Association Between HIV Symptom Burden and Inflammatory Cytokines: An Analysis by Sex and Menopause Stage

Rebecca Schnall, PhD, MPH, RN-BC, FAAN, Haomiao Jia, PhD, and Nancy Reame, PhD, MSN, RN, FAAN

Abstract

Introduction: There is a growing body of knowledge characterizing the menopause experience in those with HIV. The primary goal of this study was to assess inflammatory cytokine associations with symptoms and sex-specific differences, and the secondary focus was to assess differences among women by menopause status.

Materials and Methods: One hundred persons living with HIV (PLWH) (25 men and 75 women recruited by menopause stage) completed a blood draw for hormones and cytokines and study questions on demographics, height and weight, reproductive health status, HIV symptoms, PROMIS-29 measures, and most recent viral load; study visits were synchronized to the early follicular phase in women with regular cycles.

Results: In both sexes, the most burdensome HIV symptoms were muscle aches/joint pain, difficulty falling asleep, fatigue, and neuropathy. Three of the five symptoms where burden scores differed by menopause stage were related to pain with highest scores in the premenopause group; the postmenopause group also demonstrated a similar burden for muscle aches/joint pain while scores for men and perimenopause women were lowest. Pain intensity scores on the PROMIS-29 also varied significantly by groups. After controlling for sex, menopause stage and body mass index, significant differences were noted in C-reactive protein (CRP), interleukin (IL)-6, and IL-8 for PLWH who reported muscle aches/joint pain.

Conclusions: Our findings suggest enhanced burden for HIV-related symptoms in women in the early follicular phase, possibly owing to menstruation. This supports the need for more targeted investigations in younger cycling women with HIV at multiple phases across the menstrual cycle. Muscle aches/pain are strongly associated with decreased CRP and IL-8 levels and increased IL-6 levels suggesting the need for further investigation of the biological pathways contributing to pain in PLWH. Finally, there is evidence to support that in PLWH, systemic inflammation is heightened above recommended clinical guidelines even when viral load is undetectable supporting the need for further study of the effects of persistent elevated inflammation on health outcomes.

Keywords: muscle aches, menopause, PROMIS-29, TNF α , IL-8, CRP

Introduction

WITH IMPROVEMENTS IN antiretroviral therapies (ART), persons living with HIV (PLWH) are successfully aging into midlife,¹ with nearly half of the population now older than age 50.² As the HIV population ages, more women are experiencing menopause and its consequential symptoms such as hot flashes, poor sleep, muscle aches and pains, and mood disturbances.^{3,4} Given their overlap with common HIV symptoms⁵ such as depression,^{6,7} anxiety,⁸ sleep distur-

bance,⁹ and neuropathic pain,¹⁰ health care providers may have an especially difficult time determining the origins of symptoms in midlife women with HIV.¹¹

A previous study from our laboratory provides evidence for the view that sex differences in HIV symptoms may be related to menopause. We found that females experienced greater symptom burden for many of the most common HIV symptoms compared to males, even when controlling for important demographic and clinical variables.¹² In that study, scores for fatigue, muscle aches/joint pain, shortness of

breath, thirst, and nausea were significantly higher in women versus men with a trend for worse sleep problems.¹² Interestingly, when data from female respondents were examined, postmenopause women demonstrated significantly higher burden scores for muscle aches/joint pains, fatigue, and difficulty falling asleep, compared with premenopause women.¹² These findings suggest that menopause may be an independent predictor of enhanced burden of HIV symptoms in women.¹²

At the same time, despite adequate viral control, PLWH demonstrate alterations in inflammatory cytokines and coagulation markers, suggesting the persistence of chronic low-grade inflammation.¹⁵ The majority of studies of biomarkers of inflammation and mortality in HIV-infection have been performed in randomized clinical trials of ART initiation and interruption,¹⁴ and the association of cytokine levels and mortality is not well understood. Related to this is the need to better understand the role of sex differences, given their influence on cytokines and symptom expression in conditions of chronic inflammation.^{15–17}

Furthermore, understanding the influence of biomarkers associated with HIV symptoms may be useful in clarifying some of their physiological underpinnings, which is especially salient in the case of the most frequent and bothersome symptoms, namely neuropathy and muscle aches and pains. It has been well accepted that even among virologically suppressed HIV-infected persons, systemic inflammation may persist as evidenced by significantly higher plasma levels of interleukin (IL)-6,^{13,18} tumor necrosis factor (TNF) α , IL-8, and C-reactive protein (CRP),¹⁹ and so a better understanding of how these inflammatory cytokines relate to the symptom experience of PLWH is timely and needed. Despite well-documented sex differences and menopause effects on cytokine activity in healthy men and women, there has been limited study of whether such differences are present in PLWH where chronic inflammation may be present. The primary goal of this study was to assess inflammatory cytokine associations with symptoms and sex-specific differences, and the secondary focus was to assess differences among women by menopause status in a cohort of HIV-infected urban residents.

Materials and Methods

All study activities were approved by the Columbia University Medical Center Institutional Review Board. PLWH were recruited from a community-based organizations and an outpatient clinic in New York City serving HIV patients with the goal of including 25 men matched on age with 75 women stratified into the following 3 menopause stages: (1) premenopause women; (2) perimenopause women; and (3) postmenopause women. Data collection took place from April 2017 through March 2018.

Inclusion criteria

Participants needed to be aged 18 years or older, HIV+, self-identify as Black and/or Latino (a), taking ART, and able to read and provide written informed consent in English or Spanish. For females, a history of regular menstrual cycles at some point after menarche was required. To reduce complexity, only participants who self-identified as cisgender were included.

