JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Markers of microbial translocation during pregnancy: differences among HIV+ women of African and European provenance

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Abstract

Introduction: Microbial translocation (MT) markers are indicators of HIV-related immune activation, but reference values are mostly derived from European or North American populations and could be substantially different in populations living in developing countries. Here we evaluate possible differences in MT markers levels in HIV+ pregnant women of different geographical provenance.

Methodology: This study is nested within an observational study of pregnant women with HIV in Italy. Women were dichotomized on the basis of provenance in two groups of European (n = 14) and African (n = 26) origin. Soluble CD14, lipopolysaccharide-binding protein (LBP) and intestinal-fatty acid binding protein (I-FABP) were measured in plasma samples collected between the first and second trimester of pregnancy.

Results: Demographic and viroimmunological characteristics were similar between groups, although European women were more commonly smokers and HCV-coinfected. Irrespective of origin, LBP plasma levels were positively correlated with I-FABP (r = 0.467, p = 0.004) and sCD14 levels (r = 0.312 p = 0.060). Significantly higher levels of sCD14 (1885 vs. 1208 ng/mL, p = 0.005) LBP (28.5 vs. 25.3 µg/mL, p = 0.050) and I-FABP (573.4 vs. 358.2 pg/mL, p = 0.002) were observed in European compared with African women. A multivariable linear regression analysis, adjusted for smoking and HCV coinfection confirmed the association between sCD14 levels and women provenance (p = 0.03).

Conclusions: Our observations indicate significant differences in soluble markers among women of different provenance. In the design and analysis of studies evaluating MT markers, population-specific reference values should be considered.

Key words: HIV; microbial translocation; biomarker; pregnancy; geographical provenance.

J Infect Dev Ctries 2020; 14(2):184-190. doi:10.3855/jidc.11652

(Received 13 May 2019 – Accepted 05 September 2019)

Introduction

The systemic immune activation in HIV+ individuals is considered, together with HIV replication, the driving force leading to CD4 cell depletion and HIV progression [1]. Antiretroviral therapy can reduce this inflammatory status, that, however, may persist even after many years of successful viral suppression [2].

One of the causes of HIV-related systemic immune activation is represented by the circulation of microbial products, mostly originating from the gastrointestinal tract. HIV replicates vigorously in gut-associated lymphoid tissue (GALT), causing damage to the intestinal barrier through several mechanisms, and allows systemic dissemination of microbial products, resulting in secretion of inflammatory mediators. Circulating level of lipopolysaccharides (LPS) have been identified as a valuable indicator of immune inflammation [1,3], but since the measurement of LPS concentration in plasma is a technically complex task, other reliable biomarkers of microbial translocation have been identified and validated: elevated plasma levels of LPS binding protein (LBP), index of LPS exposure, intestinal fatty acid-binding protein (I-FABP), marker of enterocytes damage, and soluble CD14 (sCD14), that correctly reflects the degree of LPS-induced inflammation in HIV+ individuals [4].

Increased sCD14 levels in HIV patients have been correlated to disease progression [5], neurocognitive impairment, and increased risk of cardiovascular disease [6,7].

Despite the potential utility of microbial translocation markers as indicators of HIV-related inflammatory status, only a few studies have characterized their plasma levels according to provenance/ethnicity, gender or other particular

physiological conditions, such as pregnancy. There is some evidence showing that markers of immune activation have higher levels in HIV populations living in resource-limited settings [8,9], although lower sCD14 levels have been described in African versus European populations living in the same geographical regions [10,11]. Whether the differences observed are a result of environmental and/or genetic factors is uncertain. Moreover, most of these studies were performed on a general adult population, and not in pregnancy, when other factors such as hormonal changes and immune tolerance can alter the immunological and inflammatory profile [12].

Inflammation during pregnancy can increase the risk of preterm delivery and intrauterine growth retardation [9,13,14] in HIV pregnant women elevated levels of sCD14 have been associated to risk of maternal to child transmission (MTCT) [15], preterm delivery and low birth weight [16,17]. Since most of HIV women of childbearing age live in low-income countries [18], investigating the biomarkers profile of immune activation of these populations should be a priority, also considering the profound impact that maternal inflammation could have on neonatal outcomes [19].

