Retraction Notice

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Chronic Immune Activation and Sexual Exposure among HIV Serodiscordant Couples in Senegal

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Abstract

Purpose: In Sub-Saharan Africa, an important proportion of incident HIV cases occur among heterosexual serodiscordant couples (HSDC) but the majority of HIV negative partners can remain seronegative. These are called HIV-exposed seronegative (HESN). We aimed to compare immune activation (IA) levels between HESN, their HIV infected counterparts (HIV+ partners) and HIV unexposed uninfected individuals (HIV-neg Controls) and to evaluate the association between sexual exposure to HIV (SEHIV) and IA. **Methods:** We conducted a cross-sectional study in Dakar, Senegal on 148 participants recruited between November 2013 and February 2014: 40 HIV-neg Controls, 54 HESN and 54 HIV+ Partners. SEHIV was evaluated individually using questionnaires. IA level was measured by plasma level of β_2-microglobulin (β_2m). Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the different associations. **Results:** The median levels of β_2m were 1.57 mg/l (IQR: 1.37 - 1.77), 2.14 mg/l (IQR: 1.76 - 2.43) and 2.24 mg/l (IQR: 1.80 - 3.17) for HIV-neg Controls, HESN and HIV+ partners, respectively. After adjustment, HESN had similar levels of IA with HIV+ partners but significantly higher than that of HIV-neg Controls (adjusted OR = 6.28; 95% CI: [2.19 - 18.00]). The association between IA and SEHIV was evaluated

in the HIV negative individuals. High frequency of SEHIV was associated with a β_2m > 2.2 mg/l (OR = 6.56; 95% CI: [1.71 - 25.21]); significantly more than median cut off value of >1.81 mg/l. **Conclusions:** Our study shows that, despite being uninfected with HIV, HESN individuals show a high level of IA, which was depended on the level of SEHIV.

Keywords

HIV, Serodiscordant Couples, Immune Activation, Sexual Exposure

1. Background

At-risk populations such as people who inject drugs (PWID), sex workers (SW), men who have sex with men (MSM) and HIV serodiscordant couples are highly exposed to HIV infection. However, despite multiple exposures, many of these individuals remain HIV seronegative, they are referred to as HIV-exposed seronegative (HESN). In sub-Saharan Africa, an important proportion of new HIV infections occur among heterosexual serodiscordant couples (HSDC) [1] [2]. These populations are of particular interest to identify markers of susceptibility to HIV or protection against infection. One of these markers may be the level of immune activation (IA), which is known to drive infection [3] [4] [5] [6].

IA corresponds to the activation of the cellular components of the immune system, which results in systemic inflammation. It is usually assessed by the expression of cellular or soluble markers derived from reactions of innate or adaptive immune responses [7] [8]. The role of IA in the pathogenesis of HIV infection has been studied, it was found to be associated with HIV disease progression, increased morbidity and mortality [7] [9] [10]. Mucosal and systemic IA have also been demonstrated in HESN [11], although its role and impact on HIV susceptibility are not elucidated. It is suggested that level of sexual exposure despite being refractory to infection generates IA in HESN [11] [12] [13]. Compared to people living with HIV (PLHIV), similar or lower levels of IA were reported in HESN [14] [15] [16] [17]. Compared to HIV-neg Controls, some studies reported higher levels of IA in HESN [11] [16] [18] [19] while others reported lower levels [20]-[25], To gain further insight into IA in serodiscordant couples, we aimed to compare the levels (using beta 2 microglobulin (β_2m) as a soluble marker of IA) between HESN, their HIV+ counterparts and HIV-neg Controls; then analyze the association of IA levels and sexual exposure to HIV (SEHIV).

2. Methods

2.1. Context

This study was conducted in Senegal, a West African country with a concentrated HIV epidemic: HIV prevalence is low (0.7%) among pregnant women but high in at-risk populations, reaching 9.4% among PWID, 17.8% among MSM

and 18.5% among FSW [26] [27] [28] [29]. Heterosexual serodiscordant couples (HSDC) in Senegal are followed in the same health facilities as other people living with HIV (PLHIV) and a serodiscordant cohort was established from the Fann Teaching hospital. The HIV+ partners of HSDC are systematically put under ART similar to other PLHIV when CD4 cell count become <500/mm^3. They also receive counsel to guard on protected sex and no formal recommendation is given for procreation. After ART initiation, they undergo clinical examinations every two months and undertake biological assessments (including blood cell count, transaminases, creatinine, CD4 cell count and plasma viral load [pVL]) every six months.

2.2. Subjects' Recruitment and Data Collection

HSCD and HIV-neg controls were recruited in a cross-sectional study between November 2013 and February 2014 at the Fann University teaching hospital. In this hospital, there are two national reference centers for the care of PLHIV from where the Serodiscordant Cohort was established: the "Department of infectious and tropical diseases/Regional Research and Training Center for the care of HIV and associated diseases (SMIT/CRCF)" and the "Ambulatory Treatment Center (CTA)". Individuals were eligible if 1) they were aged ≥ 18 years, 2) they were married or living with an HIV serodiscordant partner for ≥six months, 3) one partner was confirmed HIV seropositive; 4) the other partner was confirmed HIV seronegative; 5) everyone signed an informed consent form. A group of HIV negative seroconcordant couples was also recruited as Controls (HIV-neg Controls) in the same facilities. They were recruited consecutively from couples who presented to the lab for HIV testing. They were eligible if: 1) they were aged ≥ 18 years, 2) they were married or living together for more than six months, 3) both partners were confirmed HIV seronegative; 4) everyone signed an informed consent form. The cross-sectional study population therefore constituted three groups: HIV-neg Controls (HIV- seroconcordant couples), HESN (HIV- partners from discordant couples) and HIV+ Partners (HIV+ partners from discordant couples). Questionnaires on sociodemographic characteristics and sexual practices were administered for all study participants accompanied by routine clinical examinations and blood sampling.

