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CD4-T Cells as a Predictor of Immune Status and Its Outcomes Following Second-Line Combination Antiretroviral Therapy in Adult HIV-1 Infected Patients Attending Apin/Juth HIV Clinic in Jos, Plateau State, Nigeria

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Abstract
Deficiency in immune cell number or activity is a cardinal feature of HIV. Second line antiretroviral therapy is geared towards improving immune cell activity and improving treatment outcomes. More people are now accessing free combination antiretroviral therapy through public health programmes in resource limited settings. There is currently no third line therapy for patients failing second line therapy in most of these programmes and data on effectiveness of second line antiretroviral therapy are limited. To adequately address and prepare for this scenario, critical assessments of the outcomes of second-line cART are needed. This is a retrospective cohort study of patients accessing second line cART at the APIN/ JUTH, Jos adult HIV clinic from 2004 to 2018, to determine the proportion of patients failing second line cART, to evaluate time to immunologic failure, time to lost to follow up and time to death using Kaplan Meier estimates. Immunological failure occurs when there is a fall of CD4 counts to pre-therapy baseline (or below) or 50% fall from the on-treatment peak value (if known) or persistent CD4 levels below 100 cells/mm^3 6 months after ART initiation. A total of 285 patients were included in the study, with a mean age of 45±9.5 years. Females where 194 (68.1%) All the patients were on boosted protease inhibitor, the predominant combination antiretroviral therapy for second line regimen was Lopinavir boosted with ritonavir in combination with Tenofovir, Lamivudine and Zidovudine (43.9%). The baseline CD4 count was 134 (IQR 54-272). The CD4 count increased to 339 (IQR213-498) at 72 weeks. In conclusion, Second line cART immunologic failure rates are low in our cohort and patient stay longer on cART before failure.
Keywords: CD4 cells, Immunologic failure, Antiretroviral therapy
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Introduction

CD4 is a type of protein found on certain immune cells like T-cells, macrophages, and monocytes on the other hand T cells are a subset of white cells in immune-surveillance. CD4 T cells are termed helper T cells as they do not neutralize the invading organism but it activates other immune cells as well as create an enabling environment for the neutralization of the pathogen.

One of the quandaries of human immunodeficiency virus (HIV) infection is that the very cells meant to initiate an immune defence are the same ones targeted for infection by the virus. (Mahnke, Beddall & Roederer, 2013)

In the process of infection, the virus attaches to the helper cells, and then empties its genetic material within them altering the host's genetic code to produce other HIV virions. In doing so, the host CD4 cell is killed. The infected person's ability to trigger an immune response is compromised thus rendering the person susceptible to opportunistic infections. The normal CD4 count ranges from 500 to 1500 cells/ cubic millimetres, when it falls below 200 the disease is classified as AIDS.

The CD4 count was used in the past to consider commencement of ART. This is not the case post 2016 as it is now recommended that therapy is initiated on diagnosis. This position was guided by studies which showed that people with very low CD4 counts had a difficult time reconstituting their CD4 counts to normal levels, particularly after a severe bout of illness. (Seng et al, 2015)

HIV is said to have affected virtually all parts of the world, with an estimated 36.7 million people worldwide living with the infection. Sub-Sahara Africa (SSA) where Nigeria is situated carries 64% burden of the global HIV epidemic. Nigeria is the most populous country in SSA, and so it has a high absolute number of people living with HIV (PLHIV) despite a relatively low HIV prevalence of 3.2%. In addition, Nigeria has the highest new incidence rate and the second highest epidemic of 3.2 million worldwide.

HIV prevalence is highest in Nigeria's South-South Zone at 5.5%. It is lowest in the South-East Zone where there is a prevalence of 1.8%. There are higher rates of HIV infection in rural areas (4%) than in urban ones (3%).Approximately 160,000 people died from AIDS-related illnesses in Nigeria in 2016 (UNAIDS, 2017)

Providing antiretroviral treatment (ART) for people living with HIV (PLHIV) substantially reduces morbidity and mortality and increases their life expectancy. It does this by reducing levels of viraemia and decreasing T cell destruction thus leading to an increase in CD4 count.

