



Cytokine levels of interleukin-2 and 7 amongst antiretroviral therapy success and failure HIV patients attending the University Teaching Hospital, Yaoundé, Cameroon

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ABSTRACT

Immune reconstitution complications (IRC) are major problems faced by HIV treated patients worldwide. Interleukin (IL)-2 and IL-7 play vital roles in peripheral T-cell homeostasis. Our study objective was to measure and compare the blood plasma levels of IL-2 and IL-7 amongst antiretroviral therapy (ART) patients attending the Yaoundé University Teaching Hospital, Cameroon. We performed a cross-sectional study with 296 HIV positive patients enrolled between July 2017 and May 2018 at the Yaoundé University Teaching Hospital. IL-2, IL-7, T-cell profile counts and plasma viral load were measured on whole blood specimens. Data obtained were analyzed using Graph Pad Prism 5.0 and Epi info 7.0 Software. IL-2 and IL-7 plasma concentration levels were higher in patients with ART failure compared to ART success, with a mean \pm SD of 19.4 ± 8 and 17.1 ± 6 pg/ml, 35.26 ± 11 and 21.5 ± 5 pg/ml, with $p < 0.001$ and < 0.001 . There was a direct and significant correlation between viral load, IL-2 and IL-7 with p values = 0.028, and 0.020, respectively. There was an association between IL-2, IL-7 and viral load in relation to the duration on treatment (DT), with p values = 0.003 ($R^2 = 0.041$, CI= 0.069 – 0.34), 0.017 ($R^2 = 0.027$, CI= -0.30 – 0.030), and 0.001 ($R^2 = 0.048$, CI= -0.047 – 0.76). Considering that limited surrogate markers are available for monitoring immune reconstitution and high associated mortality rates, IL-2 and IL-7 could be a good immunological predictor for ART failure and success in HIV infected individuals.

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INTRODUCTION

Antiretroviral therapy (ART) has significantly reduced AIDS-associated mortality by controlling viral replication and

preventing severe immune suppression in HIV-infected patients. Approximately 66% of HIV diagnosed patients are receiving ART worldwide (Deeks et al., 2004). The

monitoring of T-cell homeostasis is important for the management of HIV-infected patients on ART. Neglect in monitoring patient immunity could lead to Immune reconstitution complications (IRC) due to the exacerbation of opportunistic infections and other non-AIDS pathologies. It is known that about 10 - 25% of HIV patients and about 20 - 40% co-infected patients on ART are at risk of developing IRC, with a high mortality rate of 2 - 3% (Catalfamo et al., 2008; Lindi et al., 2010). Many countries have adapted to the recent diagnose and treat strategy proposed by WHO in 2017 that recommends the use of viral loads as standard markers for the monitoring of patients on ART (Katherine and Suzanne, 2001; Kinter et al., 2008). That approach does not consider the risk that could arise due to limited information on the homeostatic state of T-cells.

Studies have shown the involvement of interleukin-2 (IL-2) and IL-7 in HIV pathogenesis and their associated switch from T-helper type 1 (Th1) to T-helper type 2 (Th2) responses. IL-2 and IL-7 are known to play essential roles in peripheral T-cell homeostasis and survival (Bruce and Andrew, 2012; Sébastien et al., 2017). Further studies have equally shown the use of these interleukins as immune adjuvants to help stabilize the homeostatic impact of T-cell as indicators of the T-cell immune response (Kinter et al., 2008; Bruce and Andrew, 2012).

IL-2 is a monomeric glycoprotein Type 1 cytokine produced by activated CD4+ and CD8+ T-cells. Its principal role is to stimulate the activation and proliferation of T-lymphocytes and improve antigen processing, secretion and release of other cytokines (Li, 2004). IL-7 is produced by stromal cells in the bone marrow, thymus and lymph node. They are important for T-cell thymopoiesis, and stimulation by IL-7 in the double-negative thymocytes leads to phosphorylation and inactivation of Bad, a pro-apoptotic protein, which depends on the PI3K pathway (Dzhagalov et al., 2008).

