EFFECT OF HIV SEROTYPES AND DURATION OF HAART USE ON THE LEVEL OF CD4 COUNTS AMONG HIV PATIENTS IN BENIN CITY, NIGERIA

Omoregie, R.[*, 1, 2, 3] and Okojie, R. O.[3]

[1]Medical Microbiology Division, Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Nigeria, [2]School of Medical Laboratory Sciences, University of Benin Teaching Hospital, Benin City, Nigeria, and [3]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

*Corresponding Author's e-mail: richyomos@yahoo.com; richard.omoregie@ubth.org

Tel: +23408105543055

Received: 3rd November, 2022 **Accepted:** 5th December, 2022 **Published:** 8th December, 2022

ABSTRACT

Background: CD4 count is a marker for HIV disease progression, an important factor for initiating treatment as well as for monitoring effectiveness of treatment of antiretroviral therapy. A number of factors affect the rate of CD4 recovery during treatment.

Aim: This study aims to determine the effect of HIV serotypes on CD4 count as well as the effect of duration of HAART on CD4 count. A cross-sectional study was conducted.

Methods: A total of 335 HIV patients (251 HIV patients on HAART and 84 HAART-naïve) were recruited for this study. Blood specimens were collected from each patient used to determine CD4 count and HIV serotype using flow cytometry and immunochromatographic methods respectively.

Results: The CD4 count of HAART-naïve HIV patients were significantly lower than that of their counterparts on HAART ($p<0.0001$). Generally, patients infected with HIV-1 had significantly higher CD4 count than those dually infected with HIV1/2 ($p=0.0187$). There was a significant correlation between duration of HAART use and CD4 count resulting in increased CD4 count with increasing duration of HAART use ($r = 0.2347$: $p<0.001$). In terms of HIV serotypes, it was observed only for HIV-1 ($r=0.3271$, $p<0.001$) and not among HIV-1/2 patients were an insignificantly weak correlation was observed ($r=-0.0089$: $p>0.05$). There was no significant association between HIV serotypes and immunosuppression as measured by CD4 count < 200cells/ µL ($p> 0.05$).

Conclusion: CD4 counts were higher among HIV-1 patients and this increase was associated with duration of HAART use. Use of appropriate HAART agents to improve CD4 count of HIV-1/2 infected patients is advocated.

Keywords: CD4 count recovery; HIV serotypes; Duration of HAART; Nigeria.

INTRODUCTION

Among those with human immunodeficiency virus (HIV) infection, the CD4+ T-lymphocyte count is the major indicator of immunodeficiency (Lifson *et al.,* 2011). A central feature in the pathogenesis of HIV infection is the depletion of CD4+ T-lymphocytes, and it is generally accepted that CD4 count < 200 cells/µL predisposes HIV patients to opportunistic infections (Oguntibeju *et al.,* 2006). CD4 count is an important factor in initiating treatment with combination antiretroviral therapy (also known as highly active antiretroviral therapy [HAART]) as well as monitoring the effectiveness of the treatment (Lifson *et al.,* 2011). The introduction of HAART has decreased the morbidity and mortality associated with HIV infection (Engsig *et al.,* 2014) and HAART has been reported to increase CD4 count (Odunukwe *et al.,* 2005). However, failure to restore a normal CD4+ count following HAART is associated with increased morbidity and mortality (Baker *et al.,* 2008; Lawn *et al.,* 2009).

A number of factors affect the rate of CD4 recovery. Indeed, white ethnicity, pre–HAART viral load, pre–HAART CD4 count, duration of HAART use, age, gender, amongst others, have been reported as factors that affect CD4 count (Smith *et al.,* 2004; Lok *et al.,* 2010; Lifson *et al.,* 2011; Engsig *et al.,* 2014). The effect of duration of HAART use on CD4 count reveals conflicting reports in the literatures. Some authors report that CD4 increases with duration of HAART use for 4 to 6 years and stops (Moore and Keruly, 2007; Lifson *et al.,* 2011) while others report higher CD4 count for up to 7 years or more (Guihot *et al.,* 2010; Lok *et al.,* 2010). These studies have focused on HIV-1. Although HIV-2 results in slower decline of CD4 count than HIV-1 (Gottlieb *et al.,* 2011), no study has looked at the effect of HIV serotypes on CD4 count levels and recovery. Against this background, this study aims to determine the effect of HIV serotypes on CD4 count as well as the effect of duration of HAART on CD4 count.