The Nigerian National Guidelines for HIV prevention and treatment is an adoption of the World Health Organization (WHO) recommendation of using a combination of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) with a Non-nucleoside reverse transcriptase inhibitor (NNRTI) as first line regimen (1L). Occasionally 1L regimen may fail due to development of resistance or other factors such as drug interactions, poor adherence, and adverse drug reaction for those who fail to achieve sustained virologic suppression with immunological cells falling persistently below pre-therapy levels or remaining at 50% below the on treatment peak or even having CD4 counts falling persistently below 100 cells/ cmm^3. This is considered to be treatment failure and thus a second line (2L) which is a combination of 2nucleoside/nucleotide reverse transcriptase inhibitors with ritonavir boosted protease inhibitors (PI/r) is then introduced (WHO, 2019)

Worldwide about 19.5million people are on cART, that is more than 50% of all PLHIV. In Nigeria about 780,000 are on cART as at 2016.

The adoption of the target 90-90-90 by UNAIDS in 2014 to eliminate HIV epidemic by 2020 has led to significant progress and acceleration of the AIDS response. AIDS-related deaths are said to have reduced by 32% and incidence by 16% globally between 2010 and 2016. There has been a global increase in awareness of HIV testing and counselling with an increase in number of people knowing their status and enrolling in treatment programs. However this is not the picture in West and central Africa where only 42% know their status, 83% are on ART and 73% of them being immune reconstituted.(UNAIDS, 2017)

Nigeria has a more terrible picture with only 34% of PLHIV who know their status and just 30% of all people living with HIV receiving treatment in 2016. Only 21% of children living with HIV are receiving ART, and 68% of pregnant women living with HIV are not on ART. Among people on ART less than a quarter are immune reconstituted.

The large population of people who do not know they have HIV and those on cART that are not immune reconstituted or virally suppressed are a potential source for sustaining the HIV epidemic and increasing resistance, which will slow down progress towards achieving the goal of ending the HIV epidemic by 2020.

High efficacy of second line regimen has been documented in randomized trials. However, data from low- and middle-income countries (LMIC) have demonstrated high rates of persistent CD4 cell depletion among patients on second-line regimens, predominantly driven by suboptimal adherence and prolonged exposure to previous failing regimens. These rates of immunological failure tend to increase the longer the patient is on second line regimen.

For patients failing second-line therapy, treatment options are largely non-existent in most developing countries including Nigeria. Current WHO guidelines provide some guidance for treatment in the case of second-line failure, but these are prefaced with the caveat that many low and middle income countries have financial constraints that will limit the adoption of third-line options. Thus, there is a need to determine the treatment outcome in HIV patients on second-line regimens in our environment and determine the factors responsible for 2L ART failure. (Musana *et al*, 2021)

METHODS

STUDY AREA

The study was conducted at the outpatient HIV/AIDS Clinic in of the Jos University Teaching Hospital, Jos Plateau State.

The centre commenced ART services in 1997, and in 2001 became one of the Government of Nigeria (GON) ART site. The centre has been receiving PEPFAR support since 2004; initially through the Harvard PEPFAR, and now through APIN public health initiatives. To date the center has cumulatively enrolled over 26,000 patients.

STUDY POPULATION

This study included all patients on second line ART for at least 6 months; they must also have CD4 count record at least at 6months after commencement of second line ART. Those with incomplete or missing records that cannot fit into analysis were excluded from the study.

STUDY DESIGN

A retrospective cohort analysis of patients who were switched to second line cART in JUTH adult HIV clinic was done.

Secondary data was utilized for the study; it was collected in a longitudinal manner and stored in the clinic's database (FileMaker Pro, v10; FileMaker, Inc, Santa Clara, California, USA).