The study protocol was approved by the institutional ethical and research review boards of the participating institutions in Senegal: (*Comité national d'éthique pour la recherche en santé* (CNERS) of the Ministry of Health) and in Canada (Research Ethical Board of Sainte-Justine University Hospital, Montreal).

2.3. Sexual Exposure to HIV

SEHIV was represented by the average monthly number of unprotected sexual acts (calculated through the average monthly number of sexual acts and the frequency of condom use). The presence (absence if the number of unprotected sex

with an HIV+ partner per month = 0 and presence if this number was >0) and the frequency (high ≥ 2 per month versus low if <2 per month) of SEHIV were studied as exposure variables.

2.4. Beta-$_2$-Microglobulin, CD4, CD8 and Viral Load Measurement

Plasma levels of β_2m were measured using the integrated automated Abbott Architect ci4100 system (Abbott Laboratories, Wiesbaden, Germany) in accordance with the instructions of the manufacturer; using Quantia β_2m reagents under calibrated conditions. Thawed plasma samples that were collected in EDTA tubes were used for a single time point assessment. The results are expressed in mg/L of β_2m based on the WHO International Standard [30].

CD4+ and CD8+ T cell counts of all participants were determined using the FACSCount System (BD Biosciences). HIV-1 and HIV-2 RNA levels were measured by the Biocentric ultrasensitive RNA viral load assay with a lower detection limit of 50 copies/mL.

2.5. Statistical Analysis

The different characteristics of the study population were described by HIV exposure and infection (*HIV-neg Controls*, *HESN and HIV+ groups*). Comparisons of categorical variables were done using Chi^2 test. The continuous variables were compared between two groups by t-test (all the expected number > 30 or one expected number < 30 with a normal distribution of the variable) or Mann Whitney/Wilcoxon test (one expected number < 30 with a non-normal distribution of the variable). The comparisons of continuous variables between three groups were done using Anova (normal distribution of the variables and equal variances) or Krusskal Wallis test elsewhere.

The association between study groups and β_2m level was analysed by a logistic regression model (cut-off point = median of β_2m was 2.02 mg/L). We estimated crude and adjusted measures of associations (Odd ratios) with 95% confidence intervals (CI). Confounding was controlled using a 10% change in estimate method (variables that change the estimate by ≥|10%| were included in the model) among the following potential confounders: age (18 - 32/33 - 39/40 - 49/50 - 68), sex (male vs female), education level (absence/elementary/high school/university), MRDR-estimated glomerular filtration rate (eGFR) (continuous), presence of comorbidity (yes/no), HBs antigen positivity (yes/no). Age, sex, presence of comorbidity and HBs antigen positivity were tested as effect modifiers. We also used the same methodology to evaluate the association using linear regression.

In the second part, the association between SEHIV and β_2m level was evaluated among HIV susceptible individuals (HIV-neg Controls and HESN). We used different logistic regression models with SEHIV as exposure variable (presence versus absence and high versus low) and β_2m level as outcome variable (cut-off points = median (1.48 mg/L) and third quartile (2.20 mg/L)) including previous potential confounders as well as the practice of anal sex (yes/no), the practice of oral sex (yes or no), the existence of occasional sexual(s) partner(s)

(yes/no) and the HIV status of the partner (HIV- or HIV+); and in the latter case: duration of HIV infection (continuous), WHO clinical stage (1 or 2 or 3 or 4), pVL (<50 or ≥50), history of therapeutic failure (yes or no). Age, sex, practice of anal sex and existence of occasional sexual partners were tested as effect modifiers. We also repeated the analysis using linear regression.

3. Results

3.1. Study Population

Table 1 shows the characteristics of study population: 54 HSDC (108 participants) and 20 HIV negative seroconcordant couples (40 participants). The mean ages of HESN and HIV+ were similar but were higher than those of HIV-neg Controls. Among the HSDC, the male partner was less likely to be HIV+ (40.7% versus 59.3%; p = 0.054). All the HIV+ participants were receiving antiretroviral therapy (ART). Among them, three had experienced therapeutic failure and were switched on second-line therapy.

Figure 1 describes median β_2m levels by study group. The respective β_2m median levels were 1.57 mg/l (IQR = 1.37 - 1.77) for HIV-neg Controls, 2.14 mg/l (IQR = 1.76 - 2.43) for HESN and 2.24 mg/l (IQR = 1.80 - 3.17) for HIV+ (Figure 1). Median β_2m levels were significantly lower in the HIV-neg Controls than both in HESN and HIV+ but were not different between HESN and HIV+. The mean levels (with standard deviation, ±SD) were 1.61 mg/l ± 0.33, 2.18 mg/l ± 0.88 and 3.03 mg/l ± 1.86 for HIV-neg Controls, HESN and HIV+ respectively.

