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# Body mass index, proteinuria and total lymphocyte counts in predicting treatment responses among HIV-infected individuals initiated on antiretroviral treatment in Dar es Salaam, Tanzania, 2019: a cohort study

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**Keywords**: monitoring ART treatment; HIV/AIDS; CD4 count; BMI in HIV; proteinuria in HIV; viral suppression

## Abstract

**Objectives**: To explore the potential use of body mass index, proteinuria, and total lymphocyte count changes in predicting immunological and virological response in HIV-infected individuals initiated on antiretroviral therapy (ART).

**Design**: Prospective cohort study.

**Setting**: Three urban HIV care and treatment centres (CTC) in Dar es Salaam.

**Participants**: HIV-infected individuals initiating ART.

**Outcome measures**: HIV viral load <1000 copies/ml (virally suppressed) at six months after ART initiation.

**Results**: Of 215 (out of 220 enrolled) participants who returned for evaluation at six months, 147 (66.8%) were female. At six months of follow-up, 89.4% (76/85) of participants with sustained weight gain were virally suppressed compared to 31.8% (7/22) with sustained loss, p<0.001. In participants who were lymphopaenic at baseline, an increase to normal total lymphocyte counts at six months was associated with an increase in CD4 count compared to participants who remained lymphopaenic, 96.2% (50/52) vs. 54.8% (17/31), p<0.001. At baseline, 50.0% (110/220) had proteinuria. In participants without proteinuria from baseline to six months, 89.8% (79/88) were virally suppressed compared to participants with proteinuria at baseline and/or three months, 85.6% (77/90), those with persistent proteinuria, 30.8% (8/26), and proteinuria at six months only, 45.5% (5/11), p<0.001. In modified Poisson regression, the independent predictors other than CD4 cell counts for viral non-suppression at six months among HIV-infected individuals initiating on ART were BMI loss >5% from baseline to six months, {adjusted RR 2.73, 95% CI (1.36-5.47)},

lymphopaenia at six months, {adjusted RR = 4.54, 95% CI (2.19-9.39)}, and proteinuria at six months {adjusted RR = 2.63, 95% CI (1.25-5.54)}.

**Conclusions**: Changes in body mass index, total lymphocyte count, and presence of proteinuria can monitor and predict ART response and may be particularly helpful in settings when CD4 counts and viral load monitoring are unavailable.

## Article Summary

Strengths and limitations of this study

- We had complete data on 98% of the originally enrolled participants.
- In resource-constrained situations, when viral load and CD4 testing are not easily available, models such as ours with locally determined easily computable prediction cut-offs can be utilized by clinicians to make clinical decisions.
- Our findings require validation in a study with larger sample size.
- Local (and time-varying) conditions and treatment standards may influence some of the patterns we observed, both in prevalence and in effect.

## Introduction

In 2019 about 38 million people were living with HIV globally, with 1.7 million in Tanzania accounting for an estimated prevalence of 4.8% in the Tanzanian population aged 15-49 years (1). Viral load testing is the recommended method for monitoring HIV treatment response (2). However, viral load testing in resource-constrained settings is challenged by limited access, high costs, unavailability at district levels, and in areas where available, a shortage of reagents, compounded by challenges with equipment maintenance (3), as happened during the COVID-19 pandemic.

There is no doubt that viral load testing is effective in monitoring patient treatment adherence and HIV resistance. However, in resource-constrained areas that may not always be able to perform viral load testing, there is a need for readily available and routinely assessed objective measures that may predict early viral non-suppression or measures that may help with interim evaluation of patients suspected to have treatment failure who will thereafter need additional follow up with viral load testing. HIV-infected patients are routinely assessed for weight, height, renal function, and complete blood counts before initiation of combined antiretroviral treatment (ART). These assessments are repeated at intervals of three months, six months and biannually after ART initiation. Adverse changes in such parameters from baseline or subsequently at follow-up visits provides useful information about treatment responses and may identify a targeted group of patients to be prioritized for viral load testing before a decision to switch the ART regimen.

Routine parameters such as body mass index, total lymphocyte counts, and protein in urine are easily available and can be evaluated in combination for HIV treatment monitoring. Weight loss is one of the major manifestations of advanced HIV infection. Factors hypothesized to contribute

to weight loss include; metabolic alterations, anorexia, malabsorptive disorders, hypogonadism, and excessive cytokine production (4)

Weight gain following ART initiation may reflect slowed resting energy expenditure resulting from viral suppression and a decrease in HIV enteropathy (5). Weight gain, especially among individuals with low BMI, is associated with improved survival and decreased risk of clinical failure (6). ART responses depend on adherence (7), nutritional status at baseline (8), HIV subtype (9), and ART combination regimen (10). In Port Harcourt, Nigeria, among 318 participants with HIV infection aged ≥18 years initiated on ART, almost 70% and 55% of participants gained at least 1 kg weight in the first six months and one year of treatment, respectively (11). Previous studies in Tanzania have shown that a decrease in nutrition status within the first three months of ART initiation was associated with mortality (12).

HIV-associated nephropathy (HIVAN) is the most common renal biopsy finding in HIV-infected patients with a prevalence ranging from 4.7 to 38% (13). Proteinuria and elevated creatinine have been associated with AIDS-defining illness and death (14). Urine assessment for protein by dipstick can detect renal disease and progression, especially as renal biopsy, an invasive procedure, is not readily available in most resource-constrained settings.

HIV is associated with progressive destruction of CD4+ T-helper lymphocytes responsible for the profound immunodeficiency that underlies AIDS (15). As CD4 cells are a subset of lymphocytes, any significant change in CD4 cells will cause a parallel change in total lymphocyte counts (16).

This study aimed at assessing the following routinely accessible parameters: body mass index, proteinuria, and total lymphocyte counts (TLC), and their patterns of change in monitoring HIV treatment responses at six months following ART initiation.

## Methods

### Study design and population

This cohort study was conducted in three urban care and treatment centres (CTC) in Temeke district, Dar es Salaam: Temeke regional referral hospital, Mbagala Rangi-tatu district hospital, and Mbagala Kizuiani dispensary between September 2018 to April 2019. The centres were chosen due to large numbers of ART naïve clients, amounting to 60 or more per clinic per month. The sites have an organized CTC and follow up plan for clients. Participants were initiated on ART based on the Tanzanian National guidelines (17) with a default regimen of tenofovir, lamivudine and efavirenz unless contraindicated.

### Sample size estimation

To determine the minimum detectable relative risks with the power of 80% in univariate analysis for this observational study for which the sample size was determined by practical considerations, we used total number of cases between 40 and 50 and group numbers (rounded to 5) similar to the exposed groups: 115 for stable BMI, 20 for decreased BMI, 35 for lymphopaenia and proteinuria at 6 months, 80 for age over 40, 145 for female sex, 45 for secondary education or higher, 100 for employment, 115 for never married, 80 for stage greater than 1. In all cases but BMI, the size of the reference group was considered to be 215 - the number in the exposed group, except that 80 (gain) was used for the pairwise comparisons of BMI change. The minimum detectable risk ratios were 3.77, 2.56, 2.94, 2.94, 2.74, 2.59 (or <0.12), 2.47, and 2.44, respectively.

### Data collection

We used an interviewer-based structured tool to conduct face-to-face interviews to obtain socio-demographic and baseline characteristics such as age, sex, occupation, the highest level of

education attained, marital status, and clinically assessed the participant's WHO HIV clinical stage. A participant's baseline weight was measured using a SECA weighing scale recording to the nearest 0.5 kg and height using a wall stadiometer recording to the nearest 0.5 cm. Body mass index was then computed by dividing the weight in kg by the height in meters squared, the interpretation of which was adapted from WHO (18).

About 10ml of venous blood was collected aseptically from each participant; 5ml for CD4 cell counts, analysed using BD FACSCount™ (Becton Dickenson, USA) and 5ml for complete blood count to obtain the total lymphocyte counts, analyzed by an auto-analyzer (Cell DNY1800 from Abbott Diagnostics Division, USA). TLC was categorized as lymphopaenia ($<1\times10^9$/L), normal lymphocyte ($1\times10^9$/L to $4\times10^9$/L), and lymphocytosis ($>4.0\times10^9$/L). We assessed for proteinuria by collecting about 15ml of a fresh urine sample from each participant into an empty, clean, dry container and tested using CYBOW™ strips (DFI Co. Ltd, Korea). Proteinuria was categorized as negative proteinuria (no proteinuria), trace or 1+ proteinuria (equivalent to 30 to 100 mg/dl), 2+ proteinuria (equivalent to 100 to 300 mg/dl), 3+ proteinuria (equivalent to 300 to 1000 mg/dl), and 4+ proteinuria (equivalent to greater than 1000 mg/dl).

At three and six months after ART initiation, a repeat assessment of participants was done for CD4 cell counts, TLC, proteinuria, and weight. At six months, an additional 5ml of blood was collected from each study participant for viral load measurement using the Abbott RealTi*m*e HIV-1 assay. Participants were classified as virally suppressed at six months after ART initiation if their HIV viral load was <1000 copies/ml, according to Tanzania HIV treatment guidelines. Levels and changes in BMI, lymphocytes, and proteinuria were compared between participants who were HIV suppressed and that of HIV not suppressed.

BMI was considered to have changed between one time point and another if it increased or decreased by over 5%. BMI changes from ART initiation to six months were categorized into three groups as (i) loss of more than 5%, (ii) stable (change between -5% and 5%, or (iii) gain of more than 5%. The TLC were categorized as (i) lymphopaenia < $1x10^9$ cells/L, (ii) normal lymphocyte count 1-4 $x10^9$ cells/L (iii) Lymphocytosis > $4x10^9$ cells/L. The TLC pattern change was categorized as (i) lymphopaenic at 6 months, regardless of the status at baseline or three months; (ii) increased from lymphopaenic to normal or higher; (iii) stayed normal or higher (no lymphopaenia seen). Urinary protein patterns were categorized as (i) proteinuria at six months regardless of baseline proteinuria status; (ii) proteinuria at baseline and/or third month but not six months; and (iii) no proteinuria seen.

**Patient and public involvement**

Patients or members of the public were not involved in the design, or conduct, or reporting, or dissemination plans of the research.

**Statistical methods**

Data were analysed using IBM® SPSS® Statistics version 26, R, and SAS version 9.4 (Cary, NC). Categorical variables such as age group, sex, marital status, level of education, occupation, categories of BMI, CD4 count, proteinuria, TLC, BMI change, TLC change, and proteinuria change were summarized as frequencies and proportions. Continuous variables such as age, BMI, and CD4 count were summarized as means and standard deviations. When necessary, small groups were combined for analysis. To determine the association between BMI, TLC or urine protein to CD4 count, we used correlation.

To determine the relationships between individual predictors and viral non-suppression at six months, we first used modified Poisson regression for univariable analysis, to determine which variables to include in the multivariable model. For multivariable prediction, all predictors in the univariable model with a p-value of <0.2 and age, a known confounder, were entered into the modified Poisson regression model. The results of the Poisson regression model were presented as relative risk and 95% confidence interval (RR; 95% CI). To determine the test characteristics (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)) of a score based on the multivariable model, we used two cut-off levels, based on the first quartile and median of the score among the non-suppressed. The score was the sum of the rounded coefficients for the variables for which the confidence intervals did not include 1 in a model containing only these variables. Since these all rounded to 1, this is equivalent to simply counting the number of these characteristics.

Based on practices in low resourced clinics, communication with the patient and the decision to change the ART regimen depends on the patient's virological status at six months. CD4 cell counts depend on a blood sample collected at the six-month visit and are therefore unavailable for immediate decision making. We, therefore, excluded all CD4 variables from the model and used parameters available at the time of the six-month visit to predict viral non-suppression.

## Results

A total of 220 participants were enrolled in the study over a month, and each participant was followed up for six months. Two participants were lost to follow up at three months, two died before six months of follow up, and one participant, a long-distance truck driver, was out of the country at the time of the 6-month follow up. Therefore, our analysis data set includes the remaining 215 participants. Details of enrolment are shown in Fig 1.

**Figure 1. Consort diagram.**

### Baseline characteristics of study participants

Of the 215 participants analysed, the mean age (SD) was 37.1 ±11.5 years, 146 (68%) were female, 113 (53%) were never married, 45 (21%) had secondary education or higher, and 117 (54%) were unemployed (Table 1). Most participants, 59.5%, had normal BMI, while 27% were overweight, and 13% were underweight. Most participants, 113 (62%), were in WHO HIV clinical stage I, and only eight (4%) were in stage IV, though 93 (43%) had CD4 counts of 350 cells/ml or below; 83 (39%) were lymphopaenic, and 111 (52%) had proteinuria at baseline.