ART initiation and monitoring follows the Nigerian National guidelines recommendations, including both HIV-1 viral loads monitoring and CD4 count monitoring. Clinic counsellors provide adherence training before first-line ART initiation and at the time of immunological failure prior to regimen change. Adherence counselling is continuous at each contact with the facility. Adherence level of 95% and above is regarded as optimal and below 95% suboptimal.

SAMPLE SIZE CALCULATION

The sample size was determined using Open Epi epidemiological calculator (Epi info 7.2). The method for cohort studies by Fleiss with correction of continuity was used with the following assumptions: Two-sided significance level (1-alpha) of 95; power (1-beta, % chance of detecting a difference) of 80%; ratio of sample size Unexposed/exposed 1; Percentage of outcome in unexposed 25%; Risk ratio 1.8, odds ratio 2.5; Percentage of outcome in exposed 45.5%.[26] A minimum sample size of 188 was calculated.

SAMPLING TECHNIQUE

A total sampling technique was applied. All complete record of patients that presented for treatment in the Adult ART clinic from 2004 to 2018 who met the inclusion criteria were included in the study

DATA COLLECTION TECHNIQUE

The data for this study included patient information that are routinely collected at pre-assessment, starting ART, ART switch and subsequent follow up visits on standardized forms, and also at pharmacy drug pick up data.

Trained data clerks enter the information in the forms into an electronic data platform using FILEMAKER PRO software (FileMaker Inc, Santa Ana, CA, USA).

The data extracted from the electronic data base included socio demographic characteristics, WHO staging, CD4 count, Viral load, first line treatment regimen, duration on therapy and percentage adherence . The data was cleaned andcoded for statistical analysis.

To estimate adherence, a medication possession ratio was calculated by dividing the number of days that a patient submitted refill prescriptions by days since regimen initiation. Patients are routinely scheduled for clinic visits every 28 days or 56days (depending on how long and how immune-suppressed they are). At each visit, the patients were given a 30-day or 60-day supply of medication and patients accrue 2 or 4 extra days of pills at each refill. Any missed refill visit resulted in a reminder phone call or a tracking visit to the patient.

DATA ANALYSIS

Quantitative variables such as age of the study subjects are expressed as mean standard deviation (SD), and CD4+ count and viral load were expressed as median (interquartile range-IQR); Mann Whitney-U test will be used to determine the variation in median CD4+ count and viral load at baseline and at 72 weeks.

Qualitative variables such as Sex, marital status, educational level, etc. were presented using frequency tables, and expressed as percentages and Chi square test was used to determine the associations between the socio-demographic characteristics and the immunologic outcome.

Kaplan-Meier curves were generated to estimate the mean time of Immunologic failure, loss to follow up and survival at each time point and Wilcoxon rank-sum tests were used to assess significance between those with immunologic failure and Immune reconstitution.

Immunologic failure was determined as those who had less than 100 cells/mm^3 or those whose CD4 cell count fell below the Pre-therapy count.

Wilcoxon-Mann Whitney test was used for comparison of two medians. Univariate logistic regression was used to examine the association between the independent variables and outcome with the results expressed as odds ratios with their 95% CIs. Variables that were associated with treatment failure in the univariate analyses at $p<0.05$ would be fit into multivariate analysis using risk ratio and 95% CI as both point and interval estimate of the measure of effect of independent predictors of treatment failure. Stata software version 13.0 (Stata Corporation, College Station, Texas, USA) was used for analysis and a p-value of <0.05 would be considered statistically significant.

ETHICAL CONSIDERATION

HIV clinic principal investigator and PEPFAR/HARVARD have granted permission for use of the secondary data for this study.

RESULTS

CHARACTERISTICS OF THE STUDY POPULATION

Data from 387 patients on 2L cART were extracted from APIN/JUTH database between June 2004 and August 2018. A total of 285 (74.0%) met the inclusion criteria and were included in this analysis.

