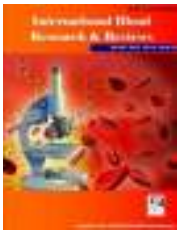

International Blood Research & Reviews
2(6): 322-334, 2014, Article no. IBRR.2014.6.007

SCIENCEDOMAIN *international*
www.sciencedomain.org

Immunological and Hematological Profiles of Human Immunodeficiency Virus Positive Patients on Antiretroviral Treatment Followed at Institute Pasteur of Ivory Coast

A. F. J. Trébissou[1,2], C. C. A. Yapo-Crezoit[2], F. A. Yapo[1*], A. Yéo[2], F. H. Yapi[1] and J. A. Djaman[1,3]

[1]Laboratory of Biochemistry-Pharmacodynamics, Faculty of Biosciences, University of Cocody-Abidjan, Ivory Coast, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2]Pole of Biology Immunity, Institute Pasteur of Ivory Coast, 01 BP490 Abidjan 01, Côte d'Ivoire.
[3]Department of Medical Biochemistry and Fundamental, Institute Pasteur of Ivory Coast, 01 BP490 Abidjan 01, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

Received 27th April 2014
Accepted 16th July 2014
Published 30th July 2014

ABSTRACT

Aims: The immune system and some of the organs of HIV positive patients may be subject of some disruptions caused by Anti-Retroviral Treatment (ART). This study has been initiated in order to assess the impact of ART on the immunological and hematological parameters of People Living with HIV (PLHIV) followed at Institute Pasteur of Ivory Coast (IPIC).
Methodology: This cross-sectional study took place from August 2012 to August 2013 on 45 HIV-positive patients under first line ART, aged from 19 to 63 years of both genders with 30 women and 15 men. Three therapeutic treatments have been used during the study and are: the determination of CD4+ T-lymphocytes and the blood cells count have been determined before the beginning of ART. Profiles for CD4+ and blood cells count were studied every 15 days (D15), 6 months (M6) and 12 months (M12) after taking ART.

**Corresponding author: E-mail: fyapo@yahoo.fr;*

Results: This study showed that CD4+ T-lymphocytes count was lower at first than 200 cells/mm^3, has increased significantly from (P< 0.0001) to values greater than 350 cells/mm^3 and restored the level of hemoglobin to its standard values after one year of antiretroviral treatment. With the exception of the TDF+FTC+EFV, all therapeutic combinations showed a neutropenia and a macrocytosis. These anomalies could be related to the presence of lamivudine (3TC) in the therapeutic combinations.
Conclusion: This study showed that the ART restore the immune system, but could be a cause of anemia in the long term. Consequently, their use by HIV positives patients should be accompanied by some anti-anaemic treatment.

Keywords: HIV; anti-retroviral treatment; CD4+; blood cells count; Institute Pasteur of Ivory Coast.

1. INTRODUCTION

In the world and particularly in Sub-Saharan Africa, HIV/AIDS is a real public health problem. The number of people living with human immunodeficiency virus (HIV) worldwide was estimated to be 34 million in 2011 [1]. In 2011, more than 69% of HIV positives patients were residing in sub-Saharan Africa, a region that represents only 12% of the world population [2]. The sub-Saharan Africa therefore remains the region most severely affected in the world [3]. In Ivory Coast, the prevalence of HIV/AIDS is of 3.4% [4].

The Highly Active Antiretroviral Therapy (HAART) formed with the combination of ART drugs constitutes the current therapeutic arsenal [2]. The role of these ART is to make undetectable the viral load in plasma below the threshold of detection (50 cells/ml), in order to avoid the opportunistic diseases and the resistors [5]. However, morbidity from other chronic conditions such as kidney, liver and heart disease is increasing [6,7]. The use of ART by people living with HIV (PLHIV) often causes disturbances on immune system and certain organsas the spinal cord, the liver and the kidney pulling certain diseases such as anemia, neutropenia, lymphopenia, thrombocytopenia, hepatitis and renal insufficiency.

In Ivory Coast, the government in its fight against this pandemic set up a national policy of coverage of HIV-positives patients [8] by using regimens recommended in National Guideline for Adult HIV and AIDS Treatment and Care. So, the other structures and non-governmental organization directed their fight in this sense [9]. This coverage is only based on the biological and psychosocial follow-ups of the PLHIV as well as therapeutic. According to the ministry of Health of Ivory Coast, a HIV-positive patient is eligible in the antiretroviral treatment (ART) when CD4+ T-lymphocytes countis lower than 350 cells/mm^3 [8] or patient makes one or several opportunist diseases such as tuberculosis, zoster etc. The Institute Pasteur of Ivory Coast used six first-line therapeutic combinations for the follow-up of adults HIV-positives patients (more than 18 years) of period from August 2012 to August 2013. Among these HAARTs, four belonged to the family of 2 nucleoside reverse transcriptase inhibitors (2NRTI) + 1 non-nucleoside inhibitor reverse transcriptase inhibitor (1NNRTI) used only for the treatment of PLHIV1. A combination belonged to the family of 2 nucleoside reverse transcriptase inhibitors (2NRTI) + 1 protease inhibitor (1IP). The last combination belonged to the family of 3 nucleoside reverse transcriptase inhibitors (3NRTI). These two last regimens are used both for the treatment of HIV-2 [8] and HIV-1. These therapeutic regimens change according to the health of PLHIV as he made renal insufficiency, anaemia, hepatitis, etc.

