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# CD38 as Surrogate Marker for HIV Infection in Antiretroviral Naive and Antiretroviral Experienced Patients in Kenya

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**Abstract**

Human Immunodeficiency Virus (HIV) patient management continues to be a challenge all over the world. CD4 absolute counts and viral load are the gold standard tools for monitoring of HIV-1 disease. However, the use of CD4 counts cannot be used solely to determine the overall status of immune system. It requires the additional measurement of viral load. Determination of viral load is also expensive in many places that are limited with resources. Therefore, there is need for identification of other markers for management of HIV. CD38 is one such candidate marker. The main the correlation between CD38 antibody binding capacity (ABC) and viral load. A a negative correlation was established for participants not on drugs, whereas a positive correlation was exhibited between CD4 and viral load for group on drugs. There was a significant correlation between CD38 ABC and viral load. CD38 levels for the group not on drugs was elevated the same way viral load was, whereas for the group on drugs CD38 levels were lowered the same way as viral load. There was no significant correlation between ages with the outcome from the two groups. Quantification of CD38 may therefore be an affordable test that can serve as an extra tool in HIV-1 management. However, more studies are required to justify the use of CD38 as a surrogate marker for HIV patients on ART.

**Keywords:** CD38; CD8; Viral load; HIV-1; ARV treatment; CD38 Antibody binding capacity; CD38 ABC

## Introduction

Measurements of cluster of differentiation (CD) 4 (CD4) absolute counts and viral load are the two common tools used to monitor disease progression in HIV-1-infected patients on drug therapy [1,2]. However, there are certain limitations in the use of these tools. Although patients on highly active antiretroviral treatment (HAART) will often exhibit suppressed viral load within the first three weeks of treatment, this is commonly not necessarily accompanied by rapid changes in absolute CD4 counts, thus rendering measurement of levels of CD4 cells unreliable indicators of efficacy of the anti-retroviral treatment at the early stages of intervention. Currently, only viral load determination offers a reliable prognostic indicator for antiretroviral (ARV) treatment. However, the cost of estimating viral load is prohibitive, making it difficult for adoption as a routine test. There is need therefore to identify other markers whose levels change rapidly following ARV treatment. Previous studies conducted in Ivory Coast on HIV-1 indicate that CD8+/CD38+ activation molecule can be a sensitive and independent marker [3]. The possible association between CD8+ T lymphocyte subsets defined by CD38 antibody binding capacity (ABC) expression, and immunological and virological parameters in the course of HIV infection has, to date, received little attention [4,5]. Moreover, the link between inflammation, coagulation, and activation of T cells is not established. However, it is suggested that they can be used as predictors of disease progression in patients with human immunodeficiency virus (HIV) and being managed with combination antiretroviral therapy (cART) [6,7]. Evidence of involvement of inflammation/coagulation has been associated with mortality and morbidity in non-AIDs patients. In these conditions, there were no significant associations with the disease outcomes. It is also now known that CD38 is a T cell activation marker. A significant correlation between CD38+CD8+ T cells with disease progression in untreated HIV infection has also been reported [8,9]. However, suppression of CD38+CD8+ T cells by the use of cART suggests that it has no impact on their levels; they remain elevated abnormality [10]. The prognostic value of CD38+ has never been clear [11,12]. To date, it is still remains unclear on the association of T cells with increased morbidity and mortality of patients using ART. There is also need for development of novel interventions that will manage excessive inflammatory and immune activations when using ART. However, the potential for their application as surrogate markers for disease progression has not beed determined and findings are still inconclusive as a result of the mixed findings. For instance, Tenorio et al. [6] and Hunt et al. [7] did not find any association between CD38 expressions on CD8+ T cells with disease outcome as opposed to existing literature.

Therefore this study aims to determine the association between CD38+ and disease outcomes in untreated and treated patients of HIV by investigating their levels.

Thus we have here addressed this question by performing a cross-sectional study involving untreated and treated patients, and by investigating levels of these parameters at one point.

