

Fungal Translocation Is Associated with Immune Activation and Systemic Inflammation in Treated HIV

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Abstract

The mechanisms causing HIV-associated immune activation remain incompletely understood. Alteration of intestinal integrity with subsequent translocation of bacterial products appears to play an important role; however, little is known about the impact of fungal translocation. We assessed the effect of fungal translocation and its association with immune activation in people living with HIV (PLWH) compared with uninfected controls. We measured serum levels of β -D-glucan (BDG) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) immunoglobulin G (IgG) and immunoglobulin A (IgA) and markers of systemic inflammation and immune activation in virally suppressed PLWH on antiretroviral therapy (ART) and uninfected controls. T-test and Mann-Whitney tests were used to compare markers by HIV status and correlation and regression analyses were used to assess associations of fungal translocation markers with markers of inflammation. One hundred seventy-six participants were included (128 HIV+ and 48 HIV-); 72% male, 65% African American, median age was 50 years, and CD4 was 710 cells/cm³. Levels of BDG tended to be lower in HIV+ when compared with controls ($p = .05$). No significant difference in levels of ASCA IgG and IgA was seen between groups ($p > .75$). There was a significant correlation between BDG and several markers of inflammation and immune activation in PLWH, not seen in uninfected controls. In contrast, no correlations were seen between levels of ASCA IgG and IgA with inflammatory markers. PLWH on ART do not have higher levels of BDG or ASCA when compared with uninfected controls, however, the association found between BDG and several inflammation markers suggests a potential role of fungal translocation in the heightened immune activation seen in treated HIV.

Keywords: HIV, fungi, inflammation, gastrointestinal microbiome, biomarkers

Introduction

DESPITE VIROLOGIC CONTROL on antiretroviral therapy (ART), people living with HIV (PLWH) continue to have overall increased mortality and decreased life expectancy when compared with the general population.¹⁻³ Heightened systemic inflammation, as demonstrated by activation of both cellular and innate immunities, remains persistently elevated in PLWH despite viral suppression.^{4,5} This persistent inflammation has been correlated to subclinical cardiovascular disease (CVD) and an increase in the prevalence of age-associated noncommunicable comorbidities among PLWH.⁶⁻⁸ The etiology of this heightened in-

flammation is likely multifactorial and remains incompletely understood, but the role of alteration of intestinal integrity and the resultant translocation of bacterial products through the intestinal lumen to the systemic circulation appears to be a central factor.^{9,10}

Compromise of the integrity of the intestinal mucosa secondary to HIV infection^{11,12} allows for translocation of bacterial products such as lipopolysaccharide (LPS), which stimulates the downstream transcription and activation of proinflammatory mediators.¹³ Although fungi are an integral part of the human microbiota, their contribution to systemic inflammation remains largely unknown. Markers such as β -D-glucan (BDG) and anti-*Saccharomyces cerevisiae*

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antibodies (ASCA) have been demonstrated in the serum of individuals with invasive fungal disease or in those with compromised intestinal integrity, such as occurring with inflammatory bowel disease (IBD).^{14,15} Through interaction with receptors belonging to the C-type lectin superfamily, fungal markers such as BDG are known activators of proinflammatory cascades.^{16,17}

Our objective was to compare the fungal translocation markers BDG and ASCA immunoglobulin G (IgG) and immunoglobulin A (IgA) in treated PLWH versus uninfected controls and study their relationship with systemic inflammation and cellular and innate immune activation.

Materials and Methods

Study design and population

This is a cross-sectional analysis using a cohort of PLWH and uninfected healthy participants of similar age, both cohorts prospectively enrolled at University Hospitals Cleveland Medical Center, Cleveland, Ohio. PLWH were included as part of a prospective cross-sectional assessment and were seen during their routine HIV care over a 2-year period. Healthy controls were recruited as volunteer University Hospital employees and/or family members of PLWH. The study was approved by the local institutional review board and written informed consent was obtained from each participant.

In both groups, participants were at least 18 years of age without a known history of CVD or other pre-existing inflammatory conditions. All PLWH had an HIV-1 RNA viral load less than 1,000 copies/mL in the past 4 months, were on stable ART for at least 3 months, and had cumulative ART use of at least 6 months. Participants in both groups were excluded if they had active bacterial or fungal infection, were pregnant or lactating, or on antifungal agents.

Study evaluation

Medical history and self-reported demographics were obtained at the initial screening visit as well as anthropometric data. Blood samples were obtained following a fasting period of at least 8 h and samples were prepared as previously described,¹⁸ with plasma and serum cryopreserved for measurement of markers of monocyte and cellular activation, systemic inflammation, and coagulation. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated.¹⁹ HIV parameters, including RNA levels and CD4⁺ cell counts, were obtained through routine care.

Biomarkers of inflammation, immune activation, and fungal translocation

Soluble markers of inflammation interleukin-6 (IL-6), soluble vascular cell adhesion molecule-1 (sVCAM-1), markers of immune activation (sCD14, sCD163, CD4⁺CD38⁺DR⁺, CD8⁺CD38⁺DR⁺), soluble tumor necrosis factor- α receptors I and II (sTNFR-I and sTNFR-II, respectively), interferon- γ -inducible protein 10 (IP-10), high-sensitivity C-reactive protein (hs-CRP), and D-dimer were determined using an enzyme-linked immunosorbent assay (ELISA) (R&D Systems; Minneapolis, MN). As a measure of oxidant stress, oxidized low-density lipoprotein (LDL) was determined via ELISA (Mercodia, Uppsala, Sweden) as well as measure-

ments for the concentrations of lipoprotein-associated phospholipase A2 (Lp-PLA2) (PLAC test; diaDexus, South San Francisco, CA). Using cryopreserved serum, the markers of fungal translocation BDG and ASCA were measured by ELISA (BDG: MyBioSource, San Diego, CA; ASCA IgG and IgA: Orgentec Diagnostika GmbH, Mainz, Germany).

Statistical analysis

The main objectives of this study were to compare levels of BDG and ASCA in PLWH with uninfected controls, and to assess the relationship between these gut markers and markers of systemic inflammation and immune activation in PLWH and uninfected controls.