**Table 1. Baseline characteristics of 215 study participants initiating ART, Dar es Salaam, Tanzania, 2019.**

| Characteristic | n (%) | Mean ± SD |
|---|---|---|
| **Age** (years) | | 37.1 ± 11.5 |
| Age group (years) | | |
| 18 – 30 | 69 (32.1%) | |

| | | |
|---|---|---|
| 31 – 40 | 72 (33.5%) | |
| 41 – 50 | 45 (20.9%) | |
| >51 | 29 (13.5%) | |
| **Sex** | | |
| Female | 146 (67.9%) | |
| Male | 69 (32.1 %) | |
| **Level of education** | | |
| No education | 10 (4.7%) | |
| Primary education | 160 (74.4%) | |
| Secondary education | 42 (19.5%) | |
| Higher education | 3 (1.4%) | |
| **Employment Status** | | |
| Not employed | 117 (54.4%) | |
| Employed | 98 (45.6%) | |
| **Marital status** | | |
| Ever married | 102 (47.4%) | |
| Never married | 113 (52.6%) | |
| **Body mass index** (kg/m$^2$) | | 22.9 ± 4.3 |
| Underweight | 28 (13.0%) | |
| Normal weight | 128 (59.5%) | |
| Overweight/Obese | 59 (27.4%) | |
| **WHO HIV clinical stages** | | |
| Stage I | 133 (61.9%) | |
| Stage II | 30 (14.0%) | |
| Stage III | 44 (20.5%) | |
| Stage IV | 8 (3.7%) | |
| **CD4 cell counts** (cells/mm$^3$) | | 401 ± 253 |
| <200 | 55 (25.6%) | |
| 200-350 | 38 (17.7%) | |
| 351-500 | 39 (18.1%) | |
| >500 | 83 (38.6%) | |
| **Lymphocyte counts** (x10$^9$cells/L) | | 1.6 ± 1.2 |
| <1 | 83 (38.6%) | |
| 1-4 | 126 (58.6%) | |
| >4 | 6 (2.8%) | |
| **Proteinuria** | | |
| No proteinuria | 104 (48.4 %) | |
| 1+ (30 – 100 mg/dl) | 80 (37.2%) | |
| 2+ (100 – 300 mg/dl) | 27 (12.6%) | |
| 3+ (300 – 1000 mg/dl) | 4 (1.9%) | |

206 CD4: Cluster of differentiation 4; HIV: Human Immunodeficiency Virus; SD: Standard Deviation

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209 **Table 2. Predictors of HIV viral load non-suppression at six months among 215**
210 **participants initiating ART, Dar es Salaam, Tanzania, 2019.**

| Variable | Total | HIV non-suppression at six months n (%) | RR (95% CI) | Adjusted RR (95% CI) |
|---|---|---|---|---|
| **Age** (years) | | | | |
| < 40 | 136 | 26 (19%) | 1 | 1 |
| ≥40 | 79 | 20 (25%) | 1.32 (0.79-2.21) | 1.43 (0.91-2.26) |
| **Sex** | | | | |
| Female | 146 | 35 (24%) | 1.50 (0.81-2.78) | 1.27 (0.73-2.20) |
| Male | 69 | 11 (16%) | 1 | 1 |
| **Level of Education** | | | | |
| Primary or less | 170 | 37 (22%) | 1 | |
| Secondary or higher | 45 | 9 (20%) | 0.92 (0.48-1.76) | |
| **Employment Status** | | | | |
| Not employed | 117 | 24 (21%) | 1 | |
| Employed | 98 | 22 (22%) | 1.09 (0.66-1.83) | |
| **Marital status** | | | | |
| Never married | 113 | 19 (17%) | 1 | 1 |
| Ever married | 102 | 27 (26%) | 1.57 (0.93-2.66) | 1.34 (0.84-2.16) |
| **Body mass index** | | | | |
| Change from baseline to three months | | | | |
| Loss >5% | 28 | 17 (61%) | 4.93 (2.41-10.09) | |
| Stable | 122 | 21 (17%) | 1.40 (0.66-2.99) | |
| Gain >5 % | 65 | 8 (12%) | 1 | |
| Change from baseline to six months | | | | |
| Loss >5% | 20 | 16 (80%) | 7.11 (3.69-13.69) | 2.73 (1.36-5.47) |
| Stable | 115 | 21 (18%) | 1.62 (0.78-3.36) | 1.87 (0.95-3.68) |
| Gain >5 % | 80 | 9 (11%) | 1 | 1 |
| **HIV clinical stage** | | | | |
| I | 133 | 24 (18%) | 1 | 1 |
| II | 30 | 8 (27%) | 1.48 (0.74-2.97) | 1.14 (0.63-2.08) |
| III and IV | 52 | 14 (27%) | 1.49 (0.84-2.66) | 0.82 (0.51-1.31) |
| **Total lymphocyte count change from baseline to six months** | | | | |

| | | | | |
|---|---|---|---|---|
| Ended lymphopaenic | 37 | 27 (73%) | 7.66 (4.32-13.60) | 4.54 (2.19-9.39) |
| Lymphopaenic to normal | 52 | 7 (13%) | 1.41 (0.59-3.40) | 1.59 (0.66-3.80) |
| Lymphopaenia not seen | 126 | 12 (10%) | 1 | 1 |
| **Pattern of change in proteinuria** | | | | |
| Proteinuria at 6 months regardless of baseline proteinuria status | 37 | 24 (65%) | 6.73 (3.34-13.58) | 2.63 (1.25-5.54) |
| Proteinuria at baseline and/or 3 months but not 6 months | 95 | 14 (15%) | 1.53 (0.67-3.47) | 1.26 (0.62-2.57) |
| No proteinuria seen | 83 | 8 (10%) | 1 | 1 |

CI: confidence interval; RR: relative risk; ART: antiretroviral therapy.

Univariable and multivariable analysis by modified Poisson regression.

BMI and CD4 count were directly correlated at baseline, 3, and 6 months. TLC and CD4 count were moderately positively correlated; while urine protein and CD4 count were inversely correlated (see Supplementary Figures 1, 2, and 3 in Additional file 1).

**Predictors of viral non-suppression at six months among HIV-infected participants initiated on ART**

Only 46 participants (21%) were not virally suppressed at six months. The strongest clinical predictors of viral non-suppression in the multivariable analysis were lymphopaenia at six months irrespective of baseline lymphocyte status, with 73% of participants with lymphopaenia at six months not being suppressed. After adjusting for other factors, lymphopaenia at six months was associated with HIV non-suppression {RR = 4.54, 95% CI (2.19-9.39)}. Among participants with a drop in body mass index at six months of 5% or more from baseline, 80% were non-suppressed {RR = 2.73; 95% CI (1.36-5.47)}. The risk of HIV non-suppression at six months was higher

among participants with proteinuria at six months {RR = 2.63; 95% CI (1.25-5.54)}, Table 2. The area under the Receiver Operating Characteristic (ROC) curve for the multivariable model was 0.895 (Fig 2). Baseline HIV stage was not related to HIV non-suppression at six months, {adjusted RR = 1.14, 95% CI (0.63-2.08)} for baseline WHO HIV clinical stage II and {adjusted RR = 0.82, 95% CI (0.51-1.31)} for advanced baseline WHO HIV clinical stages (III and IV)}.

Using the rounded coefficients of the three variables in a model containing only these variables, which all rounded to 1, we made a "prediction score" with values 0 (n=154, of which 10 were non-suppressed), 1 (n=36, 13 non-suppressed), 2 (n=17, 15 non-suppressed), and 3 (n=8, all non-suppressed). The median value of this score among the non-suppressed was 1.5 and the first quartile value was 1. Thus, having any 2 of the risk factors would be a conservative predictor of non-suppression, and having any one would be less conservative.

**Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among HIV-infected individuals initiated on ART in Dar es Salaam, Tanzania, 2019**

Using the median score among the non-suppressed as a cut-off (equivalent to having any two of the components), the sensitivity predicting non-suppression was 0.50, and the specificity was 0.99. Only 12% of the study population met this criterion. When we lowered the cut-off scores to the first quartile value among the non-suppressed (i.e. any one of the components), the sensitivity was 0.78 and the specificity was 0.85, with 28% of the study population meeting this criterion.

## Discussion

This cohort study recruited ART naïve HIV-infected individuals from three care and treatment centres in Dar es Salaam, Tanzania, with the aim of assessing the utility of total lymphocyte count, body mass index, and proteinuria in predicting ART responses at six months. The intention of this study is not to replace CD4 cell count testing and HIV viral load monitoring but to assist clinicians when faced with decision making if these standard monitoring parameters are not easily accessible. Contrary to earlier studies done when the ART medications were not as effective as the current ones (12), patient characteristics at ART initiation did not affect the probability of viral non-suppression at six months, whereas patterns of change and the patient's status at 6 months were highly predictive.

Baseline WHO HIV clinical stage did not help predict HIV non-suppression at six months, possibly because under the current "Test and Treat" strategy (19), most individuals initiating ART are in WHO HIV clinical stages I and II (76% of our sample). Current therapy is highly effective except for a few patients whose disease is so advanced that they die before the medication can improve their immune status (2 patients in this study). Symptomatic individuals with advanced HIV will likely adhere to treatment to alleviate their symptoms. Perhaps patients with advanced disease fear death and therefore are motivated to adhere to ART, with eventual viral suppression. Advanced HIV disease has been shown to be linked with ART adherence (20). Some studies, however, indicate that early HIV stages are linked with high ART adherence and viral suppression (21).

Lymphopaenia at six months, a decrease in body mass index of 5% or more from baseline, and proteinuria predicted HIV non-suppression at six months. Lymphopaenia at six months was the strongest predictor for HIV non -suppression at six months. Lymphopaenia at six months predicted

HIV non-suppression irrespective of a participant's lymphocyte count (normal or low) before ART initiation. CD4 cells are a subset of lymphocytes and therefore lymphopaenia could be indicative of a decline in CD4 cells, as a result of high HIV replication with high viral load signifying viral non-suppression with consequent CD4 cell destruction. Lymphopaenia was significantly associated with CD4 <500 cells/mm$^3$ at all time points. In this study, an increase in total lymphocyte cell count from lymphopaenia to normal lymphocyte counts from baseline to six months was significantly associated with an increase in CD4 cell count (Additional file 1). Total lymphocyte count is sensitive and specific in predicting CD4 cell counts (16,23) though there have been contradictory reports (23). The assessment of total lymphocyte counts among patients on ART, therefore, could serve as an alternative, especially in settings with limited availability of CD4 cell count measurement. A drop in total lymphocyte count in such a setting can alert a clinician to the likelihood of immunological failure.

Weight loss with consequent BMI drop of 5% or more at six months while on ART from weight prior to ART initiation, predicts HIV non-suppression, though HIV non-suppression was not associated with being underweight prior to ART initiation, perhaps because of the low prevalence of underweight leading to low power. In this study, sustained weight gain was significantly associated with viral suppression and sustained weight loss was associated with viral non-suppression at six months of ART. An increase in weight and hence BMI may be a sign of immune status improvement signalling a return to health (24) and improved survival (25), while a decrease in weight and hence BMI may be a sign of HIV progression or decline in CD4 cell counts (5,11,27). Weight loss may be due to HIV itself, opportunistic infections, or HIV-associated tumours. Weight loss in both ART naïve and exposed patients has been associated with increased morbidity and mortality (28,29,30). A study in England observed that each log10 increase in HIV viral load was

associated with a 0.92 kg decrease in body weight. However, a decrease in viral load was not significantly associated with weight gain, contrary to our study (30). Since weight changes correlate with the virological response, losing weight should be viewed as an alarming sign of virological failure. Monitoring of weight and body mass index prior to ART initiation and during follow up is a valuable inexpensive way of identifying individuals with possible treatment failure.

Half of the ART-naïve study participants had proteinuria, similar to a report from Zimbabwe (31). The presence of proteinuria was associated with HIV non-suppression. Proteinuria at six months was a strong predictor for HIV non-suppression. Proteinuria in HIV-infected individuals is attributed to a direct effect of HIV on the glomerular and tubular epithelial cells with the progression of HIV disease to AIDS, nephrotoxicity from ART, the progression of chronic kidney disease and death (14,33). The higher the viral load, the greater the damage to the kidney (33). We observed a significant proportion of participants with proteinuria of 2+ or above in WHO HIV clinical stage IV. This underscores the fact that, as HIV disease advances, the greater the damage to the kidneys as evidenced by increasing proteinuria. Therefore, routine screening for renal disease may serve not only as a follow-up of renal disease progression but also for HIV treatment response monitoring.

## Conclusion

A drop in BMI by more than 5%, persistent lymphopaenia or decrease in total lymphocyte count to lymphopaenia, and proteinuria at six months are associated with HIV non-suppression at 6 months after ART initiation. Scores based on these parameters can serve as alternatives to CD4 cell counts and viral load assessment in facilities with scarcity.

## List of abbreviations

AIDS: Acquired immunodeficiency syndrome

ART: Antiretroviral therapy

BMI: Body mass index

CD4: Cluster of differentiation 4

HIV: Human immunodeficiency virus

TLC: Total lymphocyte counts

WHO: World Health Organization

## Acknowledgements

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## Author Contributions

Study design: LJ and PM; data collection: LJ and PM; Data analysis and interpretation: LJ, PM, BT and EH; Manuscript writing: LJ, PM, BT and EH. All the authors gave final approval of the manuscript.

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## Competing interests

None declared.

## Patient consent for publication

Not applicable.

## Ethics approval

Ethical approval was obtained from the Research and Publications Committee of Muhimbili University of Health and Allied Sciences (Ref. No. DA.287/298/01A). Permission to conduct the study was obtained from Temeke Municipal Hospital administration. Participants were enrolled after providing written informed consent. The confidentiality of patient information was ensured. Participants without viral suppression at the 6$^{th}$ month of follow up were managed according to Tanzania National Guidelines for management of HIV and AIDS.

## Data availability statement

The dataset analysed during the current study is available upon reasonable request to the corresponding author.

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## Ethics Statement

Muhimbili University of Health and Allied Sciences Instituional Review Board with reference number DA287/298/01A/

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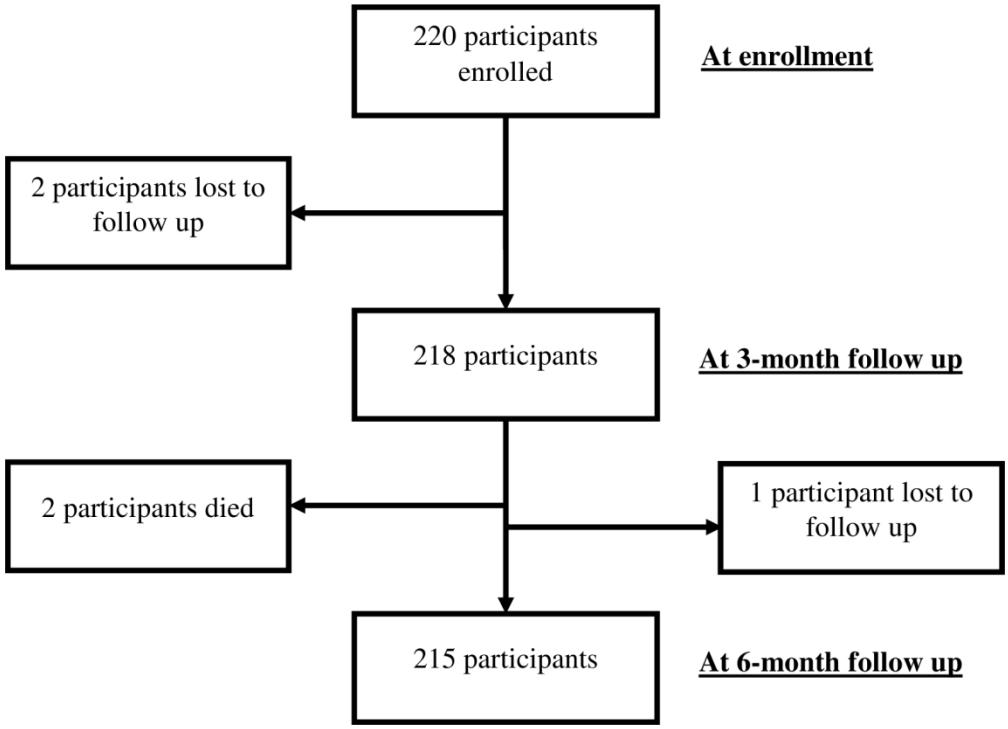



Figure 1. Consort diagram.