## Materials and Methods

### Study area and population

Th study population comprised of regular adult patients attending Mbagathi District Hospital HIV clinic, Nairobi, Kenya. Thes participants in this study were either on antiretroviral therapy or antiretroviral naive returning to clinic for routine checkup. A total of 84 study participants who were HIV-1 positive were enrolled. 44 were on antiretroviral therapy, whereas 40 were not placed on any treatments of ARVs. Thes were patient who regularly visited Mbagathi district hospital HIV clinic.

### Ethical considerations

Th study was conducted under protocol approved by Kenya Medical Research Institute (KEMRI) scientifi steering committee (SCC No.1035).

### Collection of fresh whole blood

Approximately 3-5 ml of whole blood from consenting patient was collected in Ethylene di-amine tetra acetic acid (EDTA). Approximately 100 µl of blood was used for determining CD4 absolute count and CD38 antibodies bound per cell. Th remaining blood was centrifuged at 604 g for fiv minutes. Th plasma that was separated was then used to determine the overall viral load in the patients.

### Determination of CD4+ T cell absolute count

Th CD4+ T cell absolute count was determined using Becton Dickinson (BD) multitest reagents and Tru Count tube according to manufacturer's instructions. To 20 µl of multitest reagent CD45 PerCP, CD3 FITC, CD4 APC, CD8 PE monoclonal antibody catalog number 340491, 50 µl of whole blood was added, vortexed and incubated in the dark for 15 minutes. It was then fixe and lysed for a further 15 minutes in the dark room. Finally acquisition and analysis was done on multiset™ softwar using BD FACS calibur instrument (Becton Dickinson, USA) Catalog No. 342975.

### Determination of CD38 activation marker

To determine the CD38 activation marker 5 µl (BD multitest CD8 FITC/ CD38 PE/ CD3 PerCP/Anti-HLA DR APC) monoclonal antibodies, were added to the bottom of BD falcon disposable 12 75 mm capped polystyrene test tubes. To this, 50 µl of well-mixed EDTA and fresh whole blood was added. Afte vortexing gently, incubation was done in dark room for 15 min. BD FACS lysing solution 450 µl 1X was added and further incubated in dark confinemen for 15 minutes. Acquisition and analysis was performed on a BD FACS calibur instrument using cellquest™ software

Quantibrite beads (acquired on the same instrument settings as the sample [BD Quantibrite PE tubes catalog No. 340495]) were reconstituted using 0.5 ml of phosphate buffe (Ph7) with 0.5% azide BSA. In this study CD8+/CD38+ are reported as CD38 ABC.

Gate R2 that represents CD3+CD8+ (Figures 1 and 2) was applied on Figure 3 histogram. Histogram statistics were then generated to give geometric mean fluorescenc emanating from CD3/CD8/CD38 population, which was then used to determine the levels of CD38 ABC using a standard Quantibrite histogram peaks (Figure 4) as follows:

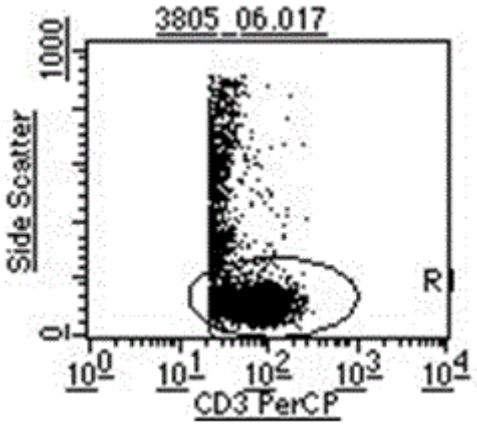


**Figure 1:** Scatter diagram for total lymphocytes showing CD3+ sub-population of T cells gated as R1. Th cycled region represents the sub population of the T cells.

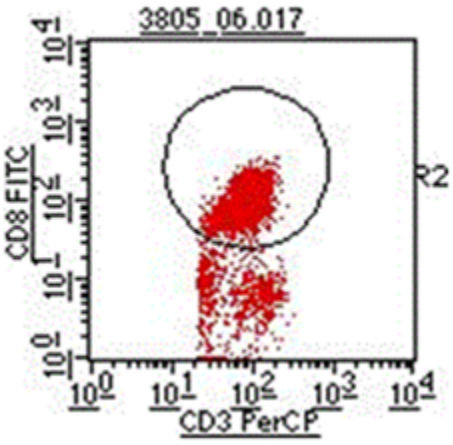


**Figure 2:** Scatter diagram for total T lymphocytes with CD3+CD8+ gated as R2. Th cycled region represents the sub population of the T cells.