This study is therefore proposed to clarify the influence of ART on the immunological and haematological parameters of HIV-positives patients from the Institute Pasteur of Ivory Coast.

2. MATERIALS AND METHODS

2.1 The Study Population

The study population was constituted by the HIV-positive patients and followed in the unit of Clinical Investigation (UIC) of the Institute Pasteur of Ivory Coast. This study took place from August 2012 to August 2013 on 45 HIV-positives patients under antiretroviral treatment. Among these patients, there were 15 men and 30 women is a sex ratio of 1/2. The average age of the patients was 38.37±9.93 with extremes of 19 and 63 years. The age group between 31-40 years was represented in 42.22% of the cases. The clinical and immunological stages of HIV patients were not considered.

On 45 studied patients, 40 patients were carriers of the HIV-1 either 88.89%, 4 patients were carriers of the HIV-2 or 8.89% and only patient was expanding of the two-tier VIH1+2 or 2.22%. Patients have received several regimens and therapeutic combinations during the study period (Table 1).

According to the Table 1, three HAART were used during the follow-up what are 2 nucleoside reverse transcriptase inhibitors + 1 non-nucleoside reverse transcriptase inhibitor (2 NRTI+1NNRTI);2 nucleoside reverse transcriptase inhibitors + 1 protease inhibitor (2 NRTI+1IP) and 3 nucleoside reverse transcriptase inhibitors (3 NRTI). The greater part of the patients was under 2NRTI+1NNRTI (86.66 %of the cases). The association of molecules AZT+3TC+NVP was the regimen the most used by the majority of the patients. These ART have been taken from the first day without ART (D0) until the 12th month (M12) after taking ART.

2.1.1 Inclusion criteria

The adults HIV-positive patients (18 years and more) diagnosed positives to HIV during the period of study were included in this study, with an initial evaluation and under antiretroviral treatment (ART).

2.1.1.1 Exclusion criteria

The patients having only data at the initial assessment and with treatment for opportunistic infections; the children (0 to 17 years) and the pregnant women were excluded from the study.

Table 1. Distribution of patients according to the drawings and therapeutic combinations used during follow up

Families	HAART	D0		D15		M6		M12	
		Effective (PLHIV)	Frequency (%)	Effective (PLHIV)	Frequency (%)	Effective (PLHIV)	Frequency (%)	Effective (PLHIV)	Frequency (%)
2NRTI + 1NNRTI	TDF+FTC+EFV	4	8.88	4	8.88	4	8.88	4	8.88
	D4T+3TC+NVP	2	4.44	2	4.44	2	4.44	2	4.44
	AZT+3TC+NVP	32	71.11	29	64.44	28	62.22	28	62.22
	AZT+3TC+EFV	4	8.88	5	11.11	5	11.11	5	11.11
2NRTI +1IP	AZT+3TC+LP/RT	2	4.44	3	6.66	4	8.88	4	8.88
3NRTI	AZT+3TC+ABC	1	2.22	2	4.44	2	4.44	2	4.44

2 NRTI +1NNRTI = 2 nucleoside reverse transcriptase inhibitors + 1 non-nucleosidereverse transcriptase inhibitor, 2 NRTI+1IP =2 nucleoside reverse transcriptase inhibitors + 1 protease inhibitor, 3NRTI = 3 nucleoside reverse transcriptase inhibitors, PLHIV = People living with HIV

2.1.2 Apparatus and reagents used

The cytometer flow BD FACSCalibur (Becton Dickinson or BD, USA) was used for enumeration of CD4+T-lymphocytes [10,11]. With respect to the determination of the serology for HIV and the serotyping, respectively have been used the reagents that are the DETERMINE® HIV-1/2 (DETERMINE, USA) and the GENIE™ III HIV-1/HIV-2 (USA) [12]. The blood count has been done on the globular counter SYSMEX XT-1800i (Sysmex GmbH, Germany).

2.2 Sampling and Determination of the Serology for HIV

Between 7:00 and 9:00 am venous blood samples of starved HIV-positives patients, were taken in dry tubes, in EDTA tubes and in oxalate fluoride tubes to the first day before taking the ART(D0) and to 15 days (D15), 6 months (M6) and 12 months (M12) after taking ART. The sample room was kept at 20±2ºC.

The Whole blood in dry tubes was centrifuged rapidly in a centrifuge JOUAN BR4i® brand (Buckinghamshire, England) at 3000 rev/min for 10 min to obtain serum. A part of this serum was used for the detection of antibodies to HIV-1 and antibodies against HIV-2 with the test immune-chromatographic DETERMINE® HIV-1/2 [13]. The serum has also been used to test of HIV serotyping, with GENIE™ III HIV-1/HIV-2 test. This test uses the immune-chromatography and immune-concentration in combination [13]. The other part of serum was aliquoted and stored at -80ºC for further studies.

