Inflammatory Biomarkers Decline but Do Not Normalize after 10 Years of cART

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Abstract 312

BACKGROUND

- HIV infection is characterized by a state of chronic inflammation.
- The degree to which combination antiretroviral therapy (cART) contributes to or ameliorates chronic inflammation over time is unclear.

METHODS

- We measured levels of several inflammatory biomarkers (IB) in the 10 year period following initiation of cART in 336 patients participating in the Study to Understand the Natural History of HIV Infection in the Era of HAART (SUN Study).
- IB levels were also measured in specimens taken at baseline among 40 cART-naïve SUN Study patients, and from a single specimen obtained from each of 30 case-matched HIV-negative controls.
- Frozen serum specimens were analyzed for RANTES, TNF-α, MIP-1α, MIP-1β, MCP-1, and Interleukin (IL)-2, IL-6, IL-7, IL-10, and IL-15 using Quansys Biosystems (Logan, UT) Multiplex ELISA array. Soluble ST2, CD14, and CD163 were measured in serum samples by ELISA, using R&D Systems (Minneapolis, MN) DuoSet kits and ancillary products. Data was analyzed by GraphPad Prism (San Diego, CA). Cryopreserved PMBCs were analyzed by flow cytometry using antibodies from eBioscience (San Diego, CA); CD33 APC, HLA-DR FITC), D14), and CD16 PE). Analysis was performed on a BD FACSCanto utilizing BD FACSDiva software (BD Biosciences, San Jose, CA).
- In the cART-experienced group, IBs were measured at study entry, 2 years and 6 years.
- We stratified the 3 IB samples by number of years continuously receiving cART.
- We used the Kruskal-Wallis test of medians to compare IB levels from the cART-experienced vs. the cART-naive and HIV-negative control groups with SAS version 9.4 (Cary, NC).

RESULTS

- Baseline demographic characteristics were similar amongst the three groups with the exception of a lower percentage of white/non-Hispanic patients in the cART-experienced group (58.9% vs. 72.5% and 73.3%) (Table 1).
- Over the 10 years following cART initiation, levels of several IBs in the cART-experienced group were significantly higher vs. those of HIV-negative controls ($p < 0.05$) (Table 2, Figures 1 & 2). IL-7 was not significantly higher vs. HIV-negative controls after 3 years of cART. IL-2 and IL-10 levels were indistinguishable from HIV-negative control specimens throughout the 10 year period (not shown).
- In the first 4-5 years of cART use, IB levels in the cART-experienced group initially rose above levels measured in cART-naïve patients but thereafter declined to levels below those of the cART-naive group. Over the entire 10 year period, IB levels never declined to those measured in the HIV-negative controls with the exception of IL-7 and ST-2 (Table 2, Figures 1 & 2).
- For each year, there were no significant differences in IBs in the PI vs. NNRTI groups (not shown).
- The HIV and cardio-protective markers: RANTES, MIP-1α, and MIP-1β in cART-experienced patients rose to levels above those of cART-naïve patients and remained so with the exception of MIP-1α. ST-2, a biomarker associated with cardiovascular disease declined to levels below those of both cART-naïve patients and HIV-negative controls for the 10-years since initiation of cART (Table 2, Figure 2).
- Median CD4+ cell count increased from 362 to 660 cells/mm^3 from the first to the tenth year of cART use (Figure 3).

Table 1: Patient characteristics of SUN Study cART Experienced, Naïve, and HIV-negative controls

Patient Characteristic	SUN Study HIV-positive ARV-naives (N=40)	SUN Study HIV-positive ARV-experienced (N=336)	HIV-negative controls (N=30)
Age, median (IQR) years	41 (35-45)	42 (37-49)	42 (36-45)
Male sex, n (%)	32 (80.0)	264 (78.6)	22 (73.3)
Race and ethnicity, n (%)			
White, non-Hispanic	29 (72.5)	198 (58.9)	22 (73.3)
Black, non-Hispanic	10 (25.0)	98 (29.2)	5 (16.7)
Hispanic	0 (0.0)	28 (8.3)	0 (0.0)
Other	1 (2.5)	12 (3.6)	1 (3.3)
Baseline* CD4+ cell count, median (IQR) cells/mm^3	418 (307-557)	478 (330-653)	
Baseline* viral load, median (IQR) copies/mL	14750 (3690-51181)	38 (25-72)	

*Baseline is visit where first set of inflammatory biomarkers were measured.

