Title

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IMPACT OF HEPATITIS C VIRUS ON THE CIRCULATING LEVELS OF IL-7 IN HIV-1 CO-INFECTED WOMEN

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Abstract

Objectives—Hepatitis C virus (HCV) infection causes an alteration in T cell maturation and activation in patients co-infected with Human Immunodeficiency Virus (HIV). As interleukin 7 (IL-7) is a major cytokine controlling T cell homeostasis, we analyzed the potential influence of HCV co-infection on circulating IL-7 levels in HIV-infected women before and after highly active antiretroviral therapy (HAART).

Design and methods—This prospective study included 56 HIV mono-infected, 55 HIV/HCV co-infected without HCV viremia, 132 HIV/HCV co-infected with HCV viremia, and 61 HIV/HCV-uninfected women for whom plasma levels of IL-7 were determined by ELISA at one or more follow-up visits pre- and post-HAART. Cross-sectional analyses of the associations between

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plasma IL-7 levels and HCV infection, demographic, clinical, and immunologic characteristics were evaluated using univariate and multivariate linear regression models pre- and post-HAART.

Results—In multivariate models, IL-7 levels were significantly higher in co-infected HCV viremic women than in HIV mono-infected women (multiplicative effect=1.48, 95% CI=1.01– 2.16, p=0.04) pre-HAART, but were similar between these two groups among women post-HAART. In addition to HCV viremia, higher IL-7 levels were associated with older age (p=0.02), lower CD4$^+$ T cell count (p=0.0007), and higher natural killer T cell (NKT) count (p=0.02) in women pre-HAART. Among HAART-treated women, only lower CD4$^+$ T cell count was significantly associated with IL-7 level (p=0.006).

Conclusions—Our data demonstrate that in HIV-infected women, circulating levels of IL-7 are strongly associated with CD4$^+$ T cell depletion both pre- and post-HAART. Our data also demonstrate that HCV viremia increases circulating IL-7 levels pre-HAART but not post-HAART in co-infected women. This suggests that the effect of HCV on lymphopenia is abrogated by HAART.

INTRODUCTION

Human immunodeficiency virus (HIV) infection induces a disruption of T cell homeostasis characterized by progressive CD4$^+$ T cell depletion, CD8$^+$ T cell expansion, and chronic immune activation [1, 2]. This leads to immune dysfunction and clinical disease progression including opportunistic infections and other manifestations of the acquired immunodeficiency syndrome (AIDS). In addition, HIV infection alters the architecture of the thymus and impairs the production of naïve T-cells essential for the generation of a complete T-cell repertoire [3].

An estimated 34 million people are currently infected with HIV worldwide, and approximately 20–30% are co-infected with hepatitis C virus (HCV), another persistent viral infection in which the immune system ineffectively controls viral replication [4]. Our previous studies and those of others have shown that persons co-infected with HIV and HCV have an increased risk of AIDS and AIDS-related death compared to HIV mono-infected individuals [5–12]. Studies have also shown that HCV infection negatively impacts response to highly active antiretroviral therapy (HAART) [6–8, 12] and causes alterations in T cell homeostasis in HIV-infected (HIV$^+$) individuals, including increases in primed activated T cells and percentage of activated CD8$^+$ T-cells, and alterations in cytokine production and naïve and memory populations [7, 12, 13].

There is increasing evidence that interleukin-7 (IL-7) is the major cytokine regulating T cell homeostasis and differentiation in humans [14–16]. IL-7 is produced by several cell types including stromal cells in the bone marrow and thymus. This cytokine belongs to the common γ chain (γc) cytokine family (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) and signals through a heterodimer receptor composed of IL-7Rα (CD127) and common gamma chain

receptor (CD132) [17]. IL-7 / IL-7Rα binding results in a cascade of signals important for T-cell development within the thymus and survival, by blocking T cell autophagy within the periphery [14–16]. Knockout mice which genetically lack IL-7 receptor exhibit thymic atrophy, arrest of T-cell development at the double positive stage, and severe lymphopenia [18]. In contrast, administration of IL-7 to mice results in an increase in recent thymic emigrants, increases in B and T cells, and increased recovery of T cells after bone marrow transplantation [19].

Several studies have reported elevated circulating IL-7 levels in HIV+ individuals, correlating with the levels of both CD4+ T cell depletion and IL-7Rα loss [3, 14, 20–22]. The increased concentration of IL-7 found in T cell-depleted individuals is considered to be a homeostatic response to T cell depletion, which may accelerate thymic output and promote peripheral T cell survival, differentiation and proliferation [14, 22, 23].