Flow-cytometry profile demonstrating CD38 and on CD4+ and CD8+ T cells in an HIV-sera-positive individual. Samples were firs gated on the CD3+ then CD8+ T cell population, and the percentage of CD38+ cells was determined. Preset gating for CD38 (Figure 3) and HLA-DR expression on CD8+ T cells was used for all analysis and quantificatio of CD38 expression. Figures 3 and 4 represents the conversion of CD38 expression to absolute counts.

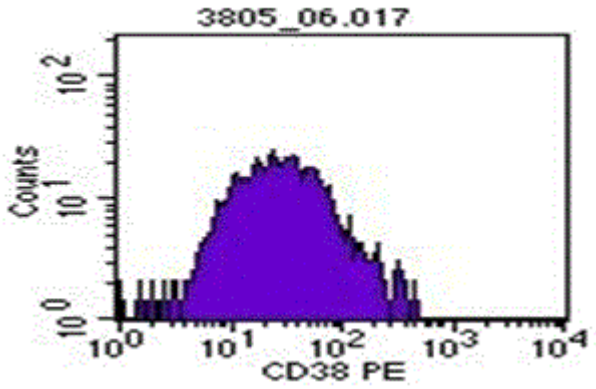


**Figure 3:** Histogram representing CD8+CD38+ cells.

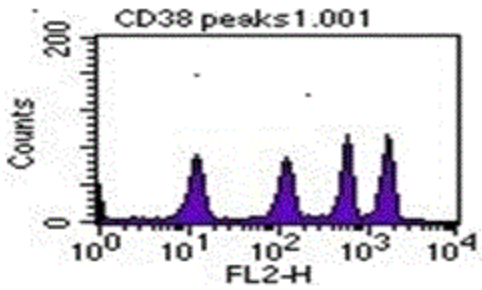


**Figure 4:** Histogram plot of QuantiBRITE PE beads on a FACScalibur flow cytometer set up with CaliBRITE calibration beads for lyse/no wash procedure

The geometric mean fluorescence was converted to $\log_{10}$ for each PE molecule per bead. Additionally, a lot specific value for PE molecules per bead from the kit was also converted to $\log_{10}$. A linear regression of $\log_{10}$ PE molecules per bead against $\log_{10}$ fluorescence using the equation y=mx + c was plotted on a spreadsheet and used for calculation of CD38 ABC for all the participants (Figure 5).

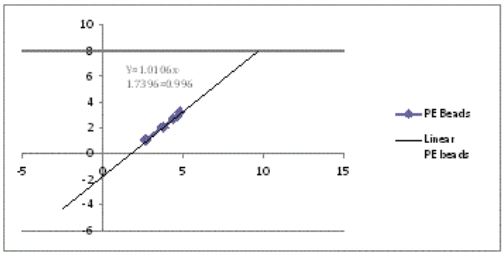


**Figure 5:** Regression curve that was used to convert CD38 fluorescence to CD38 ABC

## Determination of viral load

This was determined using the comprehensive bio-analytical system (COBAS) Amplicor™ HIV-1 Test, Version 1.5. This is a qualitative in vitro test for the direct detection of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. The COBAS Amplicor HIV-1 Test, v1.5 uses polymerase chain reaction (PCR) technology to achieve maximum sensitivity for the detection of HIV-1-RNA in plasma samples. The plasma used was stored at -80°C for viral load determination.

## Data Analysis

The data was analyzed using statistical package for social sciences (SPSS) version 17.0. Pearson's a non-parametric test was used for correlation between CD4, viral load and CD38+. P values <0.005 were considered significant.

