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MAJOR ARTICLE

Altered Intestinal Permeability and Fungal Translocation in Ugandan Children With Human Immunodeficiency Virus

Sahera Dirajlal-Fargo,[1,2,3,a] Vanessa El-Kamari,[3,a] Lukasz Weiner,[2] Lingpeng Shan,[3] Abdus Sattar,[3] Manjusha Kulkarni,[4] Nicholas Funderburg,[4] Rashidah Nazzinda,[5] Christine Karungi,[5] Cissy Kityo,[5] Victor Musiime,[5,6] and Grace A. Mccomsey[1,2,3]

[1]University Hospitals Cleveland Medical Center, [2] Rainbow Babies and Children's Hospital, [3]Case Western Reserve University, and [4]Ohio State University School of Health and Rehabilitation Sciences, Columbus; [5]Joint Clinical Research Centre, and [6]Department of Paediatrics and Child Health, Makerere University, Kampala, Uganda

Background. Children with perinatally acquired human immunodeficiency virus (HIV; PHIVs) face a lifelong cumulative exposure to HIV and antiretroviral therapy (ART). The relationship between gut integrity, microbial translocation, and inflammation in PHIV is poorly understood.

Methods. This is a cross-sectional study in 57 PHIVs, 59 HIV-exposed but uninfected children, and 56 HIV-unexposed and -uninfected children aged 2–10 years old in Uganda. PHIVs were on stable ART with HIV-1 RNA <400 copies/mL. We measured markers of systemic inflammation, monocyte activation, and gut integrity. Kruskal-Wallis tests were used to compare markers by group and the Spearman correlation was used to assess correlations between biomarkers.

Results. The mean age of all participants was 7 years and 55% were girls. Among PHIVs, the mean CD4 % was 34%, 93% had a viral load ≤20 copies/mL, and 79% were on a nonnucleoside reverse transcriptase inhibitor regimen. Soluble cluster of differentiation 14 (sCD14), beta-D-glucan (BDG), and zonulin were higher in the PHIV group ($P \leq .01$). Intestinal fatty acid binding protein (I-FABP) and lipopolysaccharide binding protein (LBP) did not differ between groups ($P > .05$). Among PHIVs who were breastfed, levels of sCD163 and interleukin 6 (IL6) were higher than levels in PHIV who were not breastfed ($P < .05$). Additionally, in PHIVs with a history of breastfeeding, sCD14, BDG, LBP, zonulin, and I-FABP correlated with several markers of systemic inflammation, including high-sensitivity C-reactive protein, IL6, d-dimer, and systemic tumor necrosis factor receptors I and II ($P \leq .05$).

Conclusions. Despite viral suppression, PHIVs have evidence of altered gut permeability and fungal translocation. Intestinal damage and the resultant bacterial and fungal translocations in PHIVs may play a role in the persistent inflammation that leads to many end-organ diseases in adults.

Keywords. children with HIV; HIV-exposed uninfected infants; gut integrity; inflammation; translocation.

Antiretroviral therapy (ART) decreases inflammation and immune activation [1, 2], in human immunodeficiency virus (HIV) infection; however, this decrease is incomplete, and levels of inflammatory markers remain elevated despite virologic suppression. Adults living with HIV (ALHIV) on ART with virologic control have elevated markers of systemic inflammation, coagulation, and immune activation, compared to HIV-uninfected individuals [3]. Several studies have also suggested that children with perinatally acquired HIV (PHIV) have higher levels of inflammation when compared to levels in uninfected controls [4–6]. Sustained immune activation also persists in PHIV children, despite ART and viral suppression [7, 8].

The role of alterations of intestinal integrity and the resultant translocation of microbial products from the intestinal lumen to the systemic circulation appears to be a central factor in HIV-associated chronic immune activation [9, 10]. Recently, several studies have suggested that intestinal dysbiosis—specifically, alteration of the gut mycobiome—plays an integral part in host-microbiota interactions, including through fungal translocation [11]. Our group has also found that markers of fungal translocation are associated with immune activation and systemic inflammation in virally suppressed ALHIV [12].

Limited data exist on the role of microbial translocation and ongoing inflammation and immune activation in PHIV and HIV-exposed but uninfected (HEU) children.

In this study, we assessed biomarkers of systemic inflammation, immune activation, and microbial and fungal translocation, as well as gut integrity, in PHIV, HEU, and HIV-unexposed and -uninfected (HIV−) Ugandan children. The primary objective of this study was to determine whether these selected

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[a]S. D.-F. and V. E.-K. contributed equally to this manuscript.

Correspondence: G. A. McComsey, University Hospitals Cleveland Medical Center and Case School of Medicine, 2061 Cornell Rd, Mail Stop 5083, Cleveland, OH 44106 (Grace.mccomsey@uhhospitals.org).

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markers were different between the groups; the secondary objective was to determine whether ongoing inflammation and immune activation were associated with alterations in gut integrity and translocations of fungal and bacterial products.

METHODS

Study Design

This is an observational cohort of PHIV, HEU, and HIV− children who were prospectively enrolled at the Joint Clinical Research Center in Kampala, Uganda. The study was approved by the Research Ethics Committee in Uganda, the Ugandan National Council of Science and Technology, and the Internal Review Board of the University Hospitals Cleveland Medical Center in Cleveland, Ohio. Caregivers gave written informed consent; older children aware of their HIV status also gave informed assent, following national guidelines. All participants were 2–10 years of age. PHIV participants were on stable ART for at least 6 months, with HIV-1 RNA <400 copies/mL. HEU and HIV− children were tested during their clinic visit to confirm their HIV seronegative status. Children with evidence of an acute infection (malaria, tuberculosis, heminthiasis, pneumonia, meningitis) in the last 3 months, or with moderate or severe malnutrition and diarrhea in the last 3 months, were excluded. Those with known diabetes or cardiovascular disease were also excluded.