Exclusion criteria

Exclusion criteria included the following: history of alcohol abuse or report of current intake of more than 3 U of alcohol daily, untreated depression, schizophrenia, bipolar disorder, taking interferon medication in the last 6 months to treat hepatitis C, and if female, any form of hormonal birth control or hormone replacement therapy within the last 3 months, pregnant or breastfeeding within the last 3 months, and history of hysterectomy.

Procedures

Documentation of informed consent was obtained before the start of study procedures. For women who reported a period in the past 3 months, the study visit was synchronized to fall between days 1 and 6 of the menstrual cycle. Following a blood draw to determine concentrations of sex steroids and cytokines, participants completed a survey on demographics, height and weight, menopause stage, HIV symptoms, and most recent viral load. We verified the viral load data for half of our study participants through their medical record.

Study instruments

Menopause stage was determined based on most recent menstrual bleeding and amenorrhea history using the Staging of Reproductive Aging in Women (STRAW) criteria.²⁰

The HIV symptom index. The HIV symptom index, a 20-item, self-completed instrument was used to assess HIV symptoms. It is considered to be comprehensive and comprehensible with demonstrated construct validity and strong associations between physical and mental health summary scores and disease severity.^{21,22} Each symptom is scored on a 5-point Likert scale as: 0=I do not have the symptom, 1=It does not bother me, 2=It bothers me a little, 3=It bothers me, 4=It bothers me a lot.

The PROMIS-29. The PROMIS-29 measures health-related quality of life.²³ and has been validated in a large U.S. sample of persons living with the disease.²⁴ Raw scores are transformed using the *T* score metric based on the item response theory calibrations, in which scores have a mean of 50 and standard deviation (SD) of 10 for the general population in the United States. A higher PROMIS *T*-score represents more of the concept being measured. For negatively worded concepts (*e.g.*, sleep disturbance), a *T*-score of 60 is one SD below average.^{25,26} The single pain intensity item is reported on its raw score (*e.g.*, 0–10).

Blood collection

Blood was collected in vacutainer red top serum tubes. Serum was separated and stored at -80°C and thawed on ice immediately before analysis. We selected a common set of inflammatory markers reported to be elevated in PLWH: CRP, TNF α , IL-1 α , IL-1 β , IL-4, IL-6, and IL-8. All cytokines were analyzed by a bead-based ELISA method by Milliplex xMAP technology (Millipore, Billerica, MA) using a Luminex 200 analyzer (Luminex Corporation, Austin, TX). High-sensitivity (hs) CRP was analyzed using turbidimetric (Cobas Integra 400 Plus; Roche Diagnostics, Indianapolis, IN). All visits of each subject were analyzed simultaneously

on the same plate to eliminate interassay variability and were run in duplicate. Using a standard kit, values for IL-1 α , IL-1 β , IL-4, and IL-6 were below the detectable levels (<3.2 pg/mL) in all but 12, 10, 20, and 21 samples, respectively. As a result, samples were rerun using a hs kit for IL-4 and IL-6, but this was not available for IL-1 α and IL-1 β . The lower detectable levels for IL-4, IL-6, and CRP were 3.66, 0.37, and 0.3 pg/mL, respectively. However, even with the hs kits, IL-4 was only detectable in 60 of the 100 study samples. Thus, we elected to only include data in the final analysis for IL-6, IL-8, CRP, and TNF α , which demonstrated measurably detectable values for 91%, 89%, 95%, and 100% of the study samples, respectively. Liquid chromatography/tandem mass spectrometry was used to measure estradiol and testosterone levels using the Xevo TQS made by Waters (Millford, MA).

Self-reported menstrual bleeding patterns in association with a measure of plasma follicle stimulating hormone (FSH) and estradiol drawn between cycle day 1 and 7 were used to assign participants to one of the following three menopause stages: (1) premenopause (regular and predictable periods, FSH=<10 mIU/mL; estradiol=>20 pg/mL); (2) perimenopause (irregular unpredictable periods within the last 3 months to no period in the last 3–11 months); FSH >10 mIU/mL; estradiol=>20 pg/mL; and (3) postmenopause (ranging from early=no period in the last 12–48 months to late=no period in the last 4 years); FSH >25 mIU/mL; estradiol <20 pg/mL.²⁷

Statistical analysis

Descriptive statistics were used to calculate the demographic characteristics. Symptom scores were determined for each of the 20 symptoms ranging from 0 to 4, with higher

values indicating a more severe (worse) symptom. We calculated mean values as well as corresponding standard errors for symptom scores, PROMIS-29, and biomarkers (log-transformed scores, base=10) according to sex and menopause stage. We calculated mean values for each of symptom scores by sex and menopause subgroups and assessed mean score difference between male and female subgroup and across the four subgroups (male, premenopause, perimenopause, and postmenopause). Multiple linear regression models were used to examine the correlation between biomarkers and symptom scores and biomarkers and PROMIS scores, while controlling for sex, menopause stage, and body mass index (BMI). In each model, the dependent variable was the symptom score for each of the 20 HIV symptoms, and main independent variables were log-transformed (base = 10) biomarkers.

Results

The final sample of 100 participants included 25 men and 75 women categorized by menopause stage (10 premenopause, 20 perimenopause, and 45 postmenopause). As a group, the majority of participants were black non-Hispanic ($n=74$; 74%), middle-aged (mean $\pm$ SD = 51.5 $\pm$ 7.7 years), with at least some high school education (60%), and reporting less than \$20,000 in annual income (67%). Most (57%) were nonsmokers. Viral load was suppressed in most participants (86%) and sexual orientation differed by sex, with a higher proportion of women identifying as heterosexual. Mean BMI was higher in females (Table 1).

