

# Association of Soluble CD14 and Inflammatory Biomarkers With HIV-2 Disease Progression

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**Background.** Human immunodeficiency virus type 2 (HIV-2) infection is characterized by a slower progression than HIV type 1. It is not known whether markers of inflammation such as high-sensitivity C-reactive protein (hsCRP), interleukin 6 (IL-6), and soluble CD14 (sCD14) may predict disease progression among HIV-2 patients.

**Methods.** We performed longitudinal retrospective analysis using 384 samples from 71 patients included in the HIV-2 French cohort ANRS CO5 and followed for a median of 8 years. Baseline was the time of the first available measurement. Disease progression was defined by the occurrence of death, Centers for Disease Control and Prevention B/C stage HIV-related event, drop in CD4 <350 cells/μL, and HIV-2 RNA detection. Cox regression models and mixed models were used for statistical analyses.

**Results.** At baseline, 75% of patients were asymptomatic, 34% were treated; 30% had detectable HIV-2 RNA load, and median CD4 cell count was 415/μL. The 3 biomarkers were positively related to each other. In adjusted analyses, sCD14 was the main factor explaining variation of hsCRP and IL-6 ( $P < .001$ ). Lower CD4, older age, and advanced clinical stage were associated with higher sCD14. The biomarkers were correlated with HIV-2 RNA in unadjusted analyses only. Patients with baseline levels above either the median values (hsCRP = 1.38 mg/L; IL-6 = 1.97 pg/mL) or the highest quartile (sCD14 = 1.74 μg/mL) had a higher risk of disease progression (all  $P < .003$ ). After adjustment for CD4 count, only sCD14 remained significantly associated with disease progression (hazard ratio, 3.59;  $P = .004$ ).

**Conclusions.** In this cohort of HIV-2-infected patients, sCD14 represents a better predictive biomarker of disease progression than hsCRP or IL-6, independent of CD4.

Human immunodeficiency virus (HIV) type 2 (HIV-2) is known to be less pathogenic and far less prevalent than HIV type 1 (HIV-1) [1–4]. However, it

affects a substantial number of patients, mainly in West Africa but also in Europe, India, and the United States [5]. HIV-2 infection is characterized by a slow disease progression [6, 7]. The clinical events documented among these patients are various and not always classified as AIDS-related [8, 9]. In HIV-1-infected patients treated by highly active antiretroviral therapy, morbidity has shifted from a majority of AIDS-defining events toward other clinical events such as cardiovascular diseases or cancers [10, 11]. Virus-associated inflammation, immune activation, and immunosenescence together with antiretroviral therapy are potential factors in the occurrence of this

Received 15 April 2012; accepted 9 August 2012.; electronically published 20 August 2012.

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**Clinical Infectious Diseases** 2012;55(10):1417–25

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DOI: 10.1093/cid/cis708

non-AIDS morbidity [12]. Recently, several studies have underlined the prognostic value of certain inflammatory biomarkers in HIV-1-infected patients [13–22], especially interleukin 6 (IL-6), high-sensitivity C-reactive protein (hsCRP), and soluble CD14 (sCD14), that are quantifiable using standard assays. IL-6 is a proinflammatory cytokine secreted in particular by lymphocyte T cells and macrophages to stimulate immune response during infection and after trauma such as tissue damage. High-sensitivity CRP is an acute-phase protein synthesized by the liver in response to IL-6 and secreted in the blood, the levels of which rise in response to inflammation. Soluble CD14 serves as a biomarker of monocyte/macrophage activation and could indirectly reflect the mechanism of microbial translocation, known to play a major role in chronic immune activation. More specifically, CD14<sup>+</sup> monocytes that bind on CD14 lipopolysaccharide (LPS), a major component of bacterial cell walls reflecting microbial translocation, are activated and secrete sCD14 [23].

In HIV-2-infected patients, data on inflammatory and activation markers are sparse [24]. Furthermore, reported predictive markers are most often limited to CD4 cell count and HIV load [8, 24–26]. Finding additional prognostic markers may help to understand the pathophysiology of the infection and could be useful for the clinical management of infected patients [27]. We hypothesized that inflammatory processes may also play an important role in HIV-2 disease progression. Thus, on the basis of studies that established the relationship between IL-6, hsCRP, and sCD14 plasma levels and HIV-1 disease progression, we assessed plasma levels of these biomarkers in patients included in the Agence Nationale de Recherches sur le SIDA et les hépatites virales (ANRS) HIV-2 CO5 cohort to determine if an inflammatory response at a given time is associated with disease progression later on in HIV-2-infected patients.

## PATIENTS AND METHODS

This is a longitudinal retrospective analysis using 384 ethylenediaminetetraacetic acid (EDTA) plasma samples from 71 patients with available stored samples in the Bichat Hospital Virology laboratory and included in the HIV-2 ANRS CO5 French cohort. The cohort, which was extensively described elsewhere [28], is an open national prospective study initiated in 1994 and currently ongoing in 111 clinical centers in France. Inclusion criteria into the cohort are HIV-2 infection only, age  $\geq 18$  years, intended residence in France for at least 1 year, and informed consent available. Baseline was defined as the measurement on the first available sample.