Demographics, clinical characteristics, fasting metabolic parameters, immune activation and inflammatory markers, measures of CVD risk, and serum gut markers were described and compared between the two study groups. The median and interquartile ranges were used to describe continuous variables, while categorical variables were reported as frequency and percentage.

Descriptive statistics, *t*-tests, and chi-square tests were used to compare baseline factors. Within and between groups, changes in BDG and ASCA were tested using Wilcoxon rank-sum test. In both groups, relationships between fungal translocation markers and inflammation and immune activation markers were assessed using Spearman correlation analysis.

In a subgroup analysis, Spearman correlation was used to evaluate relationships between elevated levels of ASCA IgA with inflammation and immune activation markers in PLWH and healthy uninfected controls. Participants with levels greater than the median level of ASCA IgA (3.08 U/mL) were selected. Overall, 93 participants were included for this analysis: 69 PLWH and 24 from the uninfected group.

Results

Baseline characteristics

This analysis included 176 participants: 128 were PLWH and 48 were HIV-uninfected. Demographic information and baseline characteristics are described in Table 1. Overall, the median age was 50 years, 72% were males and 65% were African Americans. Among PLWH, the median CD4⁺ cell count was 710 cells/cm³ and HIV viral load 20 copies/mL. While no participants were receiving any antifungal agents, four PLWH were on antibiotics during this study, including one on trimethoprim/sulfamethoxazole, two on isoniazid, and one on benzathine penicillin injections.

Two participants from each study group were taking probiotics. There were more males in the PLWH group ($p < .001$). Waist-to-hip ratio and triglycerides were higher in PLWH ($p < .05$), however, total cholesterol and LDL were higher in controls ($p < .05$). The control group had higher levels of D-dimer ($p = 0.05$), whereas PLWH had higher levels of IP-10 ($p < .001$), sTNFR-II ($p = .049$), and T cell activation, as measured by CD4⁺ and CD8⁺ T cells expressing CD38 and human leukocyte antigen-DR isotype (HLA-DR) ($p < .001$).

Fungal translocation markers

Serum levels of BDG tended to be lower in PLWH when compared with uninfected controls (Fig. 1; $p = .05$), whereas

TABLE 1. COMPARISON OF BASELINE CHARACTERISTICS BETWEEN PEOPLE LIVING WITH HIV AND HIV-UNINFECTED GROUPS (MEDIAN VALUES [INTERQUARTILE RANGE])

Variables	PLWH, n=128	HIV uninfected, n=48	p
Demographics			
Age (years)	48.98 (43.25–54.91)	49.78 (41.7–54.07)	.562
Male (%)	105 (82)	23 (48)	<.001
African American (%)	89 (70)	26 (54)	.084
Family history of diabetes mellitus (%)	55 (43)	13 (27)	.138
Family history of hypertension (%)	87 (69)	30 (63)	.577
Current smoking (%)	75 (59)	30 (64)	.084
Alcohol (%)	52 (48)	18 (49)	.961
Current marijuana use (%)	31 (28)	6 (16)	.083
Current cocaine use (%)	7 (6)	0 (0)	.025
Cardiovascular disease risk factors			
BMI (kg/m ²)	26.08 (23.29–30.55)	27.03 (24.18–30.0)	.61
Waist-to-hip ratio	0.96 (0.9–1.01)	0.91 (0.85–0.97)	.004
Systolic blood pressure (mmHg)	131.5 (120.0–142.0)	132.0 (120.75–140.5)	.571
Diastolic blood pressure (mmHg)	82.5 (77.0–89.0)	81.0 (76.0–87.25)	.732
Framingham score	4 (2–8)	3 (1–7)	.078
Total cholesterol (mg/dL)	168.0 (143.75–193.0)	186.0 (161.5–213.25)	.004
HDL cholesterol (mg/dL)	48.0 (40.5–59.5)	53.5 (43.0–66.25)	.04
LDL cholesterol (mg/dL)	88.5 (71.0–108.0)	106.0 (82.75–131.25)	.002
Triglycerides (mg/dL)	107.0 (81.75–166)	85.5 (64.75–115)	.004
Pro-BNP (pg/mL)	30 (15.25–64)	44.5 (21.75–82.75)	.064
HOMA-IR	2.46 (1.41–4.28)	2.09 (1.63–3.14)	.365
Systemic markers of inflammation			
IP-10 (pg/mL)	141.67 (84.95–235.46)	58.77 (34.26–87.16)	<.001
Lp-PLA (ng/mL)	163 (133–198.25)	169.5 (143–190.75)	.943
IL-6 (µg/mL)	2.19 (1.35–3.18)	1.66 (1.08–2.8)	.101
hs-CRP (µg/mL)	1727.01 (403.55–4376.33)	1430.1 (599.08–3535.84)	.995
Oxidized LDL (U/L)	43.45 (34.25–54.14)	47.17 (38.87–66.04)	.062
sTNFR-I (pg/mL)	766.47 (536.91–1118.53)	719.03 (575.81–966)	.126
sTNFR-II (pg/mL)	2482.08 (2075.65–3224.77)	2144.11 (1908.8–2818.78)	.049
D-dimer (µg/mL)	292.74 (179.87–524.6)	419.76 (245.74–568.06)	.05
sVCAM-1 (pg/mL)	762.35 (627.44–928.01)	753.68 (594.98–920.18)	.55
Markers of innate and cellular immune activation			
sCD14 (ng/mL)	1359 (843–2010)	1396 (579–1920)	.337
sCD163 (ng/mL)	676.08 (427.7–954.32)	746.83 (504.46–1221.05)	.183
CD4 ⁺ CD38 ⁺ DR ⁺ (%)	4.54 (3.21–6.29)	3.11 (2.3–3.96)	<.001
CD8 ⁺ CD38 ⁺ DR ⁺ (%)	8.02 (4.64–10.83)	3.9 (2.72–5.31)	<.001
HIV variables			
Current CD4 count (cells/µL)	710 (537.75–932.75)		
Viral load (copies/mL)	20 (20–20)		
Nadir CD4 count (cells/µL)	176 (57.75–302.5)		
ART duration (months)	113.31 (64–168.61)		
Fungal markers of translocation			
BDG (pg/mL)	149.22 (119.78–200.76)	166.13 (142.54–205.76)	.05
ASCA IgG (U/mL)	5.97 (2.88–13.28)	6.02 (4.33–11.39)	.747
ASCA IgA (U/mL)	3.3 (1.91–6.48)	3.08 (1.91–5.89)	.757