## Results

Overall, 84 patient were enrolled for the study. Out of the 40 not on drugs, 27 were female and 13 were male. Out of 44 on drugs, 29 were female and 15 were male. The majority of participants were between 34 and 35 years (Table 1).

| Indicators | Study Groups | Mean | Median | Minimum | Maximum | Std Deviation | P value (non-parametric) |
|---|---|---|---|---|---|---|---|
| Age | Not on drugs | 35 | 34 | 22 | 60 | 8 | 0.42 |
| | On drugs | 36 | 35 | 23 | 66 | 10 | |
| Weight | Not on drugs | 63 | 60 | 46 | 94 | 12 | 0.775 |
| | On drugs | 62 | 62 | 28 | 87 | 12 | |
| Viral load | Not on drugs | 41085 | 7321 | 0 | 300000 | 74740 | <0.001 |
| | On drugs | 1453 | 0 | 0 | 23000 | 4539 | |
| Cd4 | Not on drugs | 480 | 457 | 2 | 1276 | 290 | 0.002 |
| | On drugs | 311 | 287 | 61 | 1199 | 217 | |
| Cd38_abc | Not on drugs | 2584 | 1933 | 441 | 13285 | 2601 | <0.001 |
| | On drugs | 1303 | 1113 | 416 | 4792 | 817 | |

**Table 1:** Means and standard deviations of study variables of the study population

Ther was no significan correlation between age and the study outcome. Th range of weight for participants on drugs and not on drugs was 60 Kg to 62 Kg. Th ages of the participants ranged from 22-66 years and did not diffe significantl ($p$=0.420) between the two groups (Table 1). Th mean value for CD38 ABC for the group on drugs was 1303ABC, which is considerably lower compared to the naïve group at 2584 cells/µl. For the group on drugs the mean viral load was 1453 copies/µl whereas for the group not on drugs the mean viral load was 41085 copies/µl. Similarly, there was no significan correlation between CD8+ CD38+ and all the other variables on controlling for all other variables (Table 2).

| | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | 95% Confidence Interval for B | |
|---|---|---|---|---|---|---|---|
| | B | Std. Error | Beta | | | Lower Bound | Upper Bound |
| (Constant) | 2170.65 | 1818.72 | | 1.194 | 0.236 | -1451.7 | 5792.95 |
| Log_VIRA | 138.888 | 120.706 | 0.157 | 1.151 | 0.253 | -101.52 | 379.295 |
| CD4 | -0.661 | 0.854 | -0.093 | -0.774 | 0.441 | -2.362 | 1.04 |
| WEIGHT | -19.395 | 17.054 | -0.118 | -1.137 | 0.259 | -53.361 | 14.57 |
| AGE | -33.331 | 22.149 | -0.16 | -1.505 | 0.136 | -77.444 | 10.781 |
| DRUG | 429.622 | 244.632 | 0.341 | 1.756 | 0.083 | -57.605 | 916.848 |
| DRUG D | -19.102 | 26.455 | -0.097 | -0.722 | 0.472 | -71.791 | 33.587 |
| STAGE | 485.484 | 286.733 | 0.242 | 1.693 | 0.095 | -85.595 | 1056.56 |

**Table 2:** CD38 in relation to all study variable.

A positive correlation (r=0.046, p=0.766) existed between CD4 absolute count and CD38 ABC for participants on drugs whereas a negative correlation coefficien (r=-0.476, p=0.005) was observed for participants not on drugs (Figure 6). Viral load was positively correlated with CD38 ABC for group not on drugs ($r$=0.121, $p$=0.490) and negatively correlated for group on drugs($r$=-0.092, $p$=0.548) (Figure 7). However, the sample size was too small to definitivel establish whether the correlation between the viral load and the CD38 measurements was related to age. Similarly, the viral load was positively correlated with CD4 count for group on drugs (r=0.041, p=0.487) and negative correlation for group not on drugs (r=-0.498, p=0.002) (Figure 8).

## Discussion

Monitoring of the HIV epidemic as a strategy for effectiv control and management of HIV/AIDS disease remains a challenge in Africa [13]. Whereas significan effort have been made in scaling up the use of antiretroviral (ARV) drugs, including a substantial reduction in the costs of these drugs, routine monitoring of individual HIV patients to assess response to drug therapy also remains a challenge [14]. Th reasons behind this challenge are varied but range from unavailability of adequate facilities with CD4 and viral load testing equipment to the relatively expensive nature of these tests [15]. Thi study was aimed at evaluating the potential usefulness of CD38 activation molecule as a surrogate marker for HIV disease progression, specificall asking the research question of whether or not CD38 may be used, under certain circumstances, to replace the need for viral load testing.

