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Research Article

Profiles of Peripheral CD4+T Cells Count during Antiretroviral Treatment in Senegalese Adults Infected by HIV: Impact of Therapeutic Associations

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Abstract

Infection with Human Immunodeficiency Virus (HIV) remains a major public health problem despite advances in diagnosis and antiretroviral treatment. We aim to assess the immune reconstitution in Senegalese adults living with HIV. At this end, we conducted a cross-sectional study on 82 HIV-positive subjects under highly active antiretroviral therapy (HAART) to evaluate the peripheral TCD4 profiles during treatment as well as socio-demographic characteristics. Women were the most representative gender group (67%). The median age was 42 years at the inclusion and HIV-1 was predominant serotype (90%). At the beginning of HAART, median CD4 count were 250 cells/µl; 44% of patients living with HIV (PLHIV) had presented the stages III and IV as defined by WHO. During the follow-up of 20 PLHIV1, we found a significant increase of CD4 counts with Combivir + Efavirenz (363 to 444 cells/µl; p = 0.023) and the combination Combivir + Nevirapine (n = 11) (266 to 355 cells/µl (p = 0.021) and Tenolam + Efavirenz (n = 38) (258 and 465 cells/µl; p < 0.001). However, no significant difference in CD4 count was observed for PLHIV-1 under Tenolam + Nevirapine (250 to 358 cells/µl; p = 0.108).For the Combivir + Kaletra second-line treatment, median of CD4 count was 80% fold inPLHIV-2 and PLHIV-1+2 after 12 months of treatment. We also found a positive change in the median CD4 count except for PLHIV-1+2 under Kaletra + Tenolam. We did not find association between the CD4 count and the duration of treatment (rho = 0.201 and p = 0.359). Poor adherence to treatment was observed in 13% of cases. Our data have shown that CD4T cells counts is an important aspect of monitoring of HAART, suggesting that overall, the HIV-1 treatment lines used in national guideline improve live of patients through enhancement of immune reconstitution.

Keywords: HIV Infection, T CD4 count, HAART, Senegal

Introduction

Thirty-three years after its discovery, the Human Immunodeficiency Virus (HIV), responsible for the AIDS pandemic, remains a major public health problem despite advanced researches providing better diagnostic and therapeutic tools. The virus targets especially CD4+ T cells, leading to deficiency of the immune system and altering therefore defense against infections and cancer cells. Antiretroviral therapy in patient with HIV infection generally leads to a significant decrease of the viral load and immunity reconstitution through up regulation of CD4+ T cell count.

In 2015, 25.6 million people were infected by HIV in Sub-Saharan Africa and it also accounts for nearly 2/3 of new infections worldwide [1]. According to the latest estimation in Senegal, around 39,000 persons were infected by the virus [2,3]. In Senegal, decentralization policies have led to a significant increase of ART in people living with HIV (PLHIV), going from 1,855 patients in 2004 to 13,716 patients in 2013 [4]. Efforts have been made by the Senegalese government for widely implementation standards and policies in peripheral centers. Indeed, peripheral centers are now able to provide comprehensive care of HIV infection among others screening, clinical, and biological monitoring, antiretroviral drug dispensing, therapeutic education, and treatment adherence consultations. Such wide implementations of policies and standards of HIV care aim to improve health of HIV infected people and control the infection. However, there are few national studies investigating in deep how standards and policies now well established around the impact on HIV indictors. One of the important aspects of HIV interventions that deserve to be evaluated is immunological

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monitoring of antiretroviral treatment. The purpose of our study was to investigate the immune reconstitution in Senegalese adults living with HIV (PLHIV) by analyzing the dynamic of TCD4 during antiretroviral therapy.

Materials and Methods

Study population

This study included all PLHIV followed at the outpatient clinic at the Antiretroviral Treatment Center (ATC) of General Hospital of Grand-Yoff for consultations and under HAART from November 2015 to February 2016. We were also interested by the socio-demographic, immunological, and therapeutic characteristics of people theses PLHIV. All patients gave their informed consent prior participation in the study. Our study guarantees the confidentiality of the data. The samples and analyses were made free of charge for all patients as part of the national HIV / AIDS program.

Were excluded from this study all patients who were not fully followed-up for the whole duration of the study and those whose records were incomplete.

Therapy regimen

For PLHIV-1 in the first line, the regimen used included 2 NRTI (Nucleoside/Nucleotide Reverse Transcriptase Inhibitors) such as Combivir or Tenolam, and 1 NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitor) such as Efavirenz or Nevirapine.