Study Evaluations

Blood was drawn after an 8-hour fast. Blood was processed and plasma and serum were cryopreserved for shipment to Cleveland Medical Center in Cleveland, Ohio. A Material Transfer Agreement, approval from the Uganda National Council of Science and Technology, and a permit from the Center for Disease Control were obtained.

Inflammation, Soluble Immune Activation, and Gut Integrity Markers

Lipopolysaccharide binding protein (LBP; Hycult Biotech Inc.), a marker of microbial translocation, has been associated in ALHIV with cardiovascular disease risk factors such as hypertension [13], dyslipidemia [14], and platelet [15] and endothelial dysfunction [16]. Zonulin (Promocell, Germany), a marker of intestinal permeability, correlates well with more cumbersome tests of intestinal permeability in patients with autoimmune diseases [17]. Intestinal fatty acid binding protein (I-FABP, R&D Systems, Minneapolis, MN) is a marker of enterocyte damage [18]. Our group has found that pre-ART I-FABP levels were associated with changes in body composition over 2 years in ALHIV [19]. Beta-D-glucan (BDG; Mybiosource Inc.) is a polysaccharide cell wall component of most fungal species that is known to be highly immunogenic, stimulating macrophages, neutrophils, and T-cells. Soluble cluster of differentiation 14 (sCD14, R&D Systems, Minneapolis, MN) is a marker of monocyte activation, participates in the response of cells to lipopolysaccharide, and is associated with mortality and atherosclerosis [20].

The remaining biomarkers selected are markers of immune activation, systemic inflammation, and coagulation that are hallmarks of HIV infection. These markers either correlate with non–acquired immunodeficiency syndrome comorbidities or cardiometabolic complications, or drive other hallmarks of immune dysregulation in HIV. Plasma markers of monocyte activation (sCD163), systemic inflammation (systemic tumor necrosis factor receptor I and II), high-sensitivity C-reactive protein, interleukin 6 (IL6), coagulation (d-dimer), and oxidized lipids were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN; ALPCO, Salem, NH; and Mercodia, Uppsala, Sweden). The intra-assay variability ranged between 4–8% and the interassay variability was less than 10% for all markers. All assays were done at N. F.'s laboratory at Ohio State University in Columbus, Ohio. Laboratory personnel were blinded to group assignments and clinical characteristics.

Statistical Analyses

The primary objective of this analysis was to compare biomarkers of inflammatory and intestinal barrier integrity between PHIV, HEU, and HIV− children. The secondary objectives were to determine the relationship between gut markers and markers of systemic inflammation and immune activation in all 3 groups. Demographics, clinical characteristics, and gut markers were described and compared between the 3 groups. Descriptive statistics and Kruskal Wallis tests were used to compare baseline characteristics. In all 3 groups, relationships among gut markers and inflammation markers were assessed using Spearman Correlation analyses.

In subgroup analysis, *t*-tests and Wilcoxon rank sum tests were used to compare markers by sex, breastfeeding status, and history of prevention of maternal to child transmission (PMTCT) in all 3 groups. Regression analyses were used to determine whether specific ART regimens were associated with gut markers.

RESULTS

Baseline Characteristics

A summary of participant characteristics is shown in Table 1. Overall, 172 participants were enrolled: 57 PHIV, 59 HEU, and 56 HIV− children. The median age of participants was 7.8 years (interquartile range [IQR] 6.39–8.84), 55% were female, and the median body mass index was 15.2 kg/m^2 (IQR 14.38–15.81).

Most children were breastfed (82%). The median duration of breastfeeding was 9 months (IQR 6.00–18.00), and the durations were significantly different between the 3 groups, with HIV− children breastfed for the longest time (Table 1).

Among PHIVs, 93% had a viral load ≤20 copies/mL, the median CD4 % was 37 [21, 22], and 79% were on a nonnucleoside reverse transcriptase inhibitor–based regimen, mostly nevirapine.