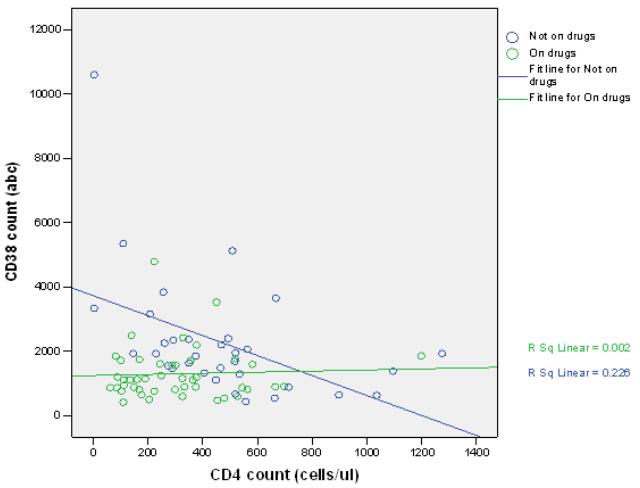


**Figure 6:** Scatter plot for correlation between CD4 and CD38 for group on drugs and group not on drugs. Th "r" value represents the correlation coefficie value while p value represents the significant A p ≤ 0.05 indicates significan association. r=0.046, p=0.766 group on drugs. r=-0.476, p=0.005 group not on drugs. Th increase in CD4 count leads to a decrease in CD38 count when patient are on ARV. Thi can be used together with CD4 to monitor drug response in patient management

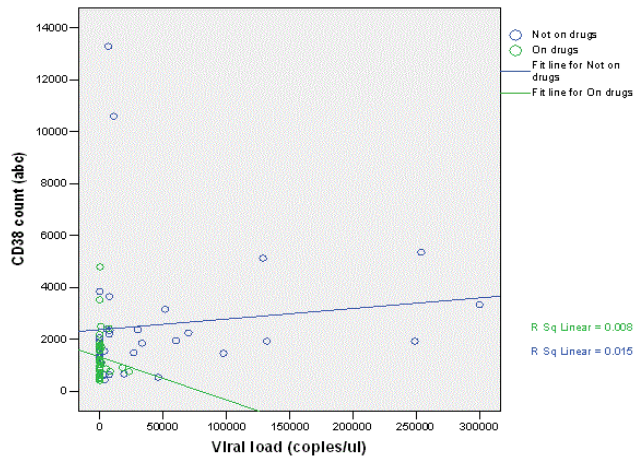


**Figure 7:** Scatter plot for correlation between CD38 and Viral load for group on drugs and group not on drugs. Th "r" value represents the correlation coefficie value while p value represents the significant A $p \leq 0.05$ indicates significan association. r=-0.092, p=0.548 for group on drugs. r=0.121, p=0.490 for group not on drugs. Th blue line is for patient who are not on drugs whereas the green line is for patient who are on ART, the results illustrate clearly that CD38 in both cases correlates with viral load.

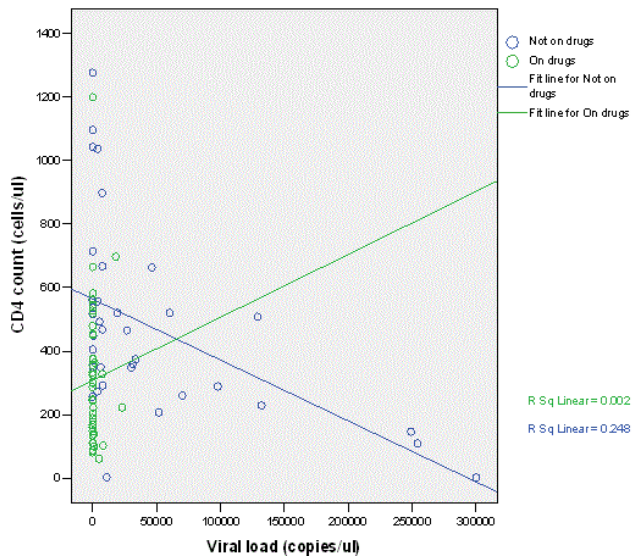


**Figure 8:** Scatter plot for correlation between CD4 cells and viral load for group not on drugs and group on drugs. Th "r" value represents the correlation coefficie value while p value represents the significant A $p \leq 0.05$ indicates significan association. r=0.041, p=0.487 for group on drugs. r=-0.498, p=0.002 for group not on drugs. Viral load increase leads to a decrease in CD4 cell count in patient who are not on ARV.