For PLHIV-2 and PLHIV-1 in the second line, the regimen used is 2 NRTI (Nucleoside/Nucleotide Reverse Transcriptase Inhibitors) such as Combivir and Tenolam, and 1 PI (Protease Inhibitor) such as Kaletra [5,6].

Data collection

Data was collected on medical fact sheets and electronically recorded using the Microsoft Office Access database management software (version 2007). Socio demographic, clinical and biological data were collected as well. Data on the treatment adherence was obtained from information collected through questionnaire and the psychological profile of the patients assessed by experienced social worker present during the consultations.

Methods

From peripheral EDTA blood, CD4 T lymphocytes count was performed by flow cytometry using Alere Pima® CD4 (Waltham, Massachusetts, USA). Data was entered and analyzed using Statview® 5.1 (SAS Institute Inc. Version 5.0). A p-value below 0.05 was considered as statistically significant. The categorical variables were expressed in proportions and the quantitative variables in medians according to their applicability conditions/ relevance. The groups were compared using the Wilcoxon rank test. The correlation between treatment duration and the dynamic of CD4 was calculated using the Spearman Rho correlation test.

Results

Socio-demographic, clinical and biological of the study population

The study included 82 patients. The study population is mainly female (67%). The median age at the onset of ART was 42 years (Min: 26 - max: 71). The median duration of the follow-up was 42 months [12-120] and the median CD4 count at inclusion was 250 cells / µL. Serological and clinical characteristics of the study population are summarized in Table 1.

Characteristics		N	%
HIV serotype	HIV1	74	90
	HIV2	4	5
	HIV1 + HIV2	4	5
WHO clinical stage			
Stage I	at diagnosis	15	18
	evaluation of treatment	72	88
Stage II	at diagnosis	20	24
	evaluation of treatment	4	5
Stage III	at diagnosis	33	40
	evaluation of treatment	4	5
Stage IV	at diagnosis	3	4
	evaluation of treatment	2	2
Uninformed		11	14
Opportunistic infections		34	41

Table 1: Serological and clinical characteristics of the study population.

Serological profile	Therapy line	Therapy regimen	N	%
HIV-1	1st	Tenolam (TDF + 3TC) + Efavirenz (EFV)	38	46
		Tenolam + Nevirapine (NVP)	3	4
		Combivir (AZT + 3TC) + Efavirenz	20	24
		Combivir + Nevirapine	11	14
	2nd	Tenolam + Kaletra (LPV/r)	2	2
HIV-2	1st	Tenolam + Kaletra	4	5
HIV-1+2	1st	Combivir + Kaletra	4	5

Therapy regimen

TDF : tenofovir disoproxil fumarate, 3TC : 2',3'-dideoxy-3'-thiacytidine (lamivudine), AZT : azidothymidine (zidovudine), LPV/r : Lopinavir/ritonavir.

Table 2: Distribution of patients with respect to their serological profile, the treatment regimen in place and their current treatment.

The most common opportunistic infections were tuberculosis (31%), shingles (21%) and, oral and / or esophageal candidiasis (10%).

Distribution of patients with respect to their serological profile, the treatment regimen in place and their current treatment

The most represented regimen of antiretroviral treatment was two nucleoside reverse transcriptase inhibitors (NRTIs) associated with a non-nucleoside reverse transcriptase inhibitor (NNRTI). Such therapeutic protocol was applied to 72 patients (88%) of the study population. The combination of Tenolam (Lamivudine / Tenofovir) + Efavirenz was the most commonly used first-line treatment in PLHIV1 (46% of the study population).

For both PLHIV-2 and PLHIV-1+2 groups, the two combinations of Combivir (Lamivudine / zidovudine) + Kaletra (Lopinavir / ritonavir) and Tenolam + Kaletra were equally used in 5% of the study population.

Regarding the HAART line, 98% of the study subjects were in the first line of therapy whilst only 2%were (all PLHIV-1) were in the second line (Tenolam + Kaletra) (Table 2).