MATERIALS AND METHODS

Study population

A total of 335 HIV positive patients (82 males and 253 females) were used for this study. The HIV patients consisted of 251 patients on HAART (60 males and 191 females) for 1 to 15 years, and 84 HAART-naive patients (22 males and 62 females). The regimens for HAART are divided into first line, alternate first line and second line drugs. The first line and alternate first line HAART regimens are as follows: zidovudine, lamivudine and nevirapine, or combivir (consisting zidovudine, lamivudine) and liponavir/ritonavir), or combivir and efavirenz, or combivir and abacavir, or abacavir, lamivudine and nevirapine. The second line HAART regimen entails any of the following combinations: abacavir, lamivudine and liponavir/ritonavir, or zidovudine, lamivudine and liponavir/ritonavir, or stavudine, lamivudine and liponavir/ritonavir, or abacavir, lamivudine and aluvir. All HIV patients were asymptomatic out-patients attending HIV clinics in the University of Benin Teaching Hospital, Benin City. Informed consent was obtained from all patients or their parents/guardian in case of children prior to specimen collection. Approval for the study was given by the Ethical Committee of the University of Benin Teaching Hospital, Benin City.

Collection and processing of specimens

Five millilitre (ml) of venous blood was collected from each patient and dispensed into ethylene diamine tetra-acetic acid (EDTA) bottles.

Determination of HIV serotypes

The HIV serotypes was determined using an immunochromatographic kit – HIV 1.2.O Rapid Test Cassette (Global Devices, USA) following the manufacturer's instruction. Briefly, 25µL of patient's plasma (obtained by centrifuging the anticoagulated blood) was added to the specimen area of the cassette device. This was followed by the addition of 40µL of reagent buffer that came with the kit. Immediately after addition of the buffer, a timer is set. The result was read at 10mins and not more than 20mins. The presence of red lines in the control and HIV-1 (T1) area was taken as HIV-1 serotype. The appearance red lines in the control and HIV-2 (T2) area was interpreted as been positive for HIV-2 serotype, while the presence of red lines in the control, T1 and T2 areas was taken as positive for HIV-1/2 serotype.

Determination of CD4 lymphocyte count

CD4 lymphocyte counts were evaluated using the flow cytometry (Partec, Gmbh, Germany) following the manufacturer's instruction as previously described (Akinbo *et al.,* 2012). Briefly, 20 µl CD4 phycoerythrin (monoclonal) antibodies was placed into a Partec test tube and 20 µl of well-mixed whole EDTA blood was added. The contents were mixed gently and incubated in the dark for 15 minutes at room temperature.

This mixture was agitated during incubation every 5 minutes. Eight hundred microlitres of CD4 buffer was added to the mixture of antibody and sample and mixed gently. The counting of the cells was done by the flow cytometer.

Statistical analysis

The parametric data were analyzed using ANOVA, unpaired t–test and Pearson's correlation. The non-parametric data were analyzed using Chi square (X^2) test or Fisher's Exact test as appropriate and odd ratio (OR) analysis. The statistical software INSTAT® (Graph Pad Software Inc., San Diego, CA, USA).was used for the analysis.

RESULTS

The CD4 count of HAART–naive HIV patients [range = 9 – 1242(cells/µL), mean ± standard error of the (SEM) = 367.18± 31.04] and those of HIV patients on HAART [range = 4 – 1586, mean ± SEM = 534.19±31.02] reveals that HIV patients on HAART had significantly ($p< 0.0001$) higher CD4 count than their HAART–naive counterparts (Table 1).