Th results from this study have demonstrated a strong positive correlation, with statistical significance between CD38 and viral load, where a rise in viral load was correlated with a rise in CD38 cells, and similarly, a drop in viral load was correlated with a drop in CD38 cells. Thi study has demonstrated a positive correlation between CD38 and viral load for group not taking antiretroviral therapy and a negative correlation for the group on drugs. It is documented that a significan distribution of HIV-specifi CD8 T-cells resides in the CD8 CD38 T-cell sub-population [16]. Increased CD38 ABC expression on CD8+ T cells in HIV infection is associated with high viral loads, disease progression and death in adults [17]. Thi is contrary to the finding made by Tenorio et al. [6] and hunt et al. [7].

Moreover, a negative correlation between CD4 and CD38 was observed on the group not on drugs which depicts the observed relation of CD4 and viral load in HIV infection. Thus these study finding are in agreement with other studies finding done on children on HAART where they did observe that good responders to antiretroviral therapy had a negative correlation of CD38 to viral load [18].

Th two study groups show significan differenc on viral load, CD4 absolute count and CD38 ABC (Table 1). Thi shows that at differen stage of HIV progression and during treatment CD38 ABC expression varies, hence need for further studies to establish normal reference ranges and follow up studies that will reflec the levels and expression of CD38ABC in HIV-1 patient at various stages of disease progression. Th finding of this study confir the possible utility of CD38 ABC as a prognostic marker in HIV-1 infected patients in Kenya. Similar study done in Uganda show up regulation of CD38 in HIV compared naïve patient [19]. In addition, the CD38 expression normal reference range values from differen countries vary significantl [20], therefore it important to establish the normal reference value for Kenya using a Kenyan population.

Thes results are similar to a previous report where a positive correlation was observed in a drug naïve pediatric population [21,22]. In another study involving long-term non-progressors, who were not receiving any antiretroviral therapy, a similarly strong positive correlation was observed between CD38 cells and viral load [23]. Thi positive correlation between CD38 and viral load has also been demonstrated even with cryopreserved cells, suggesting that CD38 expression, whether from fresh or frozen samples, may provide a useful predictor of HIV disease progression [24]. In this study, there was no association between CD38 and disease progression and hence, lacks the merit to be used as a marker for clinical disease progression.

Th results from this study also indicate that when the viral load is suppressed through use of active antiretroviral, a reduction in the number of CD38 cells is also observed. Thes results are in agreement with previous published reports demonstrating a reduction of CD8+CD38+ cells in parallel with plasma viremia, following active antiretroviral therapy [25]. Consequently, it is possible that active HIV replication has a profound impact on the expression of CD38 cells. Furthermore, the observed higher count of CD38 in untreated patients in comparison to the treated group suggests a possible application of CD38 as a potential marker of non-adherence to therapy and also drug resistance development.

In this study, CD38 cells were measured as CD38 ABC on CD8+CD38+ cells. One would argue that the observed trends in CD38 may not be due to the CD38 molecule per se, but a function of the CD8+ cells. Th role of CD8+ cells in HIV disease is well known. CD8+ T cell-mediated immune responses play an important role in host defenses against viral infections. Previous studies of immune

responses in HIV-infected individuals have suggested that CD8+ T cells play an important role in controlling viral replication, as evidenced by the emergence of HIV-specifi CD8+ cytotoxic T lymphocyte (CTL) activity coinciding with a decline in plasma viremia during primary infection [26] and a diminution of CTL responses preceding disease progression in infected individuals [27]. Further studies that have examined the capacity of CD8+ T cells to express intracellular IFN-γ in response to a broad range of HIV antigens have shown that a large proportion of infected individuals harbor significan numbers of HIV-specifi CD8+ T cells and yet fail to effectivel control viral replication, as manifested by high levels of plasma viremia [28].