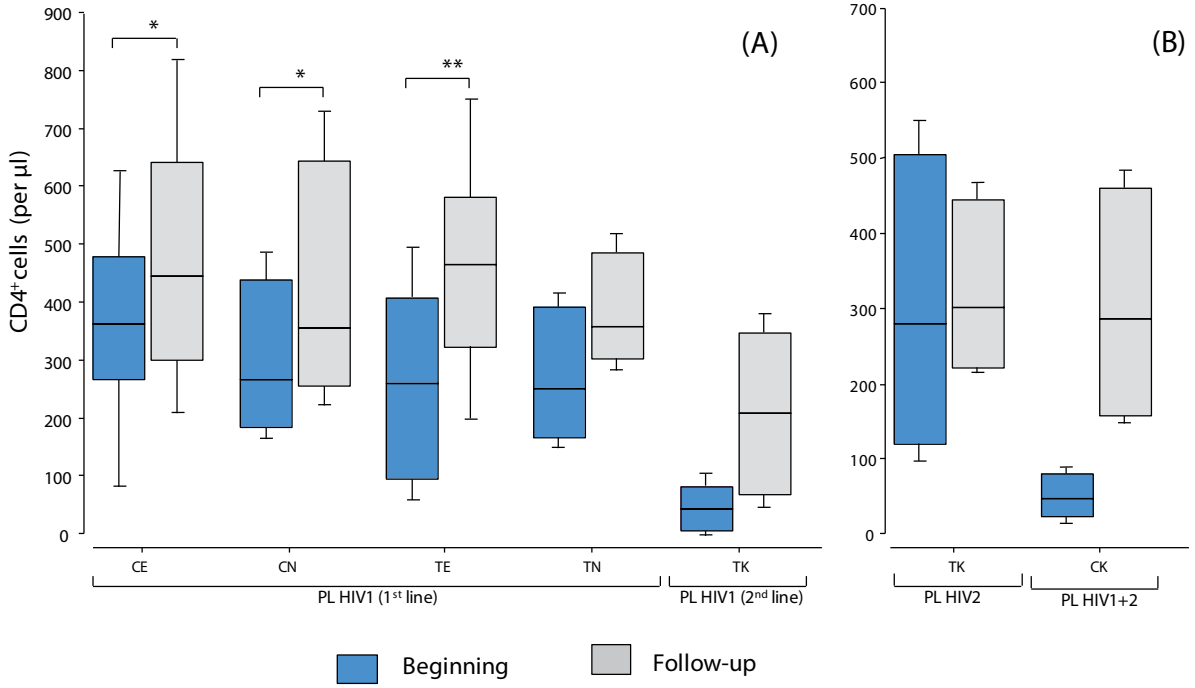

Figure 1: Evolution of CD4 under HAART. Box plots illustrating absolute CD4 count (A) in first line treatment in CE, CN, TE, TN, and TK and (B) second line treatment in TK and CK are shown. Data are shown as median values and 25%-75% interquartiles. *P*-values were calculated in Statview® 5.1using nonparametric Wilcoxon rank test and the graphing was performed using the same software. Only *P*-values for significant differences are shown in the figures. Abbreviations: CE:Combivir + Efavirenz, CN:Combivir + Nevirapine, TE:Tenolam + Efavirenz, TN:Tenolam + Nevirapine, TK:Tenolam + Kaletra, CK :Combivir + Kaletra; PLHIV: people living with HIV; **P*<0.05, ***P*<0.01

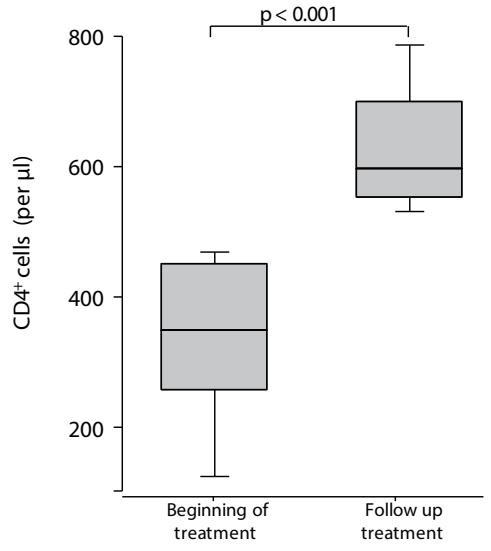

Figure 2: Evolution of CD4 to PLHIV in immune restoration. Box plots illustrating absolute CD4 count at baseline HAART (beginning of the treatment) and during follow up of HAART are shown. Data are shown as median values and 25%-75% interquartiles. *P*-values were calculated in Statview® 5.1 using nonparametric Wilcoxon rank test and the graphing was performed using the same software.