2.3 CD4+T lymphocytes Count and Complete Blood Count

The whole blood collected in EDTA tubes was used the same day within 4 hours after harvesting to make the CD4+ T-lymphocytes count with the flow cytometer in stream BD FACScalibur, [14] and the complete blood counts (CBC) using the SYSMEX XT-1800i (Sysmex GmbH, Germany). The normal values are represented in Table 2. Any variation of these standard values reflects the presence of an imbalance.

Table 2. Normal values of different biological parameters [10]

Immunological parameters and haematological	Normal values (15 Years and more)
Absolute value of CD4+	400-1750 Cells/mm3
Red blood cells (GR)	3.8 - 6.0 (10^6/ µl)
Hemoglobin (HBG)	11.5 -18 G/dl
Haematocrit (HCT)	37-54%
Mean corpuscular volume (MCV)	80-95 Fl
Mean corpuscular hemoglobin content (MCH)	27-32 PG
Mean corpuscular hemoglobin concentration (MCHC)	32-36 G/dl
platelets	150-400 (10^3/ µl)
White blood cells (WBC)	4-10 (10^3/ µl)
Absolute value of neutrophils	1.8 -7 (10^3/ µl)
Absolute value of lymphocytes	0.4 -4 (10^3/ µl)
Absolute value of monocytes	0.1 -1 (10^3/ µl)
Absolute value of eosinophils	0.1 -0.6 (10^3/ µl)
Absolute value of the Basophilic	00.1 (10^3/ µl)

Patients have received several regimens and therapeutic combinations during the study period (Table 2)

2.4 Statistical Analysis

Descriptive statistics for basic characteristics of the HIV-positives patients were carried out and univariate analyses, where Pearson chi-square tests were performed to test associations between HIV serotype, ARTs and biological parameters. Due to the small population size, the non-parametric Dunnett test was performed to assess differences between the therapeutic regimens. A multiple logistic regression analysis was used to determine the predictor for this study. The result was considered statistically significant if the p value was less than 0.05 (P<0.05). Statistical analyses were performed by using Graph Pad Prism V5.01 software (Graph Pad, Washington, USA).

3. RESULTS

3.1 Influence of HAART Used on Biological Parameters Depending on the Type of HIV

3.1.1 Influence of combinations TDF+FTC+EFV, D4T+3TC+NVP, AZT+3TC+NVP, AZT+3TC+EFV on patients HIV-1 blood cells count

The results revealed a significant increase (P<0.05) of CD4+ T-lymphocytes count (Table 3). The average rates of white blood cells, neutrophils, eosinophils and red blood cells showed non-significant decrease (Table 3). No change was found in the rate of eosinophils, monocytes and basophils. Lymphocytes, haemoglobin, haematocrit, Mean corpuscular volume (MCV), *Mean corpuscular hemoglobin* (MCH), *Mean corpuscular hemoglobin* concentration (MCHC), and the platelets have experienced a non-significant increase of their average rate (Table 3).

3.1.2 Influence of combinations AZT+3TC+LP/RT and AZT+3TC+ABC on the patients HIV-2 blood cells count

The Table 3 showed a significant increase (P<0.05) of the average rate of the CD4 T-lymphocytes induced by HAART combinations used in patients infected with HIV2. The average rates of white blood cells, neutrophils, eosinophils, lymphocytes, monocytes, the haemoglobin, MCV, MCH, MCHC, and the platelets, have experienced a non-significant increase. The red blood cells and the haematocrit have experienced a non-significant decrease. However the basophils have not experienced any variation in their average rates among these same patients (Table 3).

3.2 Influence of Different Types of HAART on the Average Rates of Immunological Parameters (CD4+T-lymphocytes)

The results showed an increase in the number of CD4+ T-lymphocytes regardless the regimen used during the follow-up. This increase was significant (P<0.0001) to M12 compared to D0 or the average number of CD4+ T-lymphocytes was less than 350cells/mm^3 (Table 4). However, the increase of CD4+ T-lymphocytes with the combination therapy D4T+3TC+NVP has been slow. Therefore, according to Table 4, it has not allowed us to reach a number greater than or equal to 350 cells/mm^3 to M12 (216 cells/mm^3).