In both sexes, the most burdensome HIV symptoms were muscle aches/joint pain, difficulty falling asleep, fatigue, and hand/foot pain (neuropathy) (Table 2). Although women as a

TABLE 1. DEMOGRAPHIC, CLINICAL, AND REPRODUCTIVE HORMONE CHARACTERISTICS BY SEX AND MENOPAUSE STATUS SUBGROUPS (PRE, PERI, AND POST)

	Male (n=25)	Female (n=75)	Pre (n=10)	Peri (n=20)	Post (n=45)	p ^a
Age, years [Mean (SD)]	51.7 (5.9)	51.4 (8.2)	36.2 (8.2)	49.2 (4.2)	55.8 (4.1)	<0.001
BMI, kg/mm ² [Mean (SD)]	26.4 (4.1)	30.5 (6.6)	30.8 (5.5)	31.8 (9.5)	29.9 (5.3)	0.03
FSH, mIU/mL [Mean (SD)]	7.3 (5.8)	59.0 (41.0)	8.1 (2.05)	44.2 (28.2)	76.9 (38.3)	<0.001
Estradiol, pg/mL [Mean (SD)]	24.5 (8.9)	15.8 (22.7)	47.4 (32.3)	21.1 (25.0)	6.2 (6.34)	<0.001
Testosterone, pg/mL [Mean (SD)]	6043.7 (2015.5)	248.7 (294.5)	497.8 (703.8)	215.5 (90.5)	208.1 (157.4)	<0.001
DHEA-S, μ g/dL [Mean (SD)]	107.0 (78.1)	65.4 (53.4)	112.2 (78.5)	70.1 (49.2)	52.9 (42.6)	0.001
	n (%)	n (%)	n (%)	n (%)	n (%)	
Race/ethnicity						
Black/non-Hispanic	20 (80)	54 (72)	6 (60)	14 (70)	34 (75.6)	0.6
Black or white/Hispanic	5 (20)	20 (26.7)	4 (40)	5 (25)	11 (24.4)	
Viral load						
<200	21 (84)	65 (87)	8 (80)	16 (80)	41 (91)	0.7
200 or higher	4 (16)	8 (10.67)	2 (20)	3 (15)	3 (6.7)	
Smoking						
Current/occasional/former smoker	15 (60)	28 (37.3)	5 (50)	7 (35)	16 (35.6)	0.2
Nonsmoker	10 (40)	47 (62.7)	5 (50)	13 (65)	29 (64.4)	
Sexual orientation						
Heterosexual	12 (48)	64 (85.3)	10 (100)	13 (65)	41 (91.1)	0.006
Bisexual/homosexual	12 (48)	10 (13.3)	0 (0)	7 (45)	3 (6.7)	

^ap-Values are for testing difference across the four sex and menopause subgroups with ANOVA *F* tests for continuous measures and chi-squared tests for categorical measures.

BMI, body mass index; FSH, follicle stimulating hormone; SD, standard deviation.

TABLE 2. A COMPARISON OF SYMPTOM BURDEN SCORES [MEAN (SD)] BY SEX AND MENOPAUSE SUBGROUPS (PRE, PERI, AND POST)

	Men (n = 25)	Women (n = 75)	Pre (n = 10)	Peri (n = 20)	Post (n = 45)	p ^a
Fatigue or loss of energy	1.5 (1.4)	1.9 (1.3)	2.6 (1.4)	1.6 (1.2)	1.9 (1.2)	0.1
Fevers, chills, or sweats	0.5 (0.9)	0.8 (1.4)	1.3 (1.6)	0.4 (1.0)	0.9 (1.4)	0.1
Feeling dizzy or lightheaded	1.1 (1.3)	1.0 (1.4)	1.7 (1.6)	0.6 (1.1)	1.0 (1.5)	0.1
Pain, numbness, or tingling in the hands or feet (neuropathy)	1.6 (1.5)	1.8 (1.5)	2.2 (1.7)	1.4 (1.4)	1.9 (1.5)	0.3
Trouble remembering	1.1 (1.4)	1.6 (1.2)	1.8 (1.2)	1.5 (1.1)	1.6 (1.3)	0.8
Nausea or vomiting	0.9 (1.2)	0.6 (1.1)	1.3 (1.4)	0.5 (0.8)	0.6 (1.1)	0.1
Diarrhea or loose bowel movements	0.8 (1.0)	0.9 (1.3)	1.9 (1.5)	0.7 (1.1)	0.8 (1.2)	0.02
Felt sad, down, or depressed	1.2 (1.3)	1.6 (1.4)	2.0 (1.8)	1.5 (1.4)	1.5 (1.3)	0.6
Felt nervous or anxious	1.3 (1.3)	1.5 (1.4)	2.6 (1.6)	1.1 (1.3)	1.4 (1.4)	0.02
Difficulty falling or staying asleep	1.5 (1.4)	2.2 (1.5)	2.8 (1.6)	2.0 (1.5)	2.1 (1.5)	0.3
Skin problems, such as rash, dryness, or itching	1.2 (1.4)	1.2 (1.4)	1.7 (1.7)	0.7 (0.9)	1.3 (1.5)	0.1
Cough or trouble catching your breath	1.0 (1.3)	1.0 (1.2)	1.4 (1.6)	1.0 (1.3)	0.9 (1.1)	0.5
Headache	1.0 (1.4)	1.1 (1.4)	2.4 (1.4)	0.8 (0.9)	1.1 (1.4)	0.004
Loss of appetite or a change in the taste of food	1.1 (1.5)	1.0 (1.3)	1.6 (1.4)	0.7 (0.9)	0.9 (1.4)	0.2
Bloating, pain, or gas in your stomach	1.0 (1.3)	1.3 (1.4)	2.5 (1.2)	1.0 (1.1)	1.2 (1.4)	0.01
Muscle aches or joint pain	1.5 (1.4)	2.2 (1.5)	2.5 (1.5)	1.5 (1.4)	2.4 (1.5)	0.04
Problems with having sex, such as loss of interest or lack of satisfaction	1.2 (1.4)	1.2 (1.5)	1.4 (1.8)	1.2 (1.4)	1.2 (1.5)	0.9
Changes in the way your body looks such as fat deposits or weight gain	1.1 (1.2)	1.7 (1.6)	2.3 (1.8)	1.4 (1.4)	1.6 (1.6)	0.3
Problems with weight loss or wasting	1.1 (1.3)	0.8 (1.3)	1.1 (1.5)	0.8 (1.3)	0.7 (1.2)	0.6
Hair loss or changes in the way your hair looks	0.7 (1.3)	1.2 (1.5)	1.6 (1.5)	1.3 (1.4)	1.0 (1.5)	0.5