Despite increasing evidence that T cell homeostasis in HIV+ individuals is impacted by HCV co-infection, variations in homeostatic factors such as IL-7 in co-infected individuals have been poorly investigated. We therefore assessed whether plasma IL-7 levels are related to HCV infection in HIV co-infected women both pre- and post-HAART. We also evaluated the associations of IL-7 with demographic, clinical, and immunologic characteristics. We hypothesized that HCV infection impacts circulating levels of IL-7 in HIV/HCV co-infected women, which may partly explain the impact of HCV on T cell maturation and function observed in HIV co-infected women.

PARTICIPANTS, MATERIALS AND METHODS

This is a sub-study of the Women's Interagency HIV Study (WIHS), a multicenter, prospective study of the natural history of HIV-1 infection and associated diseases in US women. Using stored blood samples, we evaluated 243 HIV+ women and 61 HIV-uninfected women from all WIHS sites included in a sub-study of the impact of HCV co-infection on immune function. Pre- and post-HAART samples from this sub-study were selected based on availability and tested in duplicate by ELISA in our laboratory with the Quantikine HS Human IL-7 Immunoassay (R&D Systems, Minneapolis, MN) using sodium citrate plasma specimens that were stored at −70°C or lower within 6 hours of processing. The lower limit of detection was 0.25pg/mL.

HIV-1 RNA plasma levels in WIHS were determined using the isothermal nucleic acid sequence–based amplification method (BioMérieux, Durham, NC) in laboratories that participate in and are certified by the National Institute of Allergy and Infectious Diseases Virology Quality Assurance certification program. HCV antibody status was determined at cohort entry using the Abbott EIA 2.0 and 3.0 assays. For women who were HCV antibody positive, HCV RNA levels (IU/mL) were obtained using the COBAS Amplicor Monitor 2.0 (detection range, 600–500,000 IU/mL [Roche Diagnostics]) or the COBAS TaqMan (detection range, $15 - 1 \times 10^8$ IU/mL [Roche Diagnostics]). Qualitative PCR (Amplicor 2.0; lower limit of detection, 50 IU/mL) was performed if HCV RNA was not detected, as previously reported [24].

WIHS used fresh whole blood specimens collected in EDTA tubes, with standard 3-color flow cytometry (FACSCalibur [BD Biosciences]) to determine levels of CD4$^+$ T, natural killer (NK), and natural killer T (NKT) cells in accordance with the AIDS Clinical Trials Group consensus protocol in laboratories that participated in the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS, Quality Assurance program for flow cytometry. The analysis used fluorochrome-conjugated antibodies (anti-CD4, anti-CD8, anti-CD19, anti–HLA-DR, anti-CD16, and anti-CD56 [BD Biosciences]).

Four study groups of women were compared: 1) Uninfected (HIV$^-$/HCV$^-$); 2) HIV mono-infected (HIV$^+$/HCV$^-$); 3) HIV and HCV positive without HCV viremia (HIV$^+$/HCV$^+$RNA$^-$); and 4) HIV and HCV positive with HCV viremia (HIV$^+$/HCV$^+$RNA$^+$). The HIV$^+$/HCV$^+$ RNA$^-$ women represented HCV antibody-positive women who cleared the virus and were no longer viremic. Visits were classified as "post-HAART" if they occurred after the first HAART visit (excluding visit at HAART initiation) and as "pre-HAART" if they occurred before the initiation of HAART. HIV$^+$ women with pre-HAART visits included 30 mono-infected, 55 co-infected without HCV viremia, and 127 co-infected with HCV viremia. Those with post-HAART visits included 10 mono-infected, 1 co-infected without HCV viremia, and 43 co-infected with HCV viremia.

Characteristics at first measurement visit were compared among the study groups by chi-square tests. Demographic variables included age at visit, race/ethnicity, injection drug use (IDU) and non-IDU (including cocaine, crack, meth-amphetamines, marijuana or heroin) use since last visit. Age was categorized in five-year increments from <35 to ≥45 years. Race/ethnicity was categorized as White or other, African American, or Hispanic. Clinical and immunological characteristics included HCV infection and viremia status, HIV viral load (≤80, 81–1,000, 1,001–10,000, 10,001–100,000, and >100,000 copies/mL), IL-7 (≤0.84, 0.85–1.69, 1.7–2.87, >2.87 pg/mL), CD4$^+$ T cell counts (≤200, 201–350, 351–500, >500 cells/mm^3), NK (≤45, 46–80, 81–145, >145 cells/mm^3) and NKT (≤10, 11–20, 21–41, >41 cells/mm^3) counts by quartiles, antiretroviral therapy since last visit (none, monotherapy, combination, or HAART), and previous AIDS diagnosis (yes or no), as defined by the Centers for Disease Control and Prevention (CDC). If NK and NKT counts were not available at an IL-7 measurement visit, values within six months before or after the visit were used. Even after this procedure, NK and NKT data were missing at first IL-7 visit for 20% of the uninfected and 4% (8/212) of the HIV$^+$ women with a pre-HAART visit. For the univariate and multi-variate analysis, 11% of the HIV$^+$ women had missing NK and NKT data at the pre- HAART visit. NK and NKT data were not available post-HAART.