This preliminary study aims to examine the degree of microbial translocation among HIV+ pregnant women living in Italy but of different origin. The main study objectives were: 1) to describe the degree of microbial translocation through the levels of surrogate markers in HIV-infected women during the first two trimesters of pregnancy and 2) to determine possible differences in pregnant women of different provenance, currently living in the same geographical region.

Methodology

Study setting and population

This laboratory study was nested as a single-site, immunological substudy, within the National Program on Surveillance on Antiretroviral Treatment in Pregnancy, an ongoing observational multicenter study on HIV pregnant women established in Italy in 2001 [20]. Laboratory and clinical information, including viro-immunological data, was recorded at the clinical sites during the routine visits at each trimester of pregnancy, at delivery, and during 18-month follow-up observation of mothers and infants. Gestational age at birth was determined on the basis of the last menstrual period, ultrasound biometry, or both, and maternal HIV clinical disease severity was classified according to the CDC definition [21]. Antiretroviral treatment was exclusively decided by the treating physician. The study received ethical approval by the committee of the Istituto Nazionale per la Malattie Infettive Lazzaro Spallanzani in Rome (ref. deliberations n. 578/2001 and 7/2003), and all the patients gave written informed consent to both data and sample used for the purposes of the study.

We considered eligible for this study all HIV pregnant women who had completed demographic and clinical information and had available plasma samples collected during the first and the second trimester of pregnancy All the plasma samples of this substudy were collected at the Department of Infectious Diseases and Hepatology in Parma, and sent to the central laboratory at the Istituto Superiore di Sanità (ISS) in Rome for further evaluation. Plasma samples were stored at- 80° C and thawed only at the time of testing.

Microbial translocation markers analysis

Commercially available enzyme-linked immunosorbent assays (ELISA) were used for the analysis of plasma levels of MT and enterocytes damage biomarkers. Soluble CD14 levels (sCD14, Quantikine, R&D Systems, Minneapolis, MN, USA), Intestinal fatty acid-binding protein (I-FABP, Hycult Biotech, Uden, the Netherlands) and Lipopolysaccharide-binding protein, LBP (LBP, Hycult Biotech, Uden, the Netherlands) were measured according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using SPSS software, version 23 (IBM, Somers, NY, USA). Quantitative variables were summarized as medians with interquartile ranges (IQR) and percentages. Differences between groups were evaluated using the χ^2 test or the Fisher's exact test when appropriate for categorical variables, and by the Mann Whitney U test for quantitative variables. Spearman's correlation coefficient was used to evaluate correlations between quantitative variables. The differences found among groups were tested using a linear regression analysis controlling for potential confounding factors, selected according to previous data [22] and to the results of univariate analyses. Differences were considered statistically significant when $p < 0.05$.

Results

Patient characteristics

Forty HIV-positive pregnant women were included in this study. Women were dichotomized on the basis of provenance: African (Group A n = 26, all from Sub Saharan Africa) and European (Group E n = 14, mostly

from Italy). Demographic characteristics are reported in Table 1. The two groups were similar for age, weight, HIV viremia levels and antiretroviral treatment status. HCV coinfection (n = 5) and smoking (n = 4) were observed only in Group E women. Sexual transmission was the route for HIV infection in all Group A women, and for 63.6% of Group E women, who also had a longer time interval since HIV diagnosis (64.0 vs 24.5 months, p = 0.051) and higher levels of CD4 at time of sampling (466 vs. 269 cells/mm^3, p = 0.017).

No cases of AIDS-related complications were recorded during pregnancy; no adverse pregnancy outcomes were recorded, and neonatal gestational age at birth and birth weight were similar in both groups.