ART, antiretroviral therapy; ASCA, anti-*Saccharomyces cerevisiae* antibodies; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IgA, immunoglobulin A; IgG, immunoglobulin G; IP-10, interferon- γ -inducible protein 10; LDL, low-density lipoprotein; PLWH, people living with HIV; sTNFR, soluble tumor necrosis factor- α receptors; sVCAM-1; soluble vascular cell adhesion molecule-1.

no difference was found in serum levels of ASCA IgG and IgA between the two groups (Fig. 2; $p=.75$, and Fig. 3; $p=.77$, respectively). No difference in serum levels of BDG or ASCA was observed between those with higher and lower CD4 cell counts categorized based on quartiles ($p\geq.45$). Finally, no difference in serum BDG or ASCA was observed between male and female participants ($p\geq.10$).

Relationship between fungal translocation markers, markers of systemic inflammation, and HIV variables

In univariable analyses, as shown in Figure 4, a weak but consistent correlation was observed between serum levels of BDG in PLWH and several inflammatory and monocyte activation markers, including sCD163, sCD14, IL-6, IP-10, sTNFR-II, D-dimer, and sVCAM-1. No correlation

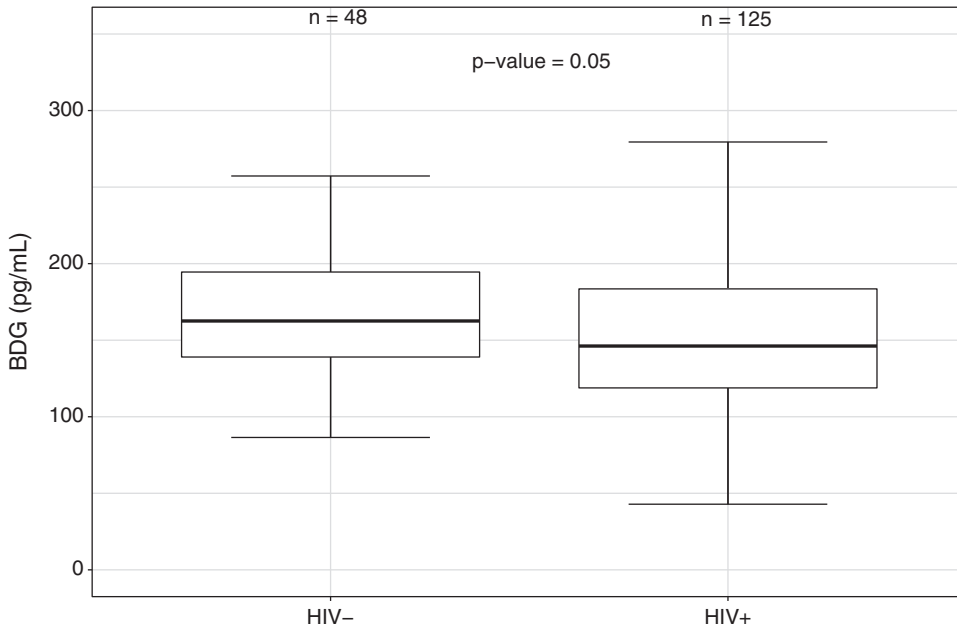


FIG. 1. Serum levels of β -D-glucan (BDG) in PLWH and in uninfected participants. PLWH, people living with human immunodeficiency virus.

was found with Lp-PLA2, oxidized LDL, hs-CRP, sTNFR-I, and activated CD4⁺ and CD8⁺ T cells ($r=0.07-0.15$; $p=.08-.49$). In addition, in these participants, only serum levels of sCD163 correlated with ASCA IgA (Fig. 5).

After adjusting for sex, IL6 and D-dimer no longer significantly correlated with BDG and sCD163 no longer correlated with ASCA IgA

In uninfected participants, BDG was found to significantly correlate only with activated CD4⁺ and CD8⁺ T cells (Fig. 6). IP-10 significantly correlated with ASCA IgA (Fig. 7).

No correlation was observed between all HIV variables studied and fungal translocation markers. No correlation was

seen between markers of fungal translocation and BMI or HOMA-IR ($p \geq .35$).

Relationship between elevated levels of ASCA IgA and markers of systemic inflammation and immune activation

We found that a total of 93 participants had ASCA IgA levels greater than 3.08 U/mL, the median value in uninfected participants, including 69 PLWH and 24 controls. As shown in Table 2, levels of ASCA IgA greater than 3.08 U/mL were found to be significantly associated with sCD14 in PLWH ($p=.012$). In uninfected controls, higher levels of ASCA IgA significantly correlated with sTNFR-I and activated CD8⁺ T cells ($p=.008$ and $p=.032$, respectively).