There are many studies that have been performed in developing countries on the potential relevance of these cytokines in

monitoring balance in T-cell homeostasis from patients on ART. In Cameroon, with a known high HIV diversity, no similar studies have been done. We hypothesise that IL-2 and IL-7 levels are higher in patients having treatment success in ART compared to failure and therefore are at risk of IRC. Our study aims to measure and compare the levels of IL-2 and IL-7 as signal markers of immune reconstitution of T-cells amongst ART patients.

MATERIALS AND METHODS

Study design

We performed a cross-sectional study with 296 enrolled HIV positive participants. They were enrolled between July 2017 and May 2018 at the Yaoundé University Teaching Hospital. All participants were consecutively recruited from daily consultation by physicians at the Internal Medicine unit at the Yaoundé University Teaching Hospital (YUTH).

Study population

The patients were classified into two groups: Treatment Success and Treatment Failure in proportions 81% and 19%, using the 2016 WHO Classification (WHO, 2016) as follows: Treatment success (VL<1000c/ml, CD4>500/μl and duration under ART > 6months,) and Failure (VL>1000c/ml with CD4 <200/μl on ART above six months). Demographic characteristics and clinical information were collected for each participant using a standard questionnaire. These included age, sex, ART option and duration of ART. All treated patients were on the first-line ART regimen considering the country guidelines, which includes the combination of two nucleoside reverse transcriptase inhibitors (NRTIs), chosen among Zidovudine (AZT), Tenofovir (TDF) and Lamivudine (3TC) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) being either Efavirenz (EFV) or Nevirapine (NVP). Ethical clearances for this study was obtained from the Cameroon National Ethical committee (reference number 044/CNE/SE/2017). Written and verbal

informed consent was given by all participants. The study was conducted according to the ethical guidelines and principles of the international Declaration of Helsinki 2013.

Sample collection and analysis site

Five ml (5ml) of whole blood was collected in EDTA anticoagulant tubes at the sample collection unit of the Yaoundé University Teaching Hospital. Blood samples were transported and analyzed at the Center for the Study and Control of Communicable Diseases (CSCCD) of the Faculty of Medicine and Biomedical Sciences (FMBS), University of Yaoundé I, Cameroon. Plasma specimens were obtained after centrifugation of whole blood at a speed of 5000 rpm for 5 minutes and stored in cryo-vials at -20 °C. Samples were analyzed within two months of the collection to avoid deterioration of cytokines over time.

Measurement of cytokines levels

Plasma levels of IL-2 and IL-7 were measured using quantitative sandwich Enzyme-linked Immune-Sorbent Assay (ELISA) kits (Invitrogen, Thermo Fisher Scientific, USA). A solid-phase Immune-Enzymatic technique was used on a microtiter plate for the quantitative determination of IL-2 and IL-7 in human plasma. Samples were analyzed according to the manufacturer's specifications. The absorbance was read using a spectrophotometer (Biotech ELx800, USA) set at a dual wavelength of 450- 550 nm. Each sample was run in duplicate. The concentration of both interleukins was determined by extrapolating the results from a standard curve generated by plotting the average absorbance (450- 550 nm) obtained for each standard level on the vertical (Y) axis compared to the corresponding IL-2 or IL-7 concentration on the horizontal (X) axis.

Determination of CD4+ and CD8+ T cells absolute count

The measurement of CD4+ T-lymphocytes was done based on the principle of immunophenotyping. Fifty μ L (50 μ L) of whole blood was used for CD4 absolute cell

counts, using the DB FACSCCount reagent kit, and automated machine (BD Biosciences, San Jose, California, USA). Samples, including quality controls, were analyzed based on the manufacturers' guidelines.

Determination of plasma HIV viral load

Determination of the HIV load was performed using the Cobas Ampli prep / Cobas Taqman 96 platform (Roche Diagnostics, Branchburg, New Jersey, USA), per manufacturer's instructions. The detection limit was <40 copies/ml.