Microbial translocation markers analysis

MT markers were analyzed at a median gestational time of 21 weeks (IQR: 16 – 23) when only 5 women (all in Group A p = 0.143) still were not receiving antiretroviral therapy, and 26 (Group A = 76.9%, Group E = 54.5%, p = 0.244) had detectable plasma HIV-RNA. The comparison between the two groups (Figure 1) showed that African women had significantly lower levels of all the biomarkers analyzed: sCD14 (Group A: 1208.0 ng/mL, IQR: 1076 – 1534; Group E: 1885 ng/mL, IQR: 1331 – 1988; p = 0.005, I-FABP (Group A: 358.2 pg/mL, IQR: 262.4 – 454.1; Group E: 573.4 pg/mL, IQR: 479.5 – 780.3, p = 0.002) and LBP (Group A: 25.3 µg/mL, IQR: 20.4 – 31.4; Group E: 28.5 µg/mL, IQR: 26.6 – 33.9, p = 0.050).

Factors associated with MT markers

The levels of MT markers were associated among each other, independent of provenance/ethnicity; LBP levels of pregnant women were significantly correlated with I-FABP levels (r = 0.467, p = 0.004) while their correlation with sCD14 had borderline statistical significance (r = 0.310, p = 0.060). No correlations between markers of MT inflammation and birth weight was recorded.

In order to find possible factors explaining the different levels of MT markers according to provenance/ethnicity, we run a multivariable linear regression analysis; maternal levels of sCD14 remained significantly dependent on origin (p = 0.026) after adjusting for potential confounding factors, including smoking and HCV coinfection. Provenance seemed also to have an impact on I-FABP and LBP levels,

Table 1. Population characteristics. Immuno-virological data refer to the time of sampling, at a median gestational time of 21 weeks. Values are expressed as medians (IQR) or percentages. Differences between groups were evaluated by the Mann Whitney U test for quantitative variables and by the Fisher exact test for categorical variables.

	African	European	P values
N	26	14	
Age (years)	32.0 (24.7 – 34.0)	34.0 (27.0 - 35.0)	0.278
Weight (Kg)	57.2 (55.0 – 80.5)	59.0 (49.8 – 68.8)	0.558
Primiparous (n, %)	19 (76.0%)	8 (57.1%)	0.194
Smokers (n, %)	0 (0%)	4 (28.6%)	**0.011**
HCV coinfection (n, %)	0 (0%)	5 (35.7%)	**0.003**
HBV coinfection (n, %)	7 (26.9%)	3 (21.4%)	1.000
CMV-Ab positive (n, %)	13 (92.9%)	26 (100)	0.341
Route of HIV transmission			
Sexual (n, %)	26 (100%)	7 (63.6%)	**0.005**
Others (n, %)	0 (0%)	4 (36.4%)	
Months from HIV diagnosis	24.5 (1.0 – 54.3)	64.0 (1.0 – 93.5)	0.051
HIV diagnosis during pregnancy (%)	9 (34.6%)	4 (28.6%)	1.000
Antiretroviral-naive at conception	5 (38.5%)	5 (35.7%)	1.000
On ART at conception (n, %)	11 (43.3%)	7 (50%)	0.744
CDC stage: A/B/C (%)	80.8/7.7/11.5	71.4/14.3/14.3	0.602
Viro-immunological data at time of analysis			
In ART at time of analysis (n, %)	21 (80.8%)	0 (100%)	0.143
CD4 (cells/mm^3)	269 (194 – 435)	466 (334- 574)	**0.017**
HIV RNA (log copies/mL)	2.70 (1.75- 4.22)	1.81 (1.69 – 2.50)	0.061
HIV RNA detectable (n, %)	20/26 (76.9%)	6/11 (54.5%)	0.244
Neonatal outcome			
Gestational age at delivery (weeks)	38 (37.0 – 38.0)	37 (36.6 – 38.0)	0.453
Neonate sex (female)	52%	50%	1.000
Neonate birthweight (g)	3010 (2778 – 3205)	2920 (2528 -3138)	0.481
Low birthweight (< 2500g) (n, %)	2/24 (8.3%)	3/12 (25.0%)	0.197