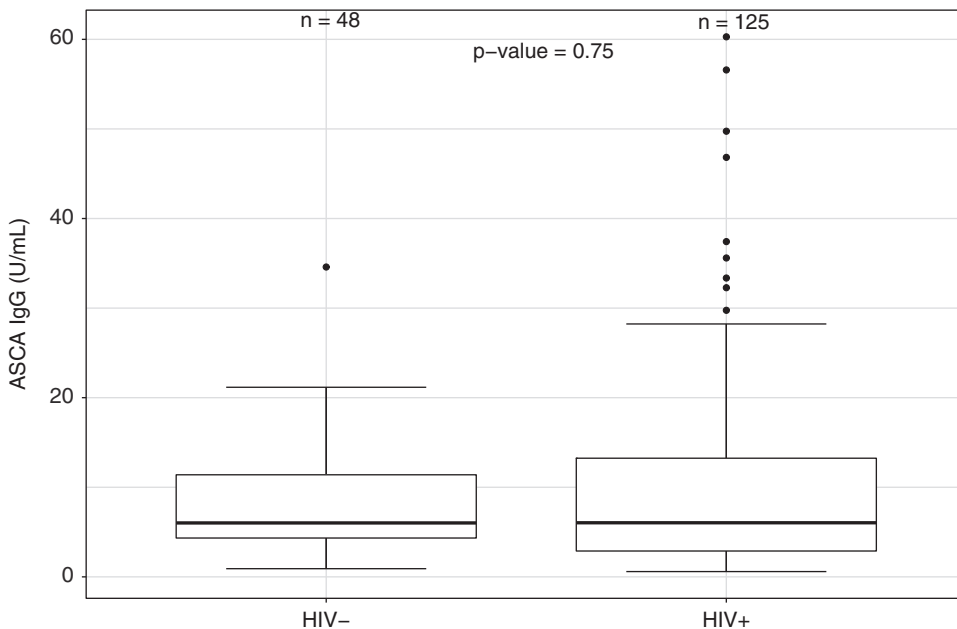


FIG. 2. Serum levels of ASCA IgG in PLWH and in uninfected participants. ASCA, anti-*Saccharomyces cerevisiae* antibodies. IgG, immunoglobulin G.

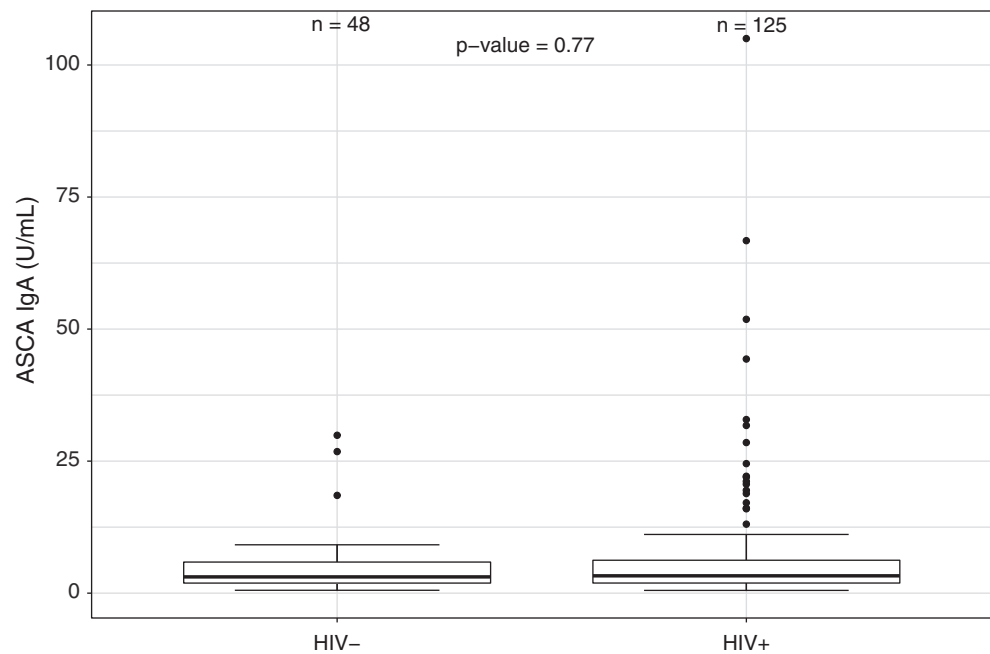


FIG. 3. Serum levels of ASCA IgA in PLWH and in uninfected participants. IgA, immunoglobulin A.

Discussion

To our knowledge, for the first time in PLWH, we investigated fungal translocation markers and their relationship with systemic inflammation and immune activation in comparison with uninfected controls. Our results suggest that overall, fungal translocation markers are not elevated in PLWH who are virally suppressed on ART when compared with levels in uninfected controls, however, this study does provide the first evidence that fungal translocation may be associated with the persistent inflammation and immune activation seen in treated HIV.

Fungal translocation in HIV

Most of the investigations in HIV describing the association between loss of gut integrity and systemic inflammation have focused on the measurement of LPS^{13,20} as a marker of bacterial translocation and a major contributor of the proinflammatory response.^{9,11,21–23} In addition, increased gastrointestinal (GI) permeability has been shown across multiple disease states, such as IBD and graft-versus-host-disease (GVHD),^{13,24} suggesting that increased gut permeability and translocation result in downstream activation of inflammation through receptor-mediated pathways.

Although fungal organisms contribute significantly to the composition of the normal GI flora, little is known regarding the translocation of fungal elements and their potential contribution to the systemic inflammatory response. The microbiome, referring specifically to the fungal component of microbiota, comprises ~0.03%–2% of total gut microorganisms.²⁵ In inflammatory conditions outside of HIV, fungal translocation has been shown to affect human health, with systemic markers of fungal translocation being associated with disease activity and at times progression. For example, serum antibody against *Saccharomyces cerevisiae* (ASCA) has been used as a serologic marker for diagnosing Crohn's disease, with more recent investigations associating the detection of BDG in the serum with disease activity.²⁶

Guo *et al.* demonstrated significantly higher levels of BDG in the serum of individuals with active Crohn's disease when compared with those in remission, and increased levels compared with those in healthy controls.²⁷ In addition, serum levels of BDG in these participants correlated significantly with disease severity and elevated levels of erythrocyte sedimentation rate. Van der Valden *et al.* demonstrated an association between the rate of development of acute GVHD and fungal colonization (demonstrated as the presence of fungal species in fecal and/or mouthwash samples of participants) in poststem cell transplant patients.²⁸ Finally, Kvehaugen *et al.* recently demonstrated a significant association between serum ASCA IgG and body fat mass and CRP in patients referred for morbid obesity, with elevated zonulin concentrations (a marker of intestinal permeability) being more frequently associated with ASCA IgG-positivity.²⁹

Although a paucity of studies have investigated the role played by the microbiome in PLWH, to our knowledge there has been no study comparing markers of fungal translocation in PLWH versus uninfected healthy individuals.