Table 1. Baseline Characteristics

Variables	Overall (N = 172)	PHIV (n = 57)	HEU (n = 59)	HIV– (n = 56)	*P* Value
Patient characteristics					
Age, years	7.89 (6.39–8.84)	7.67 (6.57–8.83)	7.90 (6.23–8.75)	7.99 (6.45–9.03)	.93
Female, %	94 (55%)	31 (54%)	33 (56%)	30 (54%)	.97
Height, m	1.22 (1.14–1.30)	1.20 (1.14–1.26)	1.23 (1.12–1.32)	1.23 (1.16–1.29)	.52
Weight, kg	22.50 (19.75–25.50)	21.75 (20.00–24.62)	22.50 (18.75–25.75)	23.00 (20.00–26.00)	.52
BMI, kg/m^2	15.02 (14.38–15.81)	14.93 (14.48–15.91)	14.92 (14.06–15.60)	15.17 (14.57–16.25)	.08
History of ART for PMTCT	61 (56%)	15 (30%)	46 (81%)	0	**<.001**
Mother's characteristics					
No history of breastfeeding, n (%)	31 (18%)	8 (15%)	23 (39%)	0 (0%)	**<.001**
Breastfeeding duration, months	9 (6–18)	9 (6–18)	3 (2–6)	18 (12–24)	**<.001**
History of ART for PMTCT, n (%)	64 (56%)	16 (29%)	48 (83%)	0	**<.001**
HIV variables					
Viral load, copies/mL	…	20 (0–20)	…	…	NA
Absolute CD4, cells/μL	…	1266 (851–1737)	…	…	NA
CD4%	…	37 (27–41)	…	…	NA
Absolute CD4 nadir, cells/μL	…	1194 (678–1641)	…	…	NA
ART duration, months	…	71.92 (63.37–76.42)	…	…	…
NRTI backbone					
Zidovudine	…	25 (50%)	…	…	…
Lamivudine	…	52 (91%)	…	…	…
Abacavir	…	29 (51%)	…	…	…
Stavudine	…	3 (5%)	…	…	NA
PI					
Lopinavir/ritonavir, n (%)	…	13 (23%)	…	…	NA
NNRTI					
Efavirenz, n (%)	…	16 (28%)	…	…	…
Nevirapine, n (%)	…	29 (51%)	…	…	…
Markers of gastrointestinal translocation and intestinal integrity					
sCD14, ng/mL	1819.84 (1545.27–2153.71)	2071.46 (1685.31–2560.62)	1824.41 (1568.31–2007.96)	1665.53 (1414.74–1854.60)	**<.001**
BDG, pg/mL	169.39 (122.43–215.57)	199.54 (178.49–242.68)	128.84 (115.55–169.71)	163.46 (131.53–207.81)	**<.001**
I-FABP, pg/mL	2677.89 (1788.93–3712.61)	2747.97 (1979.67–4043.56)	2838.78 (1738.09–3726.87)	2333.37 (1589.81–3364.01)	.15
Zonulin, ng/mL	6.74 (4.44–11.22)	10.95 (9.77–12.11)	5.42 (4.64–6.58)	5.54 (2.33–11.31)	**<.001**
LBP, ng/mL	13224.30 (5628.14–17436.99)	10829.41 (5524.94–16793.43)	13736.70 (5406.26–17501.17)	14172.99 (9744.67–19179.22)	.19

Data are shown as median (IQR), unless otherwise indicated. Bold values represent $P < .05$.

Abbreviations: ART, antiretroviral therapy; BDG, beta-D-glucan; BMI, body mass index; HEU, HIV-exposed but uninfected children; HIV, human immunodeficiency virus; HIV–, HIV-uninfected; I-FABP, intestinal fatty acid binding protein; IQR, interquartile range; LBP, lipopolysaccharide binding protein; NA, not applicable; NRTI, nucleotide reverse transcriptase inhibitor; NA, not applicable; NNRTI, nonnucleotide reverse transcriptase inhibitor; PHIV, children with perinatally acquired HIV; PI, protease inhibitor; PMTCT, prevention of maternal to child transmission; sCD14, soluble cluster of differentiation 14.

All PHIVs were on co-trimoxazole prophylaxis; participants were not on any other antibacterial or antifungal agents.

Effect of Human Immunodeficiency Virus Infection and Exposure on Gut Integrity Markers

Median levels of microbial translocation and gut integrity markers are shown in Table 1. When comparing gut markers among the 3 groups, BDG, zonulin, and sCD14 levels were higher in the PHIV group, compared to both the HEU and HIV− groups (Figure 1; all P values < .01).

No differences in gut marker levels were found between the HEU and HIV− groups, except for BDG, which was higher in the HIV− participants (P < .05).

In contrast, no significant differences in LBP and I-FABP levels were found among the 3 groups (P > .05).

We next explored whether gut markers differed in PHIV and HEU participants with a history of ART for PMTCT. HEUs with a history of maternal (n = 48) and infant (n = 46) ART for PMTCT had higher LBP levels ($P \leq .05$). None of the gut markers were higher in PHIV children with a history of maternal or infant ART for PMTCT (P > .1).

There were no correlations between gut markers and either viral load or CD4 percentages (P > .2). Protease inhibitor use was associated with lower sCD14 levels (β = −610, 95% confidence interval −1051 to −168; P < .01). Nonnucleoside reverse transcriptase inhibitor use (efavirenz or nevirapine), protease inhibitor use, or being on abacavir were not associated with any of the other biomarkers in regression analyses (P > .06).