GENERAL CHARACTERISTICS

Females constituted 194(68.1%) of the study population, Mean age of the patients was 45±9.5 years. The age group 41 to 50 constituted 107 (37.5%) of the population. Most of them were married 150 (53.9%), 116(41.7%) had tertiary education and 78(27.4%) were civil servants. The predominant mode of HIV acquisition was hetero-sexual sex 269 (97.7%).

Females constituted the majority in the younger age groups (≤30, 31-40, and 41-50), but males are majority in the older age group of (51-60 and ≥60).

Tertiary level of education was the highest attained by both sexes but more females ended in primary education than males (15(17%) Vs 39(20.5%).

Majority of the females were divorced 13 (6.9%) or single 46(24.2%) than the males 5(5.7%) divorced and 15(17.0%) single.

Most males where civil servants (40.7% Vs 21.1%) compared to females who were mostly unemployed (29.9% Vs 3.3%)

The predominant risk factor for HIV transmission was heterosexual sex in both sexes

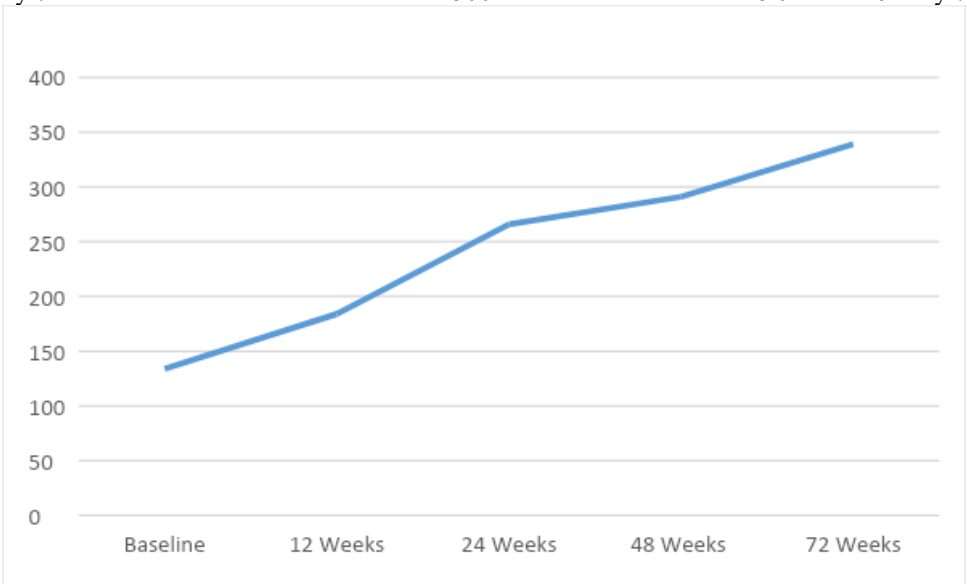
IMMUNE OUTCOME

The CD4 count increased from 134 (IQR 54-272) at baseline to 184 (IQR 105-300), 266 (IQR 173-402), 291 IQR (179-526), 339 (IQR213-498) at 12weeks, 24weeks, 48weeks and 72 weeks respectively.

The CD count for patient with virologic response increased from 156cell/ml to 328cells/ml over 72 weeks unlike virologic failure group there was a marginal increase from at 12 weeks from 112cells/ml to 173cells/ml and then it dropped to 154cells/ml at 72 weeks.

Patients with treatment success had a higher CD4 count at baseline but there was no significant difference until at 24 weeks which was maintained to 72weeks.

The proportion of patients with CD4 count<200 cells/ml at baseline line was 65.3% but decreased to 23.4% by 72wks. While those with CD4 count > 500 cells/ml increased from 6.0% to 24.9% by 72 weeks.