Table 3. Influence of ARTs on the types of HIV

Types of HIV	Average for the HIV1 (40)				Average for the HIV2 (4)			
	D0	D15	M6	M12	D0	J15	M6	M12
T lymphocytes CD4+ (cells/mm3)	145.57±123.52	207.93±150.52	276.3±217.78	488.3±333.19*	125.75±83.12	302±179.60	253.7±168.97	481.3±172.44*
White blood cells (WBC) (10^3/ul)	4.24±2.18	3.77±1.46	3.48±0.74	3.78±1.22	2.69±0.78	4.56 ±1.98	4.1±0.86	4.12±1.10
Neutrophils (10^3/ul)	1.97±1.61	1.65±1.03	1.37±0.68	1.61±0.80	1.18±0.38	2.31 ±0.75	1.7 ± 0.57	1.33 ±0.61
Lymphocytes (10^3/ul)	1.49 ±0.79	1.43±0.68	1.44±0.40	1.63±0.59	1.04±0.29	1.44 ±0.94	1.45±0.63	2.05 ±0.48
Monocytes (10^3/ul)	0.76±1.61	0.45±0.23	0.33 ±0.09	0.35±0.12	0.4±0.14	0.48 ±0.15	0.37±0.08	0.42 ±0.09
Eosinophils (10^3/ul)	0.2±0.19	0.25±0.19	0.23±0.19	0.15 ±0.13	0.07±0.02	0.32 ±0.12	0.57±0.46	0.31 ±0.29
Basophils (10^3/ul)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Red blood cells (10^6/ul)	4.16±0.93	3.56±0.74	3.55±0.62	3.73±0.68	4.85±0.70	4±0.14	4.03±0.77	4.19 ±0.98
Haemoglobin (g/dl)	10.91±2.14	9.34±1.82	11.55±1.77	11.66 ± 1.38	11.45± 0.77	10.55 ±0.35	11.6±1.16	12.1 ±1.04
Haematocrit % (HCT)	35.44±7.06	30.9±6.36	35.86±5.41	36.69 ± 4.19	39.2±4.24	32.7 ±0.42	36.82±3.86	37.63 ± 3.80
MCV (FL)	84.96±14.23	86.27±11.53	98.42±20.36	99.94±13.72	80.95± 2.89	81.8 ±1.83	92.62±10.89	91.6 ±11.84
MCH (pg)	26.68±3.96	26.43±3.21	32.55±4.48	31.84±5.03	23.7±1.20	26.4 ±1.83	29.25±4.11	46.3 ±32.78
MCHC (g/dl)	30.77±2.10	30.78±2.33	32.06 ±1.54	31.81±1.72	29.25±1.20	32.25 ±1.48	31.52±0.78	32.2 ±1.8
Platelet (10^3/ul)	216.05±88.67	273.83±79.92	228.29±54.49	249.3±143.18	119.5±23.33	307.5±54.4	227±50.83	253.33 ± 88.57

**=P< 0.05; ** = P< 0.001; *** = P<0.0001; A(n) : has indicated the combination therapy and n = the number of patients, D0= initial assessment; D15=15 days; M6 = 6 months and M12 = 12 months after taking ART, MCV = Mean corpuscular volume; MCH=Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin concentration*

Table 4. The average number of lymphocytes TCD4+ functions in the follow-up periods and of the triple therapy

Families	Molecules	CD4+ (cells/mm3)			
		Tracking periods			
		D0	D15	M6	M 12
2 NRTI+1NNRTI	TDF+FTC+EFV(4)	248.5±270.69	277±198.79	272.33 ± 133.16	896.33 ± 157.19 ***
	D4T+3TC+NVP(1.2)	91.5±127.98 [1.2]	203.5±284.96 [1.2]	208.5 ± 284.96[1.2]	216[2] ***
	AZT+3TC+NVP(28)	144.14±98.72	214.52±143.81	293.68 ± 248.52	435.95 ± 339.76 ***
	AZT+3TC+EFV(5)	110.8±91.33	102±56.15	235.2 ± 110.20	476.5 ±21.1 ***
2 NRTI+1IP	AZT+3TC+LP/RT(4)	103.75±80.88	199.33±106.60	116.33 ± 10.40	543.25 ± 121.78 ***
3 NRTI	AZT+3TC+ABC(1.2)	110.5±1.41 [1.2]	429[2]146±197.98 [1.2]	356[1] ***	

**** = P<0.0001;A(n) : has indicated the combination therapy and n the number of patients, The absence of values indicates that patients have missed their appointments, 2 NRTI +1NNRTI = 2 nucleoside reverse transcriptase inhibitors + 1 non-nucleosid reversetranscriptase inhibitor, 2 NRTI+1IP =2 nucleoside reverse transcriptase inhibitors + 1 protease inhibitor, 3NRTI = 3 nucleoside reverse transcriptase inhibitors, D0= initial assessment; D15=15 days; M6 = 6 months and M12 = 12 months after taking ART*

Table 5. Average values of haematological parameters according to therapeutic regimens AZT+3TC+LP/RT and AZT +3TC+ABC