146x105mm (300 x 300 DPI)

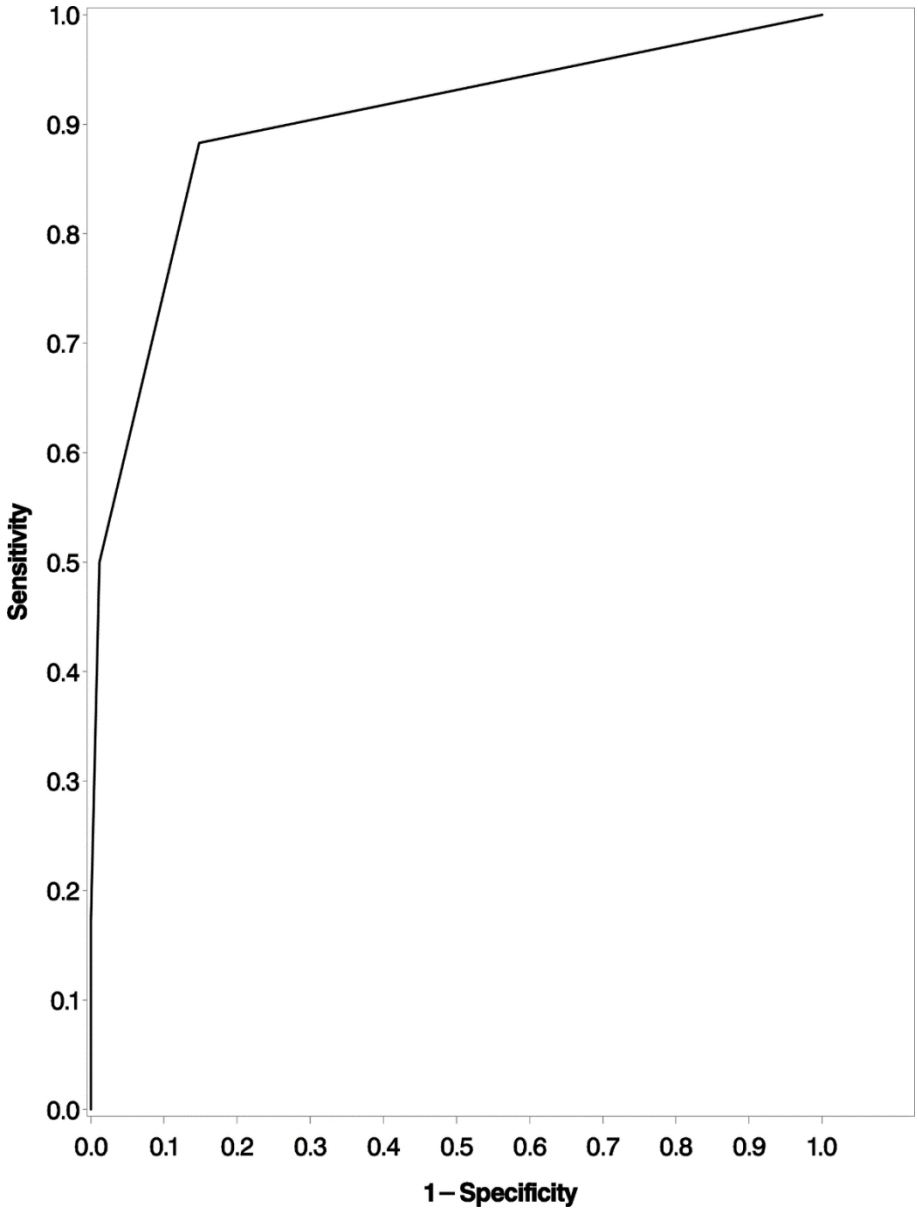
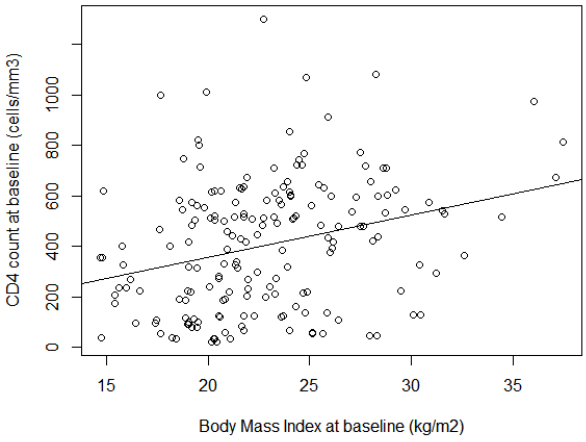


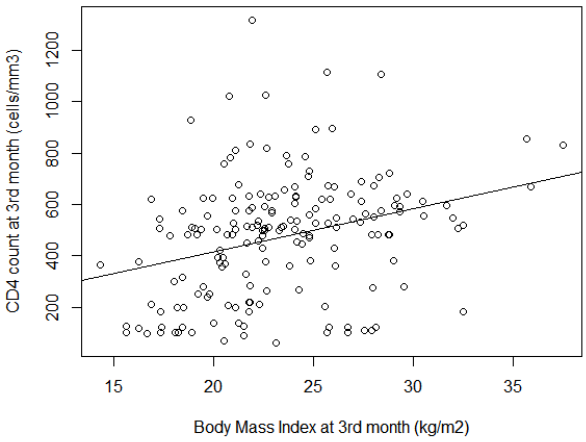

Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among HIV-infected individuals initiated on ART in Dar es Salaam, Tanzania, 2019

137x181mm (220 x 220 DPI)

**Supplementary Figure 1. Scatter plots of BMI and CD4 counts**



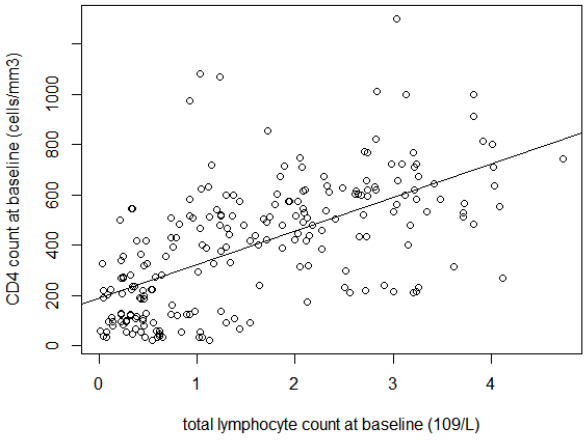

rho 0.287, p <0.0001



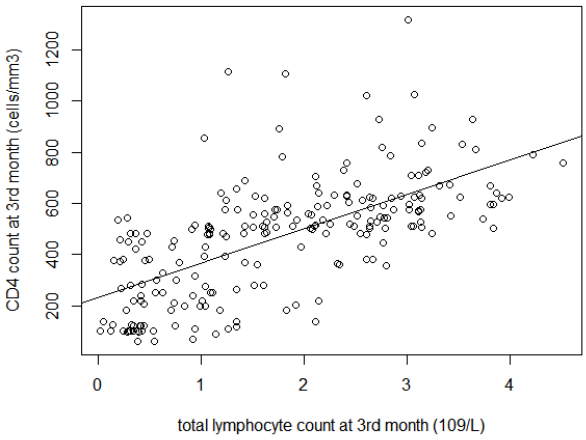

rho 0.305, p <0.0001


rho 0.373, p <0.0001

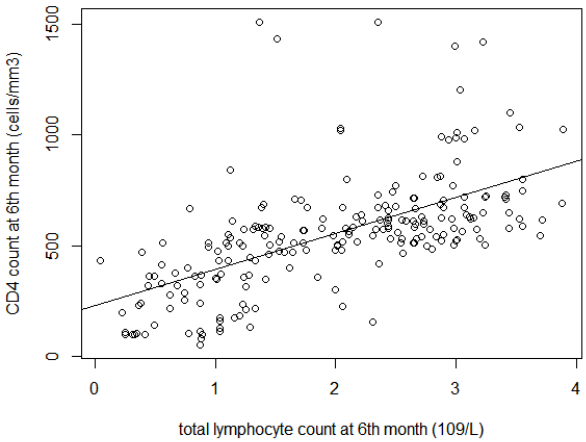
**Supplementary Figure 2. Scatter plots of total lymphocyte count and CD4 count**




rho 0.613, p <0.0001

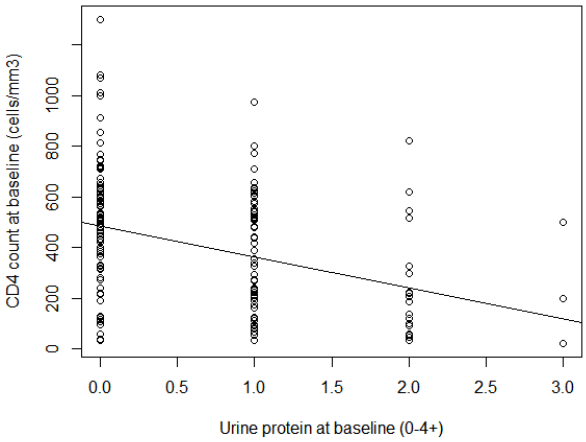



rho 0.650, p <0.0001

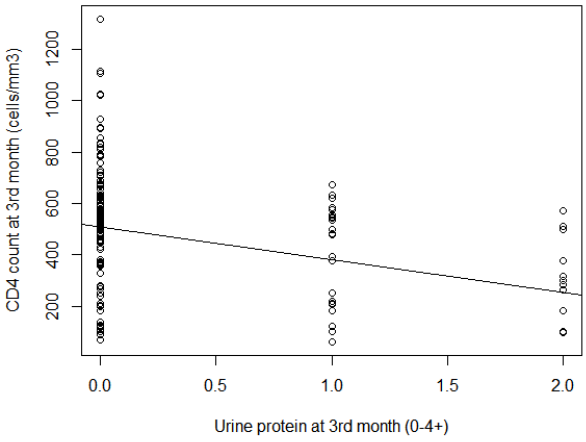



rho 0.602, p <0.0001

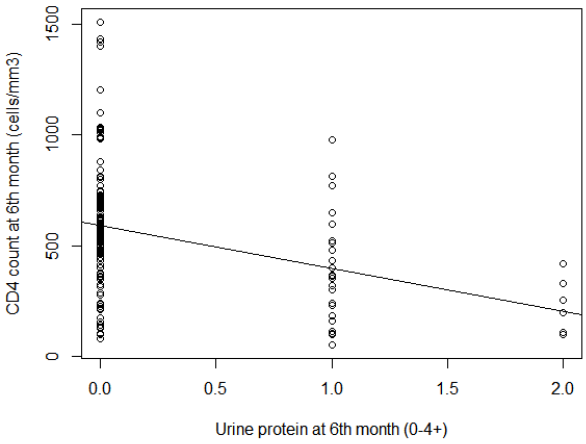
**Supplementary Figure 3. Scatter plots of urine protein and CD4 count**




rho -0.364, p <0.0001




rho -0.334, p <0.0001




rho -0.372, p <0.0001

# Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.



| | | Reporting Item | Page Number |
|---|---|---|---|
| **Title and abstract** | | | |
| Title | #1a | Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| Abstract | #1b | Provide in the abstract an informative and balanced summary | 3 |

| | | | |
|---|---|---|---|
| | | of what was done and what was found | |
| **Introduction** | | | |
| Background / rationale | #2 | Explain the scientific background and rationale for the investigation being reported | 6 |
| Objectives | #3 | State specific objectives, including any prespecified hypotheses | 7 |
| **Methods** | | | |
| Study design | #4 | Present key elements of study design early in the paper | 8 |
| Setting | #5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8-10 |
| Eligibility criteria | #6a | Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. | 8-10 |
| Eligibility criteria | #6b | For matched studies, give matching criteria and number of exposed and unexposed | - |
| Variables | #7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 9,10 |
| Data sources / measurement | #8 | For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable. | 8-10 |

| | | | |
|---|---|---|---|
| Bias | #9 | Describe any efforts to address potential sources of bias | |
| Study size | #10 | Explain how the study size was arrived at | 8 |
| Quantitative variables | #11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why | 9-11 |
| Statistical methods | #12a | Describe all statistical methods, including those used to control for confounding | 10,11 |
| Statistical methods | #12b | Describe any methods used to examine subgroups and interactions | 10, 11 |
| Statistical methods | #12c | Explain how missing data were addressed | 12 |
| Statistical methods | #12d | If applicable, explain how loss to follow-up was addressed | - |
| Statistical methods | #12e | Describe any sensitivity analyses | 11 |
| **Results** | | | |
| Participants | #13a | Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and | 12 (figure 1) |

| | | | |
|---|---|---|---|
| | | unexposed groups if applicable. | |
| Participants | #13b | Give reasons for non-participation at each stage | 12 (figure 1) |
| Participants | #13c | Consider use of a flow diagram | 12 (figure 1) |
| Descriptive data | #14a | Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. | 12,13 |
| Descriptive data | #14b | Indicate number of participants with missing data for each variable of interest | See 12 |
| Descriptive data | #14c | Summarise follow-up time (eg, average and total amount) | 12 |
| Outcome data | #15 | Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable. | 14 |
| Main results | #16a | Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 14-16 |

| | | | |
|---|---|---|---|
| Main results | #16b | Report category boundaries when continuous variables were categorized | 12-15 |
| Main results | #16c | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| - | | | |
| Other analyses | #17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 16 |
| **Discussion** | | | |
| Key results | #18 | Summarise key results with reference to study objectives | 20 |
| Limitations | #19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. | 5 |
| Interpretation | #20 | Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. | 18-20 |
| Generalisability | #21 | Discuss the generalisability (external validity) of the study results | 20 |
| **Other Information** | | | |
| Funding | #22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 22 |

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# Body mass index, proteinuria and total lymphocyte counts in predicting treatment responses among ART naïve individuals with HIV initiated on antiretroviral treatment in Dar es Salaam, Tanzania, 2019: a cohort study



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# Body mass index, proteinuria and total lymphocyte counts in predicting treatment responses among ART naïve individuals with HIV initiated on antiretroviral treatment in Dar es Salaam, Tanzania, 2019: a cohort study

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**Keywords**: monitoring ART treatment; HIV/AIDS; CD4 count; BMI in HIV; proteinuria in HIV; viral suppression

## Abstract

**Objectives**: To explore the potential use of body mass index, proteinuria, and total lymphocyte count changes in predicting immunological and virological response in individuals with HIV initiated on antiretroviral therapy (ART).

**Design**: Prospective cohort study.

**Setting**: Three urban HIV care and treatment centres (CTC) in Dar es Salaam.

**Participants**: Individuals with HIV initiating ART.

**Outcome measures**: HIV viral load ≥1000 copies/ml (viral non-suppression) at six months after ART initiation.

**Results**: Of 215 (out of 220 enrolled) participants who returned for evaluation at six months, 147 (66.8%) were female. At six months of follow-up, 89.4% (76/85) of participants with sustained weight gain were virally suppressed compared to 31.8% (7/22) with sustained loss, p<0.001. In participants who were lymphopaenic at baseline, an increase to normal total lymphocyte counts at six months was associated with an increase in CD4 count compared to participants who remained lymphopaenic, 96.2% (50/52) vs. 54.8% (17/31), p<0.001. At baseline, 50.0% (110/220) had proteinuria. In participants without proteinuria from baseline to six months, 89.8% (79/88) were virally suppressed compared to participants with proteinuria at baseline and/or three months, 85.6% (77/90), those with persistent proteinuria, 30.8% (8/26), and proteinuria at six months only, 45.5% (5/11), p<0.001. In modified Poisson regression, the independent predictors other than CD4 cell counts for viral non-suppression at six months among individuals with HIV initiating on ART were BMI loss >5% from baseline to six months, {adjusted RR 2.73, 95% CI (1.36-5.47)},

lymphopaenia at six months, {adjusted RR = 4.54, 95% CI (2.19-9.39)}, and proteinuria at six months {adjusted RR = 2.63, 95% CI (1.25-5.54)}.