^ap-Values are for testing mean score differences across sex and menopause subgroups based on ANOVA *F* tests.

group reported higher scores for symptom bother for 13 of the 20 HIV symptoms assessed, only mean scores for muscle aches and pains and difficulty falling asleep exceeded a score of 2.0 (it bothers me a little). Among men, symptom burden scores relating to weight loss/wasting, nausea, appetite change, and dizziness were higher versus women, but all fell below 2.0 (out of 4); however, after controlling for age and BMI, these sex differences (male vs. all females) were small and not statistically significant. When examined by the four sex and menopause stage subgroups, there were group variations in mean burden scores for muscle aches/joint pain, diarrhea, anxiety, stomach bloating/pain, and headache. For all five symptoms, burden scores in the premenopause group were highest; the postmenopause group also demonstrated a

similar burden for muscle aches and pains. Scores for men and perimenopause women were lowest and similar.

Table 3 illustrates mean PROMIS scores by sex and menopause stage. The group differences for physical activity, social functioning, anxiety, depression, fatigue, sleep, and pain interference were small or nonexistent and not significantly different. However, pain intensity varied greatly by groups: lowest in the men and perimenopause women and highest in the pre- and postmenopause groups.

For the total sample, no sex differences were observed in CRP, TNF α , IL-6, or IL-8 (Table 4). In contrast, when the female group was subdivided by menopause stage and compared to males, group differences were observed for TNF α and IL-8: TNF α was lowest and IL-8 was highest in

TABLE 3. A COMPARISON OF PROMIS *T*-SCORES^a BY SEX AND MENOPAUSE SUBGROUPS (PRE, PERI, AND POST)

	Men (n = 25)	Women ^b (n = 75)	Pre (n = 10)	Peri (n = 20)	Post (n = 45)	p ^c
Physical activity (4-items)	44.0 (9.2)	42.8 (8.1)	42.66 (9.0)	44.7 (8.0)	42.0 (7.9)	0.46
Anxiety (4-items)	55.7 (9.5)	56.2 (9.5)	58.93 (11.3)	56.6 (9.9)	55.4 (9.0)	0.56
Depression (4-items)	52.2 (8.6)	52.4 (8.7)	53.53 (10.0)	52.4 (10.2)	52.2 (7.9)	0.91
Fatigue (4-items)	51.1 (9.8)	51.7 (10.7)	56.52 (9.6)	50.7 (11.1)	51.1 (10.8)	0.32
Sleep (4-items)	52.4 (7.9)	54.1 (10.0)	59.90 (7.7)	52.3 (9.5)	53.6 (10.3)	0.12
Social functioning (4-items)	46.6 (7.4)	49.0 (9.5)	45.23 (5.3)	52.0 (12.2)	48.4 (8.5)	0.15
Pain interference (4-items)	56.7 (10.7)	57.4 (8.4)	57.79 (7.3)	55.7 (9.7)	58.0 (8.0)	0.59
Pain intensity (1-item)	4.5 (3.1)	5.4 (2.3)	5.8 (1.9)	4.3 (2.3)	5.9 (2.3)	0.03

The single pain intensity item is not scored but reported as its raw score (e.g., 0 to 10).

^a*T*-score of 50 represents the mean score of the general population. A higher PROMIS *T*-score represents more of the concept being measured. For positively worded concepts such as physical function, a *T*-score of 60 is one SD better than average. By comparison, a physical function *T*-score of 40 is one SD worse than average.

^bp > 0.05: men versus women on all PROMIS scores.

^cp-Value for testing mean score difference across four sex and menopause subgroups with ANOVA *F* test.

TABLE 4. A COMPARISON OF INFLAMMATORY CYTOKINES BY SEX AND MENOPAUSE SUBGROUPS (PRE, PERI, AND POST)

Cytokine	Men (n=25)	Women (n=75)	p^a (Men vs. Women)	Pre (n=10)	Peri (n=20)	Post (n=45)	p^b
hsCRP, mg/L	5.6 (12.4)	4.5 (7.0)	0.7	2.8 (3.0)	4.4 (3.5)	5.0 (8.5)	0.5
TNF α , pg/mL	2.3 (1.3)	2.1 (1.0)	0.3	1.7 (1.0)	2.4 (0.8)	2.0 (1.1)	0.0045
IL-6, pg/mL	5.1 (4.2)	4.1 (6.2)	0.4	3.8 (2.9)	3.4 (1.7)	4.4 (7.8)	0.2
IL-8, pg/mL	36.4 (59.8)	12.3 (31.7)	0.4	20.1 (26.0)	8.6 (6.0)	12.2 (38.9)	<0.001

$p = >0.05$ for comparison between men ($N=25$) versus women ($N=75$).