Generally, the CD4 count of patients infected with HIV–1 (519.45 ± 22.09) was significantly ($p = 0.0187$) higher than that of those infected with HIV–1/2 (438.38 ± 24.78). However, when the HIV patients were separated into HAART–naive and those on HAART, there was no significant difference in the CD4 count of those infected with HIV–1 and those infected with HIV–1/2 (Table 2). In both HIV–1 ($p = 0.0166$) and HIV–1/2 ($p< 0.0001$) infected patients, the CD4 count was higher among those on HAART compared to their HAART–naive counter parts (Table 2).

The effect of duration of HAART use on CD4 count is shown in Figure I and Table 3. The mean ± SEM CD4 count decrease from 367.18 ± 31.04 cells/µL in HAART–naive (0 years) to 353.05 ± 55.55 cells/µL one year after commencement of HAART and rose to 571.44 ± 23.13 cells/µL ≥ 5 years after commencement of HAART. Significant difference was observed between 0 year and ≥5 years ($p< 0.001$) and between 1 year and ≥ 5years ($p< 0.05$) (Fig I). Significant correlation ($r = 0.2347$: $p<0.001$) was observed between CD4 count and duration of HAART use generally. However, in terms of HIV serotypes, the significant correlation was observed only with HIV-1 serotype ($r=0.3271$: $p<0.001$) (Table 3).

The prevalence of HIV–1 was higher in persons with CD4 count < 200 cells/µL compared with their counterparts with CD4 count ≥ 200 cells/µL, in both HAART–naive HIV patients and those on HAART, albert, it failed to reach statistical significance ($p> 0.05$) (Table 4). The only HIV–2 case had a CD4 count ≥ 200cells/µL. An opposite trend was observed in HIV–1/2 cases. Here, the prevalence of HIV–1/2 were higher in patients with CD4 count ≥ 200 cells/µL compared with those with CD4 count < 200 cells/µL in both HAART–naive HIV patients and those on HAART. Again, the difference was not statistically significant ($p> 0.05$) (Table 4).

Table 1: Effect of HAART on CD4 count of HIV patients

Treatment type	Range*	Mean ± standard error of the mean (SEM)*	**P value**
HAART–naive (n = 84)	9 – 1242	367.18 ± 31.04	< 0.0001
On HAART (n = 251)	4 – 1586	534.24± 19.02	

*All units are in cell/µL

Table 2: Effect of HIV serotypes on CD4 count of both HAART–naive and HIV patients on HAART

Treatment status	HIV type		P value
	1	1 /2	
HAART–naive ($n_1 = 36$, $n_2 = 47$)	403.9 ± 51.04	312.26 ± 33.18	0.1373
On HAART ($n_1 = 172$, $n_2 = 79$)	543.25 ± 24.13	510.90±31.42	0.4363
General ($n_1 = 208$, $n_2 = 126$)	519.45 ± 22.09	438.38 ± 24.78	0.0152

Unit is in cells/µL. Figures are mean ± standard error of the mean; n_1 = number tested for HIV–1; n_2 = number tested for HIV–1/2

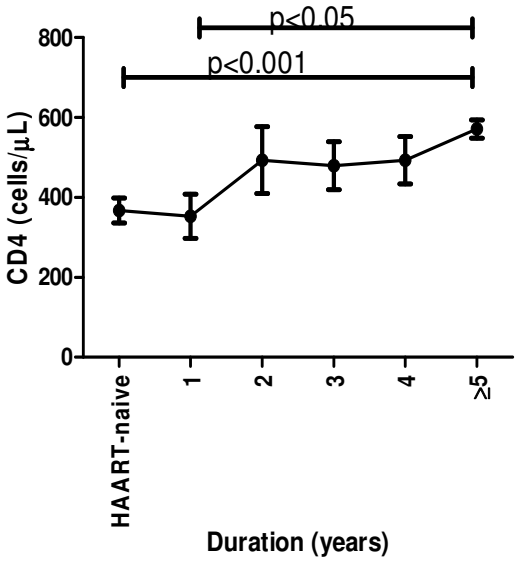

Fig I: Effect of duration of HAART use on CD4 count

Table 3: Correlation between CD4 count and duration of HAART use

HIV serotype (CD4 count)	Duration (years)
All serotype	r = 0.2347 (p<0.001)
HIV-1	r = 0.3271 (p<0.001)
HIV-1/2	r = -0.0089 (p>0.05)

