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# Association of CD4+ T cell subpopulations and psychological stress measures in women living with HIV

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## Abstract

Psychological stress is a known immunomodulator. In individuals with HIV, depression, the most common manifestation of increased psychological stress, can affect immune function with lower CD4+ T cell counts correlating with higher levels of depression. It is unknown how other forms of psychological stress can impact immune markers in people living with HIV. We conducted a cross-sectional study to determine how CD4+ T cell subpopulations correlated with different forms of psychological stress. We recruited 50 HIV-positive women as part of the Women's Interagency HIV Study. We assessed perceived stress, worry, acute anxiety, trait anxiety, and depression through self-report questionnaires and CD4+ T cell subpopulations using flow cytometry. Our sample was 96% African American with a mean ±SD age and body mass index (BMI) of 42 ±8.8 years and 36.6 ±11.5 kg/m2, respectively. The mean ±SD scores on the psychological measures were as follows: Perceived Stress Scale (PSS), 16.5 ±6.4; Penn State Worry Questionnaire (PSWQ), 47.7 ±13.8; State-Trait Anxiety Inventory – State (STAIS), 39.1 ±12.3; State-Trait Anxiety Inventory – Trait (STAIT), 40.2 ±11.4; Center for Epidemiological Studies Depression Scale (CES-D), 15.6 ±11.4. The mean +SD values for the immune parameters were as follows: regulatory T cells (Treg), 1.25% ±0.7; T helper 1 (Th1), 14.9% ±6.1; T helper 2 (Th2), 3.8% ±2; Th1/Th2 ratio, 4.6 ±3; and CD4+ T cell count (cells/$mm^3$), 493 ±251. Treg levels positively correlated with PSS, STAIS, and STAIT. CD4+ T cell count negatively correlated with PSS, PSWQ, STAIS, STAIT, and CES-D. These data suggest that immune function may be impacted by various forms of psychological stress in HIV-positive women. Interventions that target stress reduction may be useful in improving immune parameters and quality of life.

### Keywords

stress; worry; anxiety; depression; T cells

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## Introduction

Psychological stress is known to modulate immunity (Pedersen, Zachariae, & Bovbjerg, 2009; Uchakin et al., 2011; Marshall et al., 1998). The mechanisms underlying this increased risk likely involve the action of stress hormones on components of the immune system (Hall et al., 2012). We and others have shown that stress hormones decrease pro-inflammatory (anti-viral) immunity while increasing anti-inflammatory immunity (Salicru, Sams, & Marshall, 2007). Clinically, this manifests as an increase in risk for infectious diseases and an increase in the activity of inflammatory-based diseases (Hodgson et al., 2012; Ashcraft & Bonneau, 2008; Trueba, Ryan, Vogel, & Ritz, 2016).

Stress is a particular concern in people living with HIV (PLWH) with depression being the most common psychiatric condition reported among this population (Lowther, Selman, Harding, & Higginson, 2014). Depression in PLWH is associated with medication non-adherence and risky sexual behaviors (Blashill et al., 2013; Kacanek et al., 2015). Depression may also affect immune function, with higher levels of depression associated with lower CD4+ T cell counts (Kaharuza et al., 2006). There is very little data on how other forms of psychological stress (stress perception, worry, and anxiety) may affect HIV progression biomarkers. In addition, it is not known how stress can affect $CD4^+$ T cell subpopulations in PLWH.

In this study we investigated the association of CD4+ T cell subpopulations with psychological stress in a group of women that were part of the Women's Interagency HIV Study (WIHS) Mississippi site cohort.

## Materials and Methods

### Participants

We recruited 50 HIV-positive women that were part of the WIHS Study (Bacon et al., 2005). Participants were recruited after obtaining written informed consent according to a University of Mississippi Medical Center Institutional Review Board approved protocol.

### Blood Collection, PBMC Isolation, and Cryopreservation

Venous blood was collected into heparinized tubes for peripheral blood mononuclear cell (PBMC) isolation. PBMC were isolated using a Ficoll-Hypaque gradient as previously described (Rehm, Elci, Hahn, & Marshall, 2013). Cell counts were obtained using a Scepter Handheld Automated Cell Counter (Millipore). After counting, PBMC were washed and resuspended in cryopreservation media of RPMI containing 20% FBS and 10% DMSO at a concentration of $6–10 \times 10^6$ cells/ml. One milliliter aliquots were placed into cryopreservation vials, transferred to a Nalgene Cryo Freezing container, and placed at −80° C. After 24–48 hours, vials were transferred to a storage box and stored at −80°C until they were batch analyzed by flow cytometry.