Statistical analyses

Data were analyzed using the Graph Pad PRISM 5.0 software package (Graph Pad Software Inc., La Jolla, California, USA) and Epi Info 7.0 software (Epi Info™). Comparisons between IL-2 and IL-7, within the different groups, were performed using the parametric student T-test by evaluating their means and standard deviations. The correlations between IL-2, IL-7 and CD4+T-cells and HIV loads were established using the Pearson's correlation coefficient. Associations in duration on treatment time was done by using a regression coefficient and linear regression using their coefficient of determination. $p < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

We enrolled 296 HIV infected patients of which 75.6% (n= 224) were females and 24.4% (n= 72) males. Their mean age (\pm SD) was 41 ± 10 years. Patients were classified into two groups: Treatment Success 81%, n= 244, and Treatment Failure 19%, (n= 52) (Table 1).

Cytokine levels of IL-2 and IL-7 amongst different groups of patients

IL-2 and IL-7 concentration were higher in patients with ART Failure compared to ART Success, with a median of 16.08 and 12.68 pg/ml; 37.63 and 20.48 pg/ml with $p = 0.0001$ and 0.0001 respectively (Figure 1).

This effect persisted after stratification by duration of treatment (Table 2).

Correlation between Interleukins, CD4 and plasma HIV load

There was a direct correlation between IL-2, IL-7 and viral load, with Pearson's correlation coefficient $r = 0.16$ ($p = 0.0001$, 95% CI = 0.065 to 0.26) and $r = 0.11$ ($p = 0.028$, 95% CI = 0.012 to 0.21), respectively. The correlation was inversely significant between IL-2, IL-7 and CD4 count with Pearson's correlation coefficient $r = -0.29$ ($p = 0.0001$, 95% CI = -0.38 to -0.20) and $r = -0.32$ ($P = 0.0001$,

95%CI = -0.41 to -0.23), respectively (Table 4).

Linear association between variables and treatment duration

There was a significant negative association between IL-2 and IL-7 and viral load in relation to the duration on treatment (DT), with p value = 0.001 ($R^2 = 0.077$, $\beta = -2.03$, 95% CI = -2.72 – (-1.33)), 0.003 ($R^2 = 0.041$, $\beta = -0.08$, 95% CI = -0.10 – 0.27) and <0.0001 ($R^2 = 0.048$, $\beta = -2047.45$, 95% CI = -2589.90 – (-1525.01)), respectively (Table 3).

Table 1: Demographic and clinical characteristics of 296 enrolled participants at Yaoundé University Teaching Hospital between July 2017 and May 2018.

Characteristics		Treatment Failure (n=52)	Treatment Success (n=244)	Total (n=296)
		n (%)	n (%)	n (%)
Sex	Males	15(28)	57(24)	72 (24)
	Females	39(72)	185(76)	224 (76)
Age (years) (Mean±SD = 44 ± 10)	20 – 30	4(8)	1(0.4)	5 (1.7)
	31 - 40	29(50)	88(36.9)	117(39.5)
	41 - 50	11(23.1)	75(30.3)	86(29)
	51 - 60	10(19.2)	56(24.6)	76(25.6)
	61 - 70	0	13(5.7)	13(4.4)
	71 - 80	0	9(2.5)	9(3)
ART regimen	TENLAM-NVP	25(46.2)	138(56.6)	162(55)
	TENLAM-EFV	24(46.2)	72(29.5)	96(32.4)
	TENLAM-AZT	2(3.8)	0	2(0.7)
	DUOVIR-N	2(3.8)	34(13.9)	36(12)
Treatment duration(years) (mean ±SD = 6 ± 2)	1-3	16(30.8)	78(32)	94(32)
	4-6	20(38.5)	66(27)	86(29)
	7-9	10(19.2)	70(28.7)	80(27)
	10-12	4(7.7)	20(8.2)	24(8)
	13-15	2(3.8)	10(4.1)	12(4)