Z= -9.077, P<0.0001

Figure 4: Trends in median CD4 count of the study population

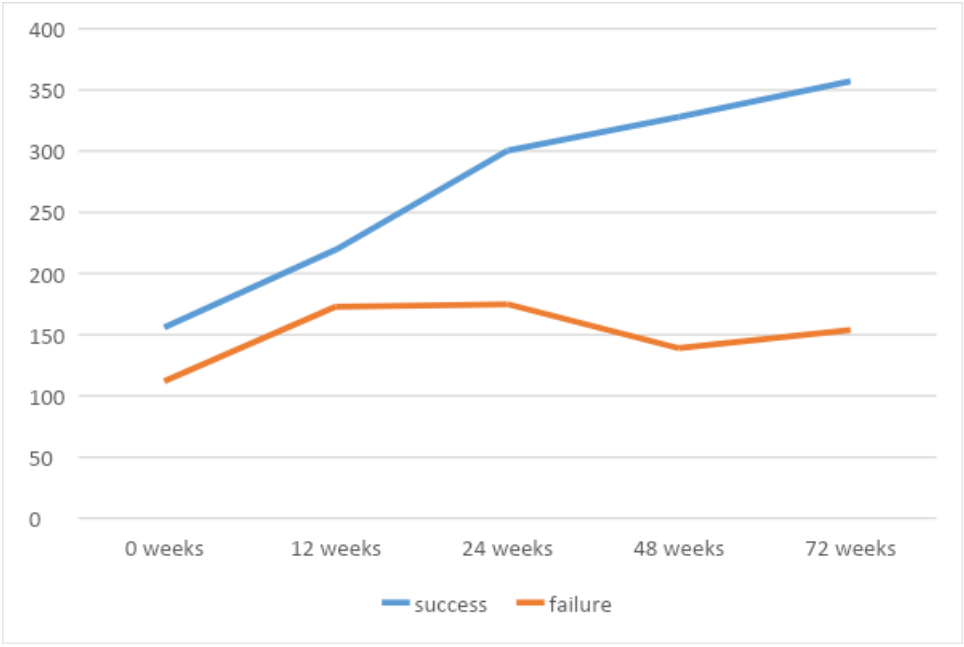


Figure 5: Median CD4 trend compared between Treatment response and Treatment failure

Table 3: Statistical Analysis for CD4 Trend for Treatment failure Vs Treatment Success

	Median CD4 (IQR)	Z (signed test)	p-value
72 weeks			
Treatment response	357 (270-565)	5.995	< 0.001
Treatment failure	154 (105-211)		
48 weeks			
Treatment response	328 (235-493)	6.684	< 0.001
Treatment failure	139 (97-192)		
24 weeks			
Treatment response	301 (195.7-446)	4.334	< 0.001
Treatment failure	175 (112-261)		
12 weeks			
Treatment response	219 (113.7-322.5)	0.524	0.601
Treatment failure	173 (145-270)		
Baseline			
Treatment response	156 (51.5-317.3)	0.781	0.435
Treatment failure	112 (61-246)		