Immunological response of patients under HAART

There was increase in the median CD4 for all participants except for a PLHIV1/2 for which only 69 cells / µL. Increase in the the median of CD4 count median was 538 cells / µLin HIV-2 infected patient under first line of treatment and Combivir + Kaletra. Fourteen patients (17% of the study population) did not respond to the treatment.

Median CD4 counts at the beginning of the treatment and during the follow-up were respectively of 363 and 444 cells/l (p = 0.023) for PLHIV-1 on Combivir + Efavirenz (n = 20), 266 and 355 cells/µl (p = 0.021) for those receiving Combivir + Nevirapine (n = 11), 258 and 465 cells/µl (p < 0.0001) for those under Tenolam + Efavirenz (n = 38), 250 and 358 cells/µl (p=0.108) for patients on Tenolam + Nevirapine (n = 3) (Figure 1).

Immune restoration

Twenty-two patients (26.8%), immunosuppressed at the beginning of the treatment, underwent an immune restoration (CD4> 500 cells / µL) during follow-up. All were infected with HIV-1 except 1 who was infected with HIV-2. Their median CD4 went from 349 cells / µL at baseline to 594 cells / µL after follow up (p <0.001) (Figure 2). All immune restoration patients were observant, had no adverse effects and had good psychological status.

The results obtained are detailed in Table 3. Distribution of patients with immune restoration is represented in Table 4.

Discussion

HIV infection remains a public health problem in Senegal. Recent progress was observed with a stabilization of the AIDS

Characteristics	N	%
Number of patients	22	100
Gender		
Females	17	77
Males	5	23
Current therapy regimen		
Tenolam + Efavirenz (TE)	12	55
Combivir + Efavirenz (CE)	5	23
Tenolam + Nevirapine (TN)	1	4
Combivir + Kaletra (CK)	1	4
Combivir + Nevirapine (CN)	3	14
Serological profile		
HIV1	21	95
HIV2	1	5
WHO clinical stage at diagnosis		
Stage I	6	27
Stage II	5	23
Stage III	7	32
Stage IV	0	0
Uninformed	4	18
Current WHO clinical stage		
Stage I	21	95
Stage II	1	5
Opportunistic Infections	4	18
Co-infections	3	14

Table 3: Characteristics of the patients with immune restoration.

epidemic with 50% of reduction for new infections between 2001 and 2012 [4]. The early management of infected individuals was facilitated by voluntary screening test and antiretroviral treatment became free with the Senegalese Initiative for Antiretroviral Access (ISAARV) [7]. In Senegal, the CD4 threshold at which PLHIV are treated was 500 cells/ µl at the time of the study. According to these improvements in the care of PLHIV, we proposed to evaluate the impact of antiretroviral treatment on the dynamic of CD4+ T cells of HIV positive adults followed at General hospital of Grand-Yoff. This work is part of the epidemiological, immunological and biological investigations conducted in collaboration between our unit and hospital treatment center. The main objective was to evaluate the immunological profile following antiretroviral therapy by measuring the dynamic of restauration of CD4 T lymphocytes.

Immunological monitoring of people living with HIV (PLHIV) is a major criteria to evaluate the response of patients to antiretroviral therapy. It's the circulating CD4 count which allows to evaluate the immunological status of the patient and the stage of the disease since it's the only immunological parameter useful for monitoring patients infected by HIV [8].

The study population was predominantly female (67%). This trend is similar that of Akilimali, et al. [9] in Congo and Diane, et al. [10] in Guinea, who respectively find 66.5% and 68.2% of women, confirming therefore the feminization of HIV infection. The median age at onset was 42 years. This result is similar to that found by Balestre, et al. [11], which is 40.6 years old, but differs from that of Ayelola, et al. [12], which is 57 years among PLHIV who suffered from stroke.

HIV-1 infection was predominant (90%) in our study. Only 5% of patients were infected with HIV-2 and 5% co-infected with both HIV-1 and HIV-2. Similar proportion was reported by Deguenonvo, et al. [13] with 90% of HIV-1, 6% HIV-2, and 4% of HIV-1- and HIV-2-infected subjects. Similar proportion of HIV-1 infection was also reported in West Africa with 93.6% in the Ivorian [14]. This broad prevalence of HIV-1 infection is consistent with the fact that HIV-1 has a higher transmission rate compared to HIV-2 and is generally less pathogenic [15,16]. Moreover, HIV-1 is found all over the world while HIV-2 is confined to West Africa [15].