Types of triple therapy	AZT+3TC+LP/RT[4]				AZT+3TC+ABC[1,2]			
	D0	D15	M6	M12	D0	D15	M6	M12
White blood cells (10^3/ul)	3.11±0.90	4.39±1.42	3.08±1.01	3.09±1.38	4.63[2]	3.16[2]	4.38±1.21[1,2]	4.24[1]
Neutrophils (10^3/ul)	1.56±1.05	1.95±1.20	1.17±0.16	1.36±0.58	2.09[2]	1.78[2]	1.98±0.60[1,2]	0.96[1]
Lymphocytes (10^3/ul)	0.93±0.30	1.34±0.71	1.01±0.38	1.62±0.79	1.43[2]	0.77[2]	1.26±1.01[1,2]	2.17[1]
Monocytes (10^3/ul)	0.47±0.12	0.58	0.39±0,05	0.37±0.10	0.39[2]	0.37[2]	0.39±0.09[1,2]	0.45[1]
Eosinophils (10^3/ul)	0.18±0.13	0.48±0.32	0.14±0.10	0.22±0.13	0.71[2]	0.24[2]	0.74±0.70[1,2]	0.66[1]
Basophils (10^3/ul)	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0±0.0	0.01[2]	0.0[2]	0.0 ± 0.0 [1,2]	0.0[1]
Red blood cells (10^6/ul)	4.18±0.87	3.68±0.59	3.2±1.04	3.9±1.24	4.17 [2]	3.9[2]	4.1±0.31[1,2]	3.78[1]
Haemoglobin (g/dl)	10.47±1.21	10.03±0.46	10.46±3.63	11.6±0.88	12.4[2]	10.8[2]	11.70±1.41[1,2]	12.8[1]
Haematocrit % (HCT)	33.77±6.78	31.1±1.65	37.53±4.03	36.8±4.04	34.2[2]	32.4[2]	36.6±3.67[1,2]	37.7[1]
MCV (FL)	80.82±4.64	85.86±13.28	102.2±23.74	98.53±21.28***	82[2]	83.1[2]	95.5±11.17[1,2]	99.7[1] ***
MCH (pg)	25.35±2.27	27.63±3.55	31.4±7.00	31.03±7.20**	29.7[2]	27.7[2]	30.55±4.17 [1,2]	83.9[1] **
MCHC (g/dl)	31.4±2.77	32.3±1.73	30.76±0.83	31.56±1.01	36.3[2]	33.3 [2]	31.95±0.63 [1,2]	34[1]
Platelets (10^3/ul)	177.75±83.35	232.66±35.0	180.33±39.5	186.33±55.58	295[2]	346[2]	265.5±20.50 [1,2]	344[1]

**=P< 0.05; ** = P< 0.001; *** = P<0.0001; A(n): has indicated the combination therapy and n = the number of patients, D0= initial assessment; D15=15 days; M6 = 6 months and M12 = 12 months after taking ART, MCV = Mean corpuscular volume; MCH =Mean corpuscular hemoglobin content; MCHC = Mean corpuscular hemoglobin concentration*

Table 6. Average values of haematological parameters according to therapeutic regimens D4T+3TC+NVP and AZT+3TC+NEV

Types of HAART	D4T+3TC+NVP[1,2]				AZT+3TC+NEV[28]			
	D0	D15	M6	M12	D0	D15	M6	M12
White blood cells (10^3/ul)	6.04±6.39[1,2]	2.25[1]	2.38[1]	3.51[2]	4.22±2.14	3.51±1.21	3.34±0.62	3.67±0.99
Neutrophils (10^3/ul)	3.19±3.35 [1,2]	1.36[1]	1.14[1]	0.68[2]	1.82±1.63	1.35±0.78	1.26±0.59	1.59±0.65
Lymphocytes (10^3/ul)	2.24±2.62[1,2]	0.41[1]	0.87[1]	2.33[2]	1.54±0.68	1.5±0.69	1.42±0.40	1.59±0.57
Monocytes (10^3/ul)	0.54±0.47[1,2]	0.25[1]	0.27[1]	0.41[2]	0.89±1.92	0.4±0.19	0.32±0.10	0.34±0.11
Eosinophils (10^3/ul)	0.05±0.06 [1,2]	0.23[1]	0.1[1]	0.09[2]	0.22±0.21	0.25±0.20	0.23±0.18	0.15±0.14
Basophils (10^3/ul)	0.01±0.01 [1,2]	0[1]	0[1]	0[2]	0±0	0±0	0±0	0±0
Red blood cells (10^6/ul)	3.42±0.79 [1,2]	3.4[1]	3.42[1]	3.97[2]	4.15±0.91	3.63±0.64	3.7±0.60	3.69±0.75
Haemoglobin (g/dl)	8.6±1.69 [1,2]	9.1[1]	8.0[1]	12.6[2]	10.94±2.03	9.56±1.40	11.4±1.95	11.78±1.42
Haematocrit % (HCT)	27.4±6.36[1,2]	29.5[1]	30.6[1]	39.1[2]	35.36±6.64	30.56±4.43	35.29±5.80	36.51±4.56
MCV (FL)	80±0.14 [1,2]	86.8[1]	89.5[1]	98.5[2] ***	84.6±16.33	85.03±10.27	98.02±23.34	101.07±14.58***
MCH (pg)	25.25±0.91[1,2]	26.8[1]	23.4[1]	31.7[2]**	26.83 ±4.14	26.58±3.08	32.97±4.82	32.6 ±5.08**
MCHC (g/dl)	31.5±1.13 [1,2]	30.8[1]	26.1[1]	32.2[2]	30.77 ±1.95	31.32±1.45	32.26±1.65	32.22±1.32
Platelets (10^3/ul)	331.5±154.85[1,2]	304[1]	276[1]	205[2]	203.73±79.46	253.9±71.26	223.27±52.25	256.55±163.70