HIV-2 RNA quantification was determined by an in-house assay with a detection limit of 250 copies/mL until 2005 [29], and 100 copies/mL afterward [30]. Levels of 3 inflammatory

biomarkers were measured in all 384 samples. High-sensitivity CRP (Human C-Reactive Protein/CRP Quantikine ELISA Kit, R&D Systems), high-sensitivity IL-6 (Human IL-6 Quantikine High Sensitivity ELISA Kit, R&D Systems), and sCD14 (Human sCD14 Quantikine ELISA Kit, R&D Systems), were measured by commercial enzyme-linked immunosorbent assay (ELISA). Lower levels of detection were 0.010  $\mu\text{g/mL}$ , 0.039  $\text{pg/mL}$ , and 0.125  $\mu\text{g/mL}$  for hsCRP, IL-6, and sCD14, respectively. In the non-HIV-infected general population aged <45 years, hsCRP values were between 0.53  $\mu\text{g/mL}$  and 3.78  $\mu\text{g/mL}$  and IL-6 values between 0.80  $\text{pg/mL}$  and 2.07  $\text{pg/mL}$  [15]. No normal values were currently defined for sCD14. Disease progression was defined by the occurrence of at least 1 of the following events during follow-up: (1) death; (2) Centers for Disease Control and Prevention (CDC) stage B or C HIV-related clinical event; (3) drop below 350 CD4 cells/ $\mu\text{L}$ ; (4) increase in plasma HIV-2 RNA level >250 copies/mL (confirmed with at least 1 additional measurement), according to the French national guidelines [27]. Progression was defined as the occurrence of any incident event, regardless of the baseline clinical and biological stage.

Time to disease progression was evaluated from the first available measurement of inflammatory biomarkers (baseline) to the first occurrence of 1 or several above-mentioned endpoints. The association of factors measured at baseline with disease progression was assessed by Kaplan-Meier estimates and log-rank tests. Adjusted analyses were performed for each inflammatory marker separately with Cox models using a forward selection strategy. Linearity of the effects was checked by categorizing the continuous explanatory variables. Quartile cutoff points were defined according to the distribution of biomarkers. For CD4 count, we looked at the threshold leading to the best model among 200, 350, and 500 cells/ $\mu\text{L}$ . For HIV-2 RNA level we used the threshold of 4  $\log_{10}$  copies/mL according to previous analyses done in the cohort [31]. Correlations between repeated measurements of inflammatory biomarkers were assessed using bivariate linear mixed models [32] imputing half of the threshold for undetectable HIV-2 RNA. Model assumptions were checked graphically. All statistical analyses were performed with SAS software, version 9.1.3 (SAS Institute, Cary, North Carolina).

## RESULTS

### Baseline Characteristics

Patient characteristics are shown in Table 1: overall 56% were women, 73% were from West Africa, and 89% were infected through heterosexual contacts. At baseline, 75% of patients were asymptomatic, 34% were treated, 30% had detectable HIV-2 RNA load with a median of 6897 copies/mL (interquartile range [IQR], 530–15 000 copies/mL), and the

**Table 1. Characteristics of Patients at First Time Point in the Study (N = 71)**

Characteristic		
Age <sup>a</sup> , years, median (IQR)	38	(31–44)
Female <sup>a</sup> , No. (%)	40	56
Region of origin <sup>a</sup>		
France	11	16
West Africa	51	73
Other	8	11
Mode of transmission <sup>a</sup>		
Heterosexual contact	63	89
Homosexual contact	1	1
Blood transfusion	3	4
Unknown	3	4
Positive HBsAg <sup>b</sup> , No. (%)	3	4
Positive anti-HCV Ab <sup>c</sup> , No. (%)	1	1
Time since HIV infection diagnosis <sup>d</sup> , years, median (IQR)	2.0	(0.5–5.8)
CDC clinical stage <sup>e</sup> , No. (%)		
A	53	(75)
B	6	(8)
C	10	(14)
Antiretroviral treatment <sup>a</sup> , No. (%)		
Yes	24	(34)
No	46	(65)
HIV-2 RNA load >250 copies/mL <sup>f</sup> , No. (%)		
Yes	21	(30)
No	41	(58)
HIV-2 RNA load, median copies/mL (IQR) in patients with detectable viremia (>250 copies/mL; n = 21)	6897	(530–15,000)
CD4 cell count <sup>g</sup> , median/μL (IQR)	415	(224–605)
CD8 cell count <sup>h</sup> , median/μL (IQR)	731	(558–881)
CD4/CD8 ratio <sup>h</sup>	0.6	(0.4–1.0)

All data are No. (%) unless otherwise specified.

Abbreviations: CDC, Centers for Disease Control and Prevention; HBsAg, hepatitis B surface antigen; HCV Ab, hepatitis C antibody; HIV, human immunodeficiency virus; IQR, interquartile range.

<sup>a</sup> Missing data: n = 1.

<sup>b</sup> Missing data: n = 4.

<sup>c</sup> Missing data: n = 6.

<sup>d</sup> Missing data: n = 5.

<sup>e</sup> Missing data: n = 2.

<sup>f</sup> Missing data: n = 9.

<sup>g</sup> Missing data: n = 9.

<sup>h</sup> Missing data: n = 21.

median CD4 cell count was 415/μL (IQR, 224–605 cells/μL). The median duration between HIV infection diagnosis and study baseline was 2 years (IQR, 1–6 years). The median duration of follow-up was 8 years (IQR, 4–11 years). During the study period, 18 patients (25%) initiated an antiretroviral treatment.

Compared with the other 678 patients currently included in the French ANRS CO5 HIV-2 cohort, the 71 patients included in this study had similar clinical and biological characteristics with the exception of age. Participants were younger than the CO5 cohort's HIV-2-infected patients not included in this study (37 vs 40 years,  $P = .004$ ).