**Conclusions**: Change in body mass index, total lymphocyte count, and presence of proteinuria can monitor and predict ART response and may be particularly helpful in settings when CD4 counts and viral load monitoring are unavailable.

## Article Summary

Strengths and limitations of this study

- We had complete data on 98% of the originally enrolled participants.
- In resource-constrained situations, when viral load and CD4 testing are not always easily available, models such as ours with locally determined easily computable prediction cut-offs can be utilized by clinicians to make clinical decisions.
- Our findings require validation in a study with larger sample size.
- Local conditions and treatment standards may influence some of the patterns we observed, both in prevalence and in effect.

## Introduction

In 2019 about 38 million people were living with HIV globally, with 1.7 million in Tanzania accounting for an estimated prevalence of 4.8% in the Tanzanian population aged 15-49 years [1]. Viral load testing is the recommended method for monitoring HIV treatment response [2]. However, viral load testing in resource-constrained settings is challenged by limited access, high costs, unavailability at district levels, and in areas where available, sometimes a shortage of reagents, compounded by challenges with equipment maintenance [3], as happened during the COVID-19 pandemic.

There is no doubt that viral load testing is effective in monitoring patient treatment adherence and HIV resistance, as per WHO guidelines. However, in resource-constrained areas that may not always be able to perform viral load testing in a timely manner, there is a need for readily available and routinely assessed objective measures that may predict early viral non-suppression or measures that may help with interim evaluation of patients suspected to have treatment failure who will thereafter need additional follow up with viral load testing. Individuals with HIV are routinely assessed for weight, height, renal function, and complete blood counts before initiation of combined antiretroviral treatment (ART) in resource constrained settings including Tanzania. These assessments are repeated at intervals of three months, six months and biannually after ART initiation. Adverse changes in such parameters from treatment initiation (baseline) or subsequently at follow-up visits provides useful information about treatment responses and may identify a targeted group of patients to be prioritized for viral load testing before a decision to switch the ART regimen.

Routine parameters such as body mass index, total lymphocyte counts, and protein in urine are easily available and can be evaluated in combination for HIV treatment monitoring. Weight loss

is one of the major manifestations of advanced HIV infection. Factors hypothesized to contribute to weight loss include metabolic alterations, anorexia, malabsorptive disorders, hypogonadism, and excessive cytokine production [4]

Weight gain following ART initiation may reflect slowed resting energy expenditure resulting from viral suppression and a decrease in HIV enteropathy [5]. Weight gain, especially among individuals with low BMI, is associated with improved survival and decreased risk of clinical failure [6]. ART responses depend on adherence [7], nutritional status at baseline [8], HIV subtype [9], and ART combination regimen [10]. In Port Harcourt, Nigeria, among 318 participants with HIV infection aged ≥18 years initiated on ART, almost 70% and 55% of participants gained at least 1 kg weight in the first six months and one year of treatment, respectively [11]. Previous studies in Tanzania have shown that a decrease in nutrition status within the first three months of ART initiation was associated with mortality [12].

HIV-associated nephropathy (HIVAN) is the most common renal biopsy finding in individuals with HIV with a prevalence ranging from 4.7 to 38% [13]. Proteinuria and elevated creatinine have been associated with AIDS-defining illness and death [14]. Urine assessment for protein by dipstick can detect renal disease and progression, especially as renal biopsy, an invasive procedure, is not readily available in most resource-constrained settings.

HIV is associated with progressive destruction of CD4+ T-helper lymphocytes responsible for the profound immunodeficiency that underlies AIDS [15]. As CD4 cells are a subset of lymphocytes, any significant change in CD4 cells will cause a parallel change in total lymphocyte counts [16].

This study aimed at assessing the following routinely accessible parameters: body mass index, proteinuria, and total lymphocyte counts (TLC), and their patterns of change in monitoring HIV treatment responses at six months following ART initiation.

## Methods

### Study design and population

This cohort study was conducted in three urban care and treatment centres (CTC) in Temeke district, Dar es Salaam: Temeke regional referral hospital, Mbagala Rangi-tatu district hospital, and Mbagala Kizuiani dispensary between September 2018 and April 2019. The centres were chosen due to large numbers of ART naïve clients, amounting to 60 or more per clinic per month. The sites have an organized CTC and follow up plan for clients. Participants were included in the study if they were newly diagnosed with HIV and were ART naïve aged 18 years or older and were able to provide written informed consent. Participants were initiated on ART based on the Tanzanian National guidelines [17] with a default regimen of tenofovir, lamivudine and efavirenz unless contraindicated.

### Sample size estimation

To determine the minimal detectable relative risks for the study variables, we considered two-sample tests of the expected highest risk category versus the expected lowest risk category. For the dichotomous potential risk factors, we assumed a total number of 215 subjects, split roughly as our actual data are split (with numbers rounded to the nearest 5 to mimic a pre-study power calculation). For BMI change we used 80 for the reference group (gain), 125 for stable, and 20 for the loss group. The minimum detectable risk ratios were 3.77 for decreased BMI, 2.56 for stable BMI, 2.94 for lymphopenia and for proteinuria, 2.47 for stage greater than 1, 2.47 for age of 40, years and above 2.59 (or < 0.11) for female sex, 2.74 for secondary or higher education, 2.44 for unemployment and for never married.

## Data collection

We used an interviewer-based structured tool to conduct face-to-face interviews to obtain socio-demographic and baseline characteristics (at treatment initiation) such as age, sex, occupation, the highest level of education attained, marital status, and clinically assessed the participant's WHO HIV clinical stage. A participant's baseline weight was measured using a SECA weighing scale recording to the nearest 0.5 kg and height using a wall stadiometer recording to the nearest 0.5 cm. Body mass index was then computed by dividing the weight in kg by the height in meters squared, the interpretation of which was adapted from WHO [18].

About 10ml of venous blood was collected aseptically from each participant; 5ml for CD4 cell counts, analysed using BD FACSCount™ (Becton Dickenson, USA) and 5ml for complete blood count to obtain the total lymphocyte counts, analysed by an auto-analyser (Cell DNY1800 from Abbott Diagnostics Division, USA). TLC was categorized as lymphopaenia ($<1\times10^9$/L), normal lymphocyte ($1\times10^9$/L to $4\times10^9$/L), and lymphocytosis ($>4.0\times10^9$/L). We assessed for proteinuria by collecting about 15ml of a fresh urine sample from each participant into an empty, clean, dry container and tested using CYBOW™ strips (DFI Co. Ltd, Korea). Proteinuria was categorized as negative proteinuria (no proteinuria), trace or 1+ proteinuria (equivalent to 30 to 100 mg/dl), 2+ proteinuria (equivalent to 100 to 300 mg/dl), 3+ proteinuria (equivalent to 300 to 1000 mg/dl), and 4+ proteinuria (equivalent to greater than 1000 mg/dl).

At three and six months after ART initiation, a repeat assessment of participants was done for CD4 cell counts, TLC, proteinuria, and weight. At six months, an additional 5ml of blood was collected from each study participant for viral load measurement using the Abbott RealTi*m*e HIV-1 assay. Participants were classified as virally suppressed at six months after ART initiation if their HIV viral load was <1000 copies/ml, according to Tanzania HIV treatment guidelines. Levels and

changes in BMI, lymphocytes, and proteinuria were compared between participants who were HIV suppressed and that of HIV not suppressed.

BMI was considered to have changed between one time point and another if it increased or decreased by over 5%. BMI changes from ART initiation to six months were categorized into three groups as (i) loss of more than 5%, (ii) stable (change between -5% and 5%, or (iii) gain of more than 5%. The TLC were categorized as (i) lymphopaenia < $1x10^9$ cells/L, (ii) normal lymphocyte count 1-4 $x10^9$ cells/L (iii) Lymphocytosis > $4x10^9$ cells/L. The TLC pattern change was categorized as (i) lymphopaenic at 6 months, regardless of the status at baseline or three months; (ii) increased from lymphopaenic to normal or higher; (iii) stayed normal or higher (no lymphopaenia seen). Urinary protein patterns were categorized as (i) proteinuria at six months regardless of baseline proteinuria status; (ii) proteinuria at baseline and/or third month but not six months; and (iii) no proteinuria seen.

**Patient and public involvement**

Patients or members of the public were not involved in the design, or conduct, or reporting, or dissemination plans of the research.

**Statistical methods**

Data were analysed using IBM® SPSS® Statistics version 26, R, and SAS version 9.4 (Cary, NC). Categorical variables such as age group, sex, marital status, level of education, occupation, categories of BMI, CD4 count, proteinuria, TLC, BMI change, TLC change, and proteinuria change were summarized as frequencies and proportions. Continuous variables such as age, BMI, and CD4 count were summarized as means and standard deviations. When necessary, small groups

were combined for analysis. To determine the association between BMI, TLC or urine protein to CD4 count, we used correlation.

To determine the relationships between individual predictors and viral non-suppression at six months, we first used modified Poisson regression for univariable analysis with an assumption that viral non suppression is a non-rare outcome (more than 10%), to determine which variables to include in the multivariable model [19,20]. For multivariable prediction, all predictors in the univariable model with a p-value of <0.2 and age, a known confounder, were entered into the modified Poisson regression model. The results of the Poisson regression model were presented as relative risk (RR) and 95% confidence interval (RR; 95% CI). To determine the test characteristics (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)) of a score based on the multivariable model, we used two cut-off levels, based on the first quartile and median of the score among the non-suppressed. The score was the sum of the rounded coefficients for the variables for which the confidence intervals did not include 1 in a model containing only these variables. Since these all rounded to 1, this is equivalent to simply counting the number of these characteristics.

Based on practices in low resourced clinics, communication with the patient and the decision to change the ART regimen depends on the patient's virological status at six months. CD4 cell counts depend on a blood sample collected at the six-month visit and are therefore unavailable for immediate decision making. We, therefore, excluded all CD4 variables from the model and used parameters available at the time of the six-month visit to predict viral non-suppression.

## Results

During the recruitment, 220 ART naïve individuals with HIV were initiated on ART and all were enrolled in the study over a month; each participant was followed up for six months. Two participants were lost to follow up at three months; two died before six months of follow up, and one participant, a long-distance truck driver, was out of the country at the time of the 6-month follow up. Therefore, our analysis data set includes the remaining 215 participants. Details of enrolment are shown in Fig 1.

**Figure 1. Consort diagram.**

### Baseline characteristics of study participants

Of the 215 participants analysed, the mean age (SD) was 37.1 ±11.5 years, 146 (68%) were female, 113 (53%) were never married, 45 (21%) had secondary education or higher, and 117 (54%) were unemployed (Table 1). Most participants, 59.5%, had normal BMI, while 27% were overweight, and 13% were underweight. Most participants, 113 (62%), were in WHO HIV clinical stage I, and only eight (4%) were in stage IV, though 93 (43%) had CD4 counts of 350 cells/ml or below; 83 (39%) were lymphopaenic, and 111 (52%) had proteinuria at baseline.

**Table 1. Characteristics of 215 study participants at ART initiation, Dar es Salaam, Tanzania, 2019.**

| Characteristic | n (%) | Mean ± SD |
|---|---|---|
| **Age** (years) | | 37.1 ± 11.5 |

| | | |
|---|---|---|
| Age group (years) | | |
| 18 – 30 | 69 (32.1%) | |
| 31 – 40 | 72 (33.5%) | |
| 41 – 50 | 45 (20.9%) | |
| >51 | 29 (13.5%) | |
| **Sex** | | |
| Female | 146 (67.9%) | |
| Male | 69 (32.1 %) | |
| **Level of education** | | |
| No education | 10 (4.7%) | |
| Primary education | 160 (74.4%) | |
| Secondary education | 42 (19.5%) | |
| Higher education | 3 (1.4%) | |
| **Employment Status** | | |
| Not employed | 117 (54.4%) | |
| Employed | 98 (45.6%) | |
| **Marital status** | | |
| Ever married | 102 (47.4%) | |
| Never married | 113 (52.6%) | |
| **Body mass index** (kg/m$^2$) | | 22.9 ± 4.3 |
| Underweight | 28 (13.0%) | |
| Normal weight | 128 (59.5%) | |
| Overweight/Obese | 59 (27.4%) | |
| **WHO HIV clinical stages** | | |
| Stage I | 133 (61.9%) | |
| Stage II | 30 (14.0%) | |
| Stage III | 44 (20.5%) | |
| Stage IV | 8 (3.7%) | |
| **CD4 cell counts** (cells/mm$^3$) | | 401 ± 253 |
| <200 | 55 (25.6%) | |
| 200-350 | 38 (17.7%) | |
| 351-500 | 39 (18.1%) | |
| >500 | 83 (38.6%) | |
| **Lymphocyte counts** (x10$^9$cells/L) | | 1.6 ± 1.2 |
| <1 | 83 (38.6%) | |
| 1-4 | 126 (58.6%) | |
| >4 | 6 (2.8%) | |
| **Proteinuria** | | |
| No proteinuria | 104 (48.4 %) | |
| 1+ (30 – 100 mg/dl) | 80 (37.2%) | |

| | |
|---|---|
| 2+ (100 – 300 mg/dl) | 27 (12.6%) |
| 3+ (300 – 1000 mg/dl) | 4 (1.9%) |

CD4: Cluster of differentiation 4; HIV: Human Immunodeficiency Virus; SD: Standard Deviation