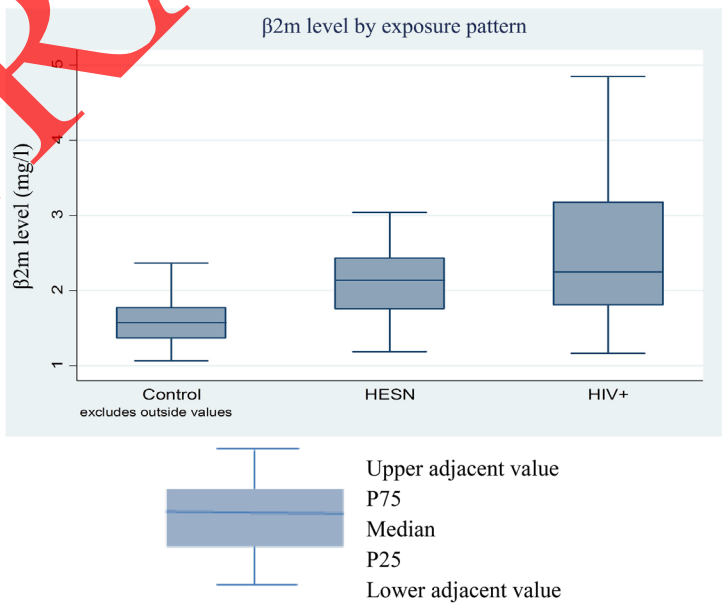

Figure 1. The median values of β_2m by study group: the HIV Negative Controls (n = 40), the Highly Exposed Seronegative (n = 54) and the HIV positive partners (n = 54). The comparisons of the median values were done by Bonferroni test after a significant ANOVA test.

Table 1. Characteristics of the study population by study group (HIV-, HESN and HIV+).

Characteristics	HIV- (N = 40) Median (IQR[a]) or Mean (SD[b]) or %	HESN (N = 54) Median IQR[a]) or Mean (SD[b]) or %	HIV+ (N = 54) Median (IQR[a]) or Mean (SD[b]) or %	p-value
β_2m level (mg/L)	1.57 (1.37 - 1.77)	2.14 (1.76 - 2.43)	2.24 (1.80 - 3.17)	<0.001[d]
Age (years)	34.5 (9.2)	45.8 (10.2)	41.6 (11.1)	<0.001[d]
Education level (%)				0.055[e]
No education	15.0	38.9	40.7	
Elementary	47.5	27.8	33.3	
High school	25.0	29.6	20.4	
University	12.5	3.7	5.6	
MDRD eGFR (ml/mn/1.73m²)	87.3 (76.0)	70.7 (19.9)	67.8 (25.0)	0.102[d]
<60	11.9	16.2	15.9	0.059[e]
HBV infection (%)				0.042[e]
No	75.0	94.0	82.0	
Yes	25.0	6.0	18.0	
Hemoglobin level (g/dL)	12.9 (1.6)	13.2 (1.5)	11.6 (2.3)	<0.001[d]
WBC (10^3/µl)	6.2 (1.3)	5.2 (1.5)	4.5 (1.2)	<0.001[d]
Lymphocytes (10^3/µl)	2.3 (0 .6)	2.1 (0.6)	1.7 (0.6)	<0.001[d]
CD4 counts (cells/µL)	1053.8 (288.6)	929.7 (284.6)	504.1 (276.0)	<0.001[d]
CD8 counts (cells/µL)	617.3 (284.3)	579.4 (304.8)	809.4 (353.2)	<0.001[d]
CD4/CD8 ratio	2.0 (0.9)	1.8 (0.8)	0.7 (0.5)	<0.001[c]
<1	10.00	11.11	78.43	<0.001[d]
Male sex (%)		59.3	40.7	0.034[d]
Time since HIV diagnosis (years)			5.4 (4.7)	
HIV serotype				
HIV-1			90.4	
HIV-2			3.8	
HIV-1 + HIV-2			5.8	
WHO clinical stage				
1			5.6	
2			16.7	
3			11.1	
4			66.7	
ARN VIH < 50 copies/ml			42.5	

[a]Interquartile range; [b]Standard deviation; [c]Krusskal Wallis test; [d]Anova; [e]Chi-square.

3.2. Association of β_2m Level and Study Group

Crude and adjusted odds ratios (OR) and 95% confidence intervals (CI)) for the association between β_2m and study group (determined by comparisons in the study subject categories) are presented in Table 2. The β_2m level was dichotomized with the median value as the cut-off point: >2.02 mg/l or ≤2.02 mg/l.

After adjustment on age and sex, Plasma β_2m was higher in exposed uninfected subjects compared to HIV negative controls but there was no difference in adjusted measures between HESN and HIV+. Comparing HIV-neg Controls, HESN were more likely to present with a β_2m level > 2.02 mg/l (aOR = 6.28; 95% CI: [2.19 - 18.00]); this is the same for HIV+ (aOR = 8.46; 95% CI: [2.90 - 24.69]). The association was not adjusted for CD4+ T cell count and CD4/CD8 ratio due to the fact that they are intermediates variables in HIV+ individuals. None of the following variables was found to modify the effect of study group on β_2m level: age (p = 0.110), sex (p = 0.665), presence of comorbidity (p = 0.435) and HBs antigen carriage (p = 0.996). All these results were similar using linear regression model (Table 3).