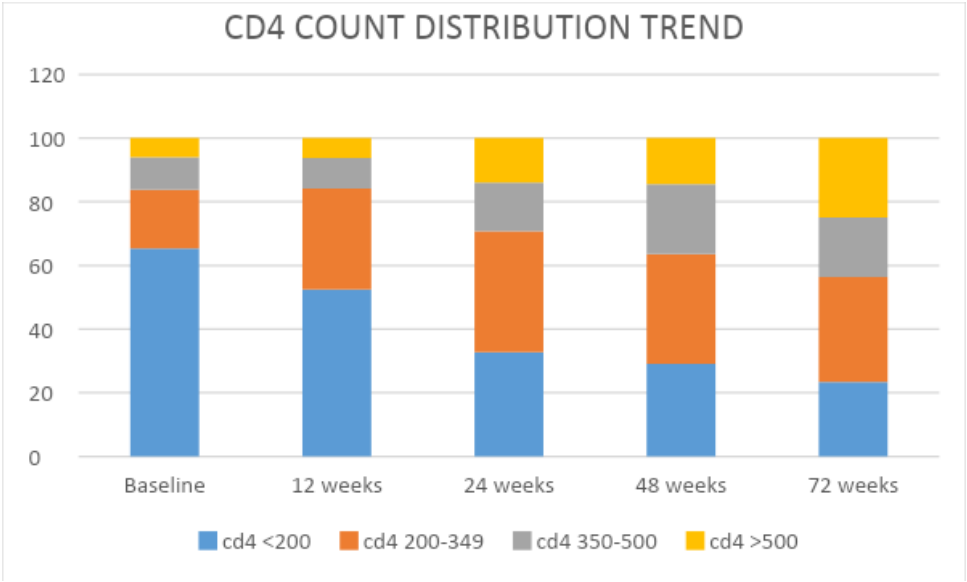


Figure 6: Trends in CD4 count distribution

TIME TO EVENT ANALYSIS on cART

Table 4: Patient clinic status on second line CART and Treatment outcome

PATIENT STATUS	FREQUENCY n(%)	TREATMENT OUTCOME		
		SUCCESS n(%)	FAILURE n(%)	p-value
Active	174(61.1)	157(90.23)	17(9.77)	0.099
Died	3(1.1)	2(66.67)	1(33.33)	
Transferred out	38(13.3)	36(94.74)	2(5.26)	
Loss to follow up	70(24.6)	57(81.43)	13(18.57)	

For the period understudy, 61.1% were active on care, 24.6% were lost to follow up and 1.1% were dead.
The mean time to failure on second line cART was 7.473± 0.269 95% CI (6.946-8.000) years.

DISCUSSION

Combination Antiretroviral Therapy (cART) has changed the prognosis of HIV infection from an ultimately fatal infection to a chronic illness. Sustained virologic suppression and immune reconstitution is the ultimate goal of cART with the attendant benefits to the individual patient and the community. (Saag et al, 2018)

A growing number of patients are expected to be on second line cART regimen in low- and middle-income countries (LMICs), and the rates of failure may differ from place to place(Wang *et al*, 2012).

This found that immune reconstitution was rapid between the 12 week and 24 weeks of cART from 34% to 69.8% had undetectable viral load (UDVL) and in-between at about 18 weeks equilibrium between suppression and viral detection was achieved and subsequently suppression rates increased marginally up to 72 weeks (69.5% to 72.1%).

Although the baseline CD4 cells at commencement of 2L cART was low there was statistically significant Immunological reconstitution among the patient on 2L cART (P›0.0001), the median CD4 cells increased from 134 to 339cells/ml by 72 weeks. The proportion of patients with CD4 count <200cell/ml decreased by 64% and the proportion with ›500cells/ml increased 4times. The increase in CD4 cell at 12 months was 157cell/ml which is well above the expected increase by 1year of 50-100cell/ml (Chima,2012).This finding is similar to that reported in Malawi, where the median increase in CD4 count was 142cells/µL (IQR 66-263) at 12 months (Ferradini *et al*,2006)

This study, among treatment responders there was significant increase in CD4 count but among those with treatment failure they had a lower CD4 count at baseline and a marginal increase from baseline to 12 weeks before plateauing at 24weeks and then a gradual decline. The initial increase in CD4 count among patients with treatment failure may suggest some treatment respond or partial response before complete failure. Issues around commencement of ART could have contributed to the eventual failure of these patients. Several factors like low CD4 count at commencement, high viral load >100000copies/ml, opportunistic infections, drug side effects, poor adherence and drug interactions among others could have contributed in the eventual failure of this patients (Fiseha *et al*,2022). Further studies may be needed to elucidate why patients have some response before failure soon after starting second line therapy.

CONCLUSION

Second line cART treatment failure rates are low according to our National guideline definition and the patients stay longer on cART before failure, but a substantial proportion of patients still remain in treatment failure with sustained CD4 cell depletion. This may affect the goal of ending the HIV epidemic.

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