Box plots were constructed to visually compare IL-7 levels at first measurement visit between study groups, stratified by HAART. Of the 243 HIV$^+$ women, 40 (16.5%) had three visits at which IL-7 was measured, 4 (1.6%) had two visits, and the remaining 199 (81.9%) had one visit. Due to the large number of women with only one visit, cross-sectional analyses were performed to assess the association between IL-7 levels and immunological/ clinical characteristics stratified by HAART treatment status. For women who had more than one visit before or after HAART, the pre-HAART or post-HAART visits closest to the HAART initiation visit were analyzed. Associations between IL-7 levels and immunologic

and clinical characteristics were determined by linear regression models separately for the 212 pre-HAART and 53 post-HAART visits. IL-7 was treated as the continuous outcome variable and log-transformed to achieve normality. All regression coefficients were back-transformed to show the multiplicative effect of the factors on IL-7. The associations were initially evaluated univariately. Multivariate models were constructed to include factors with p-values 0.20 from the univariate analyses. Backward selection was then used to arrive at the final multivariate model, using p-values <0.05 as the criteria for statistical significance. Because HCV status was a primary factor of interest in relation to IL-7 level, it was kept in the final multivariate model. All statistical analyses were conducted using STATA software (version 13).

RESULTS

Demographic and clinical characteristics of study participants at first measurement visit

The 304 women in this study included 61 (20%) HIV^-/HCV^-, 56 (18%) HIV^+/HCV^-, 55 (18%) HIV^+/HCV^+RNA^-, and 132 (43%) HIV^+/HCV^+RNA^+ (Table 1). Overall, differences between all study groups and between the HIV+ groups were observed by age at visit, race, and IDU and non-IDU. Samples selected for the co-infected women in this sub-study were more likely than the HIV mono-infected to have been collected before 1996 (71% vs. 21%), when the widespread use of HAART began. This explains the difference in HAART use observed in Table 1 between the co-infected vs. mono-infected women (4 [2%] vs. 24 [43%]). The percentage of HIV-infected women diagnosed with AIDS was highest among HIV^+/HCV^+RNA^+ women and lowest among HIV^+/HCV^+RNA^- women (19% vs. 5%). HCV-positive women were significantly (p<0.001) older than HCV-negative women (median age 40 [interquartile range: 36–44] vs. 33 [interquartile range: 28–40] years) at first IL-7 measurement and more likely to be IDU (18% vs. 1%, p<0.001) and non-IDU (36% vs. 24%, p=0.03) (data not shown).

Immunologic and virologic characteristics of study participants at first measurement visit

There were 273 women with pre-HAART visits (61 HIV^-/HCV^-, 30 HIV^+/HCV^-, 55 HIV^+/HCV^+RNA^-, and 127 HIV^+/HCV^+RNA^+) available for evaluation at first measurement visit. Overall, HIV-infected women had higher IL-7 levels and lower $CD4^+$ T, NK and NKT cell counts than HIV^-/HCV^- women (Table 2). Among the HIV^+ women, $CD4^+$ T cell counts were significantly different between infection groups (p=0.046). However, NK and NKT cell counts and plasma HIV RNA levels were not significantly different.

Post-HAART visits were only available for 10 HIV^+/HCV^-, 1 HIV^+/HCV^+RNA^- and 43 HIV^+/HCV^+RNA^+ women. The analyses and comparisons were made using 53 visits from HIV^+/HCV^- and HIV^+/HCV^+RNA^+ groups. None of the characteristics examined, including $CD4^+$ T cell count, were significantly different between the HIV^+/HCV^- and HIV^+/HCV^+RNA^+ groups (data not shown).

Plasma IL-7 levels

At the first measurement visit for uninfected and HIV$^+$ women with pre-HAART visits, plasma IL-7 levels were significantly higher in the HIV$^+$ women compared to the uninfected (mean difference = 2.04, 95% CI = (1.41–2.68), p<0.001) (Fig. 1). Mean IL-7 level was also significantly higher in the HIV$^+$/HCV$^+$RNA$^+$ compared to HIV$^+$/HCV$^-$ women (mean difference= 1.47, 95% CI= (0.47–2.48), p=0.004). However, the IL-7 levels were similar between these two groups post-HAART.