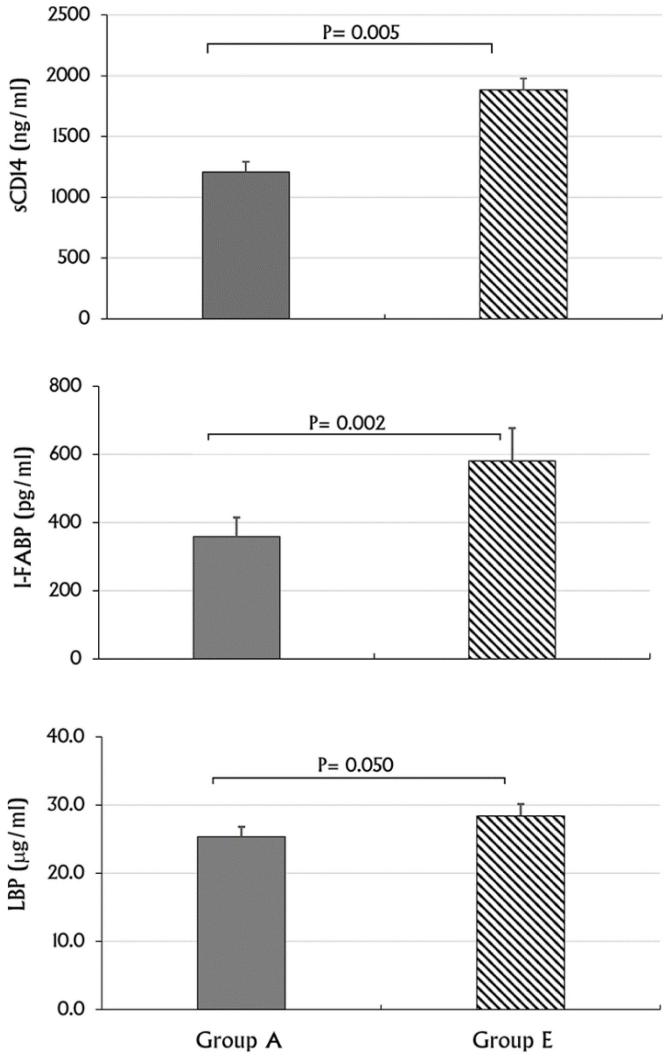
although the association did not reach statistical significance (p = 0.067 and p = 0.087, respectively).

Discussion

Our results highlight the possibility of differences in the levels of microbial translocation markers among populations of different origin but living in the same geographical area. Here we report that HIV pregnant women of African origin had lower MT markers levels when compared with European women of similar age, weight and gestational age at time of sampling.

Although antiretroviral therapy has transformed HIV infection in chronic manageable disease, unbalanced cytokine profile and subclinical inflammatory state persist in HIV patients, even many years after initiation of ART. In this view, the evaluation of biomarkers of MT in HIV patients should be considered important not only as a predictor of disease progression but particularly as a tool to understand the mechanisms underlying pathogenic processes [23]. The characterization of the inflammatory profile of HIV pregnant women is particularly important; elevated levels of sCD14 have been proposed as a risk factor for preterm delivery [16,24] and have been associated to an increased risk of mother-to-child transmission [15]. Moreover, evidence from clinical and epidemiological studies suggests that the high levels of maternal circulating pro-inflammatory cytokines, characteristic of HIV infection, can affect the maternal/fetal unit, interfering with the immunomodulatory factors which shape immune maturation in fetuses [25]. Evidence of immunological abnormalities, including functional immune defects and increased immune activation in HIV exposed uninfected (HEU) children, is widely reported [26]; HEU infants, who in African countries can represent up to 30% of newborns [18], show increased mortality rate and higher vulnerability to infections during the first years of life when compared to their HIV-unexposed counterpart [27]. Although the causes of these immunological disorders in HEU children are probably multifactorial, the severity of maternal disease has been associated with adverse infants' health outcomes [28]. While the evaluation of MT biomarkers as a measure of the degree of immune activation has provided solid evidence in HIV patients from Europe or North America [5,29] it still needs further assessment in patients of African origin. Although many studies conducted in Africa reported higher levels of inflammatory markers in HIV infected individuals with respect to their seronegative counterparts [8,30,31], others found contrasting results; no correlation between HIV progression and sCD14 and LPS levels was found in Ugandan patients, that also had concentrations of sCD14 and LPS similar to those of matched seronegative Afro-American individuals [32]; similarly, a trend toward lower levels of I-FABP in HIV-positive participants compared to HIV-uninfected individuals has been observed in a sub Saharan cohort, suggesting differential relationships among biomarkers of intestinal barrier integrity and innate immune activation [33]. In a recent study, our group found levels of sCD14 lower than expected in Malawian HIV pregnant women prior to ART treatment, but still associated to the immune virological parameters and to low birth weight [17].

