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# Circulating Markers of Immunologic Activity Reflect Adiposity in Persons with HIV on Antiretroviral Therapy

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## Abstract

**Background—**Obesity alters adipose tissue immunology, and these changes may be reflected in circulating soluble inflammatory biomarker and T cell subset profiles measured in HIV research studies.

**Methods—**We recruited 70 adults with HIV (50% obese) on efavirenz, tenofovir, and emtricitabine, virologic suppression for >2 years, and no rheumatologic or other known inflammatory conditions. We measured fasting plasma levels of several markers of innate immunity and major $CD4^+$ and $CD8^+$ T cell subsets. We assessed relationships between measurements of total adiposity (body mass index [BMI], DEXA fat mass index [FMI], and plasma leptin) and the immunologic parameters using covariate-adjusted Spearman's rank correlations.

**Results—**The cohort was 43% female, 54% non-white, and median age was 45 years. Higher BMI, FMI and plasma leptin were consistently associated with higher C-reactive protein, serum amyloid A, and interleukin (IL)-6 (p<0.01 for all), but lower IL-10 (p  0.02 for all). BMI and FMI were positively associated with soluble tumor necrosis factor-α receptor 1 levels (p<0.02 for both), and a positive correlation approached significance for all three body composition measurements with soluble CD163 (p  0.09 for all). Higher BMI and FMI were associated with lower CD38 expression on CD4+ T cells (p  0.04 for both), but higher CD69 expression (p  0.01 for BMI and FMI, p=0.07 for leptin).

**Conclusions—**Greater adiposity is associated with alterations in a limited set of circulating immune markers, potentially reflecting changes known to occur in adipose tissue with treated HIV

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infection. Measuring total fat mass radiographically did not yield substantively different results compared to BMI.

## Introduction

Relationships between circulating soluble immune mediators or T cell subsets and health outcomes among people living with HIV (PLWH) are reported in many studies.[1-8] As examples, higher circulating levels of C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are associated with increased risk of cardiovascular events, insulin resistance, and all-cause mortality among PLWH.[1,2,9-13] Similarly, a greater proportion of activated $CD8^+$ T cells is associated with poor $CD4^+$ T cell reconstitution on antiretroviral therapy (ART), subclinical carotid artery disease, and impaired arterial relaxation.[14-16] Ideally, these studies illuminate either the causal contribution of a given innate or adaptive immune actor in a pathophysiologic process, reflect an immune perturbation arising from a clinical condition, or both.

A concern in studies of biomarkers and health outcomes is confounding by participant characteristics that affect both the biomarker and outcome of interest, thus contributing to a spurious statistical relationship or a false-negative finding. In HIV-negative persons, serum levels of CRP and IL-6 increase with adiposity,[17-19] and it is estimated that adipose tissue-derived IL-6 constitutes up to 35% of circulating levels in obese individuals.[20] Furthermore, HIV-negative overweight and obese women have significantly higher $CD4^+$ and total lymphocyte counts compared to normal weight women.[21] At present there are few similar data for PLWH.

The increasing prevalence of obesity among PLWH in the US[22] raises the importance of understanding how immunologic biomarkers are affected by body composition. In this study, we characterize relationships between body fat and circulating levels of over 20 plasma markers of innate immunity and major $CD4^+$ and $CD8^+$ T cell subsets in PLWH on ART, with the goal of identifying the immune parameters most affected by adiposity as estimated by body mass index (BMI), dual energy X-ray absorptiometry (DEXA)-quantified fat mass index (FMI), and plasma leptin (an adipokine produced in proportion to fat mass).

## Methods

We enrolled 70 adults with HIV on ART from the Vanderbilt Comprehensive Care Clinic, distributed approximately equally between four BMI categories (<25.0, 25.0-29.9, 30.0-34.9, and  35.0 $kg/m^2$). Within each BMI strata, similar numbers of males and females, and whites and non-whites, were enrolled. All participants were on a single-tablet regimen of co-formulated efavirenz, tenofovir, and emtricitabine for at least 6 months, and had persistent HIV-1 RNA <50 copies/mL on ART for at least the previous 2 years. Additional inclusion criteria were $CD4^+$ T cell count >350 cells/μl at enrollment, no use of any anti-diabetic agent or statin (i.e., HMG CoA reductase inhibitor), no self-reported heavy alcohol or cocaine/amphetamine use, no active infectious condition aside from HIV, and no previously diagnosed diabetes, cardiovascular disease (CVD), rheumatologic disease, or other inflammatory condition.