#### Distribution of Plasma Levels of hsCRP, IL-6, and sCD14 at Baseline

Among the 71 study samples measured at baseline, median hsCRP, IL-6, and sCD14 levels were 1.38 μg/mL (IQR, 0.55–3.80), 1.97 pg/mL (IQR, 1.12–3.44), and 1.49 μg/mL (IQR, 1.19–1.74), respectively (Table 2). The levels of the biomarkers did not differ according to sex, CDC clinical stage, or CD8 cell count. Soluble CD14 levels were higher in older patients ( $P = .016$ ), in patients with lower CD4 cell count ( $P = .001$ ), and in antiretroviral-treated patients ( $P = .016$ ). IL-6 levels were higher in patients with lower CD4 cell count ( $P = .017$ ) and in antiretroviral-treated patients ( $P = .012$ ). The hsCRP levels were not significantly associated with baseline characteristics (Table 2).

#### Correlation Between Inflammatory, Immunological, and Virological Markers During Follow-up

A median number of 5 samples per patient (IQR, 2–8) drawn during the study period from 1992 to 2010 were available. Analysis of all 384 study samples showed that the 3 biomarkers were positively correlated with each other: hsCRP with IL-6 ( $r = 0.77$ ,  $P = .0003$ ), hsCRP with sCD14 ( $r = 0.35$ ,  $P = .026$ ), and IL-6 with sCD14 ( $r = 0.67$ ,  $P = .0005$ ).

High-sensitivity CRP plasma levels were not correlated with CD4 cell count ( $r = -0.05$ ,  $P = .74$ ), but IL-6 and sCD14 levels were negatively correlated with CD4 cell count ( $r = -0.57$ ,  $P = .002$ ; and  $r = -0.62$ ,  $P \leq 10^{-4}$ , respectively; Figure 1). In adjusted analyses using repeated measurements, higher sCD14 levels were independently associated with lower CD4 cell count ( $P = .0005$ ), older age ( $P = .04$ ), and advanced CDC B/C clinical stage ( $P = .025$ ). Higher hsCRP and higher IL-6 levels were associated with higher sCD14 only (both  $P < .0001$ ). High-sensitivity CRP, IL-6, and sCD14 were slightly correlated with HIV-2 RNA level (Figure 2;  $r = 0.34$ ,  $P = .05$ ;  $r = 0.37$ ,  $P \leq .05$ ; and  $r = 0.67$ ,  $P = .01$ , respectively), but this association was not significant after adjustment for at least 1 additional factor among sex, age, clinical stage, CD4, CD8, and antiretroviral treatment.

#### HIV-2 Disease Progression According to Plasma Biomarker Levels at Baseline

During follow-up, 41 events were observed among 30 patients who experienced disease progression. The incidence of disease progression was 7.28 (95% confidence interval [CI], 5.05–9.51) per 100 person-years. Disease progression events were

**Table 2. Distribution of the Inflammatory Biomarkers According to Baseline Characteristics**

	No.	hsCRP ( $\mu\text{g/mL}$ ), Median (IQR)	IL-6 ( $\text{pg/mL}$ ), Median (IQR)	sCD14 ( $\mu\text{g/mL}$ ), Median (IQR)
All patients	71	1.4 (0.6–3.8)	2.0 (1.1–3.4)	1.5 (1.2–1.7)
Age (years) <sup>a</sup>				
<31	18	0.8 (0.5–4.9)	1.8 (0.9–3.2)	1.4 (1.2–1.6)
31–37	17	0.8 (0.2–5.0)	1.2 (0.8–2.8)	1.3 (1.1–1.4)
38–43	17	1.2 (0.8–2.8)	2.2 (1.3–3.4)	1.6 (1.2–1.7)
>43	18	2.3 (1.6–3.4)	2.4 (1.9–3.7)	1.7 (1.4–1.9)
<i>P</i> value		.464	.113	.016
Clinical stage <sup>b</sup>				
A	53	1.2 (0.5–3.3)	1.8 (0.9–3.3)	1.4 (1.2–1.6)
B	6	1.4 (0.8–2.1)	2.1 (1.2–5.6)	1.6 (1.0–2.5)
C	10	2.9 (1.6–4.9)	2.7 (1.9–5.5)	1.8 (1.5–2.3)
<i>P</i> value		.187	.136	.069
CD4 cell count/ $\mu\text{L}$ <sup>c</sup>				
<200	13	1.6 (1.1–3.8)	2.7 (2.2–5.2)	1.9 (1.6–2.3)
200–400	15	1.4 (0.7–2.9)	2.1 (0.9–5.6)	1.6 (1.3–1.6)
401–600	18	0.8 (0.4–2.1)	1.4 (1.0–1.9)	1.3 (1.1–1.5)
>600	16	1.6 (0.6–4.6)	1.2 (0.9–2.0)	1.3 (1.0–1.6)
<i>P</i> value		.646	.017	.001
Antiretroviral treatment <sup>a</sup>				
No	46	1.0 (0.5–2.9)	1.7 (1.1–3.1)	1.4 (1.1–1.7)
Yes	24	2.3 (1.0–5.6)	2.4 (1.9–5.7)	1.6 (1.3–2.0)
<i>P</i> value		.065	.012	.016

*P* values were determined using Wilcoxon and Kruskal-Wallis statistical tests.

Abbreviations: hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IQR, interquartile range; sCD14, soluble CD14.

<sup>a</sup> Missing data:  $n = 1$ .

<sup>b</sup> Missing data:  $n = 2$ .

<sup>c</sup> Missing data:  $n = 9$ .