3.3. Association of β_2m Level and Sexual Exposure to HIV

Analysis of sexual exposure was done in the 54 HESN and 40 HIV-neg Controls. For the latter, sexual exposure to HIV was considered null; no pVL was detected in either of the HIV-neg participants. In this population, the median value of β_2m was 1.81 mg/l (IQR = 1.48 - 2.20) which is within normal ranges (24). The β_2m level was considered moderate to high if >1.81 mg/l (median) and high if >2.20 mg/l (third quartile). Among the HESN, the average number of unprotected sexual acts per month varied from 0 to 17 with a mean of 2.0 ± 3.5. It was null for the ones who reported 100% use of condom. A proportion of 21.6% reported practicing oral sex, 3.9% reported practicing anal sex and occasional sex was reported by 10%. The duration of HIV infection of the partner varied from six months to 18 years with a mean of 3.0 years ± 4.4. A proportion of 57.5% of the HIV+ partners had detectable pVL and a history of therapeutic failure found in 5.6% of them.

Among the HESN, the β_2m level was higher in case of detectable pVL of the partner (2.14 mg/L in case of detectable pVL of the partner versus 1.79 mg/L in case of pVL of the partner < 50 copies/mL; p = 0.050) but not in case of history of therapeutic failure (2.05 mg/L if the partner had experienced a therapeutic failure versus 2.19 mg/L if the partner did not experience a history of therapeutic failure; p = 0.788) of the partner. Among the 27 HESN individuals with detectable pVL in the partner, there was no statistically significant difference by the level of pVL: 2.27 versus 1.96 mg/L at a cut-off point of 1000 copies/mL (p = 0.961); 2.15 mg/L versus 2.08 mg/L at a cut-off point of 10,000 copies/mL (p = 0.898).

The results of the logistic regressions evaluating the association between a moderate to high β_2m level ($\beta_2$mmh) and SEHIV are presented in Table 4. After ad-

justment, neither the presence (aOR = 1.09; p = 0.895), nor the frequency of SEHIV (aOR = 2.08; p = 0.222) were associated with measure of β_2m mh (Table 4).

However, with the use of a second set of logistic regression models analysing the third quartile of β_2m level as the cut-off point (β_2m level was considered high if >2.2 mg/l); the association between the presence of SEHIV and a β_2m h was statistically significant (aOR = 5.36; p = 0.028) as well as the association between the frequency of SEHIV and a β_2m h (aOR = 6.56; p = 0.006) (Table 5).

The linear regression models found regression coefficients of 0.233 (p = 0.054) and 0.564 (p = 0.003), respectively (Table 6). None of the following variables was found to be an effect modifier: age (p = 0.358), sex (p = 0.603) and existence of occasional sexual partners (p = 0.802).

Table 2. Logistic regression model evaluating the association between β_2m level and study group.

β_2m (>2.025 mg/l)	Univariate Analysis		Multivariate Analysis*	
	N = 145		N = 144	
Study group	Crude OR [95% CI]	P	Adjusted OR [95% CI]	P
HIV-neg Controls	1		1	
HESN	7.40 [2.77 - 19.78]	<0.001	6.27 [2.19 - 18.00]	0.001
HIV+	8.64 [3.19 - 23.43]	<0.001	8.46 [2.90 - 24.69]	<0.001

*multivariate model adjusted for the following confounding variables (using a 10% change in estimate methods): age and sex.

Table 3. Linear regression models evaluating the association between β_2m level (continuous) and study group.

β_2m (mg/l)	Univariate Analysis		Multivariate Analysis*	
	N = 145		N = 144	
Study group	Crude β [95% CI]	P	Adjusted β [95% CI]	P
HIV-neg Controls	1		1	
HESN	0.57 [0.06 - 1.08]	0.029	0.70 [0.14 - 1.26]	0.014
HIV+	1.41 [0.89 - 1.93]	<0.001	1.47 [0.91 - 2.03]	<0.001

*multivariate model adjusted for the following confounding variables (using a 10% change in estimate methods): age and sex.

Table 4. Logistic regression model evaluating the association between a $\beta_2$mmh (moderate to high versus low using the median as the cutting point) and the SEHIV.

$\beta_2$mmh (>1.809 mg/l)	Univariate Analysis		Multivariate Analysis*	
	N = 94		N = 89	
SEHIV	Crude OR [95% CI]	p value	Adjusted OR [95% CI]	p value
No	1		1	
Yes	3.07 [1.16 - 8.12]	0.024	1.09 [0.29 - 4.19]	0.895
Low**	1		1	
High***	2.28 [0.76 - 8.81]	0.141	2.08 [0.64 - 6.75]	0.222

*multivariate model adjusted for the following confounding variables (using a 10% change in estimate methods): age and sex. ** < 2 unprotected sexual acts per month. *** ≥ 2 unprotected sexual acts per month.

Table 5. Logistic regression model evaluating the association between a β_2m h (high versus low using the up quartile as the cutting point) and the SEHIV.

