 0.11 |

[a] Model included: HSV-2 serostatus, time, sex, duration of HIV infection, baseline CD4 count
[b] Odds ratio of having an undetectable value

**Figure 2. Decline in T-cell activation, increase in T-regs over time**

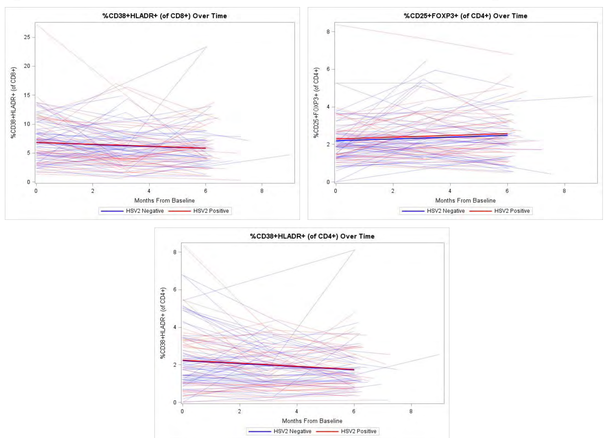


**Table 3. Decline in T-cell activation and increase in T-regs over time [a]**

| | HSV-2 | | Per month from baseline | |
|---|---|---|---|---|
| | Estimate (95% CI) | p | Estimate (95% CI) | p |
| CD25 | 0.08 (-0.42,0.58) | 0.75 | 0.05 (0.02,0.08) | <.01 |
| CD38 CD4 | 0.02 (-0.45,0.49) | 0.94 | -0.08 (-0.14,-0.02) | <.01 |
| CD38 CD8 | 0.02 (-1.31,1.35) | 0.98 | -0.16 (-0.31,-0.01) | 0.04 |

[a] Model included: HSV-2 serostatus and time only

## Results

**Table 4. No significant associations between HSV-2 serostatus and metabolic outcomes [a]**

| Outcome | HSV2 | | 6-month time point | |
|---|---|---|---|---|
| | Estimate (95% CI) | p | Estimate (95% CI) | p |
| **Glucose Metabolism** | | | | |
| Fasting glucose mmol/L | 0.05 (-0.31,0.42) | 0.78 | -0.08 (-0.24,0.08) | 0.32 |
| IFG [b] | 4.32 (0.83,22.52) | 0.08 | 1.18 (0.46,3.00) | 0.73 |
| IGT [b] | 1.05 (0.30,3.65) | 0.94 | 0.65 (0.26,1.61) | 0.35 |
| DM [b] | 0.91 (0.20,4.21) | 0.90 | 1.08 (0.41,2.83) | 0.87 |
| DM Therapy [b] | 2.50 (0.22,28.69) | 0.46 | 1.00 (1.00,1.00) | 0.65 |
| **Insulin Resistance** | | | | |
| Matsuda index | -0.81 (-3.17,1.55) | 0.50 | 0.50 (-0.98,1.98) | 0.51 |
| HOMA-IR index | -0.28 (-1.29,0.74) | 0.59 | -0.46 (-1.07,0.15) | 0.14 |
| **Framingham score** | 0.96 (-1.07,2.99) | 0.35 | -0.04 (-0.55,0.47) | 0.88 |
| **Lipid Values** | | | | |
| Total/HDL ratio | -0.20 (-0.75,0.34) | 0.47 | 0.11 (-0.14,0.36) | 0.39 |
| LDL mmol/L | -0.17 (-0.51,0.16) | 0.31 | -0.10 (-0.22,0.01) | 0.08 |
| Apolipoprotein B | -0.03 (-0.11,0.06) | 0.54 | -0.06 (-0.12,-0.01) | 0.02 |
| **Lipid-lowering therapy** | | | | |
| Meets criteria [b] | 0.67 (0.29,1.55) | 0.35 | 1.06 (0.77,1.48) | 0.71 |

[a] Model included: HSV-2 serostatus and 6-month time-point only. DM=diabetes mellitus, IFG= impaired fasting glucose, IGT = impaired glucose tolerance, HDL = high density lipoprotein, LDL = low density lipoprotein
[b] Odds ratios are presented

- **Sensitivity analyses:**
  - 24/36 HSV-2 seropositive participants were still classified as such after redefining HSV-2 seropositivity as index value ≥3.5
  - Results of immune, inflammatory and metabolic analyses were qualitatively unchanged using this definition (data not shown)

## Conclusions

- HSV-2 serostatus was not associated with immune activation, inflammatory, or glucose / lipid metabolic markers in this cohort of HIV-infected adults on suppressive ART
- Possible explanations include
  - Relatively inactive HSV-2 disease in this cohort
  - Anatomic compartmentalization of HSV-2 infection and lack of impact on systemic inflammation / metabolism
- Potential relevance of HSV-2 to HIV-related inflammation in other sub-populations (eg. incident HIV, more symptomatic HSV-2, ART initiation) warrants further study

## Acknowledgements

This work was supported by a grant from the Canadian Institutes for Health Research. DHST is supported by a CIHR Mentoring Award in Randomized Controlled Trials. , RK JR and SW are supported by OHTN Career Scientist Awards.