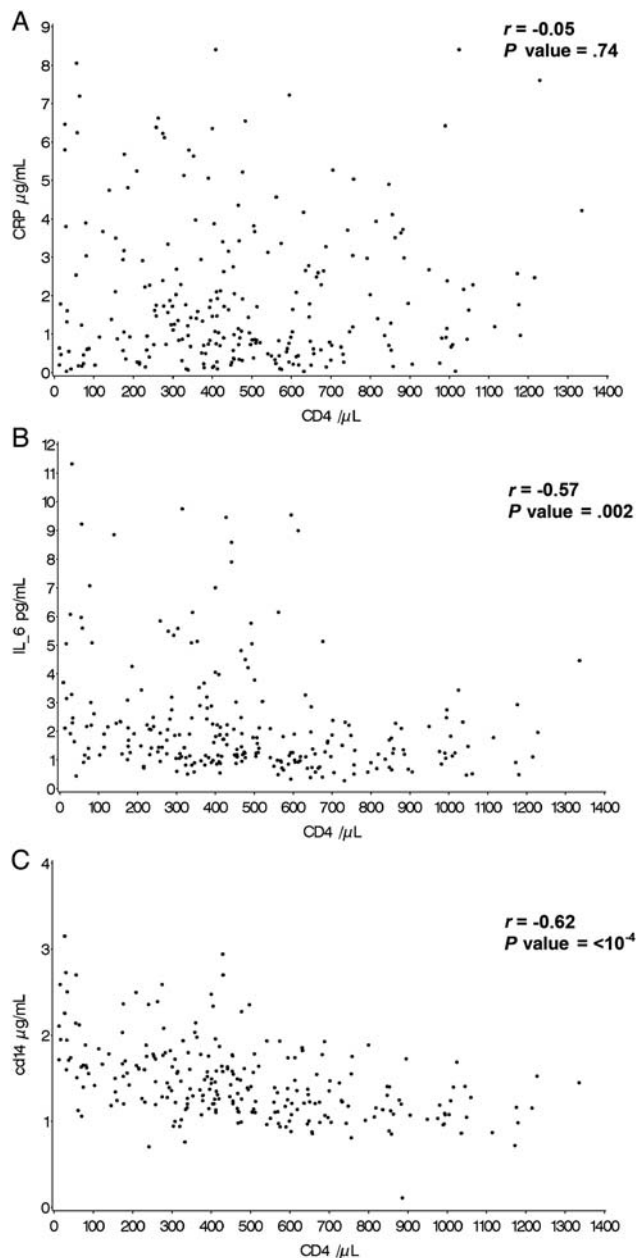
distributed as follows: (1) occurrence of CDC clinical stage B or C HIV-related events ( $n = 19$ ); (2) plasma HIV-2 RNA level becoming detectable ( $n = 7$ ); (3) CD4 cell count dropping below 350 cells/ $\mu\text{L}$  ( $n = 10$ ); and (4) death ( $n = 5$ ). Figure 3 shows the disease progression curves according to biomarker levels at baseline. Patients with biomarker levels above either the median values (hsCRP, 1.38  $\mu\text{g/mL}$ ; IL-6, 1.97  $\text{pg/mL}$ ) or the highest quartile (sCD14, 1.74  $\mu\text{g/mL}$ ) had a higher risk of disease progression (hsCRP,  $P = .02$ ; IL-6,  $P = .04$ ; and sCD14,  $P = .003$ ). Unadjusted analyses showed that higher levels of hsCRP, IL-6, and sCD14; CD4 cell count  $\leq 500/\mu\text{L}$ ; history of HIV RNA  $>4 \log_{10}$  copies/mL; CD4/CD8 ratio  $\leq 0.63$ ; and CDC B/C clinical stage were associated with disease progression, unlike sex, age, HIV transmission category, CD8 cell count, and baseline HIV RNA (Table 3). Among the 3 inflammatory biomarkers, sCD14 was the only one independently associated with disease progression in adjusted analyses (Table 3). The disease progression rate was  $>3$  times higher in patients with a level of sCD14  $>1.74 \mu\text{g/mL}$  (hazard ratio

[HR] 3.59 [CI, 1.52–8.50];  $P = .004$ ), independent of the CD4 cell count (HR, 3.13 when CD4  $<500$  cells/ $\mu\text{L}$  [CI, 1.15–8.51];  $P = .026$ ). Adjusted for sCD14 and CD4 cell count, CDC B/C clinical stage was no longer significantly associated with disease progression (HR, 1.66;  $P = .30$ , model not shown). In adjusted analyses, hsCRP was marginally associated with disease progression (HR, 2.26 [CI, .97–5.31];  $P = .061$ ), whereas IL-6 was not (HR, 1.71 [CI, .69–4.27];  $P = .25$ , Table 3).

## DISCUSSION

In this longitudinal study including 71 HIV-2-infected patients from the French ANRS HIV-2 CO5 cohort, we showed that plasma levels of hsCRP, IL-6, and sCD14 were positively correlated with each other. However, only higher sCD14 was correlated with lower CD4 cell count and also independently associated with HIV-2 disease progression measured by the





**Figure 1.** Correlation between inflammatory biomarkers and CD4 cell count. *A*, High-sensitivity C-reactive protein; *B*, Interleukin 6; *C*, Soluble CD14. Abbreviations: CRP, C-reactive protein; IL-6, interleukin 6; sCD14, soluble CD14.

occurrence of clinical events, drop in CD4 count, and viral replication.