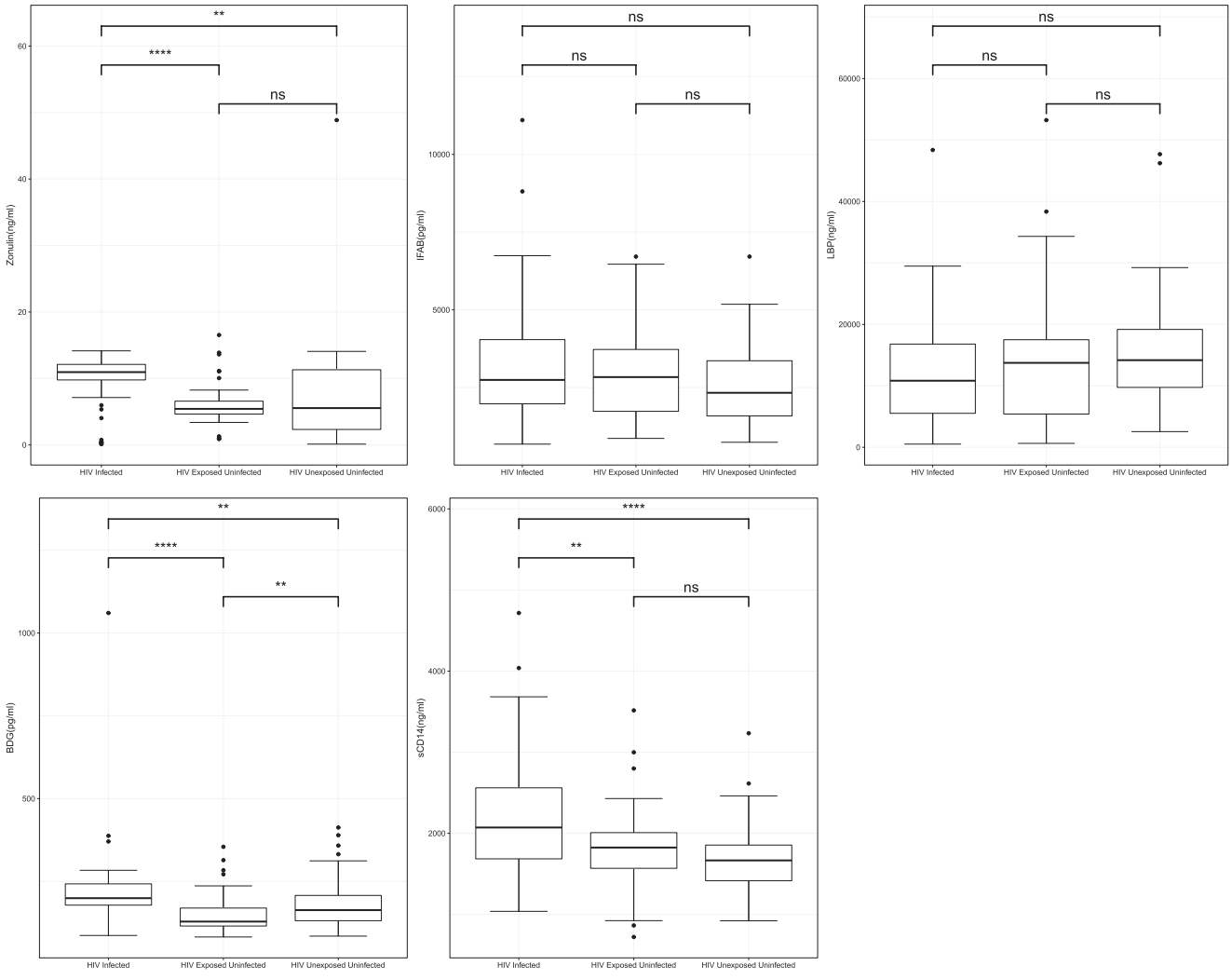

Figure 1. Comparison of gut markers between the groups. Box plots of the markers in each group. The box represents the interquartile range and whiskers represent the range. Abbreviations: BDG, beta-D-glucan; I-FABP, intestinal fatty acid binding protein; HIV, human immunodeficiency virus; LBP, lipopolysaccharide binding protein; ns, not significant; sCD, soluble cluster of differentiation. $^{**}P$ < .01; $^{****}P$ < .001.

Effect of Sex on Microbial Translocation and Gut Integrity Markers

Among PHIVs, zonulin was found to be significantly higher in females, compared to males ($P < .01$; Figure 2); however, no differences in other gut markers were found between male and female participants, either in the entire cohort or within each of the 3 groups ($P > .2$).

Effect of Breastfeeding on Inflammation, Microbial Translocation, and Gut Integrity Markers

Breastfeeding and its duration are known to play an important role in the gut microbiome. We explored whether gut markers and inflammatory markers differed between breastfed and nonbreastfed participants, both in our entire cohort and within each of the 3 groups.

All HIV− children had a history of breastfeeding; therefore, this analysis was restricted to PHIV and HEU children. No differences in gut, inflammatory, and immune activation markers were observed in the combined HEU and PHIV participants by history of breastfeeding versus no breastfeeding ($P > .05$). A within-group comparison showed that in the PHIV group, BDG levels were marginally higher in the nonbreastfed children, compared to the breastfed children ($P = .05$; Figure 3), while sCD163 and IL6 levels were significantly higher in PHIV who were breastfed (Figure 4). No significant differences in gut and inflammatory markers were observed in the HEU group by breastfeeding status ($P > .05$). Among PHIV with a history of breastfeeding, several gut integrity and translocation biomarkers were correlated with markers of systemic inflammation (Table 2). Among HEU with a history of breastfeeding, I-FABP correlated with IL6, and LBP correlated with both high-sensitivity C-reactive protein and IL6 ($P < .05$).

We also assessed the correlations among intestinal biomarkers, and found that BDG correlated with zonulin for both PHIV and HEU ($P < .05$).

DISCUSSION

Our findings suggest that, despite viral suppression, PHIV children have evidence of heightened immune activation, alterations in intestinal permeability, and fungal translocation, compared to these indices in HEU and HIV− children. Importantly, it also appears that in PHIV with a history of breastfeeding, biomarkers of intestinal damage and fungal and microbial translocation may play a role in HIV-associated chronic inflammation.