Table 4:Relationship between HIV serotypes and immunosuppression as measured by CD4 count of HIV patients

HIV type	CD4 count		P value
	< 200cells/µL	≥ 200cells/µL	
HAART–naïve	(n = 27)	(n = 57)	
HIV–1	10(37.04)	26(45.61)	0.4890
HIV–2	0(0.00)	1(1.75)	1.0000
HIV–1/2	17(62.92)	30(52.63)	0.4814
On HAART	(n = 32)	(n = 219)	
HIV–1	24(75.00)	148(67.58)	0.5413
HIV–1/2	8(25.00)	71(32.42)	0.5413

Figure in parenthesis are in percentages

DISCUSSION

The mean ± SEM of the CD4 count of HIV patients on HAART (534.24 ± 19.02cells/μL) was significantly higher ($p<0.0001$) compared with their HAART-naïve counterpart (367.18 ± 31.04cell/μL) in this study. HAART has been reported to improve immunity (Willemot and Klein, 2004; Odunukwe *et al.,* 2005; Olawumi *et al.,* 2008). This is due to viral suppression and CD4 cell recovery (CASCADE, 2000; SMART, 2006; Lohse *et al.,* 2007). The range of CD4 count observed in this study indicates that some HIV patients (both HAART–naive and those on HAART) had CD4 count outside the normal range of CD4 count for Nigerians as reported by Odiabo and Olaleye (2013). This indicates that other factors influence CD4 among HIV patients. Indeed, white ethnicity, pre–HAART viral load, pre–HAART CD4 count, duration of HAART use, age, gender, amongst others, have been reported as factors that affect CD4 count (Smith *et al.,* 2004;Lok*et al.,* 2010; Lifson *et al.,* 2011; Engsig *et al.,* 2014).

Generally, CD4 count of HIV–1 patients were significantly higher ($p = 0.0187$) compared with those with HIV–1/2. This is not in agreement with the findings of Harries *et al.* (2010) where CD4 count of HIV–1/2 dual infected patients were higher than those of HIV–1 mono-infected patients. The difference in this study and that of Harries *et al.* (2010) could be due to geographical location, study design and type of subjects used. Harries *et al.* (2010) study was carried out in Ouagadougou, Burkina Faso while this study was carried out in Benin City, Nigeria. A cross–sectional study was the study design in this study while Harries *et al.* (2010) study was a prospective study (follow–up study) for 6years. The HIV patients used in this study were all asymptomatic (WHO clinical stage 1) while that used by Harries *et al.* (2010) consisted of both asymptomatic and symptomatic HIV patients (WHO clinical stages 1 to 4). The significantly lower CD4 count among HIV–1/2 dually infected patients may support the reports that HIV–1/2 dually infected patients experience more aggressive form of infection than HIV–1 mono-infected patients (Nkengasong *et al.,* 2000; Schim van der Loeff *et al.,* 2002; Alabi *et al.,* 2003; Koblavi–Deme *et al.,* 2004). Mortality rates for HIV–1/2 dually infected patients have been reported to be higher than that of HIV–1 mono-infected patients (Holmgren *et al.,* 2007; Harries *et al.,* 2010). Also, HIV–2 and HIV–1/2 dually infected patients appear to do less well on HAART than HIV–1 patients (Harries *et al.,* 2010). Age at baseline, lower intrinsic immune recovery in HIV–2, use of ineffective HAART regimens by clinicians and poor drug adherence have been suggested as reasons for the higher mortality (Matheron *et al.,* 2006; Harries *et al.,* 2010). The CD4 counts of HIV–1 patients were higher than those of HIV–1/2 dually infected patients in irrespective of the treatment status of the HIV patients. However, the difference was not statistically significant (HAART–naive; $p = 0.1213$; On HAART; $p = 0.4363$). The reason for this is unclear as a number of factors can affect CD4 count in HAART–naive HIV patients and those on HAART.