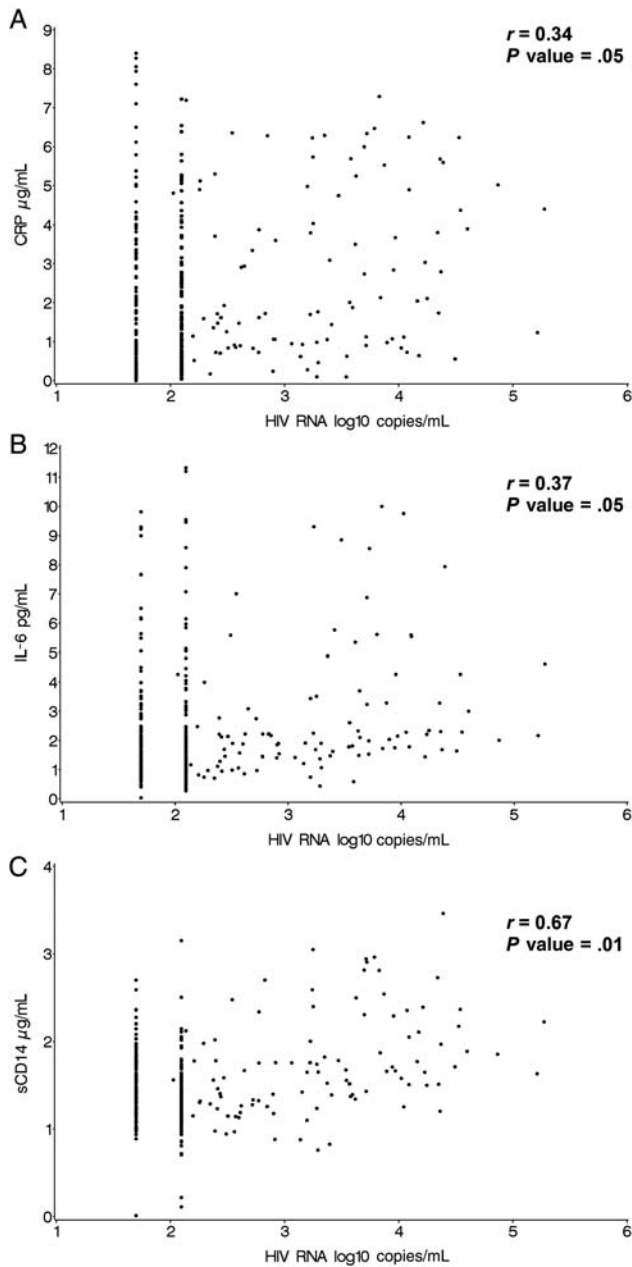
To our knowledge, this is the first study assessing such plasma biomarkers as hsCRP, IL-6, and sCD14 in the context of HIV-2 infection. Although we used an existing HIV-2 cohort initiated since 1994, which provided a long median study follow-up period of 8 years, one of the main limitations

of the present analysis is its limited sample size leading to a lack of statistical power. Therefore, we should not preclude a potential association of other markers, in particular hsCRP, with disease progression in HIV-2-infected patients. Another limitation is due to the retrospective design for the measurements of inflammatory biomarkers: we used available samples that led to unbalanced repeated measurements among patients. Therefore, we were not able to properly monitor the changes occurring in these markers over time but we used all information provided by these repeated measurements to increase the statistical power.

HIV infection is characterized by chronic immune system activation and inflammatory cytokine production with certain plasma biomarkers significantly elevated in HIV-1-infected patients when compared with the general population [13–20]. In HIV-1 infection, the hsCRP and IL-6 inflammatory biomarkers have been previously associated with mortality or opportunistic disease in large randomized clinical trials [14, 19].

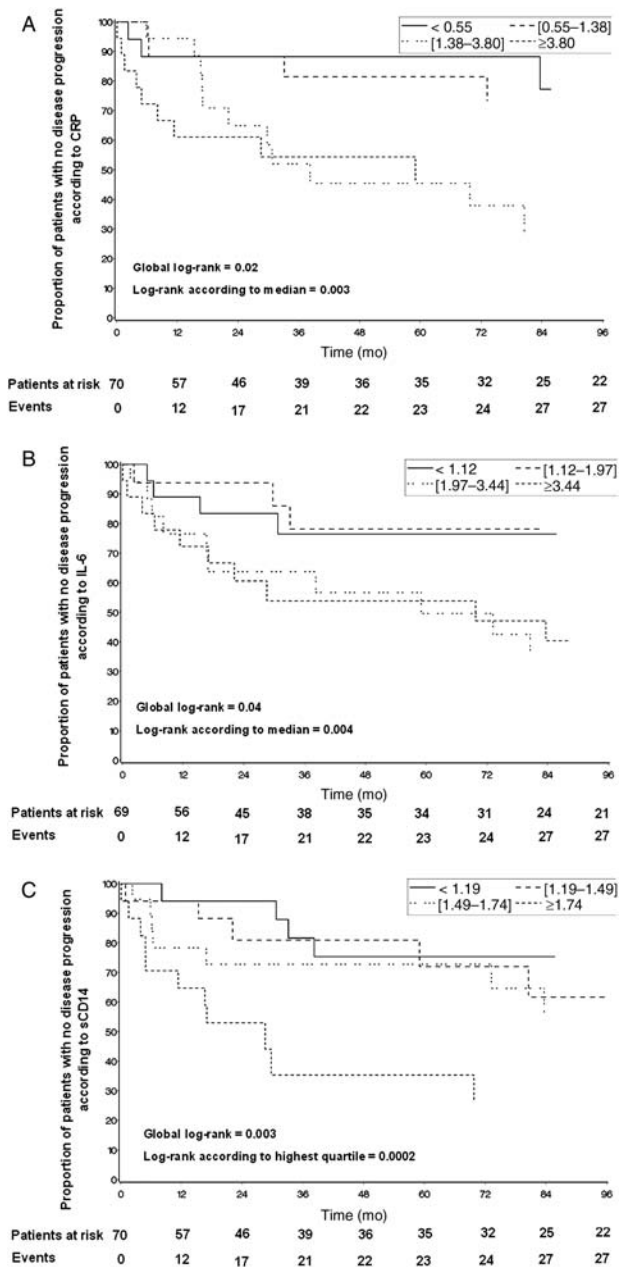
Rapid and severe gut-associated mucosal lymphoid tissue CD4 cell depletion is observed in the first stages of HIV-1 infection [20, 33]. An elevated level of microbial translocation measured by the plasma level of bacterial LPS was also reported in HIV-2 infection and correlated with disease severity, that is, decreased CD4 cell count and increased viral load [34]. Increased LPS levels resulting from disruption of gut mucosal integrity induces sCD14 secretion into plasma by monocytes/macrophages [20, 33].