Zonulin is a human protein that regulates intestinal permeability by modulating intercellular tight junctions in the gut; it increases permeability and macromolecule absorption [23]. Zonulin is increased in conditions associated with chronic inflammation, such as celiac disease and type I diabetes [17, 24]. Interestingly, lower zonulin predicted higher mortality in ALHIV who were virally suppressed [25]. In a secondary analysis of the International Maternal Pediatric Adolescent Acquired Immunodeficiency Syndrome Clinical Trial (IMPAACT) P1072 study, there was no significant difference in zonulin levels between PHIV infants and HEU infants at 3 months of age [26]. Our findings, however, suggest that PHIV children have disruptions of intestinal barrier integrity despite viral suppression, compared to age- and gender-matched HEU children and HIV− children. Factors that could account for the discrepancies

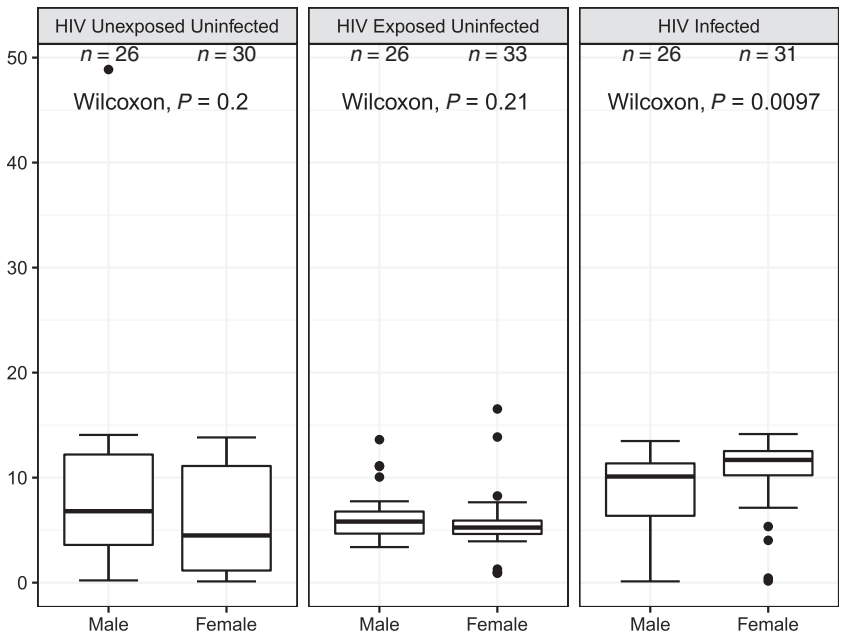

Figure 2. Comparison of zonulin between sexes in the 3 groups. Box plots of the markers in each group. The box represents the interquartile range and whiskers represent the range.