Stratified by HAART status, univariate analyses of immunologic and demographic/clinical characteristics in relation to IL-7 levels among the HIV-infected women are shown in Table 3. At the pre-HAART visit, higher IL-7 levels were associated with age (p=0.001), AIDS diagnosis (multiplicative effect= 1.54, 95% CI= (1.1–2.14), p=0.01), and lower CD4$^+$ T cell count (p=0.002). IL-7 levels were 1.47 times higher (95% CI= (1.02–2.14), p=0.04) in HIV$^+$/HCV$^+$RNA$^+$ women compared to HIV$^+$/HCV$^-$ women. IL-7 levels were not associated with HIV viral load, NK and NKT cell counts, race, or drug use. Post-HAART, IL-7 levels remained significantly associated with CD4$^+$ T-cell count (p=0.006) and AIDS diagnosis (multiplicative effect= 1.88, 95% CI= (1.14–3.09), p=0.01). In addition, IL-7 level was significantly higher for non-IDU (multiplicative effect= 1.96, 95% CI= (1.05–3.63), p=0.03). IL-7 levels did not significantly differ between HIV$^+$/HCV$^+$RNA$^+$ and HIV$^+$/HCV$^-$ women (multiplicative effect= 0.98, 95% CI= (0.52–1.82), p=0.94).

After including covariates with p-values 0.20 from univariate analyses and applying the backward selection method, the final multivariate models for pre- and post-HAART visits are shown in Table 4. Pre-HAART, higher IL-7 levels were significantly associated with older age (p=0.02), higher NKT (p=0.02), and lower CD4$^+$ T cell count (p=0.0007). IL-7 level was significantly higher in HIV$^+$/HCV$^+$RNA$^+$ compared to HIV$^+$/HCV$^-$ women (multiplicative effect=1.48, 95% CI= (1.01–2.16), p=0.04). In addition, African American women had lower IL-7 levels than Hispanic women (multiplicative effect = 0.71, 95% CI= (0.54–0.94), p=0.02). Post-HAART, higher IL-7 levels were only significantly associated with lower CD4$^+$ T cell count (p=0.006).

DISCUSSION

In the present study, we analyzed the potential influence of HCV co-infection on IL-7 levels both pre- and post-HAART in HIV$^+$ women. We found for the first time that HCV viremia positively impacts circulating IL-7 levels pre-HAART but not post-HAART in HIV$^+$ women. IL-7 levels were found to be associated with age, race, CD4$^+$ T cell depletion, and NKT cell count pre-HAART, whereas only CD4$^+$ T cell count was significant post-HAART.

IL-7 is a cytokine that plays a pivotal role in the homeostasis of T cells, promoting survival of naive T cells and generation of memory cells [14–16]. As others have also reported, we found higher circulating levels of IL-7 associated with CD4$^+$ T cell depletion in HIV-infected individuals [3, 14, 21, 22]. The mechanism underlying the elevated IL-7 concentration in HIV infected persons is not completely understood. It could result from an increased production of IL-7 by the stromal cells of the bone marrow and thymus detecting T cell depletion or to a reduced number of cells expressing IL-7Rα [14, 22].

Interestingly, we found pre-HAART plasma IL-7 levels to be significantly higher in HCV-viremic coinfected women compared to HIV mono-infected women, suggesting that HCV viremia affects IL-7 production in HIV infected women. An impact of HCV viremia on different immune parameters, such as CD4$^+$ T cell depletion, could explain this finding. Our results support this theory as we found CD4$^+$ T cell counts to be lower in co-infected HCV viremic women than HIV mono-infected women pre-HAART and to be inversely correlated with IL-7 levels. Several studies support our findings as they reported that HCV co-infection significantly increases the rates of CD4$^+$ T cell apoptosis in HIV-infected patients [25, 26].

NKT cells are a heterogeneous group of T lymphocytes that recognize lipid antigens presented by the non-classical MHC class I-like molecule CD1 [27, 28]. In our study, we found an association between IL-7 levels and NKT cell count pre-HAART. This can be related to the fact that NKT cell homeostasis is regulated by IL-7 through IL-7R. As reported by others [29], we also found NKT cell counts to be significantly decreased in HIV$^+$ compared to uninfected women but similar among the HIV mono-infected and HCV co-infected groups. These data suggest that variations in NKT cell homeostasis cannot explain differences in IL-7 levels among HIV$^+$ groups but can explain only partially those found between HIV$^+$ and healthy individuals, as NKT cells constitute only approximately 0.2% of all peripheral blood T cells.

In HIV infected individuals, HAART enables effective control over HIV viral replication and leads to recovery of CD4$^+$ T-cell counts [30], generally followed by a decrease in plasma IL-7 levels [14, 31]. Interestingly, we found similar IL-7 levels in HIV mono-infected and co-infected HCV viremic women post-HAART, indicating that HAART abrogates the effect of HCV co-infection on IL-7 levels in HIV infected persons. Our results suggest that this effect may be related to the effect of HAART on CD4$^+$ T cell reconstitution, as similar CD4$^+$ T cell counts were found in HIV mono-infected and co-infected HCV viremic women post-HAART. In addition, a strong association between IL-7 levels and CD4$^+$ T cell count remained post-HAART. Several studies support this finding as they reported an impact of HCV co-infection on CD4$^+$ T cell apoptosis in HIV-infected persons that is rapidly lost with HAART [32–34]. However, others reported that HCV infection negatively impacts immune response to HAART in HIV co-infected persons [5–7, 11].