$\beta_2$mmh (>2.2 mg/l)	Univariable Analysis		Multivariable Analysis*	
	N = 94		N = 89	
SEHIV	OR [95% CI]	p value	OR [95% CI]	p value
No	1		1	
Yes	7.13 [2.52 - 20.11]	<0.001	5.36 [1.20 - 23.88]	0.028
Low**	1		1	
High***	4.42 [1.46 - 13.40]	0.009	6.56 [1.71 - 25.21]	0.006

*multivariate model adjusted for the following confounding variables (using a 10% change in estimate methods): age and, sex. ** < 2 unprotected sexual acts per month. *** ≥ 2 unprotected sexual acts per month.

Table 6. Linear regression model evaluating the association between β_2m (continuous) and the SEHIV.

$\beta_2$mmh (>1.809 mg/l)	Univariable Analysis		Multivariable Analysis*	
	N = 94		**N = 89**	
SEHIV	Crude β [95% CI]	p value	Adjusted β [95% CI]	p value
No	1		1	
Yes	0.61 [0.27 - 0.94]	0.001	0.23 [0.0 - 0.47]	0.054
Low**	1		1	
High***	0.61 [0.22 - 1.00]	0.002	0.56 [0.20 - 0.93]	0.003

*multivariate model adjusted for the following confounding variables (using a 10% change in estimate methods): age, sex and HIV status of the partner. ** < 2 unprotected sexual acts per month. *** ≥ 2 unprotected sexual acts per month.

4. Discussion

We conducted a cross-sectional study on 54 HSDC and 20 HIV-negative seroconcordant couples in Senegal. We found that the IA levels (plasma level of β_2m) in HIV+ individuals and HESN were not different but, both were higher than the level observed in HIV unexposed uninfected people. Our study also found an association between exposure to HIV through frequency of unprotected sex with an infected partner and a high level of IA; not a moderate level.

The interpretation of our results should consider several limitations: 1) our study was cross-sectional and such design does not permit to establish the temporal sequence between SEHIV and β_2m level; although SEHIV was evaluated in the six months preceding the measurement of β_2m level; 2) SEHIV was evaluated based on information given by study subjects and they could have under or miss-reported the frequency of unprotected sex, the practice of anal sex and extra-marital sex due to social desirability; 3) in logistic regression models, β_2m levels were primarily categorized using the median levels, which fell in the normal range of β_2m (0.97 - 2.67 mg/L) [31]. However, these potential classification errors were non-differential and would only underestimate the measures of association.

The observation of higher IA in HESN than HIV negative controls and similar to those of HIV+ individuals is consistent with some previous studies such as found in HSDC [11] [18] and PWID [16]. In accordance with our results, some studies also reported that HESN such as HSDC [14], FSW [18], and MSM [19] presented with higher levels of IA than unexposed or low-risk HIV individuals. In an Amsterdam cohort, the pre-seroconversion T CD4 IA level was found lower among highly exposed MSM who remained HIV seronegative compared to their infected counterparts [17]. The similar IA levels in HESN and HIV+ as different from the normal levels in HIV-neg Control individuals was still observed following adjusted analyses that considered age and sex differences although we did not account for specific localised mucosal IA in the comparisons, which could affect IA in African populations [32] [33].

Our observations are however at variance with results showing lower levels of IA in HESN in comparison with HIV+ infected individuals [15] or in comparison with HIV-unexposed or low-risk individuals [20] [21] [22] [23] [24]. These previous studies that showed low IA in HESN did not demonstrate adjusted analyses. The reduced level of IA reported in ESN is related to quiescent status of the immune system that is less activable than in HIV-neg Controls. This phenomenon called "immune quiescence" may contribute to resistance to HIV-infection [25] [34] [35].

In our study, we categorised frequency of exposure by grouping into low exposure and high exposure based on information of a number of unprotected sex/month. Though social inhibition to divulge information about sexual activity is common in this society, we were able to analyse such available data. We found that the frequency of exposure to sexual activity to HIV+ partner (SEHIV) was associated with a high level of IA. This result suggests that there is exposure to the HIV virus and that exposure does impact on immune reaction at a certain level but the negative partner remains seronegative. There are two possible explanations. One one hand, it may be that the study population have high IA level because they are exposed to HIV but are not HIV resistant. The maintenance of an HIV seronegative status may be related to their exposure levels being low enough to allow for maintained seronegativity by chance as the per act chance of seroconversion is in the range of 3/1000. On the other hand, one proportion of the study population with low IA level irrespective of SEHIV was "immune quiescent" while the other with high IA level associated with SEHIV is at-risk of HIV infection. It will require a long term follow up to analyse if these individuals will succumb to break-through infection or that the IA is the triggered immune mechanism that protects against infection. Different studies found a relation between IA and different measures of sexual exposure to HIV: a previous report in Senegal, showed a negative correlation between CD4+/CD38+ T cell frequency and condom use among HESN [22] that did not account for duration of sexual relationship or the frequency of sex. This is similar to other reports that linked virus exposure in HIV negative individuals in various populations and

showed presence of HIV specific immune responses [11] [12] [14] [15]. However, some limitations of these studies were the use of an unreliable measurement of SEHIV, the low samples' sizes and the lack of adjustment on potential confounders.