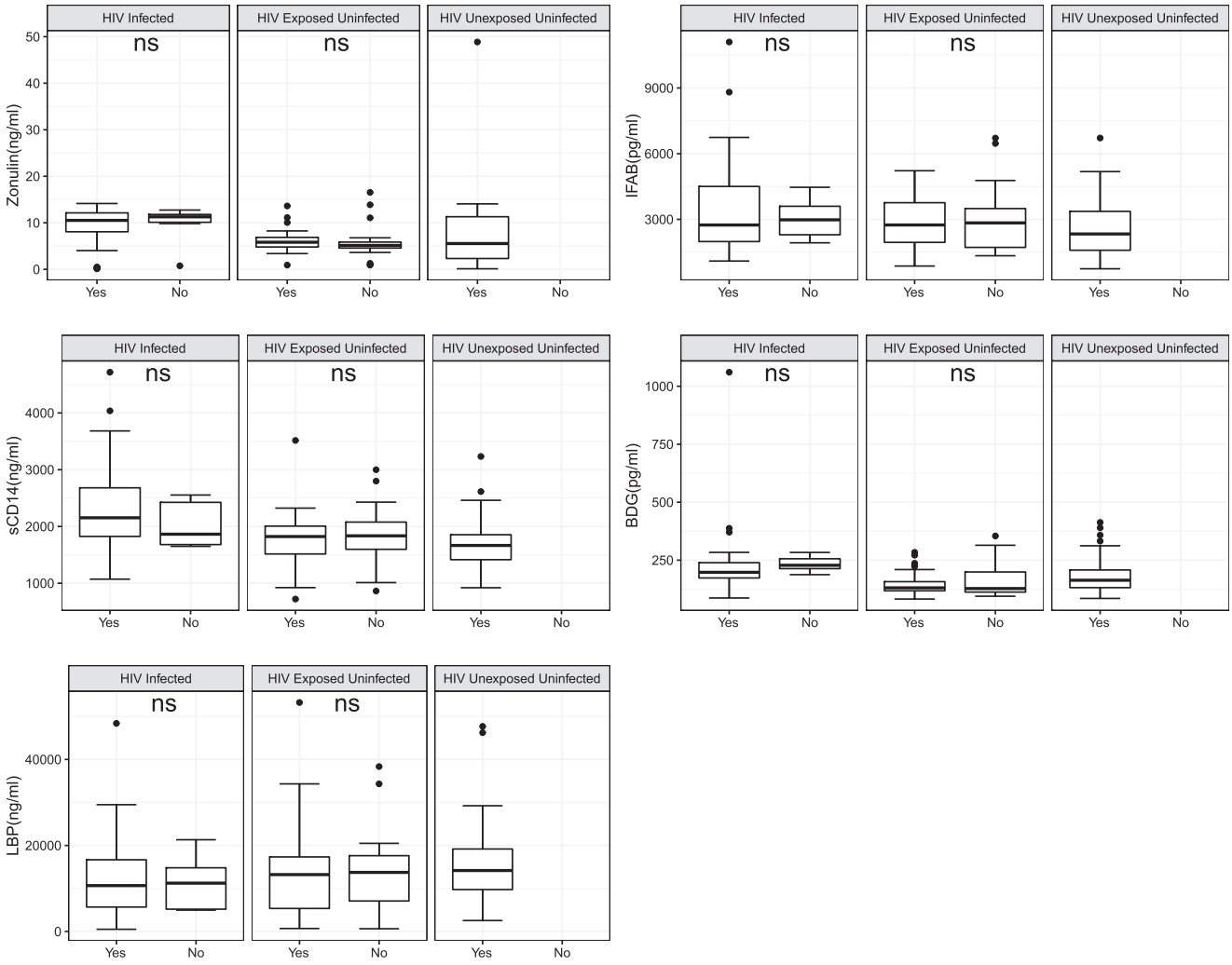

Figure 3. Comparison of gut integrity and microbial translocation markers between the group by breastfeeding status. Box plots of the markers in each group. The box represents the interquartile range and whiskers represent the range. Abbreviations: BDG, beta-D-glucan; HIV, human immunodeficiency virus; I-FABP, intestinal fatty acid binding protein; LBP, lipopolysaccharide binding protein; ns, not significant; sCD, soluble cluster of differentiation.

between findings are the differences in demographics, including age, as our patients were older, with a median age of 7 years. In the IMPAACT P1072 study, zonulin significantly increased with age in the infected infants and became significantly higher by 5 months of age, compared to HEU infants, despite early ART. This may reflect the natural gastrointestinal changes in infants, as well as the disruption in mucosal integrity that is known to occur gradually during the early stages of an HIV infection; therefore, evidence of a gut impairment may not be apparent during infancy. Similarly to in ALHIV who are untreated, intestinal damage doesn't seem to be reversible with ART and appears to persist in PHIV on ART with viral suppression [27].

I-FABP, a marker of intestinal integrity, is increased in ALHIV, as compared to controls [20, 21, 28]. Contrary to the adult literature and consistent with our findings, data from infants suggest that I-FABP is not increased in PHIV, compared to HEU and HIV− controls [26, 29]. We hypothesize that (1) I-FABP, although a marker of intestinal damage, may reflect increases in gut epithelial cell numbers; and/or (2) small intestinal damage may occur at later stages of HIV. Further longitudinal studies, in addition to intestinal mucosa sampling, are warranted to further support these hypotheses.

Despite increased intestinal permeability, we found no increased evidence of bacterial translocation in PHIV children, as measured by LBP. In low concentrations, LBP binds lipopolysaccharide and presents it to CD14 and Toll-like receptor 4 [30]. All PHIV children in our study were receiving co-trimoxazole (trimethoprim-sulfamethoxazole) prophylaxis, as recommended by the World Health Organization, which may explain why LBP was not elevated in PHIVs, compared to HEU and HIV− children. Co-trimoxazole is effective against a wide variety of aerobic gram-positive and gram-negative bacteria, *Pneumocystis jiroveci* pneumonia, and some protozoa, and therefore likely reduces the diversity and abundance of intestinal