To our knowledge, this is the first report that HCV is associated with an elevation in circulating IL-7 levels in HIV co-infected persons pre-HAART. Two previous studies suggested that HCV co-infection either does not affect the IL-7 pathway [35] or is associated with a decrease in plasma IL-7 levels in HIV-infected patients [36]. However, these two studies included both men and women, while our study included only women. A sex bias may explain these differences as higher IL-7 levels have been reported in HIV-infected women compared to HIV-infected men [22]. In addition, the study of Cianci et al. [35] included only HAART-treated patients, which can explain the reported absence of impact of HCV-co-infection on IL-7 levels. We also found this effect to be lost in women post-HAART.

In a multivariate analysis, we found pre-HAART IL-7 level to be associated with race and age, two variables reported to affect T cell homeostasis/composition in HIV infected and healthy individuals [37–39]. Some studies suggest that HAART can prevent age-related changes in $CD4^+$ T-cell composition and normalize the rate of $CD4^+$ count gain between people of diverse races [37, 40, 41]. Although several studies suggest the opposite [42–44], this HAART effect may explain, at least in part, the loss of associations of IL-7 levels with race and age in HAART-treated women.

Although our study is unique, it has several limitations. Due to the absence of an HCV mono-infected group, we were unable to determine the single versus joint effects of HIV and HCV infection on IL-7. In addition, because a large percentage of women had only one visit, we were unable to do a longitudinal assessment of the effect of immunologic, demographic, and clinical variables on IL-7. Finally, the relatively small sample of women analyzed post-HAART (n=53) limited power to detect associations. However, despite limited power, the estimate of association of HIV/HCV groups with IL7 level (multiplicative effect=0.98) post-HAART was close to the null value of 1, indicating our finding are not merely an issue of small sample size.

In conclusion, we have demonstrated that in HIV infected women, plasma IL-7 levels are strongly associated with $CD4^+$ T cell depletion. Pre-HAART, in HIV-infected women, we found that HCV viremia increases circulating IL-7 levels, probably due to the effect of HCV on lymphopenia. However, we found this effect to be lost post-HAART, probably due to the opposite effect of HAART on lymphopenia. Overall, our data provide insight regarding the impact of HCV disease on circulating IL-7 levels in HIV-infected individuals. They also raise questions regarding the use of IL-7 therapy to impair T cell depletion in HIV-infected patients, since increased IL-7 is a homeostatic response to low $CD4^+$ T cell counts in HIV-infected women.