Significant difference in CD4 count was observed between 0years (HAART–naive) and those on HAART for ≥ 5years ($p< 0.001$), and between those on HAART for 1year and those for ≥ 5years ($p < 0.05$). This may indicate that significant increase in CD4 count is observed as from 5yearsof HAART therapy. The effect of duration of HAART use on CD4 count reveals conflicting reports in the literatures. Some authors report that CD4 increases with duration of HAART use for 4 to 6 years and stops (Moore and Keruly, 2007; Lifson *et al.,* 2011) while others report higher CD4 count for up to 7 years or more (Guihot *et al.,* 2010; Lok *et al.,* 2010).

In response to HAART, it has been reported that CD4 count increases rapidly within the first 6months due in part to release of memory T–cells from lymphoid tissues, and then increases slowly during the next 3 – 4 years, reflecting reconstitution of the immune system (Pakker *et al.,* 1998; Bucy *et al.,* 2006). Age, gender, pre–HAART viral load, pre–HAART CD4 count, duration of HIV infection prior to commencement of HAART and duration of viral suppression affect CD4 recovery (Smith *et al.,* 2004; Lifson *et al.,* 2011). Data on some the above factors that influence CD4 count were not available in this study. There was no data on their baseline CD4 count on initiation of HAART; hence the trend mentioned above was not demonstrated. No cross–sectional study on effect of duration of HAART use on CD4 count was found. A significant correlation ($r = 0.2347$; $P < 0.001$) was found between duration of HAART use and CD4 count in this study. In terms of HIV serotypes, it was observed only for HIV-1 ($r=0.3271$, $p<0.001$) and not among HIV-1/2 patients were an insignificantly weak correlation was observed ($r=-0.0089$ ($p>0.05$). Approximately 94% of the HIV patients on HAART in this study were on first line drugs – zidovudine, lamivudine and nevirapine. HIV–1 readily incorporates zidovudine and is more susceptible to zidovudine than HIV–2 (Boyer *et al.,* 2006). Mutation occurs rapidly *in vitro* and *in vivo* that confer resistance to lamivudine in HIV–2 (Adje–Toure*et al.,* 2003; Van der Ende *et al.,* 2003; Jallow*et al.,* 2006; Ntemgwa *et al.,* 2009; Smith *et al.,* 2009). Nevirapine, anon-nucleoside reverse transcriptase inhibitor (NNRTI), has no activity against HIV–2 (Peterson *et al.,* 2011). These may indicate that patients on HAART, with HIV–1/2 dual infection in this study may not be responding to the HAART regimen. This may explain the findings in this study.

Among HAART–naive HIV patients, the prevalence of HIV–1/2 (62.92%) was higher among HIV patients with CD4 count < 200cells/ µL compared with those of HIV–1, while among HIV patients on HAART with CD4 count < 200cells/ µL, the seropervalence of HIV–1 (75%) was higher than that of HIV–1/2 (25%). There was no significant association between HIV serotypes and immunosuppression as measured by CD4 count < 200cells/ µL ($p> 0.05$). This is in agreement with the findings of Harries *et al.* (2010). In the Harries *et al.* (2010) study, the mean increase in CD4 count (during follow–up) was only significantly different between HIV–1 and HIV–1/2 patient at 6months post HAART, with higher increase in the CD4 count of HIV–1 patients compared with HIV–1/2 dually infected patients. Thereafter, there was no significant difference in CD4 count between both serotypes throughout the remaining follow–up period. This may indicate that differences in CD4 count between HIV serotypes are only significant within the first 6months of therapy. In this study, the duration of HAART use was ≥ 1 years. This may explain the findings in this study.

CONCLUSION

In conclusion, CD4 counts were higher among HIV-1 patients and this increase was associated with duration of use. Use of appropriate HAART agents to improve CD4 count of HIV-1/2 infected patients is advocated.