In the Strategies for Management of Anti-retroviral Therapy (SMART) trial [35], a case-control study assessing several plasma biomarkers at baseline and at the last follow-up visit before death among 85 cases and 170 matched controls showed that elevated IL-6 and hsCRP at baseline were significantly associated with mortality, after adjustment for classic HIV and cardiovascular disease risk factors [13]. Baseline or latest (before event onset) elevations of IL-6 or hsCRP were also predictive of opportunistic disease [14]. In the SMART study, patients with the highest quartile of sCD14 levels ( $>2.68 \mu\text{g/mL}$ ) had a 6-fold higher risk of death than those in the lowest quartile, after adjustment for inflammatory biomarkers, CD4 cell count, and HIV-1 RNA level [18]. Soluble CD14 was the only plasma biomarker correlated with all-cause mortality [18]. Similar results were obtained in the study of Marchetti et al assessing 379 patients in their first years of chronic infection [20]. This study reported that microbial translocation, assessed by plasma LPS levels, predicts disease progression independent of CD4 cell count and HIV-1 RNA level [20]. Thus, similar to what was described in HIV-1 infection, higher levels of sCD14 are strongly associated with HIV-2 disease progression. In our study, the median sCD14 plasma level at baseline was  $1.5 \mu\text{g/mL}$  (IQR,  $1.2$ – $1.7 \mu\text{g/mL}$ ), similar to what was observed in HIV-seronegative patients [36–39].



**Figure 2.** Correlation between inflammatory biomarkers and human immunodeficiency virus type 2 RNA level. *A*, High-sensitivity C-reactive protein; *B*, Interleukin 6; *C*, Soluble CD14. All correlations were calculated with bivariate mixed models (Spearman correlation). Abbreviations: CRP, C-reactive protein; HIV, human immunodeficiency virus; IL-6, interleukin 6; sCD14, soluble CD14.

In HIV-1-infected patients, reported median sCD14 plasma levels assessed using the same assay were higher, ranging between 2.11  $\mu\text{g/mL}$  and 3.30  $\mu\text{g/mL}$  [16, 18–20, 36–39]. Although not strictly comparable, this suggests that chronic activation might occur at lower sCD14 plasma levels in HIV-2 infection than in HIV-1 infection.



**Figure 3.** Kaplan-Meier disease progression curves for subjects with low and high biomarker plasma levels at time of first measure in the study. *A*, High-sensitivity C-reactive protein; *B*, Interleukin 6; *C*, Soluble CD14. Abbreviations: CRP, C-reactive protein; IL-6, interleukin 6; sCD14, soluble CD14.

In the context of HIV-2 infection, it has been previously reported that CD4 cell apoptosis is correlated with immune activation and disease evolution [25]. A more recent cross-sectional study including 53 HIV-2-infected antiretroviral-naïve patients from Guinea-Bissau showed that the immune activation biomarkers human leukocyte antigen-DR

**Table 3. Factors Associated With Disease Progression**

Factors	Univariable, HR (P Value)	HR (P Value) <sup>a</sup>	Multivariable, HR (P Value) <sup>b</sup>	HR (P Value) <sup>c</sup>
Female	0.85 (.66)			
Age >38 years	1.61 (.21)			
Antiretroviral treatment initiated before baseline	2.83 (.005)			
CD4/CD8 ratio ≤0.63	3.23 (.025)			
History of HIV RNA ≥4 log <sub>10</sub> copies/mL	2.70 (.014)			
CD4 ≤500 cells/μL	3.71 (.009)	2.68 (.072)	2.88 (.043)	3.13 (.026)
CDC B/C clinical stage (vs A)	2.95 (.006)	2.27 (.061)	2.30 (.060)	
IL-6 >1.97 pg/mL	3.17 (.006)	1.71 (.25)		
hsCRP >1.38 μg/mL	3.12 (.005)		2.26 (.061)	
sCD14 >1.74 μg/mL	3.74 (.0006)			3.59 (.004)

Univariable and multivariable analyses were done using Cox proportional hazard models.

Abbreviations: CDC, Centers for Disease Control and Prevention; HIV, human immunodeficiency virus; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IQR, interquartile range; sCD14, soluble CD14.

<sup>a</sup> n = 60 patients, 24 events.

<sup>b</sup> n = 61 patients, 24 events.