ACKNOWLEDGEMENTS

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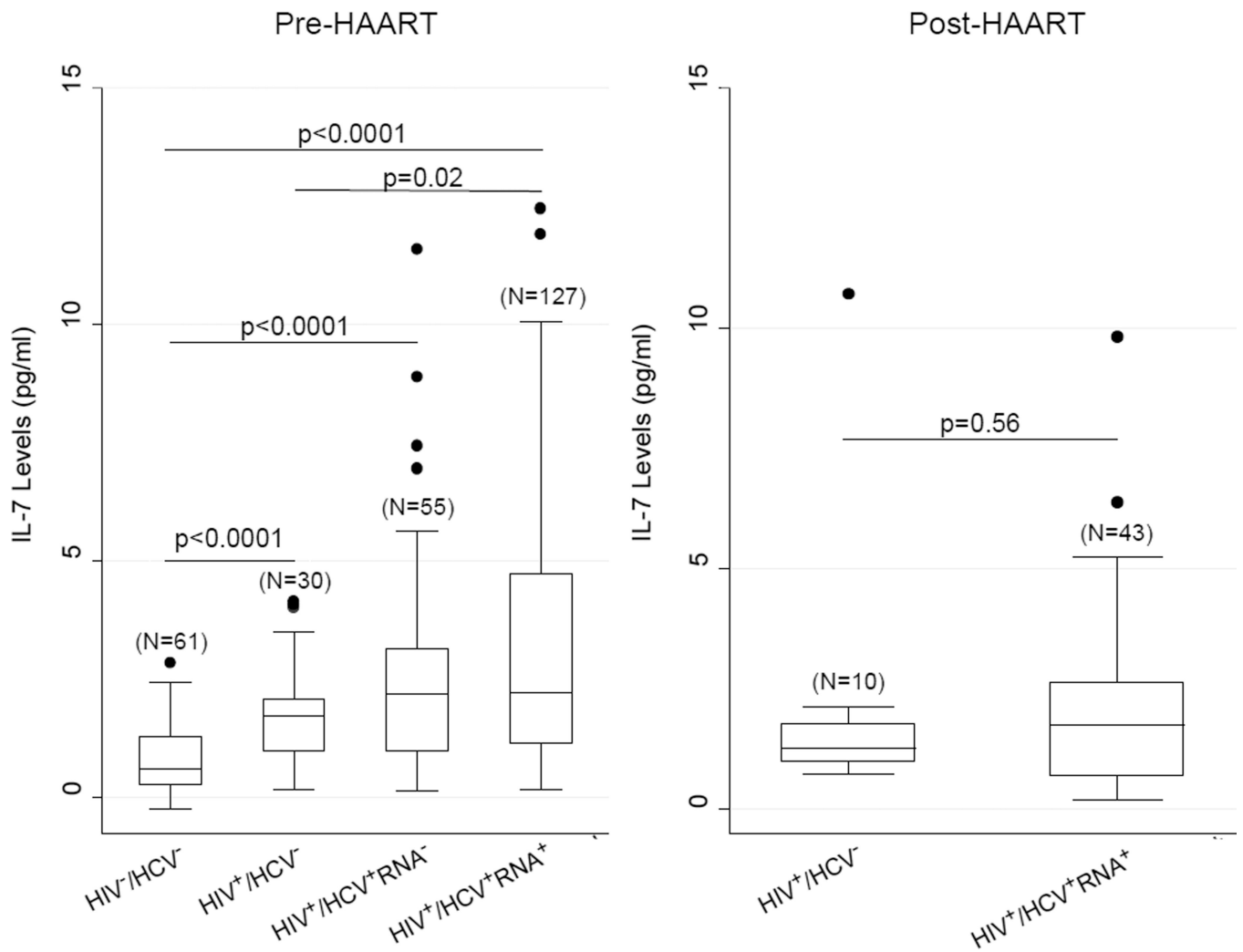
Figure 1. Comparison of IL-7 levels of 273 women at first measurement visit by HIV/HCV groups and HAART status
Plasma levels of IL-7 (pg/ml) were assessed by ELISA. P-values for group comparisons were obtained by Wilcoxon two sample tests.

Table 1

Demographic and clinical characteristics of 304 women at first measurement visit, by human immunodeficiency virus (HIV) infection status, hepatitis C virus (HCV) antibody status, and HCV RNA status.

Variable	HIV −/HCV− N=61		HIV +/HCV− N=56		HIV+/HCV+ RNA− N=55		HIV+/HCV+ RNA+ N=132		Difference between all groups	Difference between HIV+ groups
	N	Column %	N	Column %	N	Column %	N	Column %	P-value	P-value
Age at visit									<.001	<.001
Under 35	38	62%	30	54%	11	20%	29	22%		
35 to 39	11	18%	12	21%	13	24%	43	33%		
40 to 44	10	16%	7	13%	11	20%	41	31%		
45 and over	2	3%	7	13%	20	36%	19	14%		
Mean (SD)	33 (8)		36 (10)		42 (7)		39 (6)			
Min, 25th Q, Median, 75th Q, Max	19, 28, 32, 38, 55		21, 29, 34, 40, 68		28, 36, 41, 46, 68		26, 36, 39, 43, 56			
Race									<.001	<.001
White or Other	8	13%	4	7%	9	16%	22	17%		
African American	19	31%	18	32%	35	64%	76	58%		
Hispanic	34	56%	34	61%	11	20%	34	26%		
Used injection drugs since last visit									<.001	0.01
No	61	100%	55	98%	44	80%	109	83%		
Yes	0	0%	1	2%	11	20%	23	17%		
Used non-injection drugs since last visit									0.02	0.01
No	41	67%	48	86%	35	64%	85	64%		
Yes	20	33%	8	14%	20	36%	47	36%		
Antiretroviral therapy in last 6 months									N/A	<.001
None	N/A		8	14%	34	62%	69	52%		
Monotherapy	N/A		2	4%	13	24%	31	23%		
Combination	N/A		22	39%	8	15%	28	21%		
HAART	N/A		24	43%	0	0%	4	3%		

Variable	HIV −/HCV−		HIV +/ HCV−		HIV+/HCV+ RNA−		HIV+/HCV+ RNA+		Difference between all groups	Difference between HIV+ groups
	N=61		N=56		N=55		N=132			
	N	Column %	N	Column %	N	Column %	N	Column %	P-value	P-value
AIDS									N/A	0.04
No	N/A		50	89%	52	95%	107	81%		
Yes	N/A		6	11%	3	5%	25	19%		

Note: 1. P-values for group comparisons were obtained by chi-square tests.

