Effect of Intercurrent Infections and Vaccinations on Immune and Inflammatory Biomarkers Among Human Immunodeficiency Virus-Infected Adults on Suppressive Antiretroviral Therapy

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We used generalized estimating equations to quantify the impact of recent vaccination or intercurrent infections on immune and inflammatory biomarkers among 144 human immunodeficiency virus (HIV)-infected adults with HIV RNA < 50 copies/mL on antiretroviral therapy. These events were associated with a 2.244 μg/mL increase in high sensitivity C-reactive protein and should be routinely assessed in future studies.

Keywords. C-reactive protein; HIV; infections; inflammatory biomarkers; immune activation; vaccinations.

Systemic inflammation and immune activation persists in persons infected with human immunodeficiency virus (HIV), despite suppressive combination antiretroviral therapy (cART), and have been linked with adverse health outcomes [1–4]. Thus, inflammatory biomarkers are increasingly being measured in persons infected with HIV for research purposes, and these biomarkers may have potential applications in clinical practice as their value in predicting morbidity and mortality is refined. However, the extent to which common intercurrent infections and vaccinations influence these measurements is unclear.

Herpes simplex virus type 2 (HSV-2) is a common coinfection thought to contribute to increased inflammation in persons infected with HIV [5], and the availability of safe, effective, anti-HSV medications such as valacyclovir has made it an attractive candidate for study in this regard. We previously conducted 2 studies that evaluated the impact of HSV-2 coinfection and of valacyclovir, respectively, on a variety of immune and inflammatory biomarkers among HIV-infected adults with cART-induced virologic suppression, but we found no significant relationship with inflammation levels in either study [6, 7]. In this report, we present a pooled analysis of patient data from our 2 studies to evaluate the relationships between intercurrent infections and vaccinations with inflammatory biomarker levels.

METHODS

Design and Participants

We pooled individual patient data from 2 prospective studies among HIV-infected adults with plasma HIV RNA < 50 copies/mL on cART. The methods and results of the 2 studies have been previously published [6, 7]. Study 1 was a 6-month prospective cohort study among 84 participants and evaluated the impact of HSV-2 serostatus on a panel of immune and inflammatory markers, measured at baseline, 3 months and 6 months [6]. Study 2 was an 18-week randomized trial in which 60 participants were randomized 1:1:1 to low-dose valacyclovir (500 mg twice daily), high-dose valacyclovir (1 g twice daily), or placebo; measurements of the same biomarkers as in Study 1 were performed at baseline, 6, 12, and 18 weeks [7]. At each visit in both studies, participants were asked to report any vaccinations and any sexually transmitted, upper or lower respiratory tract, gastrointestinal, or other infections occurring within the preceding 2 weeks. All medications used during the studies were recorded, and the timing of vaccinations was verified. The studies were approved by the relevant Research Ethics Boards at the University Health Network (Studies 1 and 2) and St. Michael's Hospital (Study 1). All participants provided written informed consent.

Laboratory Methods

Biomarkers included measures of immune activation ($CD38^+$ $HLA\text{-}DR^+$ $CD8^+$ T cells, $CD38^+$ $HLA\text{-}DR^+$ $CD4^+$ T cells, regulatory T cells), inflammatory cytokines (interleukin [IL]-1β,

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IL-6, monocyte chemoattractant protein-1 [MCP]-1, tumor necrosis factor [TNF]), endothelial activation (angiopoietin 1:2 ratio, soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular cell adhesion molecule-1 [sVCAM-1]), and the acute-phase reactant high-sensitivity C-reactive protein (hsCRP). Full methods have been described previously [6, 7].

Statistical Analysis

We used generalized estimating equation models with exchangeable correlation matrices to estimate the effect of having any intercurrent infection or vaccination in the preceding 2 weeks on each biomarker. Biomarker levels were treated either as continuous measures (with logarithmic transformations as appropriate) or as dichotomous measures (detectable or undetectable) using identity or logit link functions, respectively. Models were adjusted for study cohort (Study 1 or Study 2) and for the number of days since the baseline study visit at the time of each specimen collection. The primary outcome of interest in both studies was the proportion of activated ($CD38^+$ $HLA\text{-}DR^+$) $CD8^+$ T cells.