^a p -Values reflect log-transformed data.

^bReflects a comparison across males and three menopause subgroups.

hsCRP, high-sensitivity C-reactive protein; IL, interleukin.

the premenopause group, although IL-8 was markedly higher in men versus all female groups. Men also demonstrated higher mean concentrations for CRP and IL-6 compared to the three female subgroups.

As presented in Table 5, after controlling for sex, menopause stage, and BMI, if IL-6 was increased by 10-fold diarrhea, loss of appetite and muscle aches and joint pain were expected to increase by 0.81, 0.72, and 0.78 points, respectively. Participants who reported more muscle aches/joint pain had lower levels of CRP and IL-8, but higher IL-6. IL-8 was more likely to decrease in those who did not report muscle aches or joint pain or neuropathy. Weight changes (lipodystrophy or weight loss/wasting) were associated with lower CRP concentrations. TNF α was lower in participants who reported problems with having sex, as was CRP. After controlling for sex, BMI, and menopause stage, there were no significant relationships between biomarkers and PROMIS scores, although a positive trend was observed between pain intensity and IL-8 ($p=0.06$; data not shown).

Discussion

Although sex hormones are known to influence immune responses between the sexes, research is scarce on how such effects might contribute to the sex differences in symptom burden. In women living with HIV (WLHIV), the magnified systemic immune activation has been postulated to be due to sex-specific responses to the virus and immunomodulatory agents, as well as by social behaviors and ovarian dysfunction, but etiology remains unclear.²⁸ To better characterize this relationship, we explored the influence of menopause stage on HIV symptoms in WLHIV in the United States and compared these and inflammatory cytokines in both sexes. Our findings

should be interpreted with caution given that we were unable to control for a number of important covariates, due to the small sample size, which have been shown to be associated with inflammatory cytokines: chronic diseases, cognitive impairment, alcohol use, smoking, and level of education.^{29–32}

In keeping with our earlier findings,¹⁰ we demonstrated that overall, both males and females reported a set of similar HIV symptoms that were most troublesome (fatigue, muscle aches/joint pain, sleep problems, neuropathy, and anxiety), but with a similarly modest mean level of burden as evidenced by ratings of less than 2.0 (bothers me a little) in most cases. These findings support earlier work by others³³ suggesting that for the majority of middle-aged PLWH well-managed with ART, a small subset of symptoms may persist with each symptom contributing a modest level of burden. To what extent their accumulated burden may significantly interfere with quality of life is not known as we did not ask about a collective symptom burden or impact on quality of life. However, others have reported an inverse relationship between quality of life and the number of symptoms in those HIV-infected individuals with mild to moderate symptom severity.³⁴

As in the previous study, burden scores for most of the 20 HIV symptoms were higher in women versus men, but mean scores for only a few symptoms were different once females were classified into subgroups by menopause stage. Moreover, muscle aches and joint pain conferred higher burden in women, even after controlling for BMI. Muscle aches and joint pains are not only common features of menopause in healthy ethnically diverse populations^{6,35} but also, when studied as menopause symptoms in WLHIV, have demonstrated greater prevalence and severity versus uninfected peers in most investigations.^{4,36}

TABLE 5. RELATIONSHIP BETWEEN INFLAMMATORY BIOMARKERS AND SYMPTOMS (CONTROLLING FOR SEX, MENOPAUSE STATUS, AND BODY MASS INDEX)

Biomarker (predictor)	Symptom (outcome)	β	S.E.	p
hsCRP, mg/L	Muscle aches or joint pain	−1.40	0.39	<0.001
	Lipodystrophy	−1.22	0.43	0.004
	Weight loss/wasting	−0.860	0.363	0.02
	Problems with having sex	−0.91	0.43	0.04
IL-6, pg/mL	Diarrhea	0.81	0.30	0.012
	Loss of appetite	0.72	0.35	0.04
	Muscle aches or joint pain	0.78	0.36	0.04
	Muscle aches or joint pain	−1.082	0.471	0.02
IL-8, pg/mL	Neuropathy	−1.214	0.543	0.03
	Problems with having sex	−2.37	1.15	0.04
TNF α , pg/mL				

However, in contrast to our previous work where the postmenopause group (as compared to the menstruating group) accounted for sex differences after adjusting for confounds, the present study revealed consistently highest symptom burden in the premenopause group when defined by menstrual bleeding histories combined with serum hormone markers and women were assessed during the early follicular phase. Indeed, in the premenopause subgroup, burden scores were rated as 2.5 or more (out of a total of 4) for five symptoms, whereas no symptoms were rated this high in the other groups. For five symptoms, the variation in burden across subgroups (including men) was significant: muscle aches/joint pain, headache, anxiety, stomach pain/bloating/gas, and diarrhea, all showing the highest burden in the premenopause group. The next highest group was postmenopause women, but only muscle aches and pain scores approached the level of burden observed in the premenopause group (2.5 vs. 2.4). In contrast, perimenopause women had the mildest symptom burden across the three menopause stages, not unlike the level of burden reported by men. Whether the younger age or higher estrogen levels, both implicated as protective factors in musculoskeletal pain disorders, have contributed to the lower HIV symptom burden in the peri- versus postmenopause group is not clear, but other symptom investigations of menopause stage in HIV women would support this.^{4,36}