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Venous blood was drawn in the morning between 8 and 11am after a minimum 8 hour fast. Samples were collected in an EDTA-containing vacutainer, centrifuged for 10 minutes at 4°C, and the plasma removed and immediately frozen at −80°C. High-sensitivity CRP (hs-CRP) was measured by nephelometry in the Vanderbilt Clinical Chemistry Laboratory. Plasma levels of soluble CD14 (sCD14) and CD163 (sCD163), two surface markers released into circulation by activated macrophages, were measured using ELISA (R&D Systems, Minneapolis, MN). Other plasma cytokines including interleukins, serum amyloid A, interferon-γ, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 α and β (MIP-1α/β), TNF-α, and soluble TNF-α receptors 1 and 2 (sTNFRI and sTNFRII) were measured in duplicate using a standard multiple immunoassay panel (MesoScale, Rockville, MD).

Peripheral blood mononuclear cells (PBMCs) were obtained from fasting whole blood samples collected in EDTA, separated by Ficoll-Paque Plus density gradient, and cryopreserved in FBS with 10% DMSO. After study enrollment was completed, PBMC aliquots were thawed, stained, and run on a Fortessa (Becton Dickson Biosciences, San Jose, CA) flow cytometer. We used three different fluorochrome panels incorporating CD8-APC-A750 (Life Technologies, Carlsbad, CA); CD4-PcP-Cy5.5, CD3-BV711, CD14-V500, CD19-V500, CD57-FITC, PD-1-PE, CD69-APC, CD38-PE-Cy7, HLA-DR-V450A, CD25-PE, CD27-PE-Cy7, CD28-APC, CCR7-BV421, (Becton Dickson Biosciences); and CD45RO-PETxR, CD127-PE-Cy5.5 (Beckman Coulter, Pasadena, CA). We measured the proportion of $CD4^+$ and $CD8^+$ T cells expressing activation (CD38, HLA-DR, and CD69), senescence (CD57), and exhaustion (PD-1) markers. We used memory (CCR7, CD45RO, CD27) surface markers to markers identify naïve ($CD45RO^-$, $CCR7^+$, $CD27^+$), central memory (Tcm; $CD45RO^+$, $CCR7^+$, $CD27^+$), transitional memory ($CD45RO^+$, $CCR7^-$, $CD27^+$), effector memory (Tem; $CD45RO^+$, $CCR7^-$, $CD27^-$), and effector memory RA+ (TemRA; $CD45RO^-$, $CCR7^-$, $CD27^-$) T cell phenotypes. Lastly, in the $CD4^+$ population, we also measured the percentage of regulatory ($CD25^{high}$, $CD127^-$) T cells.

Height and weight were measured in duplicate to calculate BMI. A full body DEXA (GE Lunar Prodigy, GE Healthcare, Little Chalfont, United Kingdom) measured total fat mass to calculate FMI (total fat in kilograms divided by height in meters, squared). FMI is a variant of BMI that accounts for individual variability in the ratio of fat to lean mass.[23] Lastly, plasma leptin was measured in duplicate using an immunoassay (MesoScale, Rockville, MD).

### Statistical analyses

Demographic, clinical, and body composition characteristics were compared between BMI categories using Kruskal-Wallis rank sum or chi-square tests.