REFERENCES

Adje–Toure, C.A., Cheingsong, R., Garcia–Lerma, J.E., Eholie, S., Borget, M.–Y., Bouchez, J.–M., Otten, R.A., Maurice, C., Sassan–Morokro, M., Ekpini, R.E., Nolan, M., Chorba, T., Walid, H. and Nkengasong, J.N. (2003). Antiretroviral therapy in HIV–2–infected patients: changes in plasma viral load, CD4 + cell counts, and drug resistance profiles of patients treated in Abidjan, Cote d'Ivorie. *AIDS 17* (Suppl 3): 49 – 54.

Akinbo, F.O., Okaka, C.E. and Omoregie, R. (2012).*Plasmodium falciparum* and intestinal parasitic co-infection in HIV-infected patients in Benin City, Edo State, Nigeria.*Journal of Infections in Developing Countries 6* (5): 430 – 435.

Alabi, A.S., Jaffar, S., Ariyoshi, K., Blanchard, T., Schim van der Loeff, M., Awasana, A.A., Corrah, T., Sabally, S., Sarge–Njie, R., Cham–Jallow, F., Jaye, A., Berry, N. and Whittle, H. (2003). Plasma viral load, CD4 cell percentage.HLA and survival of HIV–1, HIV–2, and dually infected Gambian patients.*AIDS 17*: 1513 – 1520.

Baker, J.V., Peng, G., Rapkin, J., Krason, D., Reilly, C., Cavert, W.P., Abrams, D.I., MacArthur, R.D., Henry, K., Neaton, J.D. and Terry, B. (2008). Community programs for clinical research on AIDS (CPCRA): poor initial CD4+ recovery with antiretroviral therapy prolongs immune depletion and increases risk for AIDS and non-AIDS diseases. *Journal of Acquired Immune Deficiency Syndrome 48*: 541 - 546.

Boyer, P.L., Saraflanos, S.G., Clark, P.K., Arnold, E.and Hughes, S.H. (2006). Why do HIV–1 and HIV–2 use different pathways to develop AZT resistance? *PLoS Pathogen 2(2):* e10 doi:10.1371/journal.ppat.0020010.

Bucy, R.P., Hockett, R.D., Derdeyn, C.A., Saag, M.S., Squires, K., Sillers, M., Mitsuyasu, R.T. and Kilby, J.M. (1999). Initial increase in blood CD4+ lymphocytes after HIV antiretroviral therapy reflects redistribution from lymphoid tissues. *Journal of Clinical Investigation. 103*:1391 – 1398.

Engsig, F.N., Zangerie, R., Katsarou, O., Dabis, F., Reiss, P., Gill, J., Porter, K.Salan, C., Riordan, A., Fatkenheuer, G., Gutierrez, F., Raff, F., Kirk, O., Mary – Krause, M., Stephen, C., de Olalla, P.G., Guest, J., Samji, H., Castagna, A., Monforte, A.A., Skaletz – Romwaki, A., Ramos, J., Lapadula G., Mussini, C., Force, L., Mayer, L., Lampe, F., Boufassa, F, Bucher, H.G., De Wit, S., Burkholder, G.N., Terra, R., Justice, A.C., Sterting, T.R., Crane, H.M., Gerstoft, J., Grarup, J., May, M., Chene, G., Ingle, S.M., Sterne, J. and Obel, N.(2014). Long – term mortality in HIV – positive individuals virally suppressed for > 3 years with incomplete CD4 recovery. *Clinical Infectious Diseases. 58* (9):1312 – 1321.

Gottlieb, G.S., Smith, R.A., Badiane, N.M.D., Ba, S., Hawes, S.E., Toure, M., Starling, A.K., Traore, F., Sall, F., Cherne, S.L., Stern, J., Wong, K.G., Lu, P., Kim, M., Raugi, D.N., Lam, A., Mullins, J.I., Kiviat, N.B. and Sow, P.S. (2011). HIV-2 integrase variation inhibitor-naïve adults in Senegal, West Africa.*Plos ONE* 6(7): e22204. doi: 10.1371/journal.pone.0022204.

Guihot, A., Tubiana, R., Breton, G., Marcelin, A.G., Samri, A., Assouinou, L., Ganclaves, E., Bricaire, F., Costagliola, D., Calves, V., Rouzioux, C., Autran, B., katlama, C. and carcelain, G. (2010). Immune and Virological benefits 10years of permanent viral control with antiretroviral therapy. *AIDS 24:* 614 – 617.