To what extent the higher burden scores for HIV symptoms reported by the premenopause group during the early follicular week were exacerbated by concurrent menstrual cramps or other menstrual symptoms is not known as we did not simultaneously ask about these specifically in an effort to avoid symptom conflation. Although participants were asked to assess symptoms in the last 4 weeks, and not just over the week of study, this possibility cannot be ruled out, given the similarities between features of menstrual distress (abdominal pain, gastric distress, mood changes)³⁷ and common HIV symptoms.^{10,38–40} It is noteworthy that three of the five HIV symptoms, where sex/menopause stage differences were present, involved pain (headache, muscle aches/joint pain, and gastric pain) and neuropathic pain burden was highest in the premenopause women, although not different versus other groups. At the same time, the only group difference observed in the PROMIS measures was for pain intensity, with the highest scores exhibited by premenopause women. Taken together, these lines of evidence point to the view of a menstruation-specific enhancement of burden for HIV-related pain symptoms in the follicular phase, and support the need for more targeted investigations in younger cycling women with HIV at multiple phases across the menstrual cycle.^{37,41}

We also found group differences in two of the inflammatory markers that were sex and menopause stage-dependent. Although studies are rare even in healthy women, menopause has been linked to alterations in immune function owing to a depletion of estrogen.⁴² In contrast, the significant subgroup differences we observed in mean TNF α and IL-8 were in no particular direction and did not correspond directly to the notable declines in follicular phase levels of estrogen, the marker of ovarian aging or Dehydroepiandrosterone sulfate (DHEAS), an aging biomarker, across the three female groups, suggesting other moderators of inflammation may play a more central role in women with HIV. The variability

in subgroup mean levels for IL-8 is especially notable, given the marked elevation in the premenopause women versus their counterparts in the later stages of menopause. Whether this difference can be attributed to the co-occurrence of menses—now presumed to be a state of cyclic, low-grade inflammation⁴³—requires further study of both healthy and HIV-infected women.

Although seldom studied in the context of HIV, it is noteworthy that CRP, IL-6, and IL-8 were differentially linked to muscle aches and joint pain, and neuropathy in the case of IL-8 after controlling for sex, menopause stage, and BMI: CRP and IL-8 were negatively associated with these pain symptoms, while IL-6 was positively linked. Past studies suggest that nonspecific markers of inflammation such as CRP and IL-6 are strongly associated with the loss of muscle mass and decline in performance in the general population, partially supporting the relationship between these markers and symptoms of muscle aches and pain noted by our study participants.⁴⁴ Evidence for a relationship between inflammatory biomarkers and pain symptoms across a variety of chronic pain conditions, although growing, is still relatively unsettled. Evidence is conflicting with respect to the magnitude and direction of change in both proinflammatory and anti-inflammatory cytokines as well as in response to interventions, due, in part, to a lack of consistency in design, outcome measures, assay methods, and the failure to simultaneously assess anti-inflammatory cytokines, such as IL-4, IL-10, and receptors, for TNF α which were not measured here.⁴⁵ How inflammation associated with HIV infection further alters these relationships will require additional studies incorporating a broader array of inflammatory markers.

With respect to pain symptoms, we also observed consistently higher symptom burden in females versus males, yet, the reverse tended to be true for the inflammatory markers. In general, women are known to experience more intense pain, and female sex is a predictor of a number of chronic pain conditions^{46,47} across a number of conditions, and this may be the case here. In one of the rare studies of pain intensity, inflammatory cytokines and sex differences in the extant literature, the higher pain report observed in women versus men after knee surgery was associated with reduced inflammation as evidenced by several inflammatory biomarkers, including IL-8,⁴⁸ thus supporting our findings in a much different population than studied here. Such intriguing findings in two disparate study populations suggest the need for greater attention to the differential effects of sex and gender when designing pain studies.

IL-6 has been well studied in PLWH, and HIV infection has long been shown to induce expression and secretion of IL-6.²⁹ It has been proposed as a keystone cytokine given its context-dependent pro- and anti-inflammatory properties as well as hormone-like characteristics that affect homeostasis.⁴⁹ In prior studies of PLWH, the IL-6 median value was 2.09–2.63 pg/mL.¹⁸ In our study, IL-6 mean values were higher ranging from 3.40 (peri-menopause) to 5.09 (men) pg/mL, but there was no significant sex/menopause stage difference, possibly owing to the small sample size. Our finding of higher IL-6 associated with diarrhea supports evidence from a past study, where IL-6 levels was higher in those HIV-infected patients with diarrhea than in HIV-infected patients without diarrhea and HIV-seronegative controls without diarrhea.⁵⁰ Interestingly, in that study,

higher levels of IL-6 were also associated with loss of appetite and muscle aches or joint pain, as was demonstrated here.

Notably, hsCRP levels in our sample were above those seen in studies of healthy controls (1.0–3.0 mg/L)^{51,52} except in the case of premenopause women whose CRP levels were slightly below the upper range of 3.0 mg/L. hsCRP concentrations in this study were also higher than those found in a study of both HIV-infected and uninfected women in Massachusetts³⁴. However, some of this difference might be due to the older age, higher BMI and advanced menopause stage in many of our participants. At any rate, our data agree with the well-documented observation that CRP is elevated in PLWH, supporting the view that even with adequate viral load suppression, a state of chronic low-grade inflammation persists.

Limitations

As our study excluded persons with a history of alcohol use and untreated depression, some of our findings may not be applicable to PLWH.^{29–32} Furthermore, given the small sample we were unable to conduct *post hoc* analyses. Since we did use convenience sampling, there is also the risk of both sample bias and selection bias, and therefore our findings may not be generalizable to all PLWH.