**=P< 0.05; ** = P< 0.001; *** = P<0.0001; A(n) : has indicated the combination therapy and n = the number of patients, D0= initial assessment; D15=15 days; M6 = 6 months and M12 = 12 months after taking ART, MCV = Mean corpuscular volume; MCH =Mean corpuscular hemoglobin content; MCHC = Mean corpuscular hemoglobin concentration*

Table 7. Average values of haematological parameters according to therapeutic regimens AZT+3TC+EFV and TDF+FTC+EFV

Types of HAART	AZT+3TC+EFV(5)				TDF+FTC+EFV(4)			
	D0	D15	M6	M12	D0	D15	M6	M12
White blood cells (10^3/ul)	3.65±1.02	3.08±1.90	3.45±0.85	2.38±0.26	3.59±0.62	3.45±1.31	4.59 ±0.75	4.97 ±1.46
Neutrophils (10^3/ul)	1.81±0.90	1.99	1.34±0.85	0.87±0.69	1.87±0.77	3.1±0.77	2.32 ±1.37	2.39 ±0.59
Lymphocytes (10^3/ul)	1.26±0.51	1.09±0.41	1.46±0.36	1.22±0.44	1.27±0.10	1.66 ±0.66	1.72±0.27	1.91±0.48
Monocytes (10^3/ul)	0.51±0.21	0.31±0.10	0.35±0.08	0.24±0.05	0.34±0.10	0.82 ±0.14	0.43 ±0.01	0.54 ±0.10
Eosinophils (10^3/ul)	0.16±0.08	0.3	0.28±0.27	0.05±0.04	0.1±0.09	0.24 ±0.27	0.11 ±0.11	0.13 ±0.07
Basophils (10^3/ul)	0±0	0.01±0.01	0.02±0	0±0	0±0	0.01 ±0	0±0	0±0
Red blood cells (10^6/ul)	4.1±0.84	3.33±2.45	3.64±0.20	3.70±0.44	4.77±1.29	3.45 ±0.40	3.77 ±0.51	4.06 ±0.26
Haemoglobin (g/dl)	11.28±1.69	8.56±6.57	12.06±0.83	12.15±0.49	11.4±3.62	8.45 ±1.44	11.8 ±1.75	10.87±0.62
Haematocrit % (HCT)	36.92±7.86	30.5±16.8	37.4±2.29	38.4±2.82	38.22±9.71	33.42 ±12.23	37.46±6.92	36.65±3.28
MVC (FL)	90.4±8.91	99.95±23.12	103.06±4.60	103.95±4.87***	80.67±7.45	82.42 ±11.81	93.6±12.45	90.5±12.19***
MCH (pg)	27.92±3.62	25.6±0.84	33,08±0,99	32.95±2.61**	24.12±4.07	24.6 ±4.29	28.56±4.28	26.85±3.39**
MCHC (g/dl)	30.86±2.06	26.45±7.00	32,14±0,71	31.65±1.06	29.9±3.85	29.28 ±2.29	30,46±0,70	29,72±2,55
Platelets (10^3/ul)	227.8±110.22	362±149.90	271,6±56,87	283±9.89	239.75±87.26	350.25 ±25.92	224 ±7,93	230,25±54,79

**=P< 0.05; ** = P< 0.001; *** = P<0.0001; A(n) : has indicated the combination therapy and n = the number of patients, D0= initial assessment; D15=15 days; M6 = 6 months and M12 = 12 months after taking ART, MCV = Mean corpuscular volume; MCH =Mean corpuscular hemoglobin content; MCHC = Mean corpuscular hemoglobin concentration*

3.3 Influence of HAART on the Average Rates of Haematological Parameters

Tables 5, 6 and 7 showed a non-significant variation of the average rates of white blood cells (GB), basophils, monocytes, eosinophils, platelets, hematocrit and MCH during the follow-up in all the therapeutic regimen used.

With the exception of the TDF+FTC+EFV, all therapeutic combinations have resulted in a decrease in the number of neutrophils (above Tables 5, 6 and 7). Table 5 showed non-significant increase of totals lymphocytes count with the therapeutic regimens 2NRTI+1IP and 3NRTI. With regard to the red cells, the results have revealed non-significant decrease of red blood cells count regardless of the therapeutic regimen. However, haemoglobin known non-significant increase reaching the interval of standard values (Tables 5, 6 and 7). The rates have increased significantly for them average MVC (P<0.0001) and MCH (P<0.001) regardless of the regimen used (Tables 5, 6 and 7).