Due to the large number of biomarkers with heterogeneous distributions characterized by high skewness for some and assay detection limits for others, we assessed the relationships between adiposity measurements (BMI, FMI, and leptin) and the immunologic parameters using covariate-adjusted Spearman's rank correlations robust for these types of data.[24] Covariates were pre-specified and included age, sex, race (white versus non-white), entry $CD4^+$ T cell count (square root transformed), ART duration, and smoking status. This

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method first fits separate cumulative probability models (with logit link functions) to each adiposity and immunological measure as a function of covariates.[25] Probability-scale residuals (PSRs) are then calculated, and Spearman's correlations computed as the correlation between PSRs.

Secondary analyses included nadir $CD4^+$ T cell count as a covariate. We also calculated adjusted Spearman's rank correlations conditional on sex to assess whether relationships between adiposity measures and immune parameters differed by sex. No adjustments were made for multiple comparisons for this exploratory study.[26] Analyses were conducted using SPSS 22.0.0 (IBM) and R Statistical Software, Version 3.4.2 (http://www.R-project.org).

## Results

Seventy PLWH were enrolled. The cohort was 43% female and 54% non-white (Supplementary Table 1). Median age was 45 years, BMI 30.3 kg/$m^2$, $CD4^+$ T cell count 701 cells/μl, and ART duration 6.2 years. Age, race, sex, smoking status, entry $CD4^+$ count, ART initiation, duration of ART treatment, and hepatitis C prevalence were similar across the BMI categories ($p>0.05$ for all comparisons).

Adjusted rank correlations between BMI, FMI, or plasma leptin and each of the immunologic parameters are shown in the heat map (Figure); correlations with a P-value $<0.05$ and $<0.10$ are indicated. Higher BMI, FMI and plasma leptin were consistently associated with higher hs-CRP, serum amyloid A, and IL-6 ($P<0.01$ for all), but lower IL-10 (p  0.02 for all; significant associations are shown in the Table). BMI and FMI were positively associated with sTNFRI levels ($p<0.02$ for both), and the correlation with plasma leptin approached significance ($p=0.07$). A positive correlation between sCD163 and adiposity approached significance for all three body composition measurements (p  0.09 for all). Adjusted correlations for all measured biomarkers are shown in Supplementary Table 2.

We observed more heterogeneity between adiposity and the $CD4^+$ and $CD8^+$ T cell subsets than the soluble markers. Higher BMI and FMI were associated with lower CD38 expression on $CD4^+$ T cells (p  0.04 for both), but higher CD69 expression (p  0.01 for BMI and FMI, $p=0.07$ for leptin). Greater BMI and leptin levels were accompanied by higher expression of CD57 on $CD4^+$ T cells, and this relationship approached significance (p  0.09 for both). In contrast, the only significant association for $CD8^+$ T cell subsets was a positive correlation between leptin and $CD8^+$ TemRA cells.

Results were not substantively different when the models were further adjusted for nadir $CD4^+$ T cell count. When the 6 participants with BMI <20 kg/$m^2$ were excluded, results were similar with the exception that CD38 expression on CD4+ T cells was no longer significantly associated with BMI or FMI. Lastly, we did not find that the adjusted correlations conditioned on sex indicated a significant difference for in any of the adiposity and immune parameter relationships for males vs. females ($p>0.05$ for all)

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## Discussion

In a cohort of PLWH on long-term ART and without known rheumatologic or other inflammatory conditions, higher levels of adiposity were associated with greater plasma levels of several markers of innate immune activation, and variable changes in $CD4^+$ and $CD8^+$ T cell subsets. Notably, while hs-CRP, serum amyloid A, IL-6, sCD163, and sTNFRI levels demonstrated similar proportional increases for BMI, FMI, and plasma leptin, the changes in circulating $CD4^+$ and $CD8^+$ T cell subsets were markedly less consistent. Notably, several of the biomarkers that changed with adiposity in our cohort are known to increase in adipose tissue in the setting of obesity and exposure to HIV,[27] while lower levels of IL-10, a cytokine with pleiotropic anti-inflammatory effects on T cells and macrophages, are linked to poor metabolic health.[28,29] We interpret these findings to indicate that levels of circulating markers of innate immune function in PLWH may be determined, in part, by changes occurring within adipose tissue in response to progressive weight gain. Of note, measuring total fat mass with DEXA to adjust for participant adiposity did not substantively alter results compared to BMI.