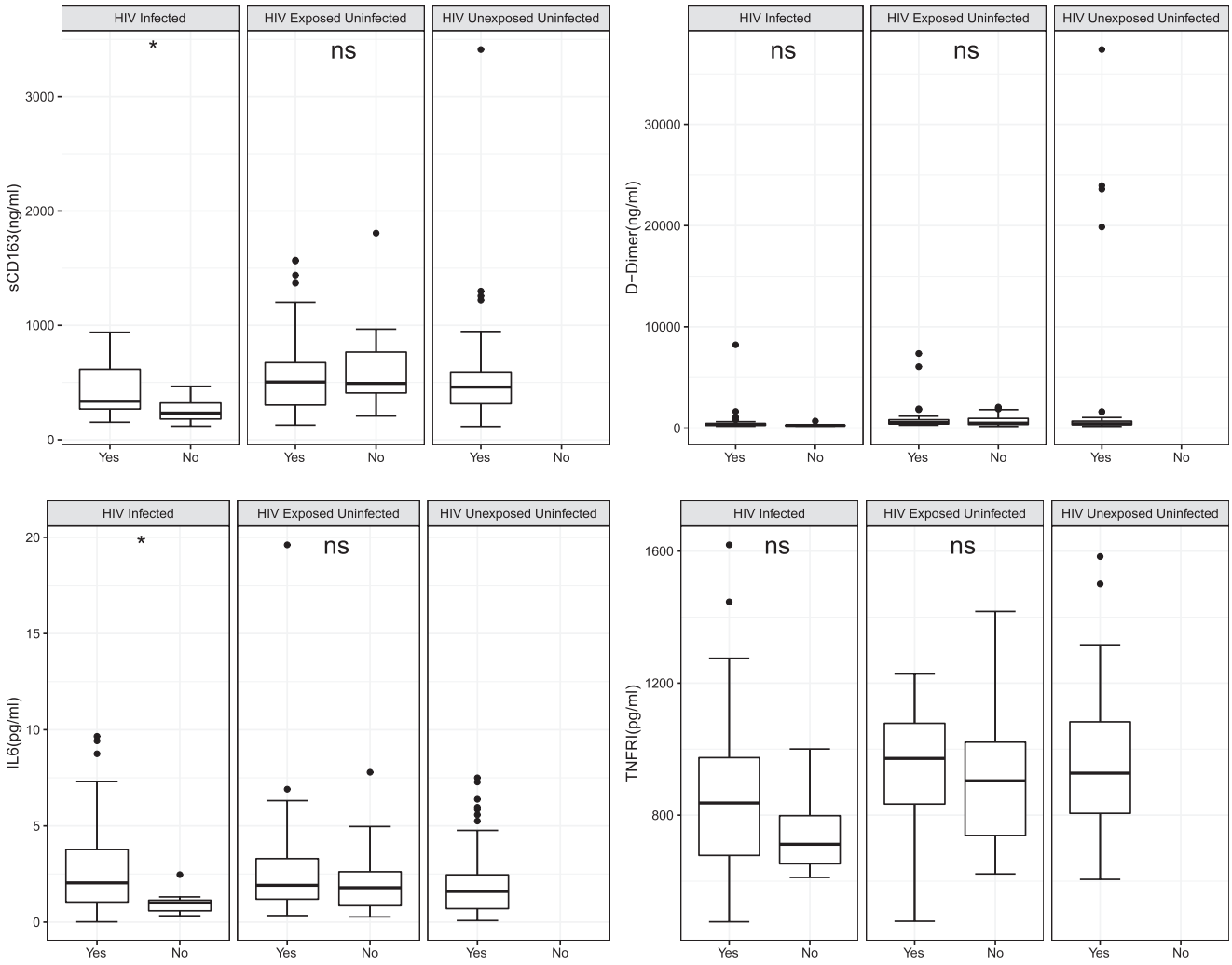

Figure 4. Comparison of inflammatory and immune activation markers between the groups by breastfeeding status. Box plots of the markers in each group. The box represents the interquartile range and whiskers represent the range. Abbreviations: HIV, human immunodeficiency virus; IL6, interleukin 6; ns, not significant; sCD, soluble cluster of differentiation; TNFRII, tumor necrosis factor receptor II. *$P < .05$.

microbiota in PHIV children. Co-trimoxazole prophylaxis in childhood is associated with reduced rates of hospitalization and mortality, as well as improved growth and reduction in anemia, irrespective of age, and CD4 cell count in settings with high prevalences of bacterial infections and/or malaria [31–33]. Some of the benefits of co-trimoxazole are likely due in part to antibacterial and antimalarial protection [34]; however, the complete mechanism of action of co-trimoxazole is unclear, as benefits have been found in children in areas with high levels of in vitro resistance to co-trimoxazole and low prevalences of *Pneumocystis jiroveci* pneumonia [31]. The role of co-trimoxazole in mitigating mortality and improving health outcomes could be attributed to its anti-inflammatory properties: in the Antiretroviral Research for Watoto trial, PHIV children who were randomly assigned to discontinue co-trimoxazole had persistent increases in several inflammatory biomarkers [35]. The benefits of co-trimoxazole may also be secondary to its ability to reduce microbial translocation across a compromised intestinal barrier [36], as highlighted in a small study of ALHIV where concomitant use of ART and co-trimoxazole reduced microbial translocation (as measured by LBP and sCD14) [37]. It is noteworthy that we found elevated levels of inflammation markers in our study despite the use of co-trimoxazole.

Our study also yielded the novel observation that fungal translocation is increased in virally suppressed PHIV children and may be a consequence of alterations in intestinal permeability. BDG is a component of the cell wall of many fungi, including, but not limited to: *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans*. Co-trimoxazole has activity against fungi, it is narrowed to Pneumocystis and Paracoccidioides, and, therefore, co-trimoxazole prophylaxis in our participants would likely not alter the mycobiome and attenuate fungal translocation. Although research has been primarily focused on microbiota in HIV, a few studies have suggested that ALHIV

Table 2. Correlation Between Systemic Inflammatory Markers and Gut and Microbial Translocation Markers

	sTNFRI (pg/mL)	sTNFRII (pg/mL)	hsCRP (ng/mL)	Ddimer (ng/mL)	sCD163 (ng/mL)	IL6 (pg/mL)
PHIV with history of breastfeeding (n = 49)						
BDG (pg/mL)	**0.329**	**0.437**	0.211	−0.009	0.027	0.028
I-FABP (pg/mL)	**0.458**	**0.380**	−0.006	−0.150	0.065	0.076
Zonulin (ng/mL)	**0.325**	0.233	0.070	−0.213	−0.116	−0.025
LBP (ng/mL)	−0.105	0.104	**0.400**	**0.320**	0.248	0.126
sCD14 (ng/mL)	**0.400**	**0.419**	**0.491**	0.282	0.051	**0.302**
HEU with a history of breastfeeding (n = 36)						
BDG (pg/mL)	−0.47	−0.197	0.001	0.178	−0.002	−0.066
I-FABP (pg/mL)	0.088	0.235	0.284	0.107	−0.233	**0.380**
Zonulin (ng/mL)	0.008	0.099	0.220	−0.69	−0.293	0.232
LBP (ng/mL)	0.187	0.113	**0.571**	0.307	−0.142	**0.343**
sCD14 (ng/mL)	0.280	0.289	0.116	0.101	−0.156	0.137