### Flow Cytometry

Cryopreserved cells were quick-thawed, washed in 10% cRPMI cell culture media, and then resuspended in 1 ml Hank's Balanced Saline Solution (HBSS) before being

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counted using the Sceptor Handheld Automated Cell Counter (Millipore). Cells were also stained with trypan blue to determine viability. Viability was determined to be ~85% (data not shown). T cell populations were analyzed by flow cytometry according to previously described methods (Rehm, Elci, Hahn, & Marshall, 2013). Data (at least 150,000 events) were collected and analyzed using the Beckman Coulter Cytomics FC500 flow cytometer. Cell populations were defined as follows, Regulatory T cells (Treg): $CD4^{+}CD25^{hi}FoxP3^{+}$ (these cells control the responses on Th1 and Th2 cells); T helper 1 cells (Th1): $CD3^{+}CD8^{-}IFN\gamma^{+}IL4^{-}$ (anti-inflammatory T cells); T helper 2 cells (Th2): $CD3^{+}CD8^{-}IFN\gamma^{-}IL4^{+}$ (pro-inflammatory T cells). CD4+ T cells coordinate the adaptive immune response. CD4+ T cell count (cells/$mm^3$) and was measured in the University of Mississippi Medical Center's Clinical Flow Cytometry Laboratory using the Beckman Coulter (Miami, FL) Cyto-Stat tetraCHROME kit which includes the following antibodies: CD45-FITC, CD4-PE, CD8-ECD, and CD3-PC5. Cells were stained using the manufacturer's instructions. Appropriate isotype controls were used to define positive and negative populations. Data were collected using a Beckman Coulter Navios Flow Cytometer and analyzed using CellQuest software.

### Measurement of Psychological Stress

To measure levels of perceived stress, worry, anxiety, and depression, participants completed a battery of questionnaires. The Perceived Stress Scale (PSS; range 0–40) was used to measure perception of acute stress over the last month (Cohen, Kamarck, & Mermelstein, 1983). The Penn State Worry Questionnaire (PSWQ; range 16–80) was used to measure tendencies to experience excessive and uncontrollable worry (Meyer, Miller, Metzger, & Borkovec, 1990). The State Trait Anxiety Inventory (STAI) was used to assess the severity of acute (STAIS; range 0–80) (i.e., at this moment) and trait (STAIT; range 0–80) anxiety (Julian, 2011). Finally, the Center for Epidemiological Studies Depression Scale (CES-D; range 0–60) (Smarr & Keefer, 2011) was used to measure depression. All measures have extensive support for their validity and reliability (Roemer, 2001).

### Statistical Analyses

Descriptive statistics were computed as mean (standard deviation) and frequencies (percentages) for continuous and categorical variables, respectively (Table 1). The relationship between outcomes of biomarkers and predictors of stress measurements (PSS, PSWQ, STAIS, STAIT, and CES-D) was evaluated using simple linear regression (Table 2). Slopes (β) and p values are reported (Table 2). Normality was tested by visual inspection of q-q plots. Regression analyses were performed after log transformations were applied to skewed data (Treg, Th1, Th2, and Th1/Th2 ratio). A p-value of $<0.05$ was deemed statistically significant. Statistical analysis was performed using SPSS v23 (Armonk, NY).

## Results

### Participant Characteristics

The characteristics of the participants are shown in Table 1. Our sample was 100% female and 96% African American with a mean (SD) age of 42 (8.8) years (range 26–58) and BMI of 36.6 (11.5) $kg/m^2$ (range 19.5–64.6). The mean (SD) min-max scores on the

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psychological measures were as follows: PSS 16.5 (6.4) 5–33; PSWQ 47.7 (13.8) 16–77; STAIS 39.1 (12.3) 20–65; STAIT 40.2 (12.7) 21–65; and CES-D 15.6 (11.4) 0–42. The mean (SD) min-max of the immune and HIV biomarkers measured were as follows: Treg ($CD4^+CD25^{hi}FoxP3^+$), 1.3% (0.7) 0.43–3.6%; Th1 ($CD3^+CD8^-IFN\gamma^+IL4^-$), 14.9% (6.1) 7–30.1%; Th2 ($CD3^+CD8^-IFN\gamma^-IL4^+$), 3.8% (2) 1.1–10.8%; Th1/Th2 ratio, 4.6 (3) 1.7–16.9; and CD4+ count, 493 cells/$mm^3$ (251) 94–994.

### Correlation between stress measures and immune biomarkers

Levels of many of the peripheral blood immune biomarkers were associated with the stress measures. The percentage of Treg cells was significantly and positively associated with scores on the PSS (β=0.014; p=0.003), STAIS (β=0.006; p=0.010), and STAIT (β=0.005; p=0.030). HIV-relevant clinical biomarkers were also associated with the stress measures. CD4+ T cell count was significantly and negatively associated with scores on all the stress measures: PSS (β=−15.6; p=0.004), PSWQ (β=−7.8; p=0.002), STAIS (β=−8.7; p=0.002), STAIT (β=−8.8; p=0.001), and CES-D (β=−7.8; p=0.011).