Harries, K., Zachariah, R., Manzi, Firmenich, P., Mathela, R. Drabo, J.,Onadja, G., Arnould, L. and Harries, A. (2010). Baseline characteristics, response to and outcome of antiretroviral therapy among patients with HIV–1, HIV–2 and dual infection in Burkina Faso.*Transactions of the Royal Society for Tropical Medicine and Hygiene 104:* 154 – 161.

Holmgren, B., da Silva, Z., Vastrap, P., Larsen, O., Anderson, S., Ravn, H. and Aaby, P. (2007). Mortality associated with HIV–1, HIV–2 and HTVL–1 single and dual infectious in a middle–age and older population in Guinea–Bissau. *Retroviralogy.* doi:10.1186/1742-4690-4-85.

Jallow, S., Alabi, A., Sarge – Njie, R., Peterson, K., Whittle, H., Corrah, T., Jaye, A., Cotton, M., Vanham, G., McConky, S.J., Rvwland – Jones, S. and Janssens W. (2009). Virological response to highly active antiretroviral therapy in patients dually infected with HIV – 1 and HIV – 2 in the Gambia and emergence of drug–resistant variants. *Journal of Clinical Microbiology. 47(7):* 2200 – 2208.

Koblavi–Deme, S., Kestens, L., Hanson, D., Otten, R.A., Borget, M.Y., Bile, C., Wiktor, S.Z., Roels, T.H., Chorba, T. and Nkangasong, J.N. (2004). Differences in HIV–2 plasma virual load and immune activation in HIV – 1 and HIV – 2 dually infected persons and those infected with HIV – 2 only in Abidjan, Cote D'Ivoire. *AIDS 18:* 413 – 419.

Lawn, S.D., Little, F., Bekker, L.G., Kaplan, R., Campbell, E., Orrell, C. and Wood, R. (2009). Changing mortality risk associated with CD4 cell response to antiretroviral therapy in South Africa. *AIDS 23*: 335 - 342.

Lifson, A.R., Krantz, E.M., Eberly, L.E., Dolan, M.J., Marconi, U.C., Weintrob, A.C., Crum – Cianflone, N.F., Ganesan, A., Grambsch, P.L. and Agan, B.K. (2011). Long – term CD4 + lymphocyte response following HAART initiation in a U.S. military prospective cohort. *AIDS Research and Therapy.* dio:10.1186/1742 – 6405 – 8 – 2.

Lohse, N., Hanson, A.B., Pederson, G., Kronborg, G., Gerstoft, J., Sorensen, H.T., Vaeth, M. and Vbel, N. (2007).Survival of persons with and without HIV infection Denmark, 1995 – 2005.*Annals of Internal Medicine 146:* 87 – 95.

Lok, J.J., Bosch, R.J. Benson, C.A., Collier, A.C., Robbins, G.K., Shafer, R.W. and Hughes, M.D. (2010). Long–term increase in CD4+ T cell counts during combination antiretroviral therapy for HIV–1 infection. *AIDS 24:* 1867 – 1876.

Matheron, S., Damond, F., Benard, A., Taieb, A. and Campa, P. (2006). CD4 recovery in treated HIV–2 infected adults is lower than expected; results from the France ANRS CO5 HIV–2 cohort. *AIDS 20:* 459 – 462.

Moore, R.D. and Keruly, J.C. (2007).CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologicsuppression.*Clinical Infectious Diseases. 44:* 441 – 446.

Nkengasong, J.N., Kestens, L., Ghys, P.D., Koblavi–Deme, S., Otten, R.A., Bile, C., Maurice, C., Kalou, M., Laga, M., Witkor, S.Z., and Greenberg, A.E. (2000). Dual infection with human immunodeficiency virus type 1 and type 2; impact on HIV type 1 viral load and immune activation markers in HIV – seropositive females sex loorkers in Abidjan, Ivory Coust. *AIDS Research and Human Retroviruses. 16:* 1371 – 1378.