Figure 1. Plasmatic levels of soluble CD14 levels (sCD14), Intestinal fatty acid-binding protein (I-FABP) and Lipopolysaccharide-binding protein, (LBP) in HIV+ women during the I-II trimester of pregnancy. Grey bars indicate women of African (Group A) and lined bars indicate women of European provenance. Mann Whitney U test was used for statistical analysis.



The interpretation of these results is difficult since the difference in inflammatory markers could be related to other factors such as hygiene and sanitation levels and/or common exposure to other gastrointestinal infections or parasites. We examined plasma concentration of MT markers in a mixed population of pregnant women living in Italy. During the first two trimesters of pregnancy, MT marker levels did not reflect the immunovirological status of pregnant women, but LBP levels were associated to sCD14 and overall I-FABP levels, supporting the hypothesis that the degree of endotoxinemia can impact on multiple drivers of inflammation. Overall we reported significant lower sCD14, LBP and I-FABP levels among HIV pregnant women of African origin. Although at the time of sampling Group A women had a significantly lower CD4 cell count with respect to group E, the populations were matched for age, gestational time and antiretroviral treatment status. Importantly, the levels of sCD14 remained significantly associated to provenance also after adjusting for factors differently distributed in the two populations, such as smoking status and HCV coinfection, that could affect levels of inflammatory markers in HIV infection [34,35]. The regression analysis also evidenced an association of borderline significance between provenance and levels of I-FABP and LBP, that should be further explored in a larger sample, due to the limited power conferred to the analysis by the small size of the sample examined.

Our results were consistent with those of an analogous study in Belgium, in which significantly lower sCD14 plasma levels but similar LBP levels were observed among HIV patients from Africa compared with a Caucasian population [11].

Both genetic and environmental factors could concur in determining the differences observed; Reiner and colleagues associated the lower levels of sCD14 in population of African origin to a lower expression of CD14 alleles [10], and another group suggested that the reduced plasmatic levels of I-FABP found in an HIV-infected Ugandan cohort could be related to different gut permeability and enterocyte turnover due to environmental adaptation in regions endemic for gut parasites [33]. In addition, the different enteric gut microbiome composition, largely dependent on environmental, nutritional and socioeconomic factors [36], might have an important role in the complex pathogenic processes leading to HIV enteropathy and in the modulation of the degree of microbial translocation [37].

This preliminary study has important limitations, including the relatively small size of the study cohort, and the lack of a control group matched for pregnancy status. We were also unable to evaluate the impact on MT markers of viral subtypes, that might have differed significantly between the two groups, being clade B strains predominant in Europe [38], compared to non-clade B predominance in Africa. Nevertheless, we showed that microbial translocation marker concentrations can be largely influenced by provenance/ethnicity, even in the context of similar environmental conditions. Taking into consideration the high prevalence of HIV infection in African countries, it should be important to consider provenance/ethnicity as a significant confounder in all the studies aimed to evaluate the degree of systemic immune activation in people with HIV.

Acknowledgements

We thank Cosimo Polizzi, Alessandra Mattei and Stefania Donnini for providing technical-secretarial aid for this study, Roberta Amici for laboratory support and Ferdinando Costa and Patrizia Cocco for technical support. We also thank Ernesto Costabile for his precious assistance as documentalist, and we are grateful to all women that participated into the study.

Ethics approval

The general study was approved by the Ethics Committee of the Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani in Rome (ref. deliberation 578/2001). The biomarker levels were evaluated in a subset of women who had given specific additional consent to collection of plasma samples for viroimmunological evaluations within a specific substudy (ref. deliberation 7/2003, same Ethics Committee).

Informed consent

All women provided informed consent to personal data collection before enrolment in the study. Data and plasma samples were collected respecting donor's confidentiality and privacy.

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Conflict of interests: No conflict of interests is declared.