2. SD: standard deviation, Q: quartile.

Table 2

Immunologic and virologic characteristics at first IL-7 measurement for 61 uninfected women and 212 HIV⁺ women with a pre-HAART visit, by HIV/HCV group.

Variable	HIV −/HCV− N=61		HIV +/HCV− N=30		HIV+/HCV+ RNA− N=55		HIV+/HCV+ RNA+ N=127		Difference between all groups P-value	Difference between HIV+ groups P-value
	N	Column %	N	Column %	N	Column %	N	Column %		
IL-7 (pg/ml)									<.001	0.08
0.84	36	59%	4	13%	9	16%	21	17%		
0.85 – 1.69	15	25%	11	37%	11	20%	27	21%		
1.7 – 2.87	10	16%	11	37%	18	33%	28	22%		
>2.87	0	0%	4	13%	17	31%	51	40%		
Mean (SD)	0.8 (0.8)		1.8 (1.1)		2.6 (2.2)		3.2 (2.7)			
Min, 25th Q, Median, 75th Q, Max	0, 0.3, 0.6, 1.3, 2.8		0.2, 1, 1.7, 2.1, 4.1		0.2, 1, 2.2, 3.1, 11.6		0.2, 1.2, 2.2, 4.7, 12.5			
Plasma HIV RNA level, copies/mL									N/A	0.07
80	N/A		0	0%	2	4%	6	5%		
81–1,000	N/A		8	27%	12	22%	20	16%		
1,001–10,000	N/A		10	33%	11	20%	33	26%		
10,001–100,000	N/A		11	37%	27	49%	44	35%		
>100,000	N/A		1	3%	3	5%	24	19%		
Mean (SD)	N/A		22645 (33217)		33017 (59009)		69678 (140k)			
Min, 25th Q, Median, 75th Q, Max			130, 1k, 6650, 25k, 120k		48, 928, 12k, 42k, 350k		48, 1856, 18822, 42k, 930k			
CD4 T cell count (cells/mm³)									<.001	0.046
200	0	0%	3	10%	8	15%	33	26%		
201–350	0	0%	7	23%	11	20%	27	21%		
351–500	1	2%	13	43%	11	20%	28	22%		
>500	60	98%	7	23%	24	44%	37	29%		
Data missing	0	0%	0	0%	1	2%	2	2%		
Mean (SD)	1072 (343)		432 (238)		483 (260)		397 (266)			
Min, 25th Q, Median, 75th Q, Max	439, 831, 1018, 1248, 2092		63, 286, 406, 496, 1331		28, 298, 475, 626, 1158		18, 198, 364, 526, 1344			

Variable	HIV −/HCV− N=61		HIV +/HCV− N=30		HIV+/HCV+ RNA− N=55		HIV+/HCV+ RNA+ N=127		Difference between all groups	Difference between HIV+ groups
	N	Column %	N	Column %	N	Column %	N	Column %	P-value	P-value
NK cell count (cells/mm³)									<.001	0.13
45	0	0%	9	30%	11	20%	49	39%		
46 – 80	5	8%	6	20%	14	25%	31	24%		
81 – 145	12	20%	4	13%	14	25%	31	24%		
>145	32	52%	6	20%	14	25%	15	12%		
Data missing	12	20%	5	17%	2	4%	1	1%		
Mean (SD)	204 (111)		111 (95)		107 (73)		82 (81)			
Min, 25th Q, Median, 75th Q, Max	64, 123, 197,266, 653		24, 44, 62, 124, 315		1, 53, 86, 149, 296		6, 38, 60, 102, 696			
NK T cell count (cells/mm³)									0.006	0.68
10	5	8%	7	23%	19	35%	33	26%		
11 – 20	7	11%	4	13%	13	24%	33	26%		
21 – 41	13	21%	7	23%	10	18%	35	28%		
>41	24	39%	7	23%	11	20%	25	20%		
Data missing	12	20%	5	17%	2	4%	1	1%		
Mean (SD)	56 (51)		36 (40)		22 (24)		30 (43)			
Min, 25th Q, Median, 75th Q, Max	0, 21, 41, 76, 261		0, 0, 22, 45, 155		0, 0, 18, 34, 100		0, 10, 19, 33, 344			

Note: 1. Plasma levels of IL-7 (pg/ml) were assessed by ELISA. P-values for group comparisons were obtained using chi-square tests.