In addition, we did not collect information on the use of anti-inflammatory medications, such as statins or nonsteroidal anti-inflammatory drugs (NSAIDs). Moreover, our sample of premenopause women was disproportionately small ($n=10$) compared to the postmenopause group ($N=45$). Recruitment of menstruating women proved to be very challenging due to the exclusion of a large number of potential candidates using hormonal birth control. Perhaps more importantly, there are a number of other cytokines, chemoattractants, and growth factors shown to help regulate chronic inflammation, especially those with anti-inflammatory activity such as IL-10 and the receptors for TNF α , which could have had important inhibitory effects on some of the proinflammatory cytokines but were not measured.⁵³

Conclusions

Our findings suggest enhanced burden for HIV-related symptoms in women in the early follicular phase, possibly owing to menstruation, which is itself a local state of inflammation. This supports the need for more targeted investigations in younger cycling women with HIV at multiple phases across the menstrual cycle. Muscle aches/pain are strongly associated with decreased CRP and IL-8 levels and increased IL-6 levels suggesting the need for further investigation of the biological pathways contributing to pain in PLWH. Finally, there is evidence to support that in PLWH, systemic inflammation is heightened above recommended clinical guidelines even when viral load is undetectable supporting the need for further study of the effects of persistent elevated inflammation on health outcomes.

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References

1. Lohse N, Hansen A, Pedersen G, et al. Survival of persons with and without HIV infection in Denmark, 1995–2005. *Ann Intern Med* 2007;146:87–95.
2. Centers for Disease Control and Prevention. HIV and Older Americans. 2018. Available at: www.cdc.gov/hiv/group/age/olderamericans/index.html Accessed August 13, 2019.
3. Miller SA, Santoro N, Lo Y, et al. Menopause symptoms in HIV-infected and drug-using women. *Menopause* 2005;12:348–356.
4. Looby SE, Shifren J, Corless I, et al. Increased hot flash severity and related interference in perimenopausal human immunodeficiency virus-infected women. *Menopause* (New York, NY) 2014;21:403–409.
5. Webel AR, Wantland D, Rose CD, et al. A cross-sectional relationship between social capital, self-compassion, and perceived HIV symptoms. *J Pain Symptom Manage* 2015;50:59–68.
6. Wilson NL, Azuero A, Vance DE, et al. Identifying symptom patterns in people living with HIV disease. *J Assoc Nurses AIDS Care* 2016;27:121–132.
7. Eller LS, Rivero-Mendez M, Voss J, et al. Depressive symptoms, self-esteem, HIV symptom management self-efficacy and self-compassion in people living with HIV. *AIDS Care* 2014;26:795–803.
8. Obadeji A, O Ogunlesi A, O Adebawale T. Prevalence and Predictors of depression in people living with HIV/AIDS attending an outpatient clinic in Nigeria. *Iran J Psychiatry Behav Sci* 2014;8:26–31.
9. Allavena C, Guimard T, Billaud E, et al. Prevalence and risk factors of sleep disturbance in a large HIV-infected adult population. *AIDS Behav* 2016;20:339–344.
10. Schnall R, Siegel K, Jia H, Olender S, Hirshfield S. Racial and socioeconomic disparities in the symptom reporting of persons living with HIV. *AIDS Care* 2018;30:774–783.
11. Tariq S, Delpech V, Anderson J. The impact of the menopause transition on the health and wellbeing of women living with HIV: A narrative review. *Maturitas* 2016;88:76–83.
12. Schnall R, Jia H, Olender S, Gradilla M, Reame N. In people living with HIV (PLWH), menopause (natural or surgical) contributes to the greater symptom burden in women: Results from an online US survey. *Menopause* 2018;25:744–752.
13. Armah KA, McGinnis K, Baker J, et al. HIV status, burden of comorbid disease, and biomarkers of inflammation, altered coagulation, and monocyte activation. *Clin Infect Dis* 2012;55:126–136.
14. Baker JV, Neuhaus J, Duprez D, et al. Inflammation predicts changes in high-density lipoprotein particles and