Ntemgwa, M.L., Toni, T.D., Brenner, B.G., Camacho, R.J. and Wainberg, W.A. (2009). Antiretroviral drug resistance in human immunodeficiency virus type 2. *Antimicrobial Agents and Chemotherapy. 53*(4):3611 – 3619.

Odaibo, G.N. and Olaleye, D.O. (2013). Laboratory profile of HIV – 2 and dual HIV – 1 / HIV – 2 associated acquired immunodeficiency syndrome in Nigeria. *World Journal of AIDS. 3*: 192 – 196.

Odunukwe, N., Idigbe, O., Kanki, P., Adewole,T., Onwujekwe, D., Audu, R., Onyewuche, J. (2005). Haematological and biochemical response to treatment of HIV-1 infection with a combination of nevirapine + stavudine + lamivudine in Lagos Nigeria.*Turkish Journal of Haematology. 22*: 125-131.

Oguntibeju, O.O., van den Heever, W.M.J. and van Schalkwyk, F.E. (2006).Effect of liquid nutritional supplement on viral load and haematological parameters in HIV-positive/AIDS patients. *British Journal of Biomedical Science. 63(3):* 134 – 139

Olawumi, H.O., Olatunji, P.O., Salami, A.K., Odeigah, L. and Iseniyi, J.O. (2008).Effect of highly active antiretroviral therapy on CD4 count and weight in AIDS patients seen at the UITH, Ilorin.*Nigerian Journal of Clinical Practice. 11*(4):312 – 315.

Pakker, N.G., Notermans, D.W., de Boer, R.J., Roos, M.T., de Wolf, F., Hill, A., Leonard, J.M., Danner, S.A, Miedema, F. and Schellekens P.T. (1998). Biphasic Kinetics of peripheral blood T cells after triple combination therapy in HIV–1 infections: a composite of redistribution and proliferation. *Nature Medicine. 4*:208 – 214.

Schim van der Loeff, M.F., Jaffar, S., Aveika, A.A., Sabally, S., Corrah, T., Harding, E., Alabi, A., Bayang, A., Ariyoshi, K. and Whittle, H.C. (2002). Mortality of HIV–1, HIV-2 and HIV–1/ HIV–2 dually infected patients in a clinic–based cohort in the Gambia. *AIDS 16:* 1775 – 1783.

SMART (The Strategies for Management of Antiretroviral Therapy) (2006). CD4+ count – guided interruption of antiretroviral treatment.*New England Journal of Medicine.335:* 2283 – 2296.

Smith, C.J., Sabin, C.A., Youle, M.S., Loes, S.K., Lampe, F.C., Madge, S., Cropley, I., Johnson, M.H. and Phillips, A.N. (2004). Factors influencing increases in CD4 all counts of HIV – positive persons receiving long–term highly active antiretroviral therapy *Journal of Infectious Diseases. 190*:1860 – 1868.

Smith, R.A., Anderson, D.J., Pyrak, C.L., Preston, B.D. and Gottlreb, G.S. (2009). Antiretroviral drug resistance in HIV–2: three amino acid changes are sufficient for classwide nucleoside analogue resistance. *Journal of Antimicrobial Chemotherapy. 57:* 709 – 713.

The CASCADE Collaboration (2000).Survival after introduction of HAART in people with known duration of HIV–1 infection. Concerted action on seroconversion to AIDS and death in Europe. *Lancet 355:* 1158 – 1159.

Van der Ende, M.E., Prins, J.M., Brinkman, K., Keuter, M., Veenstra, J., Danner, S.H., Niesters, H.G.M., Osterhaus, A.D.M.E. and Schutten, M. (2003). Clinical, immunological and virological response to different anti – retroviral regimens in a cohort of HIV – 2 infected patients. *AIDS 17(Suppl 3):* 55 – 61.

Willemot, P. and Klein, M. B. (2004).Prevention of HIV-associated opportunistic infections and diseases in the age of highly active antiretroviral therapy.*Expert Review of Anti-Infective Therapy. 2*: 521 – 531