2. SD: standard deviation, Q: quartile.

Table 3

Cross-sectional univariate analysis of demographic, clinical, and immunological characteristics associated with IL-7 among HIV-infected women by HAART treatment status

Variable	Category	Pre-HAART (n=212)				Post-HAART (n=53[*])			
		Estimate[*]	95% CI	P-value	Global P-value	Estimate[*]	95% CI	P-value	Global P-value
Age at visit					0.001				0.10
	Under 35	Ref				Ref			
	35 to 39	0.70	0.5–0.99	0.04		0.95	0.47–1.9	0.88	
	40 to 44	1.12	0.79–1.57	0.52		1.22	0.61–2.45	0.57	
	45 and over	1.42	0.99–2.03	0.06		2.17	1–4.73	0.05	
Race					0.09				0.47
	White or other	1.12	0.75–1.68	0.57		0.68	0.29–1.6	0.37	
	African American	0.79	0.59–1.06	0.11		1.15	0.69–1.92	0.57	
	Hispanic	Ref				Ref			
Used injection drugs since last visit					0.53				0.23
	No	Ref				Ref			
	Yes	0.89	0.62–1.28	0.53		1.64	0.72–3.72	0.23	
Used non-injection drugs since last visit					0.39				0.03
	No	Ref				Ref			
	Yes	0.89	0.68–1.17	0.39		1.96	1.05–3.63	0.03	
AIDS diagnosis					0.01				0.01
	No	Ref				Ref			
	Yes	1.54	1.1–2.14	0.01		1.88	1.14–3.09	0.01	
Group					0.11				0.94
	HIV+/HCV−	Ref				Ref			
	HIV+/HCV+RNA−	1.26	0.83–1.91	0.27		--	--	--	
	HIV+/HCV+RNA+	1.47	1.02–2.14	0.04		0.98	0.52–1.82	0.94	

Variable	Category	Pre-HAART (n=212)				Post-HAART (n=53)[*]			
		Estimate[*]	95% CI	P-value	Global P-value	Estimate[*]	95% CI	P-value	Global P-value
Plasma HIV RNA level, copies/mL					0.06				0.56
	80	Ref				Ref			
	81–1,000	0.92	0.49–1.71	0.79		1.36	0.59–3.11	0.46	
	1,001–10,000	0.80	0.44–1.46	0.46		1.25	0.66–2.37	0.48	
	10,001–100,000	0.98	0.55–1.76	0.95		1.21	0.59–2.49	0.59	
	>100,000	1.51	0.79–2.88	0.21		2.01	0.88–4.61	0.10	
CD4 T cell count					0.002				0.006
	200	1.99	1.41–2.82	<0.001		2.60	1.4–4.84	0.003	
	201 – 350	1.35	0.96–1.9	0.08		0.90	0.52–1.58	0.72	
	351 – 500	1.29	0.93–1.79	0.13		0.94	0.47–1.87	0.85	
	>500	Ref				Ref			
NK cell count					0.16				--
	45	1.32	0.9–1.94	0.15		--	--	--	
	46 – 80	0.91	0.62–1.34	0.65		--	--	--	
	81 – 145	1.09	0.74–1.60	0.67		--	--	--	
	>145	Ref							
NKT cell count					0.16				--
	10	0.75	0.53–1.07	0.11		--	--	--	
	11–20	0.69	0.48–1	0.05		--	--	--	
	21 – 41	0.69	0.48–1	0.05		--	--	--	
	>41	Ref							

[*] Only one woman was in the HIV[+]/HCV[+]RNA[−] group and therefore was excluded.

Note: IL-7 levels were treated as the continuous outcome variable and were log-transformed to meet normality assumption.

Table 4

Multivariate analysis of characteristics associated with IL-7 among HIV-infected women pre- and post-HAART.

Parameter	Level	Pre-HAART (N=187[*])				Post-HAART (N=52[*])			
		Estimate[*]	95% CI	P-value	Global P-value	Estimate[*]	95% CI	P-value	Global P-value
Age at visit					0.02				
	Under 35	Ref							
	35 to 39	0.82	0.59–1.15	0.25					
	40 to 44	1.21	0.88–1.68	0.24					
	45 and over	1.36	0.96–1.92	0.08					
Race					0.02				
	White or other	0.99	0.68–1.45	0.98					
	African American	0.71	0.54–0.94	0.02					
	Hispanic	Ref							
HCV group					0.12				
	HIV+/HCV−	Ref							
	HIV+/HCV+RNA−	1.31	0.87–1.98	0.19					
	HIV+/HCV+RNA+	1.48	1.01–2.16	0.04					
NKT cell count					0.02				
	10	0.65	0.47–0.9	0.01					
	11–20	0.58	0.41–0.82	0.003					
	21 – 41	0.71	0.51–0.99	0.047					
	>41	Ref							
CD4 count					0.0007				0.006
	200	2.13	1.49–3.04	<0.001		2.60	1.4–4.84	0.003	
	201–350	1.40	1.01–1.94	0.04		0.90	0.52–1.58	0.72	
	351–500	1.28	0.94–1.73	0.12		0.94	0.47–1.87	0.85	
	>500	Ref				Ref			

[*] Excludes 25 pre-HAART women and 1 post-HAART woman with data missing.