The stromal vascular fraction of adipose tissue contains a diverse mix of cells from the innate and adaptive arms of the immune system that form a complex paracrine signaling milieu, modulating local inflammation and adipocyte function. These include monocyte-derived tissue macrophages and several T cell subsets, which may infiltrate adipose tissue from the bloodstream or lymphatic system, or be tissue-resident immune cells. HIV infection intervenes on this environment at many points, including changes in adipocyte metabolic characteristics and signaling, changes in circulating monocyte and T cell populations, and potential latent infection of adipose tissue CD4+ T cells.[27]

With obesity, adipose tissue depots primarily expand through adipocyte hypertrophy rather than hyperplasia, the former of which is accompanied by a disproportionate rise in IL-6 and TNF-α.[30-32] Adipocyte hypertrophy in obesity is also accompanied by increased MCP-1 and MIP-1α expression, which promote macrophage infiltration, and increased IL-8, which promotes neutrophil chemotaxis.[33-35] Adipose tissue biopsies from obese human and animals contain higher absolute numbers of macrophages, which demonstrate greater polarization towards a pro-inflammatory M1 cytokine phenotype (characterized by high IL-6, TNF-α and inducible nitric oxide synthase production).[36-38]

In our cohort, greater adiposity, as measured by both BMI and FMI, was most strongly associated with lower CD38 expression and higher CD69 expression on $CD4^+$ T cells. A link between adiposity and cellular immunity is supported by studies from the pre-ART era demonstrating that a higher BMI was associated with slower HIV disease progression.[39-41] In the combination ART era, a higher BMI is associated with more robust $CD4^+$ T cell recovery.[42] Notably, this may not reflect an HIV-related phenomenon, as HIV-negative overweight and obese women have significantly higher $CD4^+$ and total lymphocyte counts compared to normal weight women.[21]

Strengths of this study include a wide distribution of BMI values, restriction of participants to a single ART regimen, and long-term ( 2 years) virologic suppression, which allowed for

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the inflammatory effects of plasma viremia to fade. Limitations included the use of DEXA as opposed to CT or MRI for adipose tissue quantification, and lack of adjustment for diet and exercise. The absence of an HIV-negative control group precluded the assessment of potential differences in relationships between biomarkers and body composition by HIV status. Lastly, since all participants were on efavirenz, tenofovir, and emtricitabine, our results may not be generalizable to persons on a protease inhibitors or integrase strand transfer inhibitors.

As two-thirds the US HIV population is overweight or obese, there is an acute need to understand the health outcomes of this population and, ultimately, optimize care for PLWH. Clinical studies should consider the potential effects of body composition on immunologic parameters.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| **IL** | interleukin |
| **MIP-1α/β** | macrophage inflammatory protein-1α/β |
| **MCP-1** | monocyte chemoattractant protein-1 |
| **TemRA** | T effector memory RA+ cells |
| **TNF-α** | tumor necrosis factor-α |