**Table 2. Predictors of HIV viral load non-suppression at six months among 215 ART naïve participants initiating ART, Dar es Salaam, Tanzania, 2019.**

| Variable | Total | HIV non-suppression at six months n (%) | RR (95% CI) | Adjusted RR (95% CI) |
|---|---|---|---|---|
| **Age** (years) | | | | |
| < 40 | 136 | 26 (19%) | 1 | 1 |
| ≥40 | 79 | 20 (25%) | 1.32 (0.79-2.21) | 1.43 (0.91-2.26) |
| **Sex** | | | | |
| Female | 146 | 35 (24%) | 1.50 (0.81-2.78) | 1.27 (0.73-2.20) |
| Male | 69 | 11 (16%) | 1 | 1 |
| **Level of Education** | | | | |
| Primary or less | 170 | 37 (22%) | 1 | |
| Secondary or higher | 45 | 9 (20%) | 0.92 (0.48-1.76) | |
| **Employment Status** | | | | |
| Not employed | 117 | 24 (21%) | 1 | |
| Employed | 98 | 22 (22%) | 1.09 (0.66-1.83) | |
| **Marital status** | | | | |
| Never married | 113 | 19 (17%) | 1 | 1 |
| Ever married | 102 | 27 (26%) | 1.57 (0.93-2.66) | 1.34 (0.84-2.16) |
| **Body mass index** | | | | |
| Change from baseline to three months | | | | |
| Loss >5% | 28 | 17 (61%) | 4.93 (2.41-10.09) | |
| Stable | 122 | 21 (17%) | 1.40 (0.66-2.99) | |
| Gain >5 % | 65 | 8 (12%) | 1 | |
| Change from baseline to six months | | | | |
| Loss >5% | 20 | 16 (80%) | 7.11 (3.69-13.69) | 2.73 (1.36-5.47) |
| Stable | 115 | 21 (18%) | 1.62 (0.78-3.36) | 1.87 (0.95-3.68) |
| Gain >5 % | 80 | 9 (11%) | 1 | 1 |
| **HIV clinical stage** | | | | |
| I | 133 | 24 (18%) | 1 | 1 |
| II | 30 | 8 (27%) | 1.48 (0.74-2.97) | 1.14 (0.63-2.08) |
| III and IV | 52 | 14 (27%) | 1.49 (0.84-2.66) | 0.82 (0.51-1.31) |

| | | | | |
|---|---|---|---|---|
| **Total lymphocyte count change from baseline to six months** | | | | |
| Ended lymphopaenic | 37 | 27 (73%) | 7.66 (4.32-13.60) | 4.54 (2.19-9.39) |
| Lymphopaenic to normal | 52 | 7 (13%) | 1.41 (0.59-3.40) | 1.59 (0.66-3.80) |
| Lymphopaenia not seen | 126 | 12 (10%) | 1 | 1 |
| **Pattern of change in proteinuria** | | | | |
| Proteinuria at 6 months regardless of baseline proteinuria status | 37 | 24 (65%) | 6.73 (3.34-13.58) | 2.63 (1.25-5.54) |
| Proteinuria at baseline and/or 3 months but not 6 months | 95 | 14 (15%) | 1.53 (0.67-3.47) | 1.26 (0.62-2.57) |
| No proteinuria seen | 83 | 8 (10%) | 1 | 1 |

CI: confidence interval; RR: relative risk; ART: antiretroviral therapy.

Univariable and multivariable analysis by modified Poisson regression.

BMI and CD4 count were directly correlated at baseline, 3, and 6 months. TLC and CD4 count were moderately positively correlated; while urine protein and CD4 count were inversely correlated (see Supplementary Figures 1, 2, and 3 in Additional file 1).

**Predictors of viral non-suppression at six months among individuals with HIV initiated on ART**

Only 46 participants (21%) were not virally suppressed at six months. The strongest clinical predictors of viral non-suppression in the multivariable analysis were lymphopaenia at six months irrespective of baseline lymphocyte status, with 73% of participants with lymphopaenia at six months not being suppressed. After adjusting for other factors, lymphopaenia at six months was associated with HIV non-suppression {RR = 4.54, 95% CI (2.19-9.39)}. Among participants with

a drop in body mass index at six months of 5% or more from baseline, 80% were non-suppressed {RR = 2.73; 95% CI (1.36-5.47)}. In an alternative analysis, we considered BMI changes of 10%, but only 9 of the non-suppressed participants (20%) had such large decreases. The risk of HIV non-suppression at six months was higher among participants with proteinuria at six months {RR = 2.63; 95% CI (1.25-5.54)}, Table 2. The area under the Receiver Operating Characteristic (ROC) curve for the multivariable model was 0.895 (Fig 2). Baseline HIV stage was not related to HIV non-suppression at six months, {adjusted RR = 1.14, 95% CI (0.63-2.08)} for baseline WHO HIV clinical stage II and {adjusted RR = 0.82, 95% CI (0.51-1.31)} for advanced baseline WHO HIV clinical stages (III and IV)}.

Using the rounded coefficients of the three variables in a model containing only these variables, which all rounded to 1, we made a "prediction score" with values 0 (n=154, of which 10 were non-suppressed), 1 (n=36, 13 non-suppressed), 2 (n=17, 15 non-suppressed), and 3 (n=8, all non-suppressed). The median value of this score among the non-suppressed was 1.5 and the first quartile value was 1. Thus, having any 2 of the risk factors would be a conservative predictor of non-suppression, and having any one would be less conservative.

**Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among ART naïve individuals with HIV initiated on ART in Dar es Salaam, Tanzania, 2019**

Using the median score among the non-suppressed as a cut-off (equivalent to having any two of the components), the sensitivity predicting non-suppression was 0.50, and the specificity was 0.99. Only 12% of the study population met this criterion. When we lowered the cut-off scores to the first quartile value among the non-suppressed (i.e. any one of the components), the sensitivity was 0.78 and the specificity was 0.85, with 28% of the study population meeting this criterion.

## Discussion

This cohort study recruited ART naïve individuals with HIV from three care and treatment centres in Dar es Salaam, Tanzania, with the aim of assessing the utility of total lymphocyte count, body mass index, and proteinuria in predicting ART responses at six months. The intention of this study is not to replace CD4 cell count testing and HIV viral load monitoring but to assist clinicians when faced with decision making if these standard monitoring parameters are not easily accessible. Contrary to earlier studies done when the ART medications were not as effective as the current ones [12], patient characteristics at ART initiation did not affect the probability of viral non-suppression at six months, whereas patterns of change and the patient's status at 6 months were highly predictive.

Baseline WHO HIV clinical stage did not help predict HIV non-suppression at six months, possibly because under the current "Test and Treat" strategy [21], most individuals initiating ART are in WHO HIV clinical stages I and II (76% of our sample). Current therapy is highly effective except for a few patients whose disease is so advanced that they die before the medication can improve their immune status (2 patients in this study). Symptomatic individuals with advanced HIV will likely adhere to treatment to alleviate their symptoms. Perhaps patients with advanced disease fear death and therefore are motivated to adhere to ART, with eventual viral suppression. Advanced HIV disease has been shown to be linked with ART adherence [22]. Some studies, however, indicate that early HIV stages are linked with high ART adherence and viral suppression [23].

Lymphopaenia at six months, a decrease in body mass index of 5% or more from baseline, and proteinuria predicted HIV non-suppression at six months. Lymphopaenia at six months was the strongest predictor for HIV non -suppression at six months. Lymphopaenia at six months predicted

HIV non-suppression irrespective of a participant's lymphocyte count (normal or low) before ART initiation. CD4 cells are a subset of lymphocytes and therefore lymphopaenia could be indicative of a decline in CD4 cells, as a result of high HIV replication with high viral load signifying viral non-suppression with consequent CD4 cell destruction. Lymphopaenia was significantly associated with CD4 <500 cells/mm$^3$ at all time points. In this study, an increase in total lymphocyte cell count from lymphopaenia to normal lymphocyte counts from baseline to six months was significantly associated with an increase in CD4 cell count (Additional file 1). Total lymphocyte count is sensitive and specific in predicting CD4 cell counts [16][24] though there have been contradictory reports [25]. The assessment of total lymphocyte counts among patients on ART, therefore, could serve as an alternative, especially in settings with limited availability of CD4 cell count measurement. A drop in total lymphocyte count in such a setting can alert a clinician to the likelihood of immunological failure. A drop in lymphocytes could also signal the possibility of immunological non responders, who will need primary and secondary prophylaxis for opportunistic infection.

Weight loss with consequent BMI drop of 5% or more at six months while on ART from weight prior to ART initiation, predicts HIV non-suppression, though HIV non-suppression was not associated with being underweight prior to ART initiation, perhaps because of the low prevalence of underweight leading to low power. In this study, sustained weight gain was significantly associated with viral suppression and sustained weight loss was associated with viral non-suppression at six months of ART. An increase in weight and hence BMI may be a sign of immune status improvement signalling a return to health [26] [27]and improved survival [28], while a decrease in weight and hence BMI may be a sign of HIV progression or decline in CD4 cell counts [5][11][29]. Weight loss may be due to HIV itself, opportunistic infections, or HIV-associated

tumours. Failure to gain weight has been associated with efavirenz toxicity over time as was observed in a study in South Africa [30]. Weight loss in both ART naïve and exposed patients has been associated with increased morbidity and mortality [31][32]. A study in England observed that each log10 increase in HIV viral load was associated with a 0.92 kg decrease in body weight. However, a decrease in viral load was not significantly associated with weight gain, contrary to our study [33]. Since weight changes correlate with the virological response, losing weight should be viewed as an alarming sign of virological failure. Monitoring of weight and body mass index prior to ART initiation and during follow up is a valuable inexpensive way of identifying individuals with possible treatment failure. In an alternative analysis, we considered BMI changes of 10%, but only 9 of the non-suppressed participants (20%) had such large decreases, making the 10% decrease not useful as a cut-off in our situation.

Half of the ART-naïve study participants had proteinuria, similar to a report from Zimbabwe [34]. The presence of proteinuria was associated with HIV non-suppression. Proteinuria at six months was a strong predictor of HIV non-suppression. Proteinuria in individuals with HIV is attributed to a direct effect of HIV on the glomerular and tubular epithelial cells with the progression of HIV disease to AIDS, nephrotoxicity from ART, the progression of chronic kidney disease and death [14][35] . The higher the viral load, the greater the damage to the kidney [36]. We observed a significant proportion of participants with proteinuria of 2+ or above in WHO HIV clinical stage IV. This underscores the fact that, as HIV disease advances, the greater the damage to the kidneys as evidenced by increasing proteinuria. Therefore, routine screening for renal disease may serve not only as a follow-up of renal disease progression but also for HIV treatment response monitoring.

Our findings require validation in a study with a larger sample size. Our small sample may have constrained some predictors of viral non-suppression. Similar studies conducted in different locations are also needed since local conditions and treatment standards may influence some observed patterns, both in prevalence and effect. Furthermore, use of new antiviral drugs and changes in patient characteristics at presentation may change our estimates, and possibly the important predictor variables. We recommend further studies to examine the relationship between virological response and anaemia as well as opportunistic infections and AIDS associated malignancies especially now that ART is initiated early.

One strength of our study is the cohort design with complete follow up data at three and six months for 98% of the enrolled participants. Although our scoring system is crude, it is easy to compute and is likely to be valid for a wide variety of situations, whereas a score based on more precise computations would at best work only in our location.

## Conclusion

A drop in BMI by more than 5%, persistent lymphopaenia or decrease in total lymphocyte count to lymphopaenia, and proteinuria at six months are associated with HIV non-suppression at 6 months after ART initiation. Scores based on these parameters are easy to use and can serve as alternatives to CD4 cell counts and viral load assessment in facilities with scarcity.

## List of abbreviations

AIDS: Acquired immunodeficiency syndrome

ART: Antiretroviral therapy

BMI: Body mass index

CD4: Cluster of differentiation 4

HIV: Human immunodeficiency virus

TLC: Total lymphocyte counts

WHO: World Health Organization

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**Author Contributions**

Study design: LJ and PM; data collection: LJ and PM; Data analysis and interpretation: LJ, PM, BT and EH; Manuscript writing: LJ, PM, BT and EH. All the authors gave final approval of the manuscript.

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## Competing interests

None declared.

## Patient consent for publication

Not applicable.

## Ethics approval

Ethical approval was obtained from the Research and Publications Committee of Muhimbili University of Health and Allied Sciences (Ref. No. DA.287/298/01A). Permission to conduct the study was obtained from Temeke Municipal Hospital administration. Participants were enrolled after providing written informed consent. The confidentiality of patient information was ensured. Participants without viral suppression at the 6th month of follow up were managed according to Tanzania National Guidelines for management of HIV and AIDS.

## Data availability statement

The dataset analysed during the current study is available upon reasonable request to the corresponding author.

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## Ethics Statement

Muhimbili University of Health and Allied Sciences Instituional Review Board with reference number DA287/298/01A/

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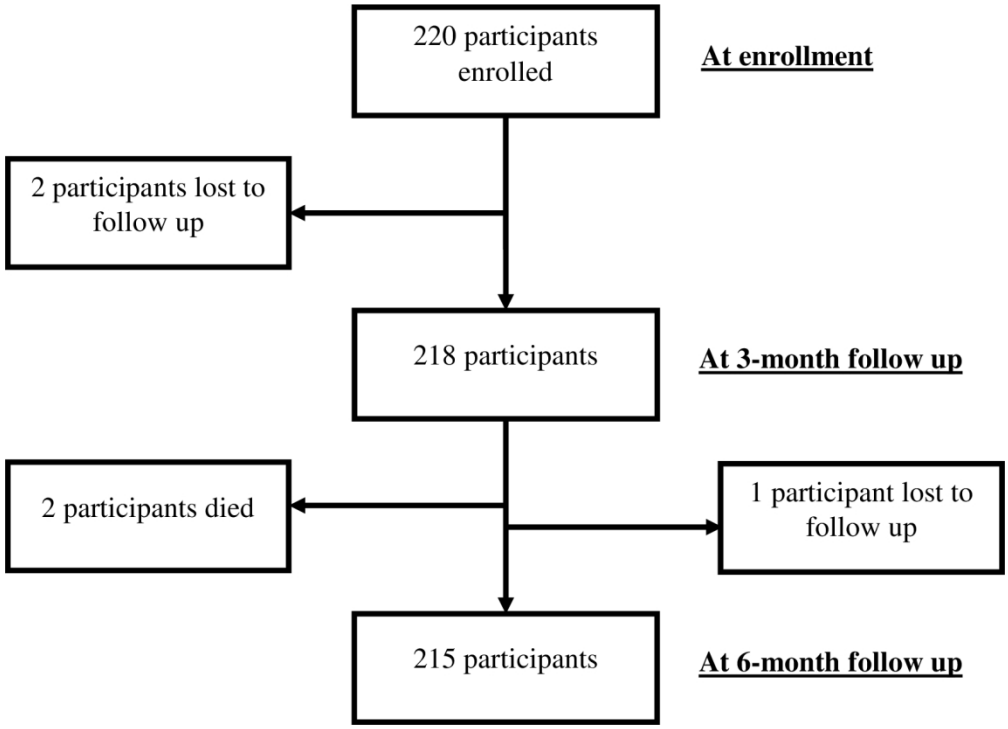



Figure 1. Consort diagram.