We hypothesized that fungal translocation markers would be elevated in PLWH compared with levels in uninfected controls, which our findings do not support. Our findings could be either because there is truly no difference in these fungal markers between virally suppressed PLWH and uninfected controls, or our controls were not selected appropriately. It is also possible that the permeability of the GI tract in virally suppressed individuals is not sufficient to allow passage of fungal moieties in most individuals. Evidence of bacterial translocation persists despite antiretroviral treatment,^{9,21} but fungal markers such as ASCA and BDG less frequently reach the threshold of detection in non-HIV disease processes, such as Crohn's disease.²⁶ The additional analysis and comparison of serum levels of LPS between our study groups may have been beneficial to demonstrate evidence of persistent gut translocation. The relationship between gut integrity and fungal moieties in HIV is likely complex and larger study sizes are needed to detect clinically significant effects.

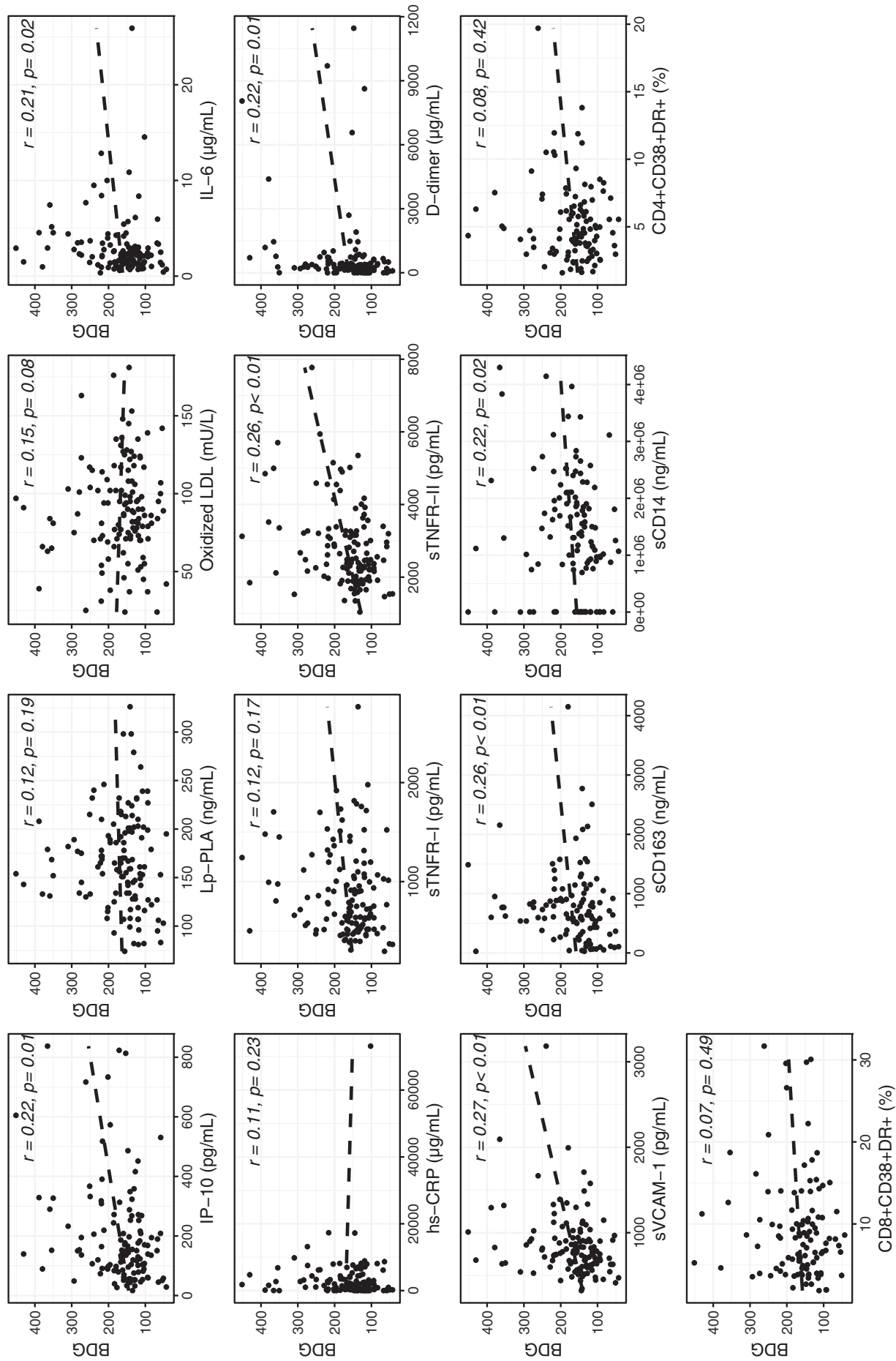


FIG. 4. Univariable analysis between serum levels of β -D-glucan (BDG) and systemic inflammation in PLWH. Correlation between serum levels of BDG and IP-10; IP-10, Lp-PLA, oxidized LDL, IL-6, hs-CRP, sTNFR-I, sTNFR-II, D-dimer, sVCAM-1, sCD163, sCD14, CD4+CD38+DR+, and CD8+CD38+DR+ T cells. IP-10, interferon gamma-induced protein-10; Lp-PLA, lipoprotein-associated phospholipase-A2; IL-6, interleukin-6; hs-CRP, high-sensitivity C-reactive protein; sTNFR-I, soluble tumor necrosis factor receptor I; sTNFR-II, soluble tumor necrosis factor receptor II; sVCAM-1, soluble vascular cell adhesion molecule; sCD163, soluble cluster of differentiation 163; sCD14, soluble cluster of differentiation 14.