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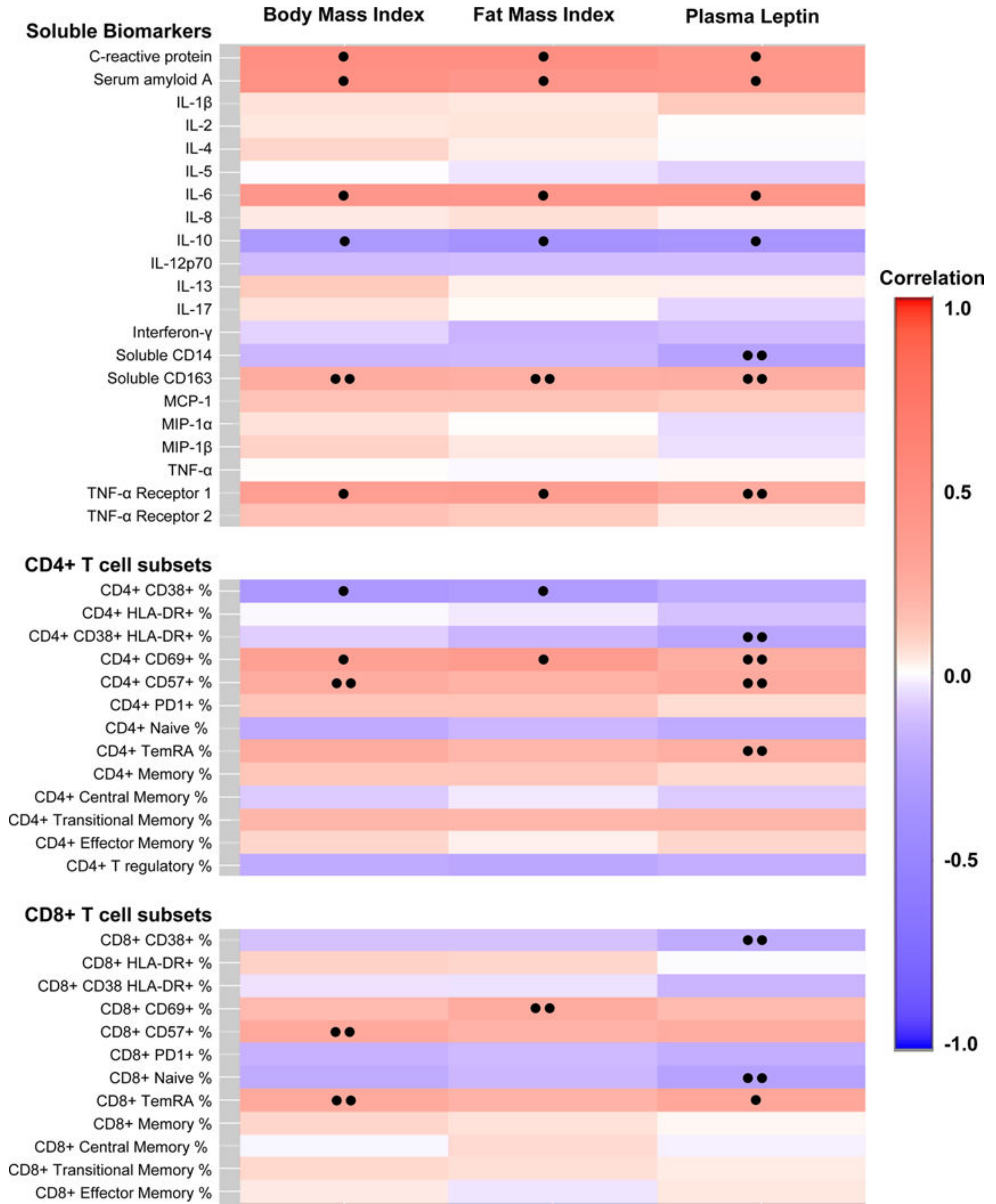


**Figure. Heat map showing adjusted correlation of body compositon measurements with immune parameters**

Relationships between adiposity measurements (BMI, FMI, and plasma leptin) and the immunologic parameters assessed using covariate-adjusted Spearman's rank correlations. Covariates included age, sex, race, CD4 count, duration of ART, and smoking status.

• $p<0.05$, •• $p<0.10$

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## Table

Adjusted correlation of selected soluble innate immune markers and T cell subsets with measures of total body adiposity