146x105mm (300 x 300 DPI)

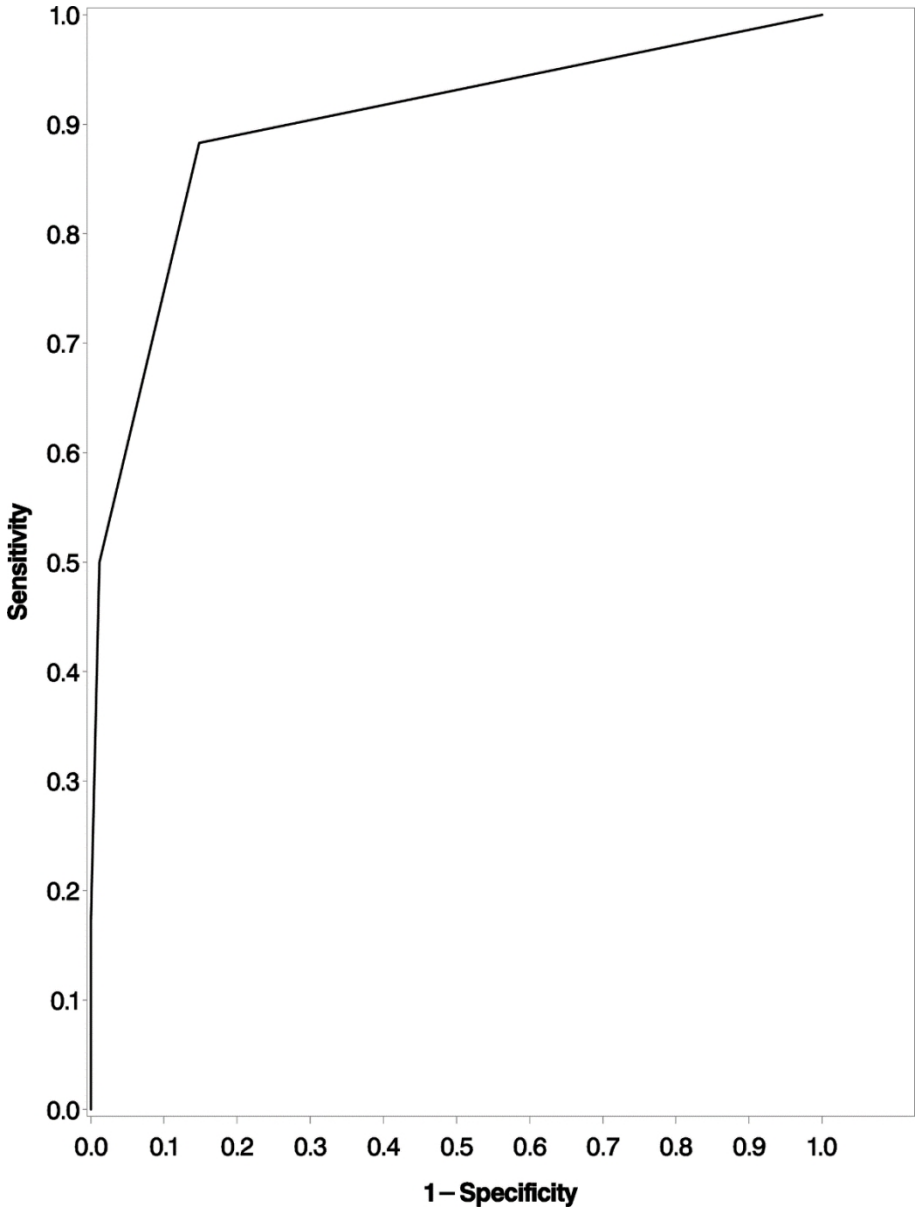
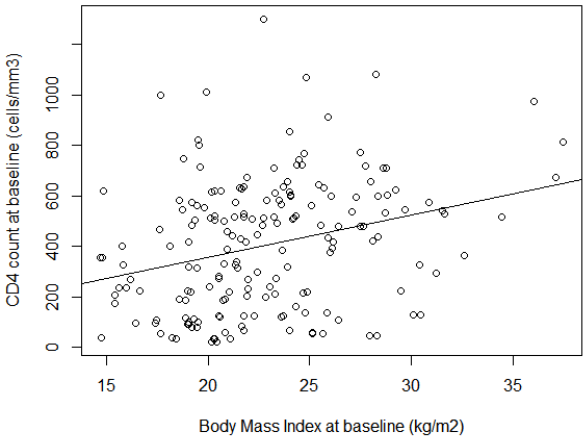


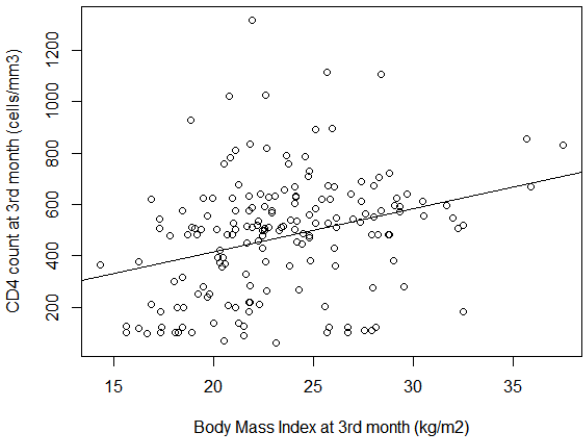

Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among HIV-infected individuals initiated on ART in Dar es Salaam, Tanzania, 2019

137x181mm (220 x 220 DPI)

**Supplementary Figure 1. Scatter plots of BMI and CD4 counts**



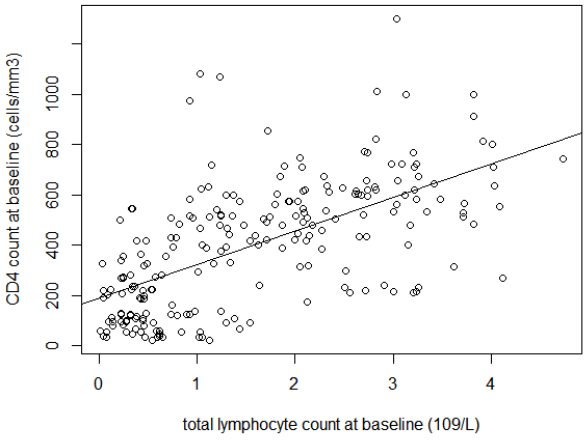

rho 0.287, p <0.0001



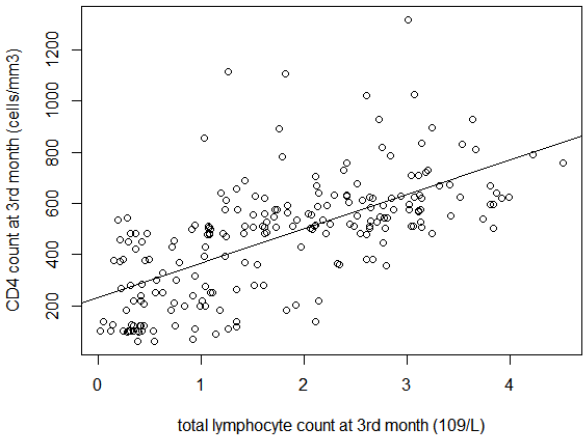

rho 0.305, p <0.0001


rho 0.373, p <0.0001

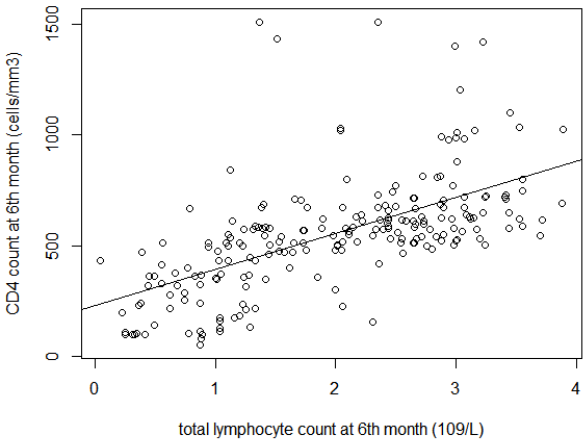
**Supplementary Figure 2. Scatter plots of total lymphocyte count and CD4 count**




rho 0.613, p <0.0001

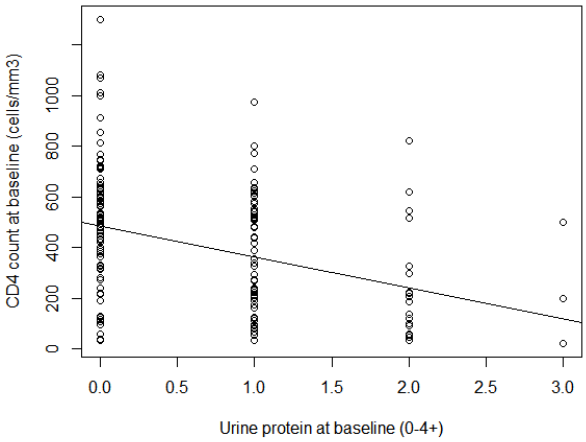



rho 0.650, p <0.0001

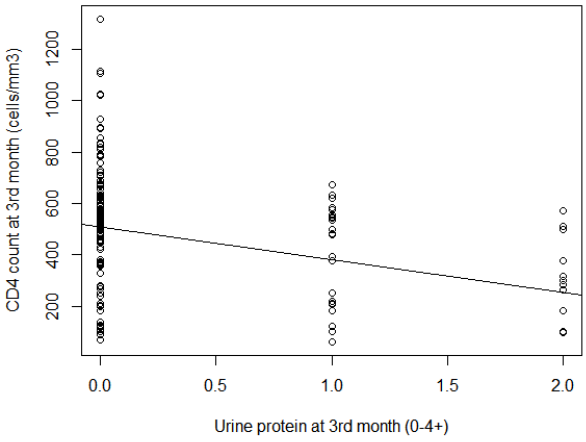



rho 0.602, p <0.0001

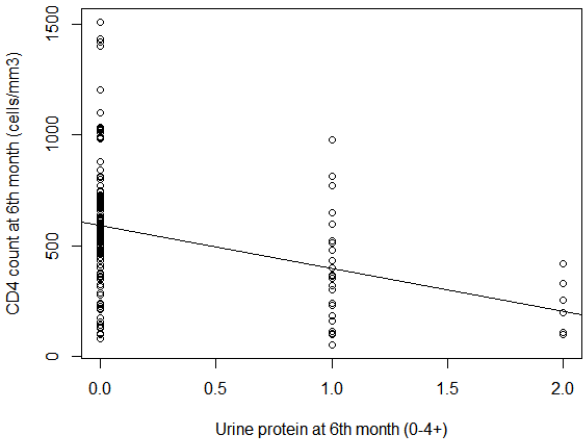
**Supplementary Figure 3. Scatter plots of urine protein and CD4 count**




rho -0.364, p <0.0001




rho -0.334, p <0.0001




rho -0.372, p <0.0001

# Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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| | | Reporting Item | Page Number |
|---|---|---|---|
| **Title and abstract** | | | |
| Title | #1a | Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| Abstract | #1b | Provide in the abstract an informative and balanced summary | 3 |

| | | | |
|---|---|---|---|
| | | of what was done and what was found | |
| **Introduction** | | | |
| Background / rationale | #2 | Explain the scientific background and rationale for the investigation being reported | 6 |
| Objectives | #3 | State specific objectives, including any prespecified hypotheses | 7 |
| **Methods** | | | |
| Study design | #4 | Present key elements of study design early in the paper | 8 |
| Setting | #5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8-10 |
| Eligibility criteria | #6a | Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. | 8-10 |
| Eligibility criteria | #6b | For matched studies, give matching criteria and number of exposed and unexposed | - |
| Variables | #7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 9,10 |
| Data sources / measurement | #8 | For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable. | 8-10 |

| | | | |
|---|---|---|---|
| Bias | #9 | Describe any efforts to address potential sources of bias | |
| Study size | #10 | Explain how the study size was arrived at | 8 |
| Quantitative variables | #11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why | 9-11 |
| Statistical methods | #12a | Describe all statistical methods, including those used to control for confounding | 10,11 |
| Statistical methods | #12b | Describe any methods used to examine subgroups and interactions | 10, 11 |
| Statistical methods | #12c | Explain how missing data were addressed | 12 |
| Statistical methods | #12d | If applicable, explain how loss to follow-up was addressed | - |
| Statistical methods | #12e | Describe any sensitivity analyses | 11 |
| **Results** | | | |
| Participants | #13a | Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and | 12 (figure 1) |

| | | | |
|---|---|---|---|
| | | unexposed groups if applicable. | |
| Participants | #13b | Give reasons for non-participation at each stage | 12 (figure 1) |
| Participants | #13c | Consider use of a flow diagram | 12 (figure 1) |
| Descriptive data | #14a | Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. | 12,13 |
| Descriptive data | #14b | Indicate number of participants with missing data for each variable of interest | See 12 |
| Descriptive data | #14c | Summarise follow-up time (eg, average and total amount) | 12 |
| Outcome data | #15 | Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable. | 14 |
| Main results | #16a | Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 14-16 |

| | | | |
|---|---|---|---|
| Main results | #16b | Report category boundaries when continuous variables were categorized | 12-15 |
| Main results | #16c | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| - | | | |
| Other analyses | #17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 16 |
| **Discussion** | | | |
| Key results | #18 | Summarise key results with reference to study objectives | 20 |
| Limitations | #19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. | 5 |
| Interpretation | #20 | Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. | 18-20 |
| Generalisability | #21 | Discuss the generalisability (external validity) of the study results | 20 |
| **Other Information** | | | |
| Funding | #22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 22 |

BMJ Open

# Body mass index, proteinuria and total lymphocyte counts in predicting treatment responses among ART naïve individuals with HIV initiated on antiretroviral treatment in Dar es Salaam, Tanzania, 2019: a cohort study



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# Body mass index, proteinuria and total lymphocyte counts in predicting treatment responses among ART naïve individuals with HIV initiated on antiretroviral treatment in Dar es Salaam, Tanzania, 2019: a cohort study

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**Keywords**: monitoring ART treatment; HIV/AIDS; CD4 count; BMI in HIV; proteinuria in HIV; viral suppression

## Abstract

**Objectives**: To explore the potential use of body mass index, proteinuria, and total lymphocyte count changes in predicting immunological and virological response in individuals with HIV initiated on antiretroviral therapy (ART).

**Design**: Prospective cohort study.

**Setting**: Three urban HIV care and treatment centres (CTC) in Dar es Salaam.

**Participants**: Individuals with HIV initiating ART.

**Outcome measures**: HIV viral load ≥1000 copies/ml (viral non-suppression) at six months after ART initiation.

**Results**: Of 215 (out of 220 enrolled) participants who returned for evaluation at six months, 147 (66.8%) were female. At six months of follow-up, 89.4% (76/85) of participants with sustained weight gain were virally suppressed compared to 31.8% (7/22) with sustained loss, p<0.001. In participants who were lymphopaenic at baseline, an increase to normal total lymphocyte counts at six months was associated with an increase in CD4 count compared to participants who remained lymphopaenic, 96.2% (50/52) vs. 54.8% (17/31), p<0.001. At baseline, 50.0% (110/220) had proteinuria. In participants without proteinuria from baseline to six months, 89.8% (79/88) were virally suppressed compared to participants with proteinuria at baseline and/or three months, 85.6% (77/90), those with persistent proteinuria, 30.8% (8/26), and proteinuria at six months only, 45.5% (5/11), p<0.001. In modified Poisson regression, the independent predictors other than CD4 cell counts for viral non-suppression at six months among individuals with HIV initiating on ART were BMI loss >5% from baseline to six months, {adjusted RR 2.73, 95% CI (1.36-5.47)},

lymphopaenia at six months, {adjusted RR = 4.54, 95% CI (2.19-9.39)}, and proteinuria at six months {adjusted RR = 2.63, 95% CI (1.25-5.54)}.