4. DISCUSSION

The analysis of the results showed a very significant increase (p<0.0001)in average rates of CD4+ T-lymphocytes for all triple combination therapies, after one year of treatment. HIV-positives patients under the combination TDF+FTC+EFV had 248.5 ± 270.69 cells/mm3 the first day before taking the ART(D0)and after twelve months under, CD4+ T-lymphocytes count was: 896.33 ± 157.19 cells/mm^3 of blood; either an increase CD4+T-lymphocytes more than 647.83 cells/mm^3 of blood (p<0.0001).Results of CD4+T-lymphocytes count of combination D4T+3TC+NVP were 91.5 ± 127.98 cells/mm^3 of blood to D0 and 208.5 ± 284.96 cells/mm^3, after six months under ART, either a significant increase from p<0.0001. In effect, these results corroborate those of Coulibaly et al. and Tumbarello et al. [15,16] which showed a significant increase of CD4+T-lymphocytes count between initial assessment and the 6th month of treatment of HIV-positives patients under D4T+3TC+NVP.This increase of CD4 T-lymphocytes count in the time would be due to the fact that the antiretroviral treatment (ART) have managed to block the replication of the virus in the body of HIV-positives patients. Consequently, ART could be stimulated the synthesis of a large number of CD4+ T-lymphocytes in the primary and secondary lymphoid organs such as the bone marrow, lymph nodes and the spleen [17]. The use of ART has enabled an immune restoration.

The white blood cells, monocytes and neutrophils counts reduced not significantly, totals lymphocytes count showed non-significant increase for all combinations. Our results are consistent with those of Nantaya et al. [18] who found a leukopenia and neutropenia in HIV-positives patients under antiretroviral treatment in Lubumbashi. The eosinophils and basophils counts showed no variation during the follow [19]. Contrary to combination TDF + FTC + EFV, white blood cells count was 3.59 x 10^3/µl±0.62 of blood to initial assessment. After twelve months under ART, their number was 4.97 x 10^3/µl ±1.46 either a not significant increase. Neutrophils, monocytes, lymphocytes, eosinophils, basophils showed also a significant increase. The decrease in average rates of white blood cells could be related to the decline of neutrophils and monocytes counts. In effect, neutrophils, monocytes, basophils, lymphocytes are white bloods cells. Combinations containing lamivudine (3TC) could be responsible for white blood cells decrease [8]. In addition, the increase in average rates of total lymphocytes could be linked to the very significant increase (p<0.0001) of CD4+ T-lymphocyte count. Therefore, the immune defenses of HIV-positives patients could be strengthened. In effect, CD4+T-lymphocytes play the role of central cells of the

immunity, because they trigger the immune response specific to the body (cellular and humoral) [20]. Thus, during the infection, the monocytes which play the role of presenter's cells of antigens (CPA) show the fragments of antigens associated with the major histocompatibility complex (MHC) class II to the CD4+T-lymphocyte. TheCD4+T-lymphocytes stimulate the secretion of cytokines (interleukins-2, 4, 5 and 6; interferon-gamma, OSB 1 and 2) which motivate the proliferation and differentiation of T-lymphocytes (response to mediation cellular) and B (response to mediation Tcell) [20].

According to the results, the HAART drugs would have no influence on the rate of eosinophils and basophils in the blood total. Therefore, the triple combination therapies used will not act on the biosynthesis of these two blood cells [19].

HAART can succeed only if anemia installed before ARV treatment was corrected because the HAART induced a decrease of the average rate of red blood cells. The reduction in red blood cells obtained in this study would be due to the fact that the ART drugs would affect the renal metabolism, especially at the level of the regulation of the hormone erythropoietin [19]. The HAART used therefore cause regenerator anemia [21,22]. Also the non-increase rate of hematocrit and the significant increase average rate of Mean corpuscular volume (MCV) found, would it be directly relate to the decrease of the average rate of red blood cells. In addition, the increase in the MVC could be responsible for a macrocytose (red blood cells of large size caused by folic acid deficiency [22].

The macrocytose occurs in patients using the combinations containing the lamivudine (3TC) [19]. This anomaly leads to long to anemia which is explained by the increase in the average rate of haemoglobin in the blood [23,24] as was shown the results of this present study.

5. CONCLUSION

The results obtained have showed that the HAART increased significantly the rate of CD4+T-lymphocytes to values greater than 350 cells/mm^3 of blood. At the level of the complete blood count, the ART have restored haemoglobin at its standard values. However, ART have misled neutropenia and a macrocytose leading to anemia in patients using the triple combination therapies containing lamivudine (3TC). Therefore, the HIV-positives patients should be also treated with anti-anemic compounds.

CONSENT

Informed consent was obtained from HIV-positives patients before taking blood samples for testing.

ETHICAL APPROVAL

This study has been examined and approved by the ethics committee of Ivory Coast through Institute Pasteur and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

This study follows the charter of Institute Pasteur based on the law established on article L.1121-2 of the Code of Public Health which states that the interest of people that are amenable to biomedical research always takes precedence over only the interests of science and society. This study is based on results of medically prescribed tests of the