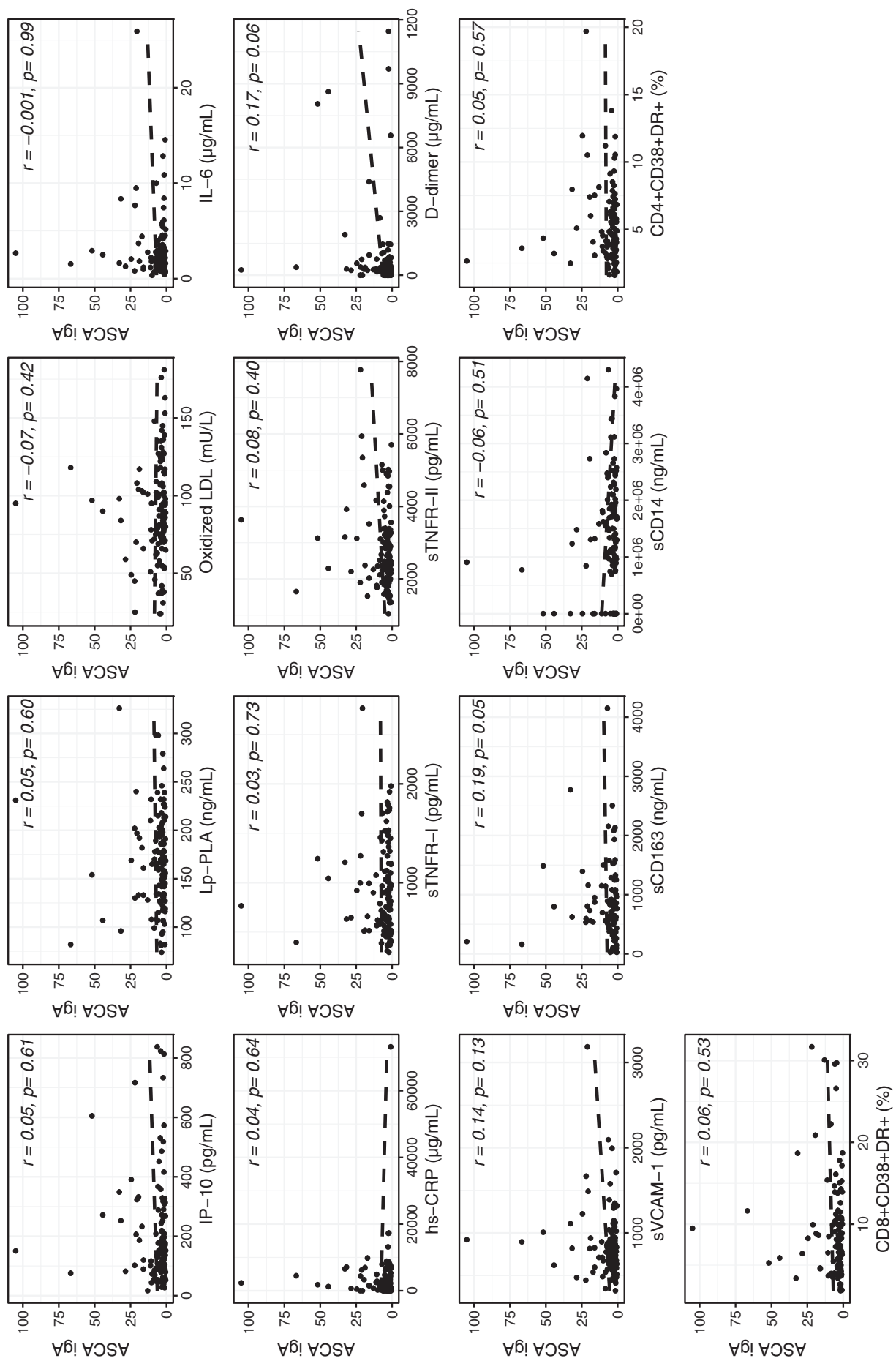


FIG. 5. Univariable analysis between serum levels of ASCA IgA and systemic inflammation in PLWH. Correlation between serum levels of ASCA IgA and IP-10: IP-10, Lp-PLA, oxidized LDL, IL-6, hs-CRP, sTNFR-I, sTNFR-II, D-dimer, sVCAM-1, sCD163, sCD14, CD4+CD38+DR+, and CD8+CD38+DR+ T cells.