<sup>c</sup> n = 62 patients, 25 events.

and CD38 expression on CD4 and CD8 cells and plasma  $\beta(2)$ -microglobulin levels were associated with disease progression [24]. In this study, criteria of disease progression were HIV-2 RNA level, CD4 cell count, body mass index <18 kg/m<sup>2</sup>, and Karnofsky score <80%. Furthermore, a consistent positive correlation was identified between immune activation and HIV-2 RNA level in these African patients [24]. Another study based on a large number (N = 348) of HIV-2-infected patients from sub-Saharan Africa, for which antiretroviral status and viral load levels were unknown, assessed the role of 3 biomarkers on HIV-2 disease progression:  $\beta(2)$ -microglobulin, neopterin, and soluble urokinase plasminogen activator receptor [40]. The biomarkers  $\beta(2)$ -microglobulin and neopterin were associated with HIV-2 clinical progression, defined as death in this study [40]. Overall, although these studies and our study assessed different populations and different biomarkers and used different criteria of disease progression, they all show the role of immune activation and inflammatory biomarkers in HIV-2 disease progression.

These inflammatory biomarkers could provide clinicians with supplementary information on the risk of disease progression. In addition, they might prove to be cost-effective tools as standardized ELISA kits are cheaper and easier to use than polymerase chain reaction-based assays. However, confirmatory studies are needed, especially in West Africa, where most HIV-2-infected patients are living and the environment is very different (eg, higher frequency of parasitic diseases).

In conclusion, in the French ANRS CO5 HIV-2 cohort, sCD14, an indirect marker of monocytes/macrophage

activation, represents a good predictive marker of disease progression, independent of other risk factors. The role of this marker, like hsCRP, remains to be confirmed in larger studies. Microbial translocation and inflammatory processes could be the driving forces behind disease progression in HIV-2 infection, as now observed during HIV-1 infection.

## Notes

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**Financial support.** This work was supported by the French National Agency for Research on AIDS and viral hepatitis (Agence Nationale de Recherches sur le SIDA et les hépatites virales [ANRS]).

**Potential conflicts of interest.** J. C. receives grant money from Janssen; is on the speakers' bureau for ViiV, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead, and Janssen, and has received travel expenses from ViiV and Janssen; C. C. is on the speakers' bureau for Bristol-Myers Squibb, ViiV Healthcare, and Janssen; G. C. has received grant money from Gilead, Tibotec, Roche, MSD, Janssen, Boehringer Ingelheim, Bristol-Myers Squibb, GlaxoSmithKline, ViiV Healthcare, Abbott, and Pfizer, and has received travel expenses from Lundbeck and Lilly; D. D. is on the board of Gilead, Janssen-Cilag, Merck, and ViiV Healthcare, and has received travel expenses from Gilead, Janssen-Cilag, Merck, and ViiV Healthcare; F. B.-V. is on the board of Gilead, Merck, and Tibotec, and has received travel expenses from Gilead and ViiV Healthcare; S. M. has grants from MSD, and has received travel expenses from Gilead, MSD, and ViiV Healthcare; R. T. is on the board of Bristol-Myers Squibb, Gilead, and Tibotec. 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.

## References

1. Marlink R, Kanki P, Thior I, et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science* **1994**; 265:1587–90.
2. Popper SJ, Sarr AD, Travers KU, et al. Lower human immunodeficiency virus (HIV) type 2 viral load reflects the difference in pathogenicity of HIV-1 and HIV-2. *J Infect Dis* **1999**; 180:1116–21.
3. MacNeil A, Sarr AD, Sankale JL, Meloni ST, Mboup S, Kanki P. Direct evidence of lower viral replication rates in vivo in human immunodeficiency virus type 2 (HIV-2) infection than in HIV-1 infection. *J Virol* **2007**; 81:5325–30.
4. Burgard M, Jasseron C, Matheron S, et al. Mother-to-child transmission of HIV-2 infection from 1986 to 2007 in the ANRS French Perinatal Cohort EPF-CO1. *Clin Infect Dis* **2010**; 51:833–43.
5. Campbell-Yesufu OT, Gandhi RT. Update on human immunodeficiency virus (HIV)-2 infection. *Clin Infect Dis* **2011**; 52:780–7.
6. Rowland-Jones SL, Whittle HC. Out of Africa: what can we learn from HIV-2 about protective immunity to HIV-1? *Nature Immunol* **2007**; 8:329–31.
7. Thiébaud R, Matheron S, Taieb A, Brun-Vezinet F, Chêne G, Autran B. Long-term nonprogressors and elite controllers in the ANRS CO5 HIV-2 cohort. *AIDS* **2010**; 25:865–7.
8. Matheron S, Pueyo S, Damond F, et al. Factors associated with clinical progression in HIV-2 infected-patients: the French ANRS cohort. *AIDS* **2003**; 17:2593–601.
9. Martinez Steele E, Awasana AA, Corrah T, et al. Is HIV-2-induced AIDS different from HIV-1-associated AIDS? Data from a West African clinic. *AIDS* **2007**; 21:317–24.
10. Bonnet F, Chene G, Thiebaut R, et al. Trends and determinants of severe morbidity in HIV-infected patients: the ANRS CO3 Aquitaine cohort, 2000–2004. *HIV Med* **2007**; 8:547–54.