Data are from the PHIV and HEU breastfeeding group. Spearman correlation coefficients. Bolded numbers represent *P* values ≤ .05.
Abbreviations: BDG, beta-D-glucan; HEU, HIV-exposed but uninfected children; HIV, human immunodeficiency virus; hsCRP, high-sensitivity C-reactive protein; I-FABP, intestinal fatty acid binding protein; IL6, interleukin 6; LBP, lipopolysaccharide binding protein; PHIV, children with perinatally acquired HIV; sCD, soluble cluster of differentiation; sTNFRI, systemic tumor necrosis factor receptor I; sTNFRII, systemic tumor necrosis factor receptor II.

may also have altered mycobiomes [38–40]. Outside of HIV, altered mycobiome diversity has been associated with inflammatory bowel disease [41], atopic dermatitis [22], and chronic hepatitis B. In addition, alterations of the mycobiome with antifungal drugs improve gastrointestinal graft-versus-host disease [42]. Surprisingly, BDG was also higher in HIV− children, compared to HEU children. A possible explanation could be socioeconomic factors or dietary habits, leading to differences in the consumption of foods rich in beta-glucan (mushroom, oats, barley, etc.) Fungal translocation may not be limited to the gut, and we cannot rule out an interaction between mycobiomes in different body sites, such as the skin and oral and nasal cavities, all of which are known to be colonized with fungi [43].

Growing evidence in ALHIV suggests that microbial translocation and gut dysbiosis are associated with persistent immune activation and inflammation despite ART [44]. In addition, evidence suggests that this may be linked to gastrointestinal mucosal damage [45]. Our group has found that in ALHIV, BDG may also play a role in driving inflammation [12]. There are conflicting data in PHIV on the persistence of microbial translocation despite ART [46–48] and its association with immune activation [47, 48]. Recent findings suggest alterations in the gut microbiome composition in PHIV children, and the relative abundance of specific bacteria are associated with ongoing immune activation and inflammation [49, 50]. In our study, we found that PHIV with a history of breastfeeding have ongoing inflammation and immune activation, compared to PHIV without a history of breastfeeding. Similarly to in ALHIV, evidence of intestinal integrity damage and microbial and fungal translocation may play a role in the heightened inflammation seen in this group. It is important to note that breastfeeding data were collected from maternal recollection and are subject to memory bias. In addition, we did not have information about the breastfeeding practices in this population (ie, exclusive vs mixed breastfeeding), which are known to be critical for child survival [51]. We hypothesize that breastfeeding may play a role in gut dysbiosis and inflammation in children who acquire HIV perinatally, either in utero, during delivery, or through breastmilk transmission from viremic mothers. This is likely compounded by alterations in intestinal permeability due to HIV infection, which appears to persist despite ART. Intestinal damage resulting in microbial translocation may drive inflammation through childhood; however, the relationship may be bidirectional, such that the increased inflammation induced by microbial translocation may further damage the already "leaky gut." Shorter durations of breastfeeding and, potentially, the early introduction of solid foods are known to change the gut microbiota in infants [52] and may also contribute to changes in intestinal integrity.

We and others have shown that HEU infants have heightened inflammation and immune activation, compared to HIV− infants [53, 54]. Our findings suggest that these biomarkers may normalize in childhood, as there were no differences in inflammatory biomarkers between HEU and HIV− children in our study.

Several studies have identified that women living with HIV have higher systemic immune activation and decreased gut integrity markers, compared to men [54–58]. We found that, among PHIVs, the only sex difference in biomarkers was higher zonulin levels in girls. This is contrary to what was found in a cohort of women living with HIV in rural Uganda [21]. Sex-related differences in immune activation in HIV may be influenced by hormonal effects on immune cell function [59, 60], sex-specificity of the microbiome [61, 62] and behavioral factors that may not become relevant until puberty.

There are a few limitations to our study, including the lack of an assessment of the gastrointestinal microbiome and mycobiome compositions. Although we excluded participants

with active infections, we did not assess potential helminthiasis or perform nutritional assessments, all of which could affect the microbiome. The cross-sectional design precluded the determination of a temporal relationship between impairments in gut integrity and immune activation. Strengths of our study include well-characterized HEU and HIV− age- and gender-matched groups from the same country, for comparison with maternal PMTCT and feeding history.

In conclusion, our study showed that children who acquired HIV perinatally had evidence of ongoing inflammation, increased intestinal permeability, and fungal translocation, despite ART. In addition, evidence of intestinal damage and microbial and fungal translocation may play a role in the increased inflammation seen in PHIVs. Further research is warranted in this population to investigate the links between intestinal barrier functions, intestinal microbiota compostions, and immune activation, as well as, importantly, the implications of lifelong exposure to the consequences of decreased gut-barrier functions on potential comorbidities in children living with HIV.

Notes

Author contributions. S. D.-F. and G. A. M. designed the study and obtained funding. G. A. M., C. Karungi, and V. M. oversaw study evaluations and monitoring. A. S., V. E.-K., and L. S. provided statistical support. M. K. and N. F. performed the biomarker assays. S. D.-F. and V. E.-K. wrote the first draft of the manuscript. All authors contributed to the data analysis and reviewed the manuscript for intellectual content.

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Potential conflicts of interest. G. A. M. served as a consultant for Gilead, Glaxo Smith Kline/Viiv, and Merck, and has received research funding from Gilead, Merck, GSK/Viiv, Roche, Astellas, Tetraphase, and Bristol Meyer Squibb. N. F. serves as a consultant for Gilead. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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