**Conclusions**: Change in body mass index, total lymphocyte count, and presence of proteinuria can monitor and predict ART response and may be particularly helpful in settings when CD4 counts and viral load monitoring are unavailable.

## Article Summary

Strengths and limitations of this study

- We had complete data on 98% of the originally enrolled participants.
- In resource-constrained situations, when viral load and CD4 testing are not always easily available, models such as ours with locally determined easily computable prediction cut-offs can be utilized by clinicians to make clinical decisions.
- Our findings require validation in a study with larger sample size.
- Local conditions and treatment standards may influence some of the patterns we observed, both in prevalence and in effect.

## Introduction

In 2019 about 38 million people were living with HIV globally, with 1.7 million in Tanzania accounting for an estimated prevalence of 4.8% in the Tanzanian population aged 15-49 years [1]. Viral load testing is the recommended method for monitoring HIV treatment response [2]. However, viral load testing in resource-constrained settings is challenged by limited access, high costs, unavailability at district levels, and in areas where available, sometimes a shortage of reagents, compounded by challenges with equipment maintenance [3], as happened during the COVID-19 pandemic.

There is no doubt that viral load testing is effective in monitoring patient treatment adherence and HIV resistance, as per WHO guidelines. However, in resource-constrained areas that may not always be able to perform viral load testing in a timely manner, there is a need for readily available and routinely assessed objective measures that may predict early viral non-suppression or measures that may help with interim evaluation of patients suspected to have treatment failure who will thereafter need additional follow up with viral load testing. Individuals with HIV are routinely assessed for weight, height, renal function, and complete blood counts before initiation of combined antiretroviral treatment (ART) in resource constrained settings including Tanzania. These assessments are repeated at intervals of three months, six months and biannually after ART initiation. Adverse changes in such parameters from treatment initiation (baseline) or subsequently at follow-up visits provides useful information about treatment responses and may identify a targeted group of patients to be prioritized for viral load testing before a decision to switch the ART regimen.

Routine parameters such as body mass index, total lymphocyte counts, and protein in urine are easily available and can be evaluated in combination for HIV treatment monitoring. Weight loss

is one of the major manifestations of advanced HIV infection. Factors hypothesized to contribute to weight loss include metabolic alterations, anorexia, malabsorptive disorders, hypogonadism, and excessive cytokine production [4]

Weight gain following ART initiation may reflect slowed resting energy expenditure resulting from viral suppression and a decrease in HIV enteropathy [5]. Weight gain, especially among individuals with low BMI, is associated with improved survival and decreased risk of clinical failure [6]. ART responses depend on adherence [7], nutritional status at baseline [8], HIV subtype [9], and ART combination regimen [10]. In Port Harcourt, Nigeria, among 318 participants with HIV infection aged ≥18 years initiated on ART, almost 70% and 55% of participants gained at least 1 kg weight in the first six months and one year of treatment, respectively [11]. Previous studies in Tanzania have shown that a decrease in nutrition status within the first three months of ART initiation was associated with mortality [12].

HIV-associated nephropathy (HIVAN) is the most common renal biopsy finding in individuals with HIV with a prevalence ranging from 4.7 to 38% [13]. Proteinuria and elevated creatinine have been associated with AIDS-defining illness and death [14]. Urine assessment for protein by dipstick can detect renal disease and progression, especially as renal biopsy, an invasive procedure, is not readily available in most resource-constrained settings.

HIV is associated with progressive destruction of CD4+ T-helper lymphocytes responsible for the profound immunodeficiency that underlies AIDS [15]. As CD4 cells are a subset of lymphocytes, any significant change in CD4 cells will cause a parallel change in total lymphocyte counts [16].

This study aimed at assessing the following routinely accessible parameters: body mass index, proteinuria, and total lymphocyte counts (TLC), and their patterns of change in monitoring HIV treatment responses at six months following ART initiation.

## Methods

### Study design and population

This cohort study was conducted in three urban care and treatment centres (CTC) in Temeke district, Dar es Salaam: Temeke regional referral hospital, Mbagala Rangi-tatu district hospital, and Mbagala Kizuiani dispensary between September 2018 and April 2019. The centres were chosen due to large numbers of ART naïve clients, amounting to 60 or more per clinic per month. The sites have an organized CTC and follow up plan for clients. Participants were included in the study if they were newly diagnosed with HIV and were ART naïve aged 18 years or older and were able to provide written informed consent. Participants were initiated on ART based on the Tanzanian National guidelines [17] with a default regimen of tenofovir, lamivudine and efavirenz unless contraindicated.

### Sample size estimation

To determine the minimal detectable relative risks for the study variables, we considered two-sample tests of the expected highest risk category versus the expected lowest risk category. For the dichotomous potential risk factors, we assumed a total number of 215 subjects, split roughly as our actual data are split (with numbers rounded to the nearest 5 to mimic a pre-study power calculation). For BMI change we used 80 for the reference group (gain), 125 for stable, and 20 for the loss group. The minimum detectable risk ratios were 3.77 for decreased BMI, 2.56 for stable BMI, 2.94 for lymphopenia and for proteinuria, 2.47 for stage greater than 1, 2.47 for age of 40, years and above 2.59 (or < 0.11) for female sex, 2.74 for secondary or higher education, 2.44 for unemployment and for never married.

## Data collection

We used an interviewer-based structured tool to conduct face-to-face interviews to obtain socio-demographic and baseline characteristics (at treatment initiation) such as age, sex, occupation, the highest level of education attained, marital status, and clinically assessed the participant's WHO HIV clinical stage. A participant's baseline weight was measured using a SECA weighing scale recording to the nearest 0.5 kg and height using a wall stadiometer recording to the nearest 0.5 cm. Body mass index was then computed by dividing the weight in kg by the height in meters squared, the interpretation of which was adapted from WHO [18].

About 10ml of venous blood was collected aseptically from each participant; 5ml for CD4 cell counts, analysed using BD FACSCount™ (Becton Dickenson, USA) and 5ml for complete blood count to obtain the total lymphocyte counts, analysed by an auto-analyser (Cell DNY1800 from Abbott Diagnostics Division, USA). TLC was categorized as lymphopaenia ($<1\times10^9$/L), normal lymphocyte ($1\times10^9$/L to $4\times10^9$/L), and lymphocytosis ($>4.0\times10^9$/L). We assessed for proteinuria by collecting about 15ml of a fresh urine sample from each participant into an empty, clean, dry container and tested using CYBOW™ strips (DFI Co. Ltd, Korea). Proteinuria was categorized as negative proteinuria (no proteinuria), trace or 1+ proteinuria (equivalent to 30 to 100 mg/dl), 2+ proteinuria (equivalent to 100 to 300 mg/dl), 3+ proteinuria (equivalent to 300 to 1000 mg/dl), and 4+ proteinuria (equivalent to greater than 1000 mg/dl).

At three and six months after ART initiation, a repeat assessment of participants was done for CD4 cell counts, TLC, proteinuria, and weight. At six months, an additional 5ml of blood was collected from each study participant for viral load measurement using the Abbott RealTi*m*e HIV-1 assay. Participants were classified as virally suppressed at six months after ART initiation if their HIV viral load was <1000 copies/ml, according to Tanzania HIV treatment guidelines. Levels and

changes in BMI, lymphocytes, and proteinuria were compared between participants who were HIV suppressed and that of HIV not suppressed.

BMI was considered to have changed between one time point and another if it increased or decreased by over 5%. BMI changes from ART initiation to six months were categorized into three groups as (i) loss of more than 5%, (ii) stable (change between -5% and 5%, or (iii) gain of more than 5%. The TLC were categorized as (i) lymphopaenia < $1x10^9$ cells/L, (ii) normal lymphocyte count 1-4 $x10^9$ cells/L (iii) Lymphocytosis > $4x10^9$ cells/L. The TLC pattern change was categorized as (i) lymphopaenic at 6 months, regardless of the status at baseline or three months; (ii) increased from lymphopaenic to normal or higher; (iii) stayed normal or higher (no lymphopaenia seen). Urinary protein patterns were categorized as (i) proteinuria at six months regardless of baseline proteinuria status; (ii) proteinuria at baseline and/or third month but not six months; and (iii) no proteinuria seen.

**Patient and public involvement**

Patients or members of the public were not involved in the design, or conduct, or reporting, or dissemination plans of the research.

**Statistical methods**

Data were analysed using IBM® SPSS® Statistics version 26, R, and SAS version 9.4 (Cary, NC). Categorical variables such as age group, sex, marital status, level of education, occupation, categories of BMI, CD4 count, proteinuria, TLC, BMI change, TLC change, and proteinuria change were summarized as frequencies and proportions. Continuous variables such as age, BMI, and CD4 count were summarized as means and standard deviations. When necessary, small groups

were combined for analysis. To determine the association between BMI, TLC or urine protein to CD4 count, we used correlation.

To determine the relationships between individual predictors and viral non-suppression at six months, we first used modified Poisson regression for univariable analysis with an assumption that viral non suppression is a non-rare outcome (more than 10%), to determine which variables to include in the multivariable model [19,20]. For multivariable prediction, all predictors in the univariable model with a p-value of <0.2 and age, a known confounder, were entered into the modified Poisson regression model. The results of the Poisson regression model were presented as relative risk (RR) and 95% confidence interval (RR; 95% CI). To determine the test characteristics (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)) of a score based on the multivariable model, we used two cut-off levels, based on the first quartile and median of the score among the non-suppressed. The score was the sum of the rounded coefficients for the variables for which the confidence intervals did not include 1 in a model containing only these variables. Since these all rounded to 1, this is equivalent to simply counting the number of these characteristics.

Based on practices in low resourced clinics, communication with the patient and the decision to change the ART regimen depends on the patient's virological status at six months. CD4 cell counts depend on a blood sample collected at the six-month visit and are therefore unavailable for immediate decision making. We, therefore, excluded all CD4 variables from the model and used parameters available at the time of the six-month visit to predict viral non-suppression.

## Results

During the recruitment, 220 ART naïve individuals with HIV were initiated on ART and all were enrolled in the study over a month; each participant was followed up for six months. Two participants were lost to follow up at three months; two died before six months of follow up, and one participant, a long-distance truck driver, was out of the country at the time of the 6-month follow up. Therefore, our analysis data set includes the remaining 215 participants. Details of enrolment are shown in Fig 1.

**Figure 1. Consort diagram.**

### Baseline characteristics of study participants

Of the 215 participants analysed, the mean age (SD) was 37.1 ±11.5 years, 146 (68%) were female, 113 (53%) were never married, 45 (21%) had secondary education or higher, and 117 (54%) were unemployed (Table 1). Most participants, 59.5%, had normal BMI, while 27% were overweight, and 13% were underweight. Most participants, 113 (62%), were in WHO HIV clinical stage I, and only eight (4%) were in stage IV, though 93 (43%) had CD4 counts of 350 cells/ml or below; 83 (39%) were lymphopaenic, and 111 (52%) had proteinuria at baseline.

**Table 1. Characteristics of 215 study participants at ART initiation, Dar es Salaam, Tanzania, 2019.**

| Characteristic | n (%) | Mean ± SD |
|---|---|---|
| **Age** (years) | | 37.1 ± 11.5 |

| | | |
|---|---|---|
| Age group (years) | | |
| 18 – 30 | 69 (32.1%) | |
| 31 – 40 | 72 (33.5%) | |
| 41 – 50 | 45 (20.9%) | |
| >51 | 29 (13.5%) | |
| **Sex** | | |
| Female | 146 (67.9%) | |
| Male | 69 (32.1 %) | |
| **Level of education** | | |
| No education | 10 (4.7%) | |
| Primary education | 160 (74.4%) | |
| Secondary education | 42 (19.5%) | |
| Higher education | 3 (1.4%) | |
| **Employment Status** | | |
| Not employed | 117 (54.4%) | |
| Employed | 98 (45.6%) | |
| **Marital status** | | |
| Ever married | 102 (47.4%) | |
| Never married | 113 (52.6%) | |
| **Body mass index** (kg/m$^2$) | | 22.9 ± 4.3 |
| Underweight | 28 (13.0%) | |
| Normal weight | 128 (59.5%) | |
| Overweight/Obese | 59 (27.4%) | |
| **WHO HIV clinical stages** | | |
| Stage I | 133 (61.9%) | |
| Stage II | 30 (14.0%) | |
| Stage III | 44 (20.5%) | |
| Stage IV | 8 (3.7%) | |
| **CD4 cell counts** (cells/mm$^3$) | | 401 ± 253 |
| <200 | 55 (25.6%) | |
| 200-350 | 38 (17.7%) | |
| 351-500 | 39 (18.1%) | |
| >500 | 83 (38.6%) | |
| **Lymphocyte counts** (x10$^9$cells/L) | | 1.6 ± 1.2 |
| <1 | 83 (38.6%) | |
| 1-4 | 126 (58.6%) | |
| >4 | 6 (2.8%) | |
| **Proteinuria** | | |
| No proteinuria | 104 (48.4 %) | |
| 1+ (30 – 100 mg/dl) | 80 (37.2%) | |

| | |
|---|---|
| 2+ (100 – 300 mg/dl) | 27 (12.6%) |
| 3+ (300 – 1000 mg/dl) | 4 (1.9%) |

CD4: Cluster of differentiation 4; HIV: Human Immunodeficiency Virus; SD: Standard Deviation

**Table 2. Predictors of HIV viral load non-suppression at six months among 215 ART naïve participants initiating ART, Dar es Salaam, Tanzania, 2019.**

| Variable | Total | HIV non-suppression at six months n (%) | RR (95% CI) | Adjusted RR (95% CI) |
|---|---|---|---|---|
| **Age** (years) | | | | |
| < 40 | 136 | 26 (19%) | 1 | 1 |
| ≥40 | 79 | 20 (25%) | 1.32 (0.79-2.21) | 1.43 (0.91-2.26) |
| **Sex** | | | | |
| Female | 146 | 35 (24%) | 1.50 (0.81-2.78) | 1.27 (0.73-2.20) |
| Male | 69 | 11 (16%) | 1 | 1 |
| **Level of Education** | | | | |
| Primary or less | 170 | 37 (22%) | 1 | |
| Secondary or higher | 45 | 9 (20%) | 0.92 (0.48-1.76) | |
| **Employment Status** | | | | |
| Not employed | 117 | 24 (21%) | 1 | |
| Employed | 98 | 22 (22%) | 1.09 (0.66-1.83) | |
| **Marital status** | | | | |
| Never married | 113 | 19 (17%) | 1 | 1 |
| Ever married | 102 | 27 (26%) | 1.57 (0.93-2.66) | 1.34 (0.84-2.16) |
| **Body mass index** | | | | |
| Change from baseline to three months | | | | |
| Loss >5% | 28 | 17 (61%) | 4.93 (2.41-10.09) | |
| Stable | 122 | 21 (17%) | 1.40 (0.66-2.99) | |
| Gain >5 % | 65 | 8 (12%) | 1 | |
| Change from baseline to six months | | | | |
| Loss >5% | 20 | 16 (80%) | 7.11 (3.69-13.69) | 2.73 (1.36-5.47) |
| Stable | 115 | 21 (18%) | 1.62 (0.78-3.36) | 1.87 (0.95-3.68) |
| Gain >5 % | 80 | 9 (11%) | 1 | 1 |
| **HIV clinical stage** | | | | |
| I | 133 | 24 (18%) | 1 | 1 |
| II | 30 | 8 (27%) | 1.48 (0.74-2.97) | 1.14 (0.63-2.08) |
| III and IV | 52 | 14 (27%) | 1.49 (0.84-2.66) | 0.82 (0.51-1.31) |

| | | | | |
|---|---|---|---|---|
| **Total lymphocyte count change from baseline to six months** | | | | |
| Ended lymphopaenic | 37 | 27 (73%) | 7.66 (4.32-13.60) | 4.54 (2.19-9.39) |
| Lymphopaenic to normal | 52 | 7 (13%) | 1.41 (0.59-3.40) | 1.59 (0.66-3.80) |
| Lymphopaenia not seen | 126 | 12 (10%) | 1 | 1 |
| **Pattern of change in proteinuria** | | | | |
| Proteinuria at 6 months regardless of baseline proteinuria status | 37 | 24 (65%) | 6.73 (3.34-13.58) | 2.63 (1.25-5.54) |
| Proteinuria at baseline and/or 3 months but not 6 months | 95 | 14 (15%) | 1.53 (0.67-3.47) | 1.26 (0.62-2.57) |
| No proteinuria seen | 83 | 8 (10%) | 1 | 1 |

CI: confidence interval; RR: relative risk; ART: antiretroviral therapy.