- apolipoprotein A1 following initiation of antiretroviral therapy. *AIDS* (London, England) 2011;25:2133–2142.
15. Han MK, Postma D, Mannino DM, et al. Gender and chronic obstructive pulmonary disease: Why it matters. *Am J Resp Crit Care Med* 2007;176:1179–1184.
 16. Greenspan JD, Craft RM, LeResche L, et al. Studying sex and gender differences in pain and analgesia: A consensus report. *Pain* 2007;132:S26–S45.
 17. Weyand CM, Schmidt D, Wagner U, Goronzy JJ. The influence of sex on the phenotype of rheumatoid arthritis. *Arthritis Rheum* 1998;41:817–822.
 18. Neuhaus J, Jacobs DR, Jr., Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis* 2010;201:1788–1795.
 19. Wada NI, Jacobson LP, Margolick JB, et al. The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. *AIDS* (London, England) 2015;29:463–471.
 20. Harlow SD, Gass M, Hall JE, et al. Executive summary of the Stages of Reproductive Aging Workshop +10: Addressing the unfinished agenda of staging reproductive aging. *Menopause* (New York, NY) 2012;19:387–395.
 21. Justice AC, Holmes W, Gifford AL, et al. Development and validation of a self-completed HIV symptom index. *J Clin Epidemiol* 2001;54:S77–S90.
 22. Edelman EJ, Gordon K, Justice AC. Patient and provider-reported symptoms in the post-cART era. *AIDS Behav* 2011;15:853–861.
 23. Cella D, Riley W, Stone A, et al. Initial Adult Health Item Banks and First Wave Testing of the Patient-Reported Outcomes Measurement Information System (PROMIS™) Network: 2005–2008. *J Clin Epidemiol* 2010;63:1179–1194.
 24. Schnall R, Liu J, Cho H, Hirshfield S, Siegel K, Olender S. A health-related quality-of-life measure for use in patients with HIV: A validation study. *AIDS Patient Care STDs* 2017;31:43–48.
 25. DeWalt DA, Rothrock N, Yount S, Stone AA, Group PC. Evaluation of item candidates: The PROMIS qualitative item review. *Med Care* 2007;45:S12–S21.
 26. Revicki D, Cella D. Health status assessment for the twenty-first century: Item response theory, item banking and computer adaptive testing. *Qual Life Res* 1997;6:595–600.
 27. Randolph JF, Jr., Zheng H, Sowers MR, et al. Change in follicle-stimulating hormone and estradiol across the menopausal transition: Effect of age at the final menstrual period. *J Clin Endocrinol Metab* 2011;96:746–754.
 28. Looby SE, Fitch KV, Srinivasa S, et al. Reduced ovarian reserve relates to monocyte activation and subclinical coronary atherosclerotic plaque in women with HIV. *AIDS* (London, England) 2016;30:383–393.
 29. Nakajima K, Martínez-Maza O, Hirano T, et al. Induction of IL-6 (B cell stimulatory factor-2/IFN-beta 2) production by HIV. *J Immunol* 1989;142:531–536.
 30. Erren M, Reinecke H, Junker R, et al. Systemic inflammatory parameters in patients with atherosclerosis of the coronary and peripheral arteries. *Arterioscler Thromb Vasc Biol* 1999;19:2355–2363.
 31. Reichenberg A, Yirmiya R, Schuld A, et al. Cytokine-associated emotional and cognitive disturbances in humans. *Arch Gen Psychiatry* 2001;58:445–452.
 32. Zeidel A, Beilin B, Yardeni I, Mayburd E, Smirnov G, Bessler H. Immune response in asymptomatic smokers. *Acta Anaesthesiol Scand* 2002;46:959–964.
 33. Keating SM, Golub ET, Nowicki M, et al. The effect of HIV infection and HAART on inflammatory biomarkers in a population-based cohort of women. *AIDS* (London, England) 2011;25:1823–1832.
 34. Dolan SE, Hadigan C, Killilea KM, et al. Increased cardiovascular disease risk indices in HIV-infected women. *J Acquir Immune Defic Syndr* 2005;39:44–54.
 35. Dugan SA, Powell LH, Kravitz HM, Rose SAE, Karavolos K, Luborsky J. Musculoskeletal pain and menopausal status. *Clin J Pain* 2006;22:325–331.
 36. Ferreira CE, Pinto-Neto AM, Conde DM, Costa-Paiva L, Morais SS, Magalhaes J. Menopause symptoms in women infected with HIV: Prevalence and associated factors. *Gynecol Endocrinol* 2007;23:198–205.
 37. Jarrett M, Cain KC, Heitkemper M, Levy RL. Relationship between gastrointestinal and dysmenorrheic symptoms at menses. *Res Nurs Health* 1996;19:45–51.
 38. Barroso J, Leserman J, Harmon JL, Hammill B, Pence BW. Fatigue in HIV-infected people: A three-year observational study. *J Pain Symptom Manage* 2015;50:69–79.
 39. Wantland DJ, Mullan JP, Holzemer WL, et al. Additive effects of numbness and muscle aches on fatigue occurrence in individuals with HIV/AIDS who are taking antiretroviral therapy. *J Pain Symptom Manage* 2011;41:469–477.
 40. Johnson MO, Stallworth T, Neilands TB. The drugs or the disease? Causal attributions of symptoms held by HIV-positive adults on HAART. *AIDS Behav* 2003;7:109–117.
 41. Massad LS, Evans CT, Minkoff H, et al. Effects of HIV infection and its treatment on self-reported menstrual abnormalities in women. *J Women's Health* 2006;15:591–598.
 42. Gameiro CM, Romão F, Castelo-Branco C. Menopause and aging: Changes in the immune system—A review. *Maturitas* 2010;67:316–320.
 43. Evans J, Salamonsen LA. Inflammation, leukocytes and menstruation. *Rev Endocr Metab Disord* 2012;13:277–288.
 44. Cesari M, Penninx BWJH, Pahor M, et al. Inflammatory markers and physical performance in older persons: The InCHIANTI study. *J Gerontol A Biol Sci Med Sci* 2004;59:M242–M248.
 45. DeVon HA, Piano MR, Rosenfeld AG, Hoppensteadt DA. The association of pain with protein inflammatory biomarkers: A review of the literature. *Nurs Res* 2014;63:51–62.
 46. Ruau D, Liu LY, Clark JD, Angst MS, Butte AJ. Sex differences in reported pain across 11,000 patients captured in electronic medical records. *J Pain* 2012;13:228–234.
 47. Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL, 3rd. Sex, gender, and pain: A review of recent clinical and experimental findings. *J Pain* 2009;10:447–485.

48. Solheim N, Östlund S, Gordh T, Rosseland LA. Women report higher pain intensity at a lower level of inflammation after knee surgery compared with men. *Pain Rep* 2017;2: e595.
 49. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol* 2015;16:448.
 50. Snijders F, Bartelsman J, Jansen J, Mevissen M, Danner SA, Reiss PJA. Diarrhoea in HIV-infected patients: No evidence of cytokine-mediated inflammation in jejunal mucosa. *AIDS* 1995;9:367–373.
 51. Ridker P, Libby P, Buring J. Risk markers and the primary prevention of cardiovascular disease. PA: Elsevier Saunders, 2015.
 52. Chernecky C, Berger B. Laboratory tests and diagnostic procedures. St. Louis, MO: Elsevier Saunders, 2013.
 53. Zhang J-M, An J. Cytokines, inflammation and pain. *Int Anesthesiol Clin* 2007;45:27.
- Address correspondence to:
Rebecca Schnall, PhD, MPH, RN-BC, FAAN
Columbia University
School of Nursing
560 West 168th Street
New York, NY 10032

E-mail: rb897@columbia.edu