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.

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