Thes studies indicate that while CD8 levels are high, the levels of plasma viremia can either be low or high, suggesting that there is no direct correlation between CD8 levels and viral load. Therefor the observed trend in CD38 cells is a true reflectio of the dynamics of these cells during HIV infection in the presence or absence of ARV therapy. Taken together, these results indicate that the CD38 cells can serve as independent indicators of the trend of viral load.

Since the early years of the HIV/AIDS epidemic, clinicians have monitored patients' CD4 cell counts as an indicator of immune function. As CD4 numbers decline, the risk of rapid disease progression become eminent. Th levels of CD4 cells, expressed both as percentages or absolute counts, are conveniently measured by flo cytometry. However, CD4 cells count alone has an inherent limitation of, among other things, not being able to defin the precise clinical presentation of a HIV infected individual. Several cases abound where patients with CD4 counts of <200 cells/μl of whole blood, as define by WHO as the stage to initiate ARV therapy, may either be symptomatic or asymptomatic. Furthermore, during ARV therapy, the changes in CD4 levels may not necessarily be rapid but will exhibit a rather gradual increase or decrease depending on the outcome of the therapy. Thes observations make it difficu for clinicians to make solid decisions on the basis of CD4 counts alone.

It is in this context that in the mid-1990s, clinicians also began to routinely monitor HIV viral load, which is a direct measure of viral replication. Viral load has since been shown to be a better predictor of the HIV disease progression or death than CD4 count. A single HIV-1 RNA measurement is also a good predictor of time to AIDS, explaining almost half of the variability in clinical relevant outcome [16]. High variance around CD4 regression lines likely accounted for the inability of any marker to explain CD4 cell decline. Th predictive value of HIV-1 RNA for time to AIDS reaffirm the central role of viremia in AIDS pathogenesis and strongly supported the use of HIV-1 RNA for patient management. In a related study, [29] investigated the relationship between HIV RNA levels and CD4 cell decline, as well as the predictive value of HIV RNA for clinical disease progression. Th results from these studies suggested that HIV viral load was the best predictor of progression to AIDS or death over the long term, but that CD4 cell count can play a role in predicting short-term 1-2 year disease progression.

Th deleterious effect of active viral replication on immunologic functions of various subsets of lymphocytes in HIV-infected individuals have been well documented [30]. Among those, expression of CD38, a surface antigen with multiple functions on CD8+ T cells [31], has been well accepted as a prognostic marker for disease progression in infected individuals who do not control viral replication [8]. In addition, it has been speculated that an increase of CD8+ T cells expressing CD38 is a reflectio of HIV-mediated immune activation and that these cells are ultimately unable to control viral replication or prevent disease progression [8]. Th results from the present study give further insight into the pathophysiologic relationship between HIV-specifi CD8+ T cells and CD38 expression that result from HIV-driven aberrant immune activation in patients who are not receiving antiretroviral therapy and those receiving antiretroviral.

## Conclusion

Early administration of ART in HIV is very significan in the management of HIV. In order to ensure a sustained suppression of HIV in ART, there is need to screen and identify specifi biomarkers be better indicators for increased surveillance for development of drug resistance, improvement in the general health of the HIV patient's, quality of life, survival rate and above all, decreased HIV transmission rates. Th use of viral load and CD4 has been used as a gold standard for a long period of time. Th correlation between the viral load and CD 38 may therefore offe an alternative indicator for monitoring of HIV-1 progression. Quantificatio of CD38 may also offe an alternative and an affordabl test that can serve as an extra tool in the management of HIV-1. Thi will possibly offe a long term solution to monitoring of adherence to ART as a critical determinant of the disease outcome, progression and improvement in health and quality of life of HIV infected patients. Moreover, a study with more factors influencin outcome of the observation and use of a larger sample size should be conducted. Thi study also suggests that T cell counts may be determined with more than one measurement so as to provide more information of the well-known variations of laboratory finding for these variables.

## Acknowledgement

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## Conflict of Interest

Th Authors declare no conflic of interest

## Funding

BD international by Nick bright

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