Figure 2: Cardio-protective Biomarker Levels Over 10 years, SUN Study

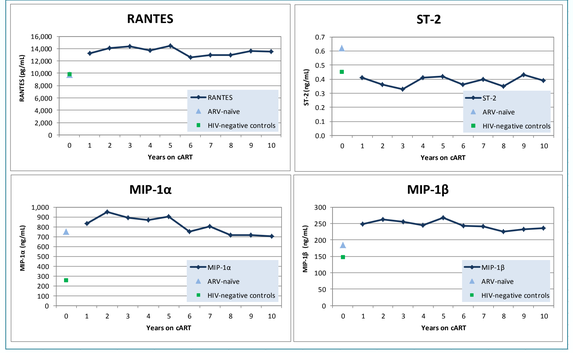
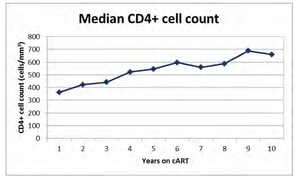


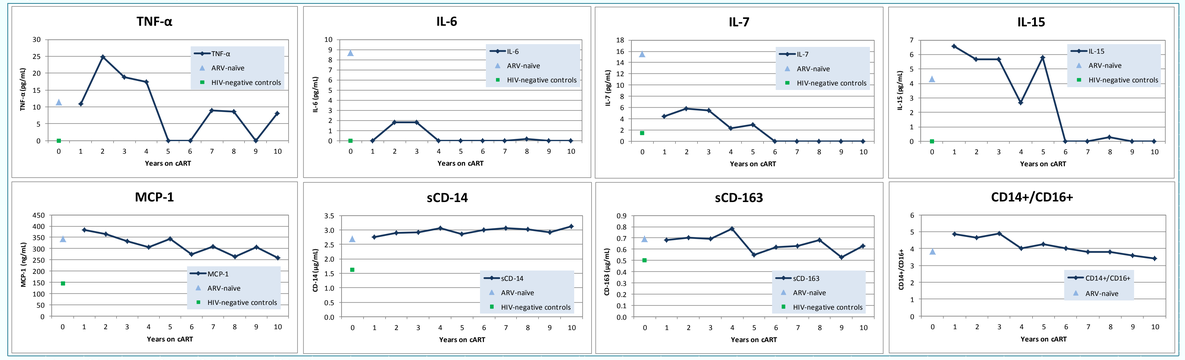
Figure 3: CD4+ cell count among 336 SUN Study patients by years of cART use



LIMITATIONS

- HIV negative control patients had levels assessed on one specimen per patient.
- cART-naïve patients' biomarker levels were those collected at baseline.
- There were no HIV-negative control specimens for CD14+/CD16+ due to different methodologies in the collections of those specimens vs. SUN Study specimens.

Figure 1: Inflammatory Biomarkers Gradually Decline to Levels Below Those of cART-Naïve Patients 4-6 Years After Initiation of cART, SUN Study



Abbreviations: sCD-14, soluble CD-14; sCD-163, soluble CD-163.

Table 2: Median biomarker values among 336 SUN Study patients who had been taking ARVs when biomarker tests were done, by years of ARV experience compared to median values among 40 ARV naives and 30 HIV-negative matched controls