Relationship between SEHIV and immune response or activation in the uninfected persons is difficult to interpret. It may require the assessment of several time point follow up of both HIV+ specific cellular responses and IA markers. Many studies showed the role of HIV+ specific responses in exposed uninfected individuals on account of triggered IA in sex workers [14] [33] [36], and MSM [37], but HIV correlates of protection still remain elusive although a growing body of evidence is supporting the phenomenon of immune quiescence [25] [34] [35].

It has been reported that IA is possible in HESN in HSDC even in the case of viral control in the HIV+ partner [15]. Though all of our HIV+ patients were under ART for viral suppression, the majority had a detectable pVL and the IA level was high in presence of viral replication of the partner. It would have been interesting to evaluate the adjusted association between pVL and IA but we were not enough powered to do it although such an association has been established by several studies [12] [15]. To what level plasma IA markers is influenced by the presence of viral load in positive partners will continue to generate interest in the context of frequencies of breakthrough infections in HESN vis-à-vis protection from infection. Understanding the influence of IA in the context of viral load may aid the identification of at-risk HIV uninfected population of potential breakthrough infection and therefore call for more preventive measures.

Moreover, from a public health perspective, it is important to identify most at risk HESN based on epidemiological characteristics in the absence of sophisticated biological parameters. These latter might not be available in several cases including occasional sex with an HIV+ partner, insufficiency of resources as it is frequently the case in some poor settings.

Our assessment of IA in HESN, though exploratory has been aided by the good sample size from our cohort and patient data that allowed adjustments for confounders in the statistical analyses. Since SEHIV is the main risk factor for HIV transmission in HSDC and IA is critical in the susceptibility to HIV infection [22] [38], it was important to explore the association between SEHIV and IA. This was our objective and our results indicate an association between SEHIV and a high level of IA.

In our study, HESN presented with a median level of β_2m similar to that of HIV+ partners but higher than that of HIV-neg controls. The level of β_2m in HIV-neg persons appears to be associated with SEHIV and the relationship seems to mirror the one between IA and HIV acquisition. However, our study was exploratory and the results must be interpreted with caution. Despite these limitations and in accordance with existing literature, our study seems to indicate two profiles of HESN: those with an immune quiescent status and those

without immune quiescence status who presented different relationship with SEHIV. In order to better understand such mechanisms and their relationship with SEHIV, the differences between these two profiles must be more studied. A longitudinal study with a deep analysis of the dynamics of SEHIV and IA would be necessary for this purpose.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Dunkle, K.L., Stephenson, R., Karita, E., Chomba, E., Kayitenkore, K., Vwalika, C., *et al.* (2008) New Heterosexually Transmitted HIV Infections in Married or Cohabiting Couples in Urban Zambia and Rwanda: An Analysis of Survey and Clinical Data. *The Lancet*, **371**, 2183-2191. https://doi.org/10.1016/S0140-6736(08)60953-8

[2] Chemaitelly, H., Cremin, I., Shelton, J., Hallett, T.B. and Abu-Raddad, L.J. (2012) Distinct HIV Discordancy Patterns by Epidemic Size in Stable Sexual Partnerships in Sub-Saharan Africa. *Sexually Transmitted Infections*, **88**, 51-57. https://doi.org/10.1136/sextrans-2011-050114

[3] Lawn, S.D., Butera, S.T. and Folks, T.M. (2001) Contribution of Immune Activation to the Pathogenesis and Transmission of Human Immunodeficiency Virus Type 1

Infection. *Clinical Microbiology Reviews*, **14**, 753-777.
https://doi.org/10.1128/CMR.14.4.753-777.2001

[4] Stevenson, M., Stanwick, T.L., Dempsey, M.P. and Lamonica, C.A. (1990) HIV-1 Replication Is Controlled at the Level of T Cell Activation and Proviral Integration. *The EMBO Journal*, **9**, 1551-1560.
https://doi.org/10.1002/j.1460-2075.1990.tb08274.x

[5] Stanley, S.K., Ostrowski, M.A., Justement, J.S., Gantt, K., Hedayati, S., Mannix, M., *et al.* (1996) Effect of Immunization with a Common Recall Antigen on Viral Expression in Patients Infected with Human Immunodeficiency Virus Type 1. *The New England Journal of Medicine*, **334**, 1222-1230.
https://doi.org/10.1056/NEJM199605093341903

[6] Shapira-Nahor, O., Kalinkovich, A., Weisman, Z., Greenberg, Z., Nahmias, J., Shapiro, M., *et al.* (1998) Increased Susceptibility to HIV-1 Infection of Peripheral Blood Mononuclear Cells from Chronically Immune-Activated Individuals. *AIDS*, **12**, 1731-1733.

