

## CONCISE COMMUNICATION

# sCD14 is not a bona-fide biomarker of microbial translocation in HIV-1-infected Africans living in Belgium

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**Objective:** To compare microbial translocation and its biomarkers in HIV-1-infected African and White patients of the Liège AIDS Reference Center.

**Design:** The study is based on a cross-sectional dataset of HIV-infected patients treated at the Liège AIDS Reference Center. Groups of white and African patients have been randomly selected to be identical for sex, age and duration of treatment.

**Methods:** sCD14, lipopolysaccharide (LPS), lipopolysaccharide-binding protein (LBP) and routine HIV-follow-up parameters were measured on plasma samples.

**Results:** High values of LPS and LBP were observed in both groups of patients without significant difference between them. High values of sCD14 were observed in 53.1% of whites and only in 18.8% of African patients ( $P=0.0042$ ). A correlation between LPS and sCD14 was observed in whites but not African patients.

**Conclusion:** Our observation suggests that factors not related to microbial translocation are responsible for lower sCD14 value in Africans.

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**Keywords:** HIV, immune activation, innate immunity, microbial translocation, soluble CD14

## Introduction

The progression of HIV disease is lead both by viral replication and by immune activation whose intensity is in fact a better predictor of progression to AIDS than virological markers [1].

It is known that HIV infection of the gut-associated lymphoid tissue (GALT) selectively depletes CD4<sup>+</sup> Th17 cells and subsequently impairs intestinal barrier function. Consequently, whole bacteria and microbial products from the intestine enter the systemic circulation, a phenomenon called microbial translocation.

Since years, microbial translocation has been viewed as a possible mechanism underlying the chronic immune activation associated with HIV infection and subsisting despite antiretroviral therapy [2,3].

Microbial translocation is usually evaluated by measuring blood concentration of bacterial lipopolysaccharide (LPS). Nevertheless, measuring LPS concentration is a technically complex process and difficult to implement in routine care. Therefore, there is a need to identify reliable biomarkers of microbial translocation easier to measure than LPS itself.

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**Table 1. Comparison of the two ethnic groups (Whites vs. Africans).**

	White (N=32)	Black African (N=32)	P value <sup>a</sup>
Male sex	17 (53)	15 (47)	0.62
Age (years)	47 ± 13	46 ± 10	0.16
BMI (kg/m <sup>2</sup> )	25.1 ± 1.5	26.5 ± 1.9	0.31
Absolute CD4 <sup>+</sup> (10 <sup>3</sup> cells/μl)	0.67 ± 0.25	0.55 ± 0.24	0.28
Absolute CD8 <sup>+</sup> (10 <sup>3</sup> cells/μl)	0.89 ± 0.44	0.67 ± 0.29	0.10
β2-microglobulin (mg/l)	1.50 ± 3.9	0.27 ± 0.48	0.74
Patients with high viral load (>200 copies/ml)	1 (1.6)	2 (3.1)	0.99 <sup>b</sup>
C-reactive protein (CRP) (mg/l)	5.1 ± 8.7	5.7 ± 10.8	0.69
Total cholesterol (g/l)	2.2 ± 0.4	2.0 ± 0.51	0.58
High-density lipoprotein cholesterol (g/l)	2.8 ± 10.2	2.5 ± 8.1	0.42
Non-high-density lipoprotein cholesterol (g/l)	1.6 ± 0.48	1.4 ± 0.5	0.47
Triglycerides (g/l)	2.2 ± 2.0	1.4 ± 1.8	0.026
Gamma glutamyl transpeptidase (GGT) (U/l)	37.8 ± 24.2	35.7 ± 20.0	0.89
Glutamate-oxaloacetate transaminase (GOT) (U/l)	25.9 ± 9.2	29.4 ± 13.8	0.17
Glutamopyruvate transferase (GPT) (U/l)	29.8 ± 22.3	30.9 ± 29.5	0.39
Alcaline phosphatase (U/l)	93.0 ± 32.5	84.4 ± 34.8	0.44
sCD14 (ng/ml)	1939 ± 440	1756 ± 806	0.0087 <sup>c</sup>
Patients with high sCD14 (≥2000 ng/ml)	17 (53.1)	6 (18.8)	0.0042 <sup>c</sup>
Lipopolysaccharides (LPS) (pg/ml)	22.8 ± 12.3	20.6 ± 11.6	0.53
Patients with high LPS (≥25.0 pg/ml)	14 (43.8)	14 (43.8)	1.00
Lipopolysaccharide-binding protein (LBP) (ng/ml)	1.9 ± 2.2	1.5 ± 1.5	0.43
Patients with high LBP (≥4.1 ng/ml)	5 (15.6)	2 (6.3)	0.43 <sup>b</sup>

<sup>a</sup>Wilcoxon rank-sum test.<sup>b</sup>χ<sup>2</sup> test or Fisher exact test.<sup>c</sup>Indicates in which aspects our populations are different.