clinical practice, from an anonymous database retrospectively evaluated, with no risks to patients, protecting the integrity and anonymity of participants and with no need of informed consents. The approval of an ethics committee of a specific institution is not needed because it was accessed only numerical values of the database (without access to the names, data source or clinic history of patients), collected specifically for research purposes by one of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. UNAIDS (a) Regional information sheet. UNAIDS communications. 2012;4.
2. Aubry P. HIV / AIDS and tropics. Med Trop. 2012;23:1-16.
3. UNAIDS (b). Results. 2012;48. Available on: www.unaids.org (15/11/2013).
4. UNAIDS (c). Estimated HIV and AIDS; 2012:1.
   Available on: http://www.unaids.org/fr/regionscountries/countries/ctedivoire
5. Gutiérrez F, Masiá M. The Role of HIV and Antiretroviral Therapy in Bone Disease. AIDS Rev. 2011;13:18-109.
6. Hilton R. Human immunodeficiency virus infection and kidney disease. J R Coll Physicians Edinb. 2013;43(3):236-9.
7. Di Benedetto F[1], D'Amico G, De Ruvo N, Cocchi S, Montalti R, Cautero N, Guerrini GP,Ballarin R, Spaggiari M, Tarantino G, Baisi B, Cappelli G, Codeluppi M, Gerunda GE. Combined liver-kidney transplantation in patients infected with human immunodeficiency virus. Transpl Infect Dis. 2011;13(5):501-6.
8. Eholié S-P, Aoussi EF, Ouattara SI, Bissagnéné E, Anglaret X. HIV treatment and care in resource-constrained environments: challenges for the next decade. JInt AIDS Soc. 2012;15(2):17334-40.
9. Kamenga C, Sawadogo Y, Zouzoua C, Seck K. Analyse situationnelle des soins et prise en charge du VIH/SIDA et des infections sexuellement transmissibles Burkina Faso, Cameroun,Cote d'Ivoire, Togo. Projet SFPS. 2001;128.
10. Wee JC, Guat BT, Ponnudurai K. Establishment of Adult Peripheral Blood Lymphocyte Subset Reference Range for an Asian Population by Single-Platform Flow Cytometry: Influence of Age, Sex, and Race and Comparison with Other Published Studies. Clin Vaccine Immunol. 2004;11(1):168-173.
11. Fumiko I, Koji K, Akihiro T, et al. Increased Populations of Regulatory T Cells in Peripheral Blood and Tumor-Infiltrating Lymphocytes in Patients with Gastric and Esophageal Cancers. Clin Cancer Res. 2003;9:4404.
12. Delaugerre C, Simon F. Les« Trod » : des outils pour le dépistage du VIH ? Virologytrod Vih. 2012 ;304(3):9.
13. Krou PE, Yessé ZN, Kouadio IK, Patrice K. Influence des Antirétroviraux (ARV) sur des paramètres biochimiques de quelques organes vitaux des personnes vivants avec le VIH en Côte d'Ivoire. J Appl Biosci. 2012;54:3848–3858.
14. WHO. Laboratory Guidelines for enumerating CD4+ T Lymphocytes in the context of HIV/AIDS. 2007;68.
15. Coulibaly SM, Oumar AA, Aboubacrine Ag S, Coulibaly S, Koumare BY, Mariko E, Traoré HA, Harama M. The clinical and biological tolerance of nevirapine among patients with AIDS under treatment at the Hospital of the Point G. Mali Med. 2007;22(3):1-4.

16. Tumbarello M, Rabagliati R, Katleen de Gaetano D. Older age does not influence CD4+ cell recovery in HIV-1 infected patients receiving Highly Active Anti Retroviral Therapy. BMC Infect Dis. 2004;4: 46.
17. Mgogwe J, Semvua H, Msangi R, Mataro C, Kajeguka D, Chilongola J. The evolution of haematological and biochemical indices in HIV patients during a six-month treatment period. Afr Health Sci. 2012;12(1):2–7.
18. Nantaya C, Prasit C, Kamolmar lV, Maitree S, Damrong C. Biochemical and hematological manifestations of HIV/AIDS in chiang Mai, Thailand. Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand. Southeast Asian J Trop med public health. 2001;32:500-503.
19. Kazadi M, Kabongo K, Kasamba I, Bilonda M, Mundongo T, Mwembo T, Balaka EM, Wembonyama S, Kalenga MK. Study of hematological abnormalities during antiretroviral therapy in HIV infected patients in lubumbashi Université Libre de Bruxelles. 2008;1:10.
Available on: www.vih.org/sites/default/.../ARTICLE%20SUR%20L'ANEMIE%201.
20. Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R. Toll-like receptors control activation of adaptive immune responses. Nature Immunology. 2001;2:947–950.
21. Semba RD, Shah N, Klein RS. Highly Active Antiretroviral Therapy Associated with Improved Anemia Among HIV-Infected Women. AIDS Patient Care and STDs. 2001;15(9):473-480.
22. Sakorn P, Pakhaporn S, Suchart K, Virat K, Prattana L, Channat P, Prasit I, Siraporn A, Pranee L. Evolution of Hematological Parameters in HIV-1-Infected Patients With and Without Thalassemia Carriages During Highly Active Antiretroviral Therapy. HIV Clin Trials. 2009;10(2):88–93.
23. Okome N, Mml, Okome ER, Obiang NGP, Okome MF. Balance clinicobiological patients infected with HIV at the Jeanne Ebori foundation Libreville (2002-2005). Med too. 2007 ;67:357-362.
24. Semba RD, Shah N, Vlahov D. Improvement of anemia among HIV-infected injection drug users receiving highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 1999;26(4):315-319.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=576&id=28&aid=5594