[7] Hsu, D.C. and Sereti, I. (2014) Chronic Immune Activation in HIV. In: Hope, T.J., Stevenson, M. and Richman, D., Eds., *Encyclopedia of AIDS*, Springer, New York, 1-10. https://doi.org/10.1007/978-1-4614-9610-6_178-1

[8] Siewe, B. and Landay, A. (2015) Cellular and Soluble Immune Activation Markers in HIV-Infected Subjects. In: Hope, T.J., Stevenson, M. and Richman, D., Eds., *Encyclopedia of AIDS*, Springer, New York, 1-8.
https://doi.org/10.1007/978-1-4614-9610-6_192-1

[9] Sereti, I. and Altfeld, M. (2016) Immune Activation and HIV: An Enduring Relationship. *Current Opinion in HIV and AIDS*, **11**, 129-130.
https://doi.org/10.1097/COH.0000000000000244

[10] Utay, N.S. and Hunt, P.W. (2016) Role of Immune Activation in Progression to AIDS. *Current Opinion in HIV and AIDS*, **11**, 131-137.
https://doi.org/10.1097/COH.0000000000000242

[11] Biasin, M., Caputo, S.L., Speciale, L., Colombo, F., Racioppi, L., Zagliani, A., *et al.* (2000) Mucosal and Systemic Immune Activation Is Present in Human Immunodeficiency Virus-Exposed Seronegative Women. *The Journal of Infectious Diseases*, **182**, 1365-1374. https://doi.org/10.1086/315873

[12] Suy, A., Castro, P., Nomdedeu, M., García, F., López, A., Fumero, E., *et al.* (2007) Immunological Profile of Heterosexual Highly HIV-Exposed Uninfected Individuals: Predominant Role of CD4 and CD8 T-Cell Activation. *The Journal of Infectious Diseases*, **196**, 1191-1201. https://doi.org/10.1086/521193

[13] Prodger, J.L., Hirbod, T., Kigozi, G., Nalugoda, F., Reynolds, S.J., Galiwango, R., *et al.* (2014) Immune Correlates of HIV Exposure without Infection in Foreskins of Men from Rakai, Uganda. *Mucosal Immunology*, **7**, 634-644.
https://doi.org/10.1038/mi.2013.83

[14] Restrepo, C., Rallón, N.I., del Romero, J., Rodríguez, C., Hernando, V., López, M., *et al.* (2010) Low-Level Exposure to HIV Induces Virus-Specific T Cell Responses and Immune Activation in Exposed HIV-Seronegative Individuals. *The Journal of Immunology*, **185**, 982-989. https://doi.org/10.4049/jimmunol.1000221

[15] Kebba, A., Kaleebu, P., Serwanga, J., Rowland, S., Yirrell, D., Downing, R., *et al.* (2004) HIV Type 1 Antigen-Responsive CD4+ T-Lymphocytes in Exposed Yet HIV Type 1 Seronegative Ugandans. *AIDS Research and Human Retroviruses*, **20**, 67-75.
https://doi.org/10.1089/088922204322749512

[16] Tran, H.K., Chartier, L., Troung, L.X., Nguyen, N.N., Fontanet, A., Barré-Sinoussi,

F.E., *et al.* (2006) Systemic Immune Activation in HIV-1-Exposed Uninfected Vietnamese Intravascular Drug Users. *AIDS Research and Human Retroviruses*, **22**, 255-261. https://doi.org/10.1089/aid.2006.22.255

[17] Koning, F.A., Otto, S.A., Hazenberg, M.D., Dekker, L., Prins, M., Miedema, F., *et al.* (2005) Low-Level CD4+ T Cell Activation Is Associated with Low Susceptibility to HIV-1 Infection. *The Journal of Immunology*, **175**, 6117-6122. https://doi.org/10.4049/jimmunol.175.9.6117

[18] Jennes, W., Sawadogo, S., Koblavi-Dème, S., Vuylsteke, B., Maurice, C., Roels, T.H., *et al.* (2003) Cellular Human Immunodeficiency Virus (HIV)-Protective Factors: A Comparison of HIV-Exposed Seronegative Female Sex Workers and Female Blood Donors in Abidjan, Côte d'Ivoire. *The Journal of Infectious Diseases*, **187**, 206-214. https://doi.org/10.1086/346049

[19] Palmer, C.D., Tomassilli, J., Sirignano, M., Romero-Tejeda, M., Arnold, K.B., Che, D., *et al.* (2014) Enhanced Immune Activation Linked to Endotoxemia in HIV-1 Seronegative MSM. *AIDS*, **28**, 2162-2166. https://doi.org/10.1097/QAD.0000000000000386

[20] Card, C.M., McLaren, P.J., Wachihi, C., Kimani, J., Plummer, F.A. and Fowke, K.R. (2009) Decreased Immune Activation in Resistance to HIV-1 Infection Is Associated with an Elevated Frequency of CD4(+)CD25(+)FOXP3(+) Regulatory T Cells. *The Journal of Infectious Diseases*, **199**, 1318-1322. https://doi.org/10.1086/597801

[21] Bégaud, E., Chartier, L., Marechal, V., Ipero, J., Léal, J., Versmisse, P., *et al.* (2006) Reduced CD4 T Cell Activation and *in Vitro* Susceptibility to HIV-1 Infection in Exposed Uninfected Central Africans. *Retrovirology*, **3**, 35. https://doi.org/10.1186/1742-4690-3-35

[22] Camara, M., Dieye, T.N., Seydi, M., Diallo, A.A., Fall, M., Diaw, P.A., *et al.* (2010) Low-Level CD4+ T Cell Activation in HIV-Exposed Seronegative Subjects: Influence of Gender and Condom Use. *The Journal of Infectious Diseases*, **201**, 835-842. https://doi.org/10.1086/651000