RESULTS

Of the 144 participants, 87.5% were male, 67% were HSV-2 coinfected, and 81% were HSV-1 coinfected. Most participants (67.4%) were white, and another 16.7% were black, 10.4% Asian, and 5.6% other ethnicities. Median (interquartile range) duration of HIV infection was 12 (8,19) years, duration of cART was 8 (5,13) years, and CD4 count at baseline was 492 (355, 648) cells/mm^3. Most participants were either current (29.9%) or former (29.2%) smokers.

At baseline, the participants in Study 2 had higher levels of $CD38^+$ $HLA\text{-}DR^+$ $CD4^+$ T cells (2.83% vs 2.02%, $P < .01$), sVCAM-1 (323 vs 268 pg/mL, $P < .01$), a higher percentage of undetectable IL-1β (97% vs 84%, $P = .02$), and undetectable MCP-1 (70% vs 51%, $P = .02$), and lower levels of $CD38^+$ $HLA\text{-}DR^+$ $CD8^+$ T cells (4.56% vs 6.77%, $P = .04$) than those in Study 1.

Recent infection or vaccination was reported for 83 of 482 (17.2%) study visits. These included upper respiratory tract (n = 48 episodes), urinary tract (n = 2), gastrointestinal (n = 2), lower respiratory tract (n = 1), and other infections (n = 5) as well as n = 18 orolabial or anogenital herpes reactivations. Antibiotics were used for 4 of these episodes (both urinary tract infections and 2 upper respiratory tract infections); none of the herpes reactivations were treated with short-course systemic antivirals. Vaccinations were for influenza (n = 13), tetanus (n = 2), and hepatitis B (n = 1). As summarized in Table 1, these events were generally not associated with any significant changes in biomarker levels in either univariable or multivariable (adjusted for Study and time since baseline) models (Table 1). The exception was $\log_{10}$ hsCRP, which was increased by 0.354 (95% confidence interval [CI], 0.234–0.473) $\log_{10}$ ng/mL and by 0.351 (95% CI, 0.233–0.470) $\log_{10}$ ng/mL in the unadjusted and adjusted analyses, respectively, corresponding to 2.259 and 2.244 μg/mL, respectively on the absolute scale. Results were qualitatively unchanged when separate models were run for infections and vaccinations, respectively (full data not shown). Four vaccinations were recorded to have occurred within 2 weeks, but the exact date could not be verified; results were also qualitatively unchanged in sensitivity analyses excluding these 4 events.

Table 1. Changes in Immune and Inflammatory Biomarkers Associated With Self-Reported Recent Infection and/or Vaccination

	Univariable		Multivariable[a]	
Biomarker	Est (95% CI)	*P* Value	Est (95% CI)	*P* Value
$CD38^+CD8^+$ T cells (%)	0.42 (−0.66–1.50)	.45	0.40 (−0.70–1.49)	.48
$CD38^+CD4^+$ T cells (%)	0.12 (−0.23–0.48)	.49	0.13 (−0.23–0.48)	.49
Regulatory T cells (%)	0.07 (−0.19–0.33)	.59	0.08 (−0.18–0.35)	.53
IL-1β (pg/mL)[b]	1.29 (0.78–2.14)	.32	1.27 (0.77–2.10)	.34
IL-6 (pg/mL)[b]	1.02 (0.68–1.54)	.92	1.02 (0.67–1.55)	.94
MCP-1 (pg/mL)[b]	1.17 (0.84–1.63)	.36	1.17 (0.83–1.66)	.37
TNF (pg/mL)[b]	0.97 (0.62–1.50)	.88	1.00 (0.65–1.55)	.99
Ang1:2 ratio Log_{10}	−0.07 (−0.16–0.01)	.09	−0.07 (−0.15–0.01)	.09
sICAM-1 (pg/mL)	8.18 (−3.38–19.7)	.17	8.46 (−3.25–20.2)	.16
sVCAM-1 (pg/mL)	10.8 (−16.1–37.7)	.43	11.1 (−15.3–37.6)	.41
hsCRP (ng/mL) Log_{10}	0.354 (0.234–0.473)	<.0001	0.351 (0.233–0.470)	<.0001

Abbreviations: Ang, angiopoietin; CI, confidence interval; Est, estimate; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; MCP, monocyte chemoattractant protein; sICAM, soluble intercellular adhesion molecule-1; sVCAM, soluble vascular cell adhesion molecule-1; TNF, tumor necrosis factor.