11. Smith C, Sabin CA, Lundgren JD, et al. Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D Study. *AIDS* **2010**; 24:1537–48.
12. Deeks SG. HIV infection, inflammation, immunosenescence, and ageing. *Annu Rev Med* **2011**; 62:141–55.
13. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS Med* **2008**; 5:e203.
14. Rodger AJ, Fox Z, Lundgren JD, et al. Activation and coagulation biomarkers are independent predictors of the development of opportunistic disease in patients with HIV infection. *J Infect Dis* **2009**; 200:973–83.
15. Neuhaus J, Jacobs DR Jr, Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis* **2010**; 201:1788–95.
16. Kamat A, Ancuta P, Blumberg RS, Gabuzda D. Serological markers for inflammatory bowel disease in AIDS patients with evidence of microbial translocation. *PLoS One* **2010**; 5:e15533.
17. Kalayjian RC, Machezano RN, Rizk N, et al. Pretreatment levels of soluble cellular receptors and interleukin-6 are associated with HIV disease progression in subjects treated with highly active antiretroviral therapy. *J Infect Dis* **2010**; 201:1796–805.
18. Sandler NG, Wand H, Roque A, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J Infect Dis* **2011**; 203:780–90.
19. Boulware DR, Hullsiek KH, Puronen CE, et al. Higher levels of CRP, D-dimer, IL-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. *J Infect Dis* **2011**; 203:1637–46.
20. Marchetti G, Cozzi-Lepri A, Merlini E, et al. Microbial translocation predicts disease progression of HIV-infected antiretroviral-naïve patients with high CD4+ cell count. *AIDS* **2011**; 25:1385–94.
21. Justice AC, Freiberg MS, Tracy R, et al. Does an index composed of clinical data reflect effects of inflammation, coagulation, and monocyte activation on mortality among those aging with HIV? *Clin Infect Dis* **2012**; 54:984–94.
22. Eastburn A, Scherzer R, Zolopa AR, et al. Association of low level viremia with inflammation and mortality in HIV-infected adults. *PLoS One* **2011**; 6:e26320.
23. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* **2006**; 12:1365–71.
24. Leligidowicz A, Feldmann J, Jaye A, et al. Direct relationship between virus load and systemic immune activation in HIV-2 infection. *J Infect Dis* **2010**; 201:114–22.
25. Michel P, Balde AT, Roussillon C, Aribot G, Sarthou JL, Gougeon ML. Reduced immune activation and T cell apoptosis in human immunodeficiency virus type 2 compared with type 1: correlation of T cell apoptosis with beta2 microglobulin concentration and disease evolution. *J Infect Dis* **2000**; 181:64–75.
26. Gourlay AJ, van Tienen C, Dave SS, et al. Clinical predictors cannot replace biological predictors in HIV-2 infection in a community setting in West Africa. *Int J Infect Dis* **2012**; 16:e337–43.
27. Yeni P. Prise en charge médicale des personnes infectées par le VIH [Medical management of HIV-infected persons]. Paris: La documentation française, **2010**:417.
28. Drylewicz J, Matheron S, Lazaro E, et al. Comparison of viro-immunological marker changes between HIV-1 and HIV-2-infected patients in France. *AIDS* **2008**; 22:457–68.
29. Damond F, Descamps D, Farfara I, et al. Quantification of proviral load of human immunodeficiency virus type 2 subtypes A and B using real-time PCR. *J Clin Microbiol* **2001**; 39:4264–8.
30. Damond F, Collin G, Descamps D, et al. Improved sensitivity of human immunodeficiency virus type 2 subtype B plasma viral load assay. *J Clin Microbiol* **2005**; 43:4234–6.
31. Vivot A, Chêne G, Thiébaud R, et al. Peak HIV-2 RNA better predicts clinical progression in HIV-2+ adults than baseline or current values or copy-years. In: Conference on Retroviruses and Opportunistic Infections. Seattle, WA, USA, **2012**.
32. Thiébaud R, Jacqmin-Gadda H, Chêne G, Lepout C, Commenges D. Bivariate linear mixed models using SAS proc MIXED. *Comput Methods Programs Biomed* **2002**; 69:249–56.
33. Brenchley JM, Schacker TW, Ruff LE, et al. CD4(+) T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* **2004**; 200:749–59.
34. Nowroozalazadeh S, Mansson F, da Silva Z, et al. Microbial translocation correlates with the severity of both HIV-1 and HIV-2 infections. *J Infect Dis* **2010**; 201:1150–4.
35. El Sadr WM, Lundgren JD, Neaton JD, et al. CD4 + count-guided interruption of antiretroviral treatment. *N Engl J Med* **2006**; 355:2283–96.
36. Sheikh V, Krishnan S, Richterman A, et al. D-dimer levels are elevated in HIV-infected LTNP despite normal C-reactive protein and sCD14 levels. In: 18th Conference on Retroviruses and Opportunistic Infections. Boston, MA, USA, **2011**.
37. Cassol E, Malfeld S, Mahasha P, et al. Persistent microbial translocation and immune activation in HIV-1-infected South Africans receiving combination antiretroviral therapy. *J Infect Dis* **2010**; 202:723–33.
38. Merlini E, Bai F, Bellistri GM, Tincati C, d'Arminio Monforte A, Marchetti G. Evidence for polymicrobial flora translocating in peripheral blood of HIV-infected patients with poor immune response to antiretroviral therapy. *PLoS One* **2011**; 6:e18580.
39. Sandler NG, Koh C, Roque A, et al. Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. *Gastroenterology* **2011**; 141:1220–30.
40. Nyamweya S, De Silva T, Van Tienen C. A study of 3 biomarkers of immune activation and disease progression: a comparison between HIV-1 and HIV-2 infections. In: 18th Conference on Retroviruses and Opportunistic Infections. Boston, MA, USA, **2011**.