## Discussion

This cross-sectional study examined the relationship between CD4+ T cell subpopulations and various forms of psychological stress (stress perception, worry, state anxiety, trait anxiety, and depression) in 50 women living with HIV. Our sample was predominantly African-American with an average BMI in the obese range. Our results show that low CD4+ counts were associated with higher levels of all stress measures. In addition, we found that that Treg levels were positively correlated with stress and anxiety. To our knowledge this is the first paper to show some of these relationships, particularly in women who are typically understudied in the HIV literature.

Psychological stress has been linked to immune dysfunction and negative health outcomes in both infectious and inflammatory-based diseases (Pedersen, Zachariae, & Bovbjerg, 2009; Uchakin et al., 2011; Marshall et al., 1998; Hodgson et al., 2012; Ashcraft & Bonneau, 2008; Trueba, Ryan, Vogel, & Ritz, 2016). Treg cells keep the immune system in check by controlling unwanted responses of Th1 and Th2 cells (Lehner, 2008). Stress-induced dysregulation of these regulatory mechanisms may result in inappropriate activation of effector mechanisms that can increase infectious disease susceptibility and inflammatory-based disease activity (Hodgson et al., 2012; Ashcraft & Bonneau, 2008; Trueba, Ryan, Vogel, & Ritz, 2016). Our results suggest that in PLWH Treg populations can be affected by stress which may lead to ineffective control of immune responses. CD4+ T cells are important in coordinating the overall adaptive immune response and a reduction in these as a result of higher stress levels can impact the overall progression of HIV.

Our study has several limitations. First, our study design was cross-sectional. Longitudinal changes to immune measures associated with the various forms of psychological stress would be of interest. Second, our sample was small, predominantly African American, and included only one site of the WIHS study, which make the generalizability of the findings difficult. Third, we did not recruit a group of HIV-negative women so we do not know if these relationships are specific to HIV or if this population (in terms of gender, age, and

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race) is just prone to stress that would impact immunity in general. Future studies should address these limitations.

In conclusion, these findings show an important relationship between immune measures and psychological stress in women with HIV. These data could have implications in the development of stress management techniques that may improve overall immune profiles. These stress reduction techniques, such as physical activity and mindfulness-based stress reduction, may delay progression to AIDS. This is an area of research that should be investigated further. Physicians should partner with local health organizations to provide stress management strategies to those most at risk.

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**Table 1.**

Participant Characteristics

| | |
|---|---|
| Age, yrs | 42 (8.8) 26–58 |
| Race (% AA) | 96% (48) |
| Gender (% Female) | 100% (50) |
| BMI, kg/m$^2$ | 36.6 (11.5) 19.5–64.6 |
| **Psychological Stress Measures** | |
| Perceived Stress Scale (PSS) | 16.5 (6.4) 5–33 |
| Penn State Worry Questionnaire (PSWQ) | 47.7 (13.8) 16–77 |
| State Trait Anxiety Inventory - State (STAIS) | 39.1 (12.3) 20–65 |
| State Trait Anxiety Inventory - Trait (STAIT) | 40.2 (12.7) 21–65 |
| Center for Epidemiological Studies Depression Scale (CES-D) | 15.6 (11.4) 0–42 |
| **Immune Biomarkers** | |
| Treg: CD4+CD25hiFoxP3+ (%) | 1.25 (0.7) 0.43–3.6 |
| Th1: CD3+CD8−IFNg+IL4− (%) | 14.9 (6.1) 7–30.1 |
| Th2: CD3+CD8−IFNg−IL4+ (%) | 3.8 (2) 1.1–10.8 |
| Th1/Th2 Ratio | 4.6 (3) 1.7–16.9 |
| CD4 Count (cells/mm3) | 493 (251) 94–994 |

Shown are mean (SD) min-max for continuous variables and % (n) for categorical variables

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**Table 2:**

Correlation between stress measures and immune biomarkers

| | PSS | PSWQ | STAIS | STAIT | CES-D |
|---|---|---|---|---|---|
| **Treg, %** [#] | 0.014 [**] | 0.004 | 0.006 [*] | 0.005 [*] | 0.004 |
| **Th1, %** [#] | 0.005 | 0.002 | 0.002 | 0.003 | 0.00002 |
| **Th2, %** [#] | 0.001 | 0.001 | −0.001 | −0.001 | −0.003 |
| **Th1/Th2** [#] | 0.003 | 0.001 | 0.003 | 0.004 | 0.003 |
| **CD4 count, cells/mm** $^3$ | −15.6 [**] | −7.8 [**] | −8.7 [**] | −8.8 [**] | −7.8 [*] |

Shown are regression coefficients (β)

[*] $p<0.05$;

[**] $p<0.01$

[#] log transformed