[a] Multivariable model includes study (Study 1 or Study 2) and the number of days since baseline study visit.

[b] Odds ratio of having an undetectable value is presented.

DISCUSSION

In this post hoc pooled analysis, intercurrent infections or vaccinations were associated with modestly increased hsCRP but not other immune and inflammatory biomarkers in this cohort of ART-treated adults. The magnitude of increase in hsCRP was estimated at approximately 2.2 μg/mL on the absolute scale. Such an increase is clinically significant, similar in magnitude to the observed difference in baseline hsCRP levels between cases who died and controls who did not in the SMART trial of continuous versus interrupted ART (4.26 vs 2.14 μg/mL) [2], or between cases who progressed to acquired immune deficiency syndrome/death and controls who did not in the FIRST trial of initial ART regimens (4.4 vs 1.8 μg/mL) [8]. This increase is also large enough to impact clinical decision making; for instance, eligibility for the seminal JUPITER trial of rosuvastatin for reducing cardiovascular risk in the general population required hsCRP levels of only ≥2 μg/mL at enrollment [9].

In contrast to our findings, a number of previous studies have found modest increases in some inflammatory markers after vaccinations, including (1) a rise in CRP of 0.20–0.75 mg/L in a cohort of generally healthy older adults that peaked 2–3 days after receiving influenza with or without pneumococcal vaccine [10] and (2) a 30% rise in IL-6 and 45% rise in CRP that peaked 5–7 days after healthy volunteers received yellow fever vaccine [11]. Possible explanations for our discrepant findings include the longer time elapsed time since vaccination in our study (up to 14 days) as well as the HIV-infected status of our participants. Individuals infected with HIV are known to have less robust immune responses to standard vaccines [12, 13], and 1 study among 6 adults infected with HIV observed a rise in IL-6 of 0.34–7.61 pg/mL that peaked only 2–6 hours after pneumococcal polysaccharide vaccine; no change was seen in TNF [14].

The lack of observed differences in biomarkers other than hsCRP may reflect the nonspecific nature of hsCRP as an inflammatory marker. High-sensitivity CRP is a sensitive marker of both acute and chronic inflammation, and levels are known to increase during infections, although considerably less so for viral than bacterial etiologies [15]. In contrast, the other parameters we assessed may have been more specific for endothelial activation, inflammatory cytokine, and T-cell activation pathways that are not altered to the same degree by common infections. Although hepatic synthesis of hsCRP is itself triggered by inflammatory cytokines such as IL-1β, IL-6, and TNF, in which we did not observe significant changes, redundancies within the pathways that stimulate acute-phase reactants may explain the lack of changes in those analytes. The kinetics of biomarker changes may also have played a role, because the rate at which biomarkers increase and decrease in response to stimuli may be different.

This study has limitations that warrant consideration. This was a post hoc analysis of data pooled from 2 different studies, and data on infections were self-reported for the preceding 2 weeks before study visits only. However, methods for processing and analyzing specimens were the same for both projects. Furthermore, different vaccines and infections may have different effects on the biomarkers we measured. The reported infections varied in severity, with antibiotics prescribed in 4 cases, potentially biasing towards the null. Finally, our sample may not have been large enough to detect small changes.

In conclusion, we observed an increase in hsCRP of approximately 2 mg/L in the setting of recent infection or vaccination but no impact on other immune and inflammatory biomarkers among HIV-infected adults with ART-induced virologic suppression. Such events should be routinely assessed in studies in which hsCRP is measured, and, as is generally recommended for measurement of plasma HIV RNA levels, bloodwork should be timed to avoid such events when possible. It may not be appropriate to base clinical decisions on a single measure, and caution should be exercised with using a single measure of hsCRP as an outcome in research studies.

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