| | Body mass index | | Fat mass index | | Plasma leptin | |
|---|---|---|---|---|---|---|
| **Soluble Biomarkers** | **Correlation (95% CI)** | **P-value** | **Correlation (95% CI)** | **P-value** | **Correlation (95% CI)** | **P-value** |
| C-reactive protein | ***0.48 (0.23, 0.67)*** | ***< 0.001*** | ***0.48 (0.22, 0.67)*** | ***< 0.001*** | ***0.40 (0.14, 0.6)*** | ***0.003*** |
| Serum amyloid A | ***0.45 (0.22, 0.64)*** | ***< 0.001*** | ***0.41 (0.17, 0.6)*** | ***0.001*** | ***0.40 (0.14, 0.6)*** | ***0.003*** |
| IL-6 | ***0.41 (0.15, 0.61)*** | ***0.002*** | ***0.39 (0.14, 0.6)*** | ***0.003*** | ***0.4 (0.18, 0.58)*** | ***< 0.001*** |
| IL-10 | ***−0.30 (−0.52, −0.05)*** | ***0.02*** | ***−0.36 (−0.57, −0.11)*** | ***0.006*** | ***−0.33 (−0.55, −0.07)*** | ***0.01*** |
| Soluble CD14 | −0.14 (−0.38, 0.11) | 0.27 | −0.14 (−0.38, 0.11) | 0.28 | **−0.24 (−0.47, 0.02)** | **0.07** |
| Soluble CD163 | **0.23 (−0.03, 0.47)** | **0.08** | **0.22 (−0.03, 0.45)** | **0.09** | **0.22 (−0.03, 0.45)** | **0.09** |
| Soluble TNF-α receptor 1 | ***0.33 (0.07, 0.55)*** | ***0.01*** | ***0.34 (0.07, 0.57)*** | ***0.02*** | **0.25 (−0.02, 0.48)** | **0.07** |
| **CD4+ T cell subsets** | | | | | | |
| CD4+ CD38+ % | ***−0.32 (−0.55, −0.05)*** | ***0.02*** | ***−0.29 (−0.52, −0.01)*** | ***0.04*** | −0.2 (−0.44, 0.07) | 0.15 |
| CD4+ CD38+ HLA-DR+ % | −0.08 (−0.35, 0.2) | 0.57 | −0.15 (−0.4, 0.11) | 0.26 | **−0.23 (−0.46, 0.03)** | **0.08** |
| CD4+ CD69+ % | ***0.32 (0.08, 0.53)*** | ***0.01*** | ***0.35 (0.12, 0.55)*** | ***0.004*** | **0.23 (−0.02, 0.44)** | **0.07** |
| CD4+ CD57+ % | **0.24 (−0.03, 0.47)** | **0.09** | 0.21 (−0.08, 0.46) | 0.15 | **0.25 (−0.03, 0.49)** | **0.07** |
| CD4+ TemRA % | 0.23 (−0.05, 0.48) | 0.10 | 0.18 (−0.09, 0.42) | 0.18 | **0.22 (−0.03, 0.45)** | **0.09** |
| **CD8+ T cell subsets** | | | | | | |
| CD8+ CD38+ % | −0.11 (−0.36, 0.15) | 0.41 | −0.11 (−0.35, 0.14) | 0.39 | **−0.19 (−0.4, 0.04)** | **0.10** |
| CD8+ CD69+ % | 0.16 (−0.1, 0.41) | 0.23 | **0.23 (−0.04, 0.48)** | **0.09** | 0.16 (−0.1, 0.4) | 0.23 |
| CD8+ CD57+ % | **0.26 (−0.02, 0.5)** | **0.07** | 0.19 (−0.11, 0.47) | 0.21 | 0.23 (−0.07, 0.49) | 0.13 |

| | Body mass index | | Fat mass index | | Plasma leptin | |
|---|---|---|---|---|---|---|
| **Soluble Biomarkers** | **Correlation (95% CI)** | **P-value** | **Correlation (95% CI)** | **P-value** | **Correlation (95% CI)** | **P-value** |
| CD8+ Naive % | −0.19 (−0.43, 0.07) | 0.15 | −0.15 (−0.39, 0.11) | 0.27 | **−0.24 (−0.46, 0.01)** | **0.06** |
| CD8+ TemRA % | **0.25 (−0.01, 0.49)** | **0.06** | 0.2 (−0.07, 0.44) | 0.14 | ***0.27 (0.01, 0.49)*** | ***0.04*** |

Table shows only biomarkers or T cell subsets with at least one correlation with p<0.10.

**Bold** indicates p-values <0.10, ***bold italics*** indicates p-values <0.05.

Abbreviations: IL, interleukin; TemRA, T effector memory RA+ cells; TNF-α, tumor necrosis factor-α.



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