CD14 is a coreceptor for LPS along with toll-like receptor-4 and myeloid differentiation factor-2 and is bound to the membrane by a glycosyl phosphatidylinositol anchor. After exposure to bacterial endotoxin, monocytes release soluble CD14 (sCD14) by a protease-dependent shedding of the membrane form [4] but also by direct secretion of the soluble form [5,6]. Hepatocytes also produce sCD14 after LPS exposure by both mechanisms [7]. Accordingly, the increase of sCD14 has been described in Gram<sup>-</sup> bacterial sepsis [8] but also in other conditions associated with microbial translocation such as insulin resistance [9], liver inflammation [10], and cardiovascular diseases [11]. Several studies have also shown increased levels of sCD14 in HIV-infected patients with a good correlation with endotoxin levels [12–14] therefore establishing sCD14 as a potential surrogate marker for microbial translocation. Most importantly, from a nested case–control study performed on patients from the SMART trial, it was demonstrated that sCD14 is an independent marker of mortality [15].

The mechanisms underlying the increase of sCD14 are nevertheless complex as exposure to cytokines such as interleukin 6 (IL-6) and IL-1 β is sufficient to trigger its release [16] suggesting that it is more a marker of monocyte activation than of microbial translocation. It is also known that the exposure to IL-6 releases sCD14 by the liver [17]. Importantly, ethnicity could also be a confounding factor weakening the link between sCD14 and microbial translocation. Reiner *et al.* measured sCD14 levels in more than 5000 non-HIV-infected individuals above 65-year old living in the United States. Interestingly, sCD14 levels were lower in blacks. The authors estimated that genetic factors (i.e. differences in frequencies of CD14

alleles associated with its lower expression) could only explain 23% of this difference [18]. The objective of this study was to confirm these data in a mixed race population of HIV-infected patients living in Belgium.

## Material and methods

The study was based on a sample of 64 chronically HIV-infected patients treated at the Liège AIDS Reference Center. There were 32 whites and 32 black Africans living in Belgium for at least 3 years (mean ± SD, 11.1 ± 8.0 years) and originating from Congo (DRC), Rwanda, Burundi, Cameroun, Ivory Coast, Guinea and Togo. The two ethnic groups were matched by age, sex and treatment. The Ethical Committee of the CHU of Liège approved the study protocol. Consent for participation was obtained in accordance with institutional review board standards.

## Microbial translocation biomarkers

Direct [LPS and lipopolysaccharide-binding protein (LBP)] and indirect (sCD14) markers of microbial translocation were measured. LPS were measured using a Human LPS ELISA kit (Cusabio, Wuhan, China). Samples were measured according to the assay protocol. Samples were stored at –80°C (193.15°K) after centrifugation. All the measurements were performed in the same run. sCD14 was measured by using enzyme-linked immunosorbent assay with the manufacturers' protocol (R&D Systems, Minneapolis, Minnesota, USA) on 100 μl of patient's serum (serum diluted to 0.5%). LBP was quantified with the manufacturers protocol (Cusabio, Wuhan, China).

## Statistical methods

Ethnic groups were compared by the Wilcoxon rank-sum test for quantitative variables and the  $\chi^2$  or Fisher exact test for qualitative findings. The Spearman correlation coefficient was calculated to measure the association between two quantitative variables. Calculations were computed on the maximum of available data, none missing values were replaced. Results were considered as significant at the 5% critical level ( $P < 0.05$ ). The analyses were carried out using SAS (version 9.4; SAS Institute Inc., Cary, North Carolina, USA) and R (version 3.0.3) statistical packages.

## Results

The two ethnic groups were similar for BMI, CD4, CD8, viral load, liver tests and cholesterol (Table 1). Triglycerides levels were higher in whites than in black African patients ( $P = 0.026$ ) and so were sCD14 values ( $1939 \pm 439$  vs.  $1756 \pm 806$  ng/ml,  $P = 0.0087$ ). High values of sCD14 (defined by a concentration higher than 2000 ng/ml) were observed in 53.1% of whites and only in 18.8% of black Africans ( $P = 0.0042$ ) (Fig. 1).