Year	N	RANTES	p: naives	p: controls	TNF-α	p: naives	p: controls	MIP-1α	p: naives	p: controls	MIP-1β	p: naives	p: controls	IL-6	p: naives	p: controls	IL-7	p: naives	p: controls	IL-15	p: naives	p: controls	MCP-1	p: naives	p: controls	ST-2	p: naives	p: controls	sCD-14	p: naives	p: controls	sCD-163	p: naives	p: controls	CD14+/CD16+	p: naives
1	65	13272	↑	↑	10.95	·	↑	836	·	↑	249	↑	↑	0.00	·	↑	4.41	↓	↑	6.57	·	↑	383	·	↑	0.41	↓	·	2.75	·	↑	0.68	·	↑	4.9	↑
2	90	14037	↑	↑	24.80	·	↑	953	·	↑	263	↑	↑	1.81	·	↑	5.82	↓	↑	5.68	·	↑	365	·	↑	0.36	↓	·	2.89	·	↑	0.70	·	↑	4.7	·
3	91	14381	↑	↑	18.88	·	↑	892	·	↑	256	↑	↑	1.81	·	↑	5.44	↓	↑	5.66	·	↑	333	·	↑	0.33	↓	·	2.92	↑	↑	0.69	·	↑	4.9	·
4	77	13689	↑	↑	17.38	·	↑	869	·	↑	245	↑	↑	0.00	·	↑	2.31	↓	·	2.65	·	↑	307	·	↑	0.41	↓	·	3.06	↑	↑	0.78	·	↑	4.0	·
5	70	14439	↑	↑	0.00	·	↑	904	·	↑	267	↑	↑	0.00	·	↑	2.98	↓	·	5.78	·	↑	343	·	↑	0.42	↓	·	2.85	·	↑	0.55	↓	·	4.3	·
6	86	12556	↑	↑	0.04	·	↑	749	·	↑	243	↑	↑	0.00	·	↑	0.00	↓	↓	0.00	·	↑	275	·	↑	0.36	↓	·	3.00	↑	↑	0.62	·	↑	4.0	·
7	109	12953	↑	↑	8.87	·	↑	801	·	↑	242	↑	↑	0.00	·	↑	0.00	↓	·	0.00	·	↑	309	·	↑	0.40	↓	·	3.06	↑	↑	0.63	·	↑	3.8	·
8	83	12993	↑	↑	8.68	·	↑	714	·	↑	225	·	↑	0.14	·	↑	0.00	↓	·	0.28	·	↑	263	·	↑	0.35	↓	·	3.01	·	↑	0.68	·	↑	3.8	·
9	67	13618	↑	↑	0.00	·	↑	717	·	↑	232	·	↑	0.00	·	↑	0.00	↓	·	0.00	·	↑	306	·	↑	0.43	·	·	2.92	↑	↑	0.53	·	·	3.6	·
10	133	13544	↑	↑	8.09	·	↑	701	↓	↑	236	·	↑	0.00	↓	↑	0.00	↓	·	0.00	↓	↑	259	·	↑	0.39	↓	·	3.13	↑	↑	0.63	·	↑	3.4	·
Naives	N=40*	9726			11.43			749			185			8.70			15.52			4.31			342			0.62			2.68			0.69			3.85	
Controls	N=30	9868			0.00			256			147			0.00			1.45			0.00			146			0.45			1.62			0.50			

Legend: ↑=p-value indicates significantly higher ($p<0.05$) median value compared to naives or controls; ↓=p-value indicates significantly lower ($p<0.05$) median value compared to naives or controls; · = non-significant difference compared to naives or controls. Statistical test used: Kruskal-Wallis test of medians. Abbreviations: sCD-14, soluble CD-14; sCD-163, soluble CD-163.

Units: RANTES, MIP-1α, MIP-1β, MCP-1, ST-2, ng/mL; TNF-α, IL-6, IL-7, IL-15, pg/mL; sCD14, sCD163, μg/mL; CD14+/CD16*, percent of monocytes.

* Naives N = 28 for ST2, sCD-14, sCD-163, CD14+/CD16+.

CONCLUSIONS

- After 10 years of cART, levels of several IBs declined but remained elevated compared to those seen in HIV-negative individuals.
- Elevation of IB levels above those of cART-naïve patients over the first 4-6 years of cART suggests a form of immune reconstitution.
- Cardio-protective biomarker levels improved in the first year of initiation of cART vs. those of cART-naïve patients and remained at those levels over the 10-year period.
- There were no significant differences in levels seen with PI vs. NNRTI based cART.

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