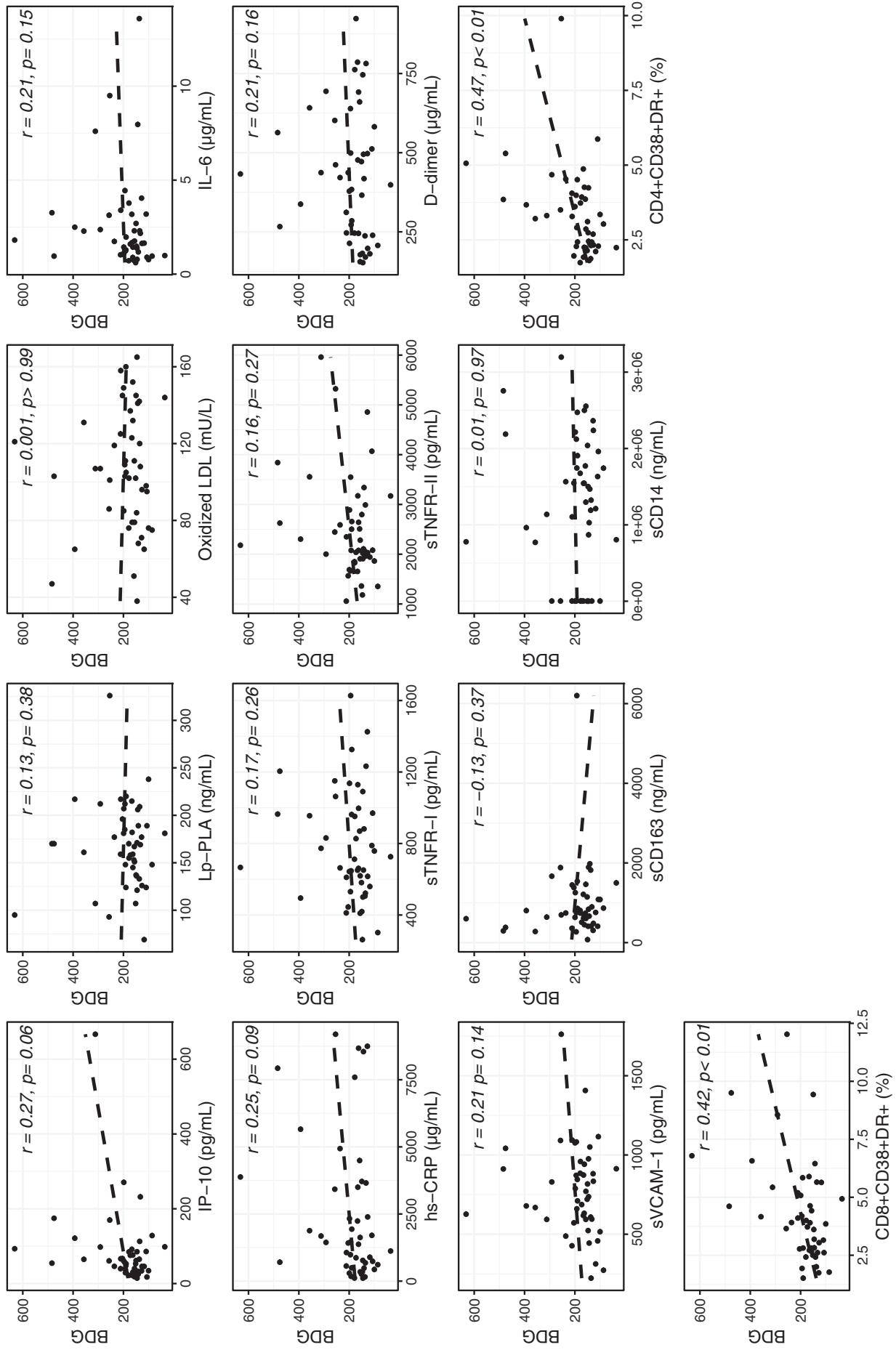


FIG. 6. Univariable analysis between serum levels of β -D-glucan (BDG) in HIV-uninfected participants. Correlation between serum levels of BDG and IP-10, Lp-PLA, oxidized LDL, IL-6, hs-CRP, sTNFR-I, sTNFR-II, D-dimer, sVCAM-1, sCD163, sCD14, CD4+CD38+DR+, and CD8+CD38+DR+ T cells.

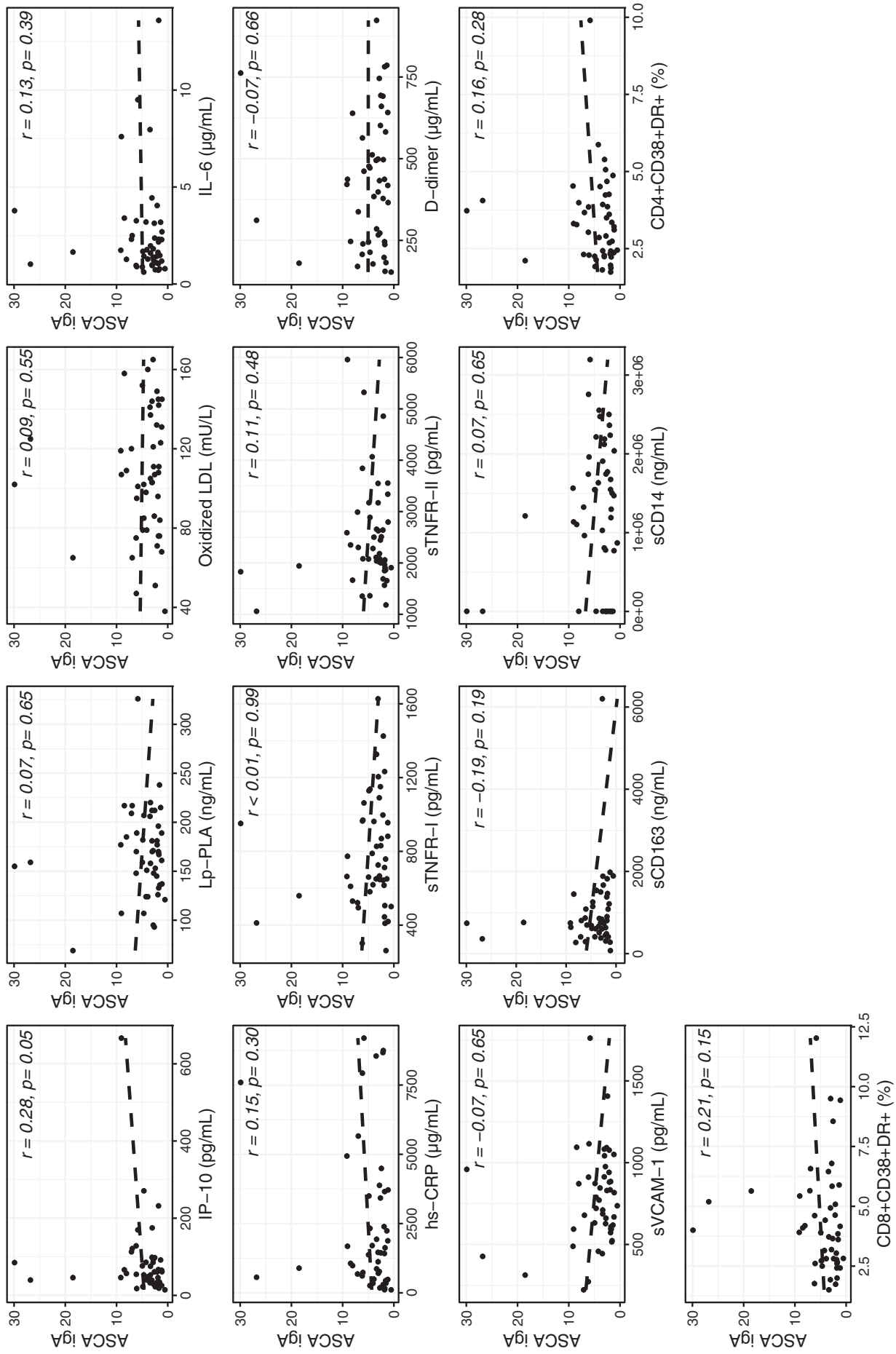


FIG. 7. Univariable analysis between serum levels of ASCA IgA and systemic inflammation in HIV-uninfected participants. Correlation between serum levels of ASCA IgA and IP-10: IP-10, Lp-PLA, oxidized LDL, IL-6, hs-CRP, sTNFR-I, sTNFR-II, D-dimer, sVCAM-1, sCD14, CD4+CD38+DR+, and CD8+CD38+DR+ T cells.