In our study 40% of patients were diagnosed at stage III of the WHO and 24% at stage II, showing delay in HIV diagnosis and management. The percentage of patients in stage I increased from 18% at baseline to 88% after HARRT follow-up, indicating significant clinical improvement following antiretroviral treatment. This shows progress in HIV management since previous data did not show such good treatment efficacy [17,18].

The most treatment line used was 2 NRTI (Nucleoside/ Nucleotide Reverse Transcriptase Inhibitors) + 1 NNRTI (Non-Nucleoside Reverse Transcriptase Inhibitor) (88% of patients), followed by the combination 2 NRTI + 1 PI (Protease Inhibitor) in 12%. These results are almost the same of those of the study conducted in Mali by Kone [19] for who the regimen 2 NRTI + 1 NNRTI concerned 95.8% of patients and the regimen 2 NRTI + 1PI 4.2% of patients. PIs were primarily used as first line treatment in PLHIV-1 + 2, and second line in PLHIV-2 and PLHIV-1; however in our study population, proportion of patients in second-line was small with only 12%.

This result is in agreement with the study carried out in West Africa by Bashi, et al. [20], who found 83% of the patients on the first line of treatment. The combination of Stavudine, Lamivudine and Nevirapine is the most used with 48.6 % of the study population.

	CE	CN	TE	TN	CK
N (%)	5 (23)	3 (14)	12 (55)	1 (4)	1 (4)
Length of ART in months med [min - max] cells/µL	60 [13 - 105]	86 [82 - 98]	23.5 [7 - 99]	48	82
CD4 count at the beginning med [min - max] cells/µL	454 [150 - 492]	465 [355 - 473]	296 [69 - 449]	437	52
CD4 count during follow-up med [min - max] cells/µL	637 [529 - 838]	716 [697 - 745]	580 [507 - 841]	527	590
CD4 in gain med [min - max] cells/µL	193 [66 - 688]	251 [224 - 390]	301 [102 - 507]	90	538

Therapy regimen
CE: Combivir + Efavirenz, CN: Combivir + Nevirapine, TE: Tenolam + Efavirenz, TN : Tenolam + Nevirapine, TK : Tenolam + Kaletra, CK : Combivir + Kaletra
ART : antiretroviral treatment

Table 4: Distribution of patients who restored their immunity.

Regarding the outcome of immunological parameters, we observed overall increase of CD4+ T cell counts in patients under HAART except for 14 patients. This constant evolution of the circulating CD4 is also found by Henard [21] on a cohort in the north and east of France.

During follow-up, 41% of patients displayed opportunistic infection, which is much lower than those reported by Kra [22] for which 74% of the study population presented opportunistic infections. However, both studies agree that the tuberculosis is the most represented opportunistic infections. In addition, Deguenonvo, et al. found tuberculosis as the most diagnosed opportunistic infections (40.9%) [23].

In our study, 22 patients with immune depression at treatment initiation underwent an immune restoration with CD4 count above 500 cells / µL after treatment. Inter country comparison of immune restitution under first-line antiretroviral therapy has shown higher immune restoration in the Northern countries (41%) than in the South (15%) [24]. As southern country, our data are consistent with those of Clement, et al. Most importantly, all the patients undergoing immune restoration were observant presented a good psychological state and did not present any adverse effects. These factors may explain why they responded well to antiretroviral therapy.

One of the limitation of our study is absence of viral load despite the recommendations of UNAIDS [25], considering viral load measurement as a key step in monitoring of antiretroviral therapy. Indeed, viral load platform does not available at HOGGY.

Conclusion

In this study, we showed that immunological monitoring of PLHIV under HAART is essential for monitoring of clinical improvement of HIV-infected patient and early identification of treatment failure in PLHIV. However, we did not find relation between increase of CD4 counts and the duration of treatment. Altogether, our data show that the national guidelines provide significant immune reconstitution in HIV infected people and therefore need to be strengthened. We plan to extend this preliminary study at HOGGY to help the national HIV program in improvement of PLHIV care.

The importance of this study lies in the fact that HIV infection is a real public health problem affecting the world. This study has also enabled us to provide essential information in the fight against HIV. It emerges that early initiation of antiretroviral therapy, as well as immunological follow-up and good adherence are essential for better care of PLHIV.

Also, we were able to make recommendations, especially on raising awareness among populations, promoting access to viral loads for all treatment sites and correlating circulating CD4 count with viral load in order to highlight eventual immuno-virologic dissociations.

Conflict of Interest

The authors confirm that this article content has no conflicts of interest.

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