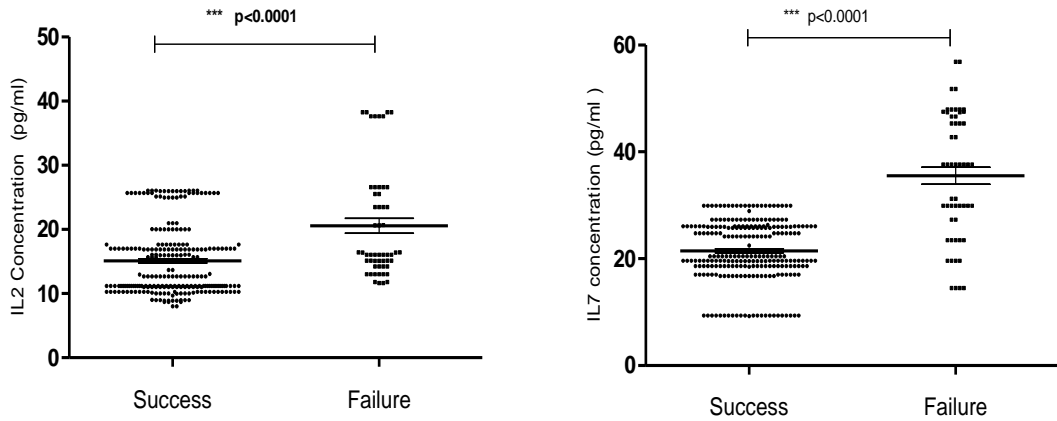


Figure 1: The dot plot illustrates the plasma levels of IL-2 and IL-7 in ART Failure and Success groups.

Table 2: The plasma levels of IL-2 and IL-7 and their linear association with treatment duration of the 296 ART patients.

Treatment Duration (years)	IL7 (pg/ml)			IL2 (pg/ml)		
	Treatment	Treatment	P- value	Treatment	Treatment	P- value
	Failure	Success		Failure	Success	
	(n=52)	(n=244)		(n=52)	(n=244)	
	Median	Median		Median	Median	
	(Range)	(Range)		(Range)	(Range)	
1-3(n= 94)	19.6(14- 57)	20.4(9 -30)	0.296	22.1(15-26)	16(8-26)	0.002
4-6(n= 86)	44(23-52)	18.6(9 - 27)	0.0001	15.6 (12-38)	12(8-26)	0.008
7-9(n= 80)	46.6 (29- 47)	24.2(16-30)	0.0001	15.1 (12-16)	12.6(8-26)	0.57
10-12(n=24)	32.6 (17-38)	25.9(20-30)	0.325	20.6 (13-37)	21(10-25)	0.06
13-15 (n= 12)	18.1 (10- 26)	-	-	-	18(10-25)	-
Total (n= 296)	31.16(10 - 57)	17.6(9 - 30)	0.0001	16(11-38)	12.6(8-26)	0.001

Table 3: Linear association between the plasma levels of IL-2 and IL-7 and the treatment duration of the 296 ART patients.

Parameters	Treatment Duration (TD)			
	IL7	IL2	CD4	Viral Load
R²	0.077	0.041	0.062	0.048
P-value	0.001	0.003	<0.0001	<0.0001
95% CI	-2.72 – (-1.33)	-0.10 – 0.27	12.01 – 27.03	-2589.90 – (-1525.01)
β	β = -2.03	β = -0.08	β = 19.52	β = -2047.45
Equation	IL7 =	IL2 =	CD4 =	VL =
model	38.12 + (-2.03*TD)	16.38+ (-0.08*TD)	387.64+19.52*TD	20215.33+(-2047.45*TD)

Table 4: Pearson Correlation between IL-2, IL-7, CD4 and plasma viral load using Pearson correlation coefficient amongst the 296 ART patients.

	IL7	IL2	CD4
IL7			
IL2	0,054		
CD4	-0,323**	-0,288**	
Viral load	0,109*	0,162*	-0,583***

DISCUSSION

We have evaluated the plasma levels of IL-2 and IL-7 from HIV Infected consented patients experiencing ART success and failure and compared their levels with HIV loads and CD4 cell counts. We have found that, IL-2 and IL-7 levels were higher in plasma of ART failure patients compared with success. We have also found a direct correlation between IL-2 and IL-7 and HIV viral loads with an inverse correlation with CD4. Information generated in this study may reinforce the usage of IL-2 and IL-7 as biomarkers for monitoring immune reconstitution in ART patients.