TABLE 2. UNIVARIABLE ANALYSIS BETWEEN ELEVATED LEVELS OF ANTI-*SACCHAROMYCES CEREVISIAE* ANTIBODIES IGA AND SYSTEMIC INFLAMMATION AND HIV VARIABLES (SPEARMAN CORRELATION FACTORS WITH ASSOCIATED *P* VALUES)

	ASCA IgA (>3.08 U/mL)	
	PLWH	HIV uninfected
Inflammatory markers		
IP-10 (pg/mL)	-0.0012 (<i>p</i> = .9922)	0.213 (<i>p</i> = .3175)
Lp-PLA (ng/mL)	-0.0314 (<i>p</i> = .8024)	-0.168 (<i>p</i> = .4327)
Oxidized LDL (mU/L)	0.1274 (<i>p</i> = .3041)	0.1975 (<i>p</i> = .3549)
IL-6 (μg/mL)	0.1184 (<i>p</i> = .3326)	-0.0165 (<i>p</i> = .9389)
hs-CRP (μg/mL)	0.219 (<i>p</i> = .0706)	0.04 (<i>p</i> = .8528)
sTNFR-I (pg/mL)	0.0053 (<i>p</i> = .9658)	-0.5235 (<i>p</i> = .0087)
sTNFR-II (pg/mL)	0.0665 (<i>p</i> = .5874)	-0.2626 (<i>p</i> = .2151)
D-dimer (μg/mL)	0.2011 (<i>p</i> = .0976)	-0.1496 (<i>p</i> = .4855)
sVCAM-1 (pg/mL)	0.0772 (<i>p</i> = .5282)	-0.1748 (<i>p</i> = .414)
sCD163 (ng/mL)	0.1468 (<i>p</i> = .263)	-0.1296 (<i>p</i> = .5462)
sCD14 (ng/mL)	-0.3171 (<i>p</i> = .012)	-0.3696 (<i>p</i> = .0755)
CD4 ⁺ CD38 ⁺ DR ⁺ (%)	-0.074 (<i>p</i> = .5708)	0.2302 (<i>p</i> = .2906)
CD8 ⁺ CD38 ⁺ DR ⁺ (%)	0.0535 (<i>p</i> = .6821)	0.4476 (<i>p</i> = .0322)
HIV variables		
Viral load (copies/mL)	-0.0051 (<i>p</i> = .9666)	
Absolute CD4 ⁺ (cells/μL)	-0.0622 (<i>p</i> = .6172)	
CD8 ⁺ count (cells/μL)	-0.1203 (<i>p</i> = .3437)	
Absolute CD4 nadir (cells/μL)	0.0712 (<i>p</i> = .5671)	
ARV duration (months)	0.0621 (<i>p</i> = .6318)	
CD4:CD8 ratio	0.0745 (<i>p</i> = .5588)	

Statistically significant values are *bold*.

ARV, antiretroviral; IgA, immunoglobulin A; IL, interleukin.

Fungal translocation and inflammatory markers

BDG is known to be highly immunogenic. In HIV, Hoenigl *et al.* showed an association between BDG and sCD14, a marker of monocyte activation.³⁰ Morris *et al.* demonstrated an association between higher levels of IL-8 and TNF- α in PLWH and higher levels of circulating BDG.³¹ Our study yields novel insights into the potential roles played by fungal markers in HIV. We demonstrated a weak, but consistent, correlation between serum levels of BDG in PLWH and several markers of systemic inflammation, including sCD163, sCD14, IL-6, sTNFR-II, D-dimer, IP-10, and sVCAM-1, that was not seen in uninfected controls. This suggests that fungal translocation from an inflamed gut mucosa may contribute to the ongoing inflammation and immune activation seen in PLWH despite viral suppression on ART.

ASCA IgG and IgA did not show the same associations with inflammatory markers as BDG in PLWH. We hypothesized that this might be secondary to the inclusion of lower ASCA values, which may not necessarily reflect poor gut integrity. Therefore, a subgroup analysis was performed to evaluate if higher levels of ASCA IgA would have a stronger correlation with markers of systemic inflammation. This analysis did not yield any additional evidence to support that higher levels of ASCA IgA correlate with markers of systemic immune activation, highlighting the fact that ASCA may be a poor marker of intestinal mucosal integrity and fungal translocation in HIV.

Study limitations

Our study has several strengths, including the comprehensive evaluation of inflammation, and both innate and

cellular arms of immune activation. Because this is a cross-sectional analysis, we cannot prove causal relationships or exclude the possibility of residual confounding. Due to lack of prior data to guide sample size calculation, our study may not have been powered adequately to truly detect a difference between the groups and/or an association with measures of inflammation. The control group, despite being healthy and without any known active inflammatory conditions, had higher levels of cholesterol and D-dimer, which may have influenced our findings. Our population was also mostly men and African Americans, so our findings may not be applicable to other PLWH.

In addition, our study assumed that serum levels of BDG originated via GI translocation of candidal species, however, fungal organisms such as *Histoplasma capsulatum* have BDG as a surface moiety and candidal colonization involves organ systems beyond the GI tract. A limitation of our study is that we were not able to include measurements of the presence of other fungal species. We were also not able to investigate the microbiome of our participants nor measurements of serum LPS or other markers of gut integrity, such as zonulin, which may have been useful in demonstrating alterations in gut mucosal permeability.

Conclusion

In conclusion, our investigation provides preliminary and novel insight into the role of fungal translocation in persistent inflammation and immune activation in treated HIV. Although BDG was not altered by HIV-serostatus, its direct relationship with inflammatory markers in PLWH suggests a possible role of fungal translocation as a mediator of inflammation in PLWH. The role of the microbiome and fungal translocation in inflammation in PLWH warrants further investigation.

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Authors' Contributions

G.A.M. designed the study, obtained funding, and oversaw study evaluations and monitoring. A.S. and L.S. provided statistical support. N.F. assisted with the inflammation and immune activation marker assays. M.R., C.L.H., and M.A.G. assisted with the fungal translocation markers. L.W. assisted in the data analysis plan and wrote the first draft of the article. All authors contributed to data analysis and reviewed the article for intellectual content.

Author Disclosure Statement

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