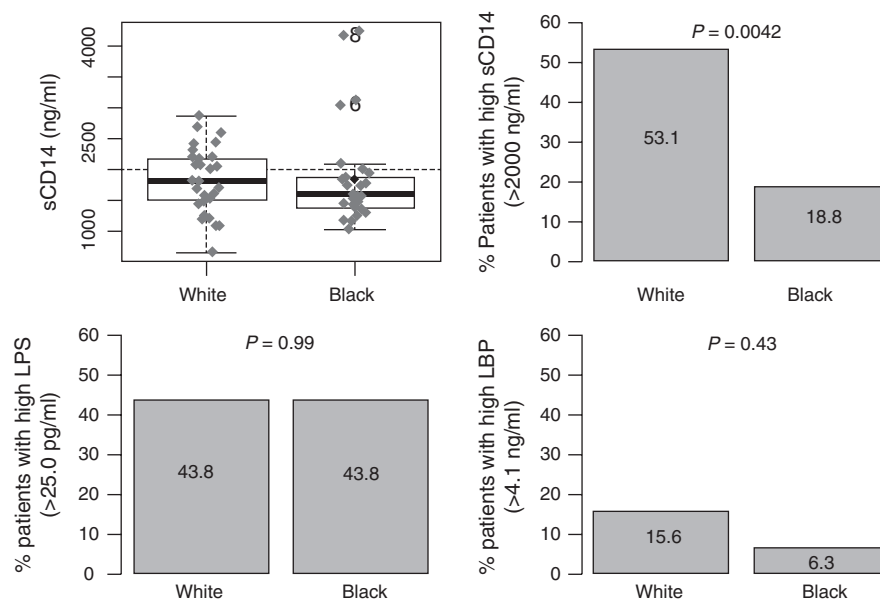
In contrast, LPS concentrations did not differ significantly between the two ethnic groups ( $22.8 \pm 12.3$  vs.  $20.6 \pm 11.6$  pg/ml,  $P = 0.53$ ) and the proportion of patients with concentrations above 25 pg/ml was comparable in both groups. Similarly, no difference was found regarding LBP concentrations ( $1.9 \pm 2.2$  vs.  $1.5 \pm 1.5$  ng/ml,  $P = 0.43$ ) and proportions of patients with values above the cut-off level.

As expected, the correlation between LPS and LBP was strong ( $r = 0.79$ ) and comparable in white ( $r = 0.79$ ) and African patients ( $r = 0.78$ ). Interestingly, the negative association between LPS and sCD14 was weaker in African patients ( $r = -0.23$ ) than in whites ( $r = -0.38$ ).

## Discussion

Microbial translocation is a central mechanism of HIV pathogenesis as it links the tropism of the virus for mucosal CD4<sup>+</sup> T cells to the long-term deleterious immune activation process associated with HIV infection. In addition to its role as a biomarker, sCD14 is also an actor in the inflammatory responses as it sensitizes tissues devoid of membrane CD14 such as endothelium [19] and platelets [20] to the effects of endotoxin. It is therefore logical that sCD14 stands as an independent marker of morbidity and mortality in various inflammatory processes including HIV infection.

In our study, we confirmed that HIV-infected Black Africans living in Belgium have lower levels of sCD14 when compared with a group of whites matched with respect to age, sex and CD4<sup>+</sup> cell count. Different levels of microbial translocation could not explain this difference as endotoxin concentrations and LBP were similar in both groups. A recent study has shown that in the general population above 65, sCD14 level is lower in black Africans, a difference only partially explained by genetics [18]. It is interesting to note that this also holds true in a younger population of HIV-infected individuals and in the context of microbial translocation, similar in



**Fig. 1. Markers of microbial translocation according to ethnicity.** High value of sCD14 (defined by a count  $>2000$  ng/ml) is observed in 53.1% of white and in 18.8% of black ethnicity patients ( $\chi^2$ ,  $P = 0.0042$ ). Proportion of patient with high LPS value cannot be considered as different ( $\chi^2$ ,  $P = 1.00$ ). Proportion of patient with high LBP value cannot be considered as different (Fisher exact,  $P = 0.43$ ). LBP, lipopolysaccharide-binding protein; LPS, lipopolysaccharide.

both groups. Smoking was recently demonstrated to be associated with higher sCD14 levels in HIV-infected individuals [21]. In our study, the proportion of current smokers was indeed higher in Europeans than in black Africans. Different enterotypes, influenced by long-term dietary habits, could also intervene as the composition of intestinal microbiome also influences sCD14 levels in the context of HIV infection [22]. IL-6 is directly involved in the upregulation of sCD14 [16,17]. Interestingly, in untreated HIV-infected patients, IL-6 concentrations are lower in blacks than in whites [23]. As CRP levels were similar in our groups of treated patients, we do not believe that a difference in the concentration of IL-6 could explain our observation. Finally, it is interesting to note that association between LPS and sCD14 concentrations is weaker in the black African patients, confirming that sCD14 is not a direct marker of microbial translocation and that confounding factors, largely influenced by environmental conditions have to be taken into account for the interpretation of abnormal sCD14 concentrations.

## Acknowledgements

### Conflicts of interest

Competing interests statement: The authors declare that they have no competing financial interests.

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