[23] Jennes, W., Evertse, D., Borget, M.Y., Vuylsteke, B., Maurice, C., Nkengasong, J.N., *et al.* (2006) Suppressed Cellular Alloimmune Responses in HIV-Exposed Seronegative Female Sex Workers. *Clinical & Experimental Immunology*, **143**, 435-444. https://doi.org/10.1111/j.1365-2249.2006.03017.x

[24] Songok, E.M., Luo, M., Liang, B., Mclaren, P., Kaefer, N., Apidi, W., *et al.* (2012) Microarray Analysis of HIV Resistant Female Sex Workers Reveal a Gene Expression Signature Pattern Reminiscent of a Lowered Immune Activation State. *PLoS ONE*, **7**, e30048. https://doi.org/10.1371/journal.pone.0030048

[25] McLaren, P.J., Ball, T.B., Wachihi, C., Jaoko, W., Kelvin, D.J., Danesh, A., *et al.* (2010) HIV-Exposed Seronegative Commercial Sex Workers Show a Quiescent Phenotype in the $CD4^+$ T Cell Compartment and Reduced Expression of HIV-Dependent Host Factors. *The Journal of Infectious Diseases*, **202**, S339-S344. https://doi.org/10.1086/655968

[26] National Agency of Statistics and Demography of Senegal (ANSD) (2012) Multiple Indicator Demographic and Health Surveys in Senegal (EDS-MICS) 2010-2011. Dakar.

[27] MSM (2014) Report Combined Survey of Men Who Have Sex with Men.

[28] Wade, A.S., Larmarange, J., Diop, A.K., Diop, O., Gueye, K., Marra, A., *et al.* (2010) Reduction in Risk-Taking Behaviors among MSM in Senegal between 2004 and 2007 and Prevalence of HIV and Other STIs. ELIHoS Project, ANRS 12139. *AIDS Care*, **22**, 409-414. https://doi.org/10.1080/09540120903253973

[29] Leprêtre, A., Ba, I., Lacombe, K., Maynart, M., Toufik, A., Ndiaye, O., *et al.* (2015) Prevalence and Behavioural Risks for HIV and HCV Infections in a Population of Drug Users of Dakar, Senegal: The ANRS 12243 UDSEN Study. *Journal of the International AIDS Society*, **18**, 19888. https://doi.org/10.7448/IAS.18.1.19888

[30] Who International Laboratory for Biological Standards (1996) The International Standard for Beta 2 Microglobulin. WHO, Medicine and Healthcare Products Regalatory Agency, Geneva.

[31] Forman, D.T. (1987) Methods in Clinical Chemistry.

[32] Cohen, C.R., Moscicki, A.B., Scott, M.E., Ma, Y., Shiboski, S., Bukusi, E., *et al.* (2010) Increased Levels of Immune Activation in the Genital Tract of Healthy Young Women from Sub-Saharan Africa. *AIDS*, **24**, 2069-2074. https://doi.org/10.1097/QAD.0b013e32833c323b

[33] Abdulhaqq, S.A., Zorrilla, C., Kang, G., Yin, X., Tamayo, V., Seaton, K.E., *et al.* (2016) HIV-1-Negative Female Sex Workers Sustain High Cervical IFNε, Low Immune Activation, and Low Expression of HIV-1-Required Host Genes. *Mucosal Immunology*, **9**, 1027-1038. https://doi.org/10.1038/mi.2015.116

[34] Card, C.M., Ball, T.B. and Fowke, K.R. (2013) Immune Quiescence: A Model of Protection against HIV Infection. *Retrovirology*, **10**, 141. https://doi.org/10.1186/1742-4690-10-141

[35] Fulcher, J.A., Romas, L., Hoffman, J.C., Elliott, J., Saunders, T., Burgener, A.D., *et al.* (2017) Highly Human Immunodeficiency Virus-Exposed Seronegative Men Have Lower Mucosal Innate Immune Reactivity. *AIDS Research and Human Retroviruses*, **33**, 788-795. https://doi.org/10.1089/aid.2017.0014

[36] Rowland-Jones, S., Sutton, J., Ariyoshi, K., Dong, T., Gotch, F., McAdam, S., *et al.* (1995) HIV-Specific Cytotoxic T-Cells in HIV-Exposed But Uninfected Gambian Women. *Nature Medicine*, **1**, 59-64. https://doi.org/10.1038/nm0195-59

[37] Erickson, A.L., Willberg, C.B., McMahan, V., Liu, A., Buchbinder, S.P., Grohskopf, L.A., *et al.* (2008) Potentially Exposed But Uninfected Individuals Produce Cytotoxic and Polyfunctional Human Immunodeficiency Virus Type 1-Specific CD8(+) T-Cell Responses Which Can Be Defined to the Epitope Level. *Clinical and Vaccine Immunology*, **15**, 1745-1748. https://doi.org/10.1128/CVI.00247-08

[38] Pedraza, M.A., del Romero, J., Roldán, F., García, S., Ayerbe, M.C., Noriega, A.R., *et al.* (1999) Heterosexual Transmission of HIV-1 Is Associated with High Plasma Viral Load Levels and a Positive Viral Isolation in the Infected Partner. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, **21**, 120-125.