Our results could be explained based on the following hypothesis. 1) The involvement of IL-2 and-7 in the process of Activated Induce Cell Death (AICD) of T-cell death through signaling mediated by their common receptors (Li, 2004; Dzhalgalov et al., 2008). 2) The pleiotropic nature of cytokines

and their substantial involvement in maintaining homeostasis and survival of T cells relates to this role. 3) The up-regulation of specific cytokines like IL-2 and IL-7 has shown their commitment to T-cell activation, proliferation, lymphopoietic and survival plays major roles in immune reconstitution of T- cells (Chetoui, 2010; Corfe et al., 2011). 4) The variability of these cytokines in treated patients has suggested an alteration of their availability at various production and targets sites (Gougeon et al., 2002; Rethi et al., 2009; Chiodi et al., 2017).

The association of these cytokines with treatment success and failure may be attributed to the levels of IL-2 and IL-7 consumption influenced by the biology of CD4 T-cells during HIV pathogenesis. The cytokines transducer signals and multiple protein kinase pathways have been involved in this process. The balance between proliferation and apoptosis to regulate the size

and diversity of mature naive and memory T-cell pools in the peripheral circulation could also contribute (Meira et al., 2004; Silas et al., 2016).

Our results were similar to studies that have shown that IL-2 and 7 could not improve thymic activity, and may be responsible in the differences in levels within the different groups as it may instead enhance the expansion of cells already in the peripheral T-cell pool (Terry et al., 2001; Huan et al., 2018). The levels of IL-7 remain stable over time and the intrinsic capacity of T-cells to respond to IL-7 signaling in vitro does not appear to diminish with time. Many other studies have also demonstrated the role of IL-2 and IL-7 in homeostasis of T-cells and response in modulating the equilibrium between proliferation and apoptotic cell death in mature naive and memory T-cell subsets (Manu, 2015; Huan et al., 2018).

We have also found that there was a significant negative association between plasma levels of IL-2 and IL-7 and the duration after initiation on ART. Reduction in these cytokines after a long treatment is also consistent with these cytokines being markers of poor immunologic status. Our results also show higher levels of CD4 T-lymphocytes in participants with treatment success compare to treatment failure, as might be expected with immune reconstitution. It is known that HIV indirectly controls uninfected CD4 cells triggering them to apoptosis and destroying them after antigenic activation (Terry et al., 2001; Roger et al., 2012). However, the increase in CD4TL, as a result of the ART, is in part due to the blood trafficking of these cells, sequestered in the lymphatic compartment and increased production rate of circulating lymphocytes resulting from the expansion of peripheral T-cells, apoptosis suppression, and new T-cell production (Ohotu et al., 2015; Susana et al., 2017).

We recognize some limitations of this study. Firstly, we have not had prospective data on each participant due to the cross-sectional study design. Secondly, we have not measured the adherence with ART, although

we did have data on HIV viral load and CD4 count which may be surrogates for adherence.

Conclusion

Our findings have the relevance of identifying markers of immune reconstitution in HIV treated patients on ART. Further studies shall be required in similar cohorts in Africa to validate our preliminary findings. Considering that limited surrogate markers are available for monitoring immune reconstitution and high associated mortality rates, IL-2 and IL-7 could be an important immunological predictor for ART failure and success in HIV infected individuals.

COMPETING INTERESTS

The authors of this paper declare they have no competing interest for this work.

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AUTHORS' CONTRIBUTIONS

CHM and GMI: Development of research concept, recruitment of patients, Laboratory analyses, interpretation of results and participated in the initial draft of the manuscript. GBJ and MCOA: Development of research concept, interpretation of results and drafting of manuscript. MM, CTT and EL contributed in laboratory analyses and drafting of the manuscript. All authors read and approved the final version of the manuscript.

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