Univariable and multivariable analysis by modified Poisson regression.

BMI and CD4 count were directly correlated at baseline, 3, and 6 months. TLC and CD4 count were moderately positively correlated; while urine protein and CD4 count were inversely correlated (see Supplementary Figures 1, 2, and 3 in Additional file 1).

**Predictors of viral non-suppression at six months among individuals with HIV initiated on ART**

Only 46 participants (21%) were not virally suppressed at six months. The strongest clinical predictors of viral non-suppression in the multivariable analysis were lymphopaenia at six months irrespective of baseline lymphocyte status, with 73% of participants with lymphopaenia at six months not being suppressed. After adjusting for other factors, lymphopaenia at six months was associated with HIV non-suppression {RR = 4.54, 95% CI (2.19-9.39)}. Among participants with

a drop in body mass index at six months of 5% or more from baseline, 80% were non-suppressed {RR = 2.73; 95% CI (1.36-5.47)}. In an alternative analysis, we considered BMI changes of 10%, but only 9 of the non-suppressed participants (20%) had such large decreases. The risk of HIV non-suppression at six months was higher among participants with proteinuria at six months {RR = 2.63; 95% CI (1.25-5.54)}, Table 2. The area under the Receiver Operating Characteristic (ROC) curve for the multivariable model was 0.895 (Fig 2). Baseline HIV stage was not related to HIV non-suppression at six months, {adjusted RR = 1.14, 95% CI (0.63-2.08)} for baseline WHO HIV clinical stage II and {adjusted RR = 0.82, 95% CI (0.51-1.31)} for advanced baseline WHO HIV clinical stages (III and IV)}.

Using the rounded coefficients of the three variables in a model containing only these variables, which all rounded to 1, we made a "prediction score" with values 0 (n=154, of which 10 were non-suppressed), 1 (n=36, 13 non-suppressed), 2 (n=17, 15 non-suppressed), and 3 (n=8, all non-suppressed). The median value of this score among the non-suppressed was 1.5 and the first quartile value was 1. Thus, having any 2 of the risk factors would be a conservative predictor of non-suppression, and having any one would be less conservative.

**Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among ART naïve individuals with HIV initiated on ART in Dar es Salaam, Tanzania, 2019**

Using the median score among the non-suppressed as a cut-off (equivalent to having any two of the components), the sensitivity predicting non-suppression was 0.50, and the specificity was 0.99. Only 12% of the study population met this criterion. When we lowered the cut-off scores to the first quartile value among the non-suppressed (i.e. any one of the components), the sensitivity was 0.78 and the specificity was 0.85, with 28% of the study population meeting this criterion.

## Discussion

This cohort study recruited ART naïve individuals with HIV from three care and treatment centres in Dar es Salaam, Tanzania, with the aim of assessing the utility of total lymphocyte count, body mass index, and proteinuria in predicting ART responses at six months. The intention of this study is not to replace CD4 cell count testing and HIV viral load monitoring but to assist clinicians when faced with decision making if these standard monitoring parameters are not easily accessible. Contrary to earlier studies done when the ART medications were not as effective as the current ones [12], patient characteristics at ART initiation did not affect the probability of viral non-suppression at six months, whereas patterns of change and the patient's status at 6 months were highly predictive.

Baseline WHO HIV clinical stage did not help predict HIV non-suppression at six months, possibly because under the current "Test and Treat" strategy [21], most individuals initiating ART are in WHO HIV clinical stages I and II (76% of our sample). Current therapy is highly effective except for a few patients whose disease is so advanced that they die before the medication can improve their immune status (2 patients in this study). Symptomatic individuals with advanced HIV will likely adhere to treatment to alleviate their symptoms. Perhaps patients with advanced disease fear death and therefore are motivated to adhere to ART, with eventual viral suppression. Advanced HIV disease has been shown to be linked with ART adherence [22]. Some studies, however, indicate that early HIV stages are linked with high ART adherence and viral suppression [23].

Lymphopaenia at six months, a decrease in body mass index of 5% or more from baseline, and proteinuria predicted HIV non-suppression at six months. Lymphopaenia at six months was the strongest predictor for HIV non -suppression at six months. Lymphopaenia at six months predicted

HIV non-suppression irrespective of a participant's lymphocyte count (normal or low) before ART initiation. CD4 cells are a subset of lymphocytes and therefore lymphopaenia could be indicative of a decline in CD4 cells, as a result of high HIV replication with high viral load signifying viral non-suppression with consequent CD4 cell destruction. Lymphopaenia was significantly associated with CD4 <500 cells/mm$^3$ at all time points. In this study, an increase in total lymphocyte cell count from lymphopaenia to normal lymphocyte counts from baseline to six months was significantly associated with an increase in CD4 cell count (Additional file 1). Total lymphocyte count is sensitive and specific in predicting CD4 cell counts [16,24] though there have been contradictory reports [25]. The assessment of total lymphocyte counts among patients on ART, therefore, could serve as an alternative, especially in settings with limited availability of CD4 cell count measurement. A drop in total lymphocyte count in such a setting can alert a clinician to the likelihood of immunological failure. A drop in lymphocytes could also signal the possibility of immunological non responders, who will need primary and secondary prophylaxis for opportunistic infection.

Weight loss with consequent BMI drop of 5% or more at six months while on ART from weight prior to ART initiation, predicts HIV non-suppression, though HIV non-suppression was not associated with being underweight prior to ART initiation, perhaps because of the low prevalence of underweight leading to low power. In this study, sustained weight gain was significantly associated with viral suppression and sustained weight loss was associated with viral non-suppression at six months of ART. An increase in weight and hence BMI may be a sign of immune status improvement signalling a return to health [26,27] and improved survival [28], while a decrease in weight and hence BMI may be a sign of HIV progression or decline in CD4 cell counts [5,11,29]. Weight loss may be due to HIV itself, opportunistic infections, or HIV-associated

tumours. Failure to gain weight has been associated with efavirenz toxicity over time as was observed in a study in South Africa [30]. Weight loss in both ART naïve and exposed patients has been associated with increased morbidity and mortality [31,32]. A study in England observed that each log10 increase in HIV viral load was associated with a 0.92 kg decrease in body weight. However, a decrease in viral load was not significantly associated with weight gain, contrary to our study [33]. Since weight changes correlate with the virological response, losing weight should be viewed as an alarming sign of HIV viral non suppression from any cause. Monitoring of weight and body mass index prior to ART initiation and during follow up is a valuable inexpensive way of identifying individuals with possible viral non suppression. In an alternative analysis, we considered BMI changes of 10%, but only 9 of the non-suppressed participants (20%) had such large decreases, making the 10% decrease not useful as a cut-off in our situation.

Half of the ART-naïve study participants had proteinuria, similar to a report from Zimbabwe [34]. The presence of proteinuria was associated with HIV non-suppression. Proteinuria at six months was a strong predictor of HIV non-suppression. Proteinuria in individuals with HIV is attributed to a direct effect of HIV on the glomerular and tubular epithelial cells with the progression of HIV disease to AIDS, nephrotoxicity from ART, the progression of chronic kidney disease and death [14,35] The higher the viral load, the greater the damage to the kidney [36]. We observed a significant proportion of participants with proteinuria of 2+ or above in WHO HIV clinical stage IV. This underscores the fact that, as HIV disease advances, the greater the damage to the kidneys as evidenced by increasing proteinuria. Therefore, routine screening for renal disease may serve not only as a follow-up of renal disease progression but also for HIV treatment response monitoring.

The presence of proteinuria, lymphopaenia, and a drop in BMI of 5% are relatively simple parameters to monitor among people living with HIV on ART especially in a setting where viral load monitoring is a challenge. The presence of any of these parameters should alert a clinician on the possibility of viral non-response and review adherence issues including individualized enhanced adherence counselling and subsequent treatment options.

Our findings require validation in a study with a larger sample size. Our small sample may have constrained some predictors of viral non-suppression. Similar studies conducted in different locations are also needed since local conditions and treatment standards may influence some observed patterns, both in prevalence and effect. Furthermore, use of new antiviral drugs and changes in patient characteristics at presentation may change our estimates, and possibly the important predictor variables. We recommend further studies with extended follow up of patients beyond six months to monitor further change in lymphopaenia, proteinuria and drop in BMI of 5% or more especially for individuals maintained on the same regimen after enhanced adherence counselling. We recommend further studies to examine the relationship between virological response and anaemia as well as opportunistic infections and AIDS associated malignancies especially now that ART is initiated early.

One strength of our study is the cohort design with complete follow up data at three and six months for 98% of the enrolled participants. Although our scoring system is crude, it is easy to compute and is likely to be valid for a wide variety of situations, whereas a score based on more precise computations would at best work only in our location.

## Conclusion

A drop in BMI by more than 5%, persistent lymphopaenia or decrease in total lymphocyte count to lymphopaenia, and proteinuria at six months are associated with HIV non-suppression at 6 months after ART initiation. Scores based on these parameters are easy to use and can serve as alternatives to CD4 cell counts and viral load assessment in facilities with scarcity.

## List of abbreviations

AIDS: Acquired immunodeficiency syndrome

ART: Antiretroviral therapy

BMI: Body mass index

CD4: Cluster of differentiation 4

HIV: Human immunodeficiency virus

TLC: Total lymphocyte counts

WHO: World Health Organization

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**Author Contributions**

Study design: LJ and PM; data collection: LJ and PM; Data analysis and interpretation: LJ, PM, BT and EH; Manuscript writing: LJ, PM, BT and EH. All the authors gave final approval of the manuscript.

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**Competing interests**

None declared.

**Patient consent for publication**

Not applicable.

**Ethics approval**

Ethical approval was obtained from the Research and Publications Committee of Muhimbili University of Health and Allied Sciences (Ref. No. DA.287/298/01A). Permission to conduct the study was obtained from Temeke Municipal Hospital administration. Participants were enrolled after providing written informed consent. The confidentiality of patient information was ensured. Participants without viral suppression at the 6$^{th}$ month of follow up were managed according to Tanzania National Guidelines for management of HIV and AIDS.

**Data availability statement**

The dataset analysed during the current study is available upon reasonable request to the corresponding author.

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**Ethics Statement**

Muhimbili University of Health and Allied Sciences Institutional Review Board with reference number DA287/298/01A/

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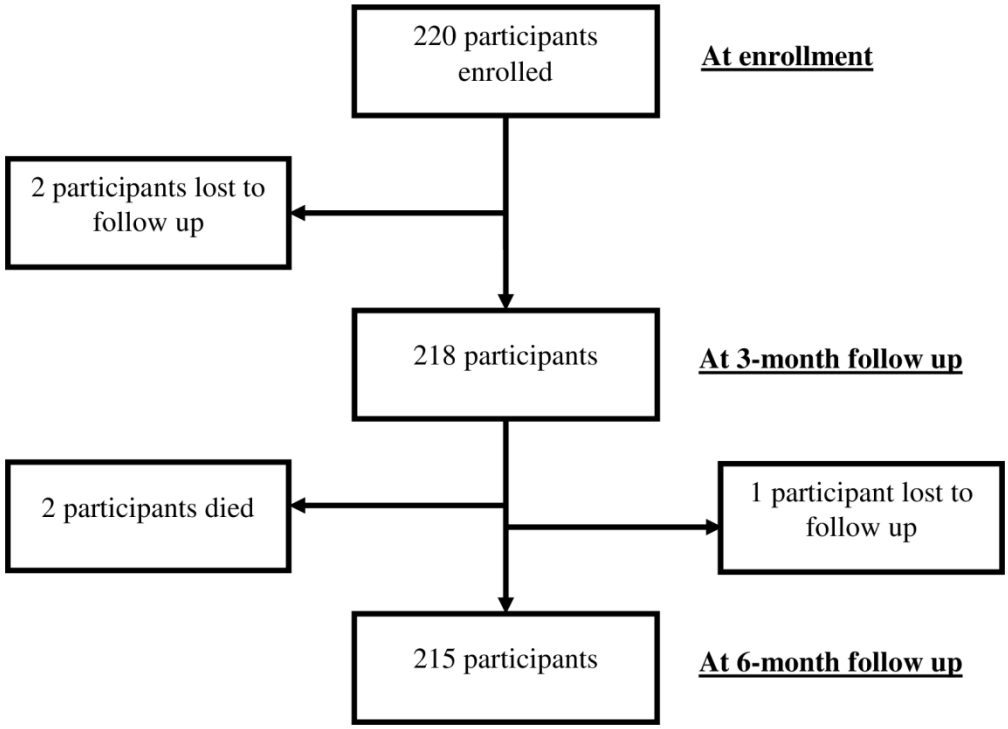



Figure 1. Consort diagram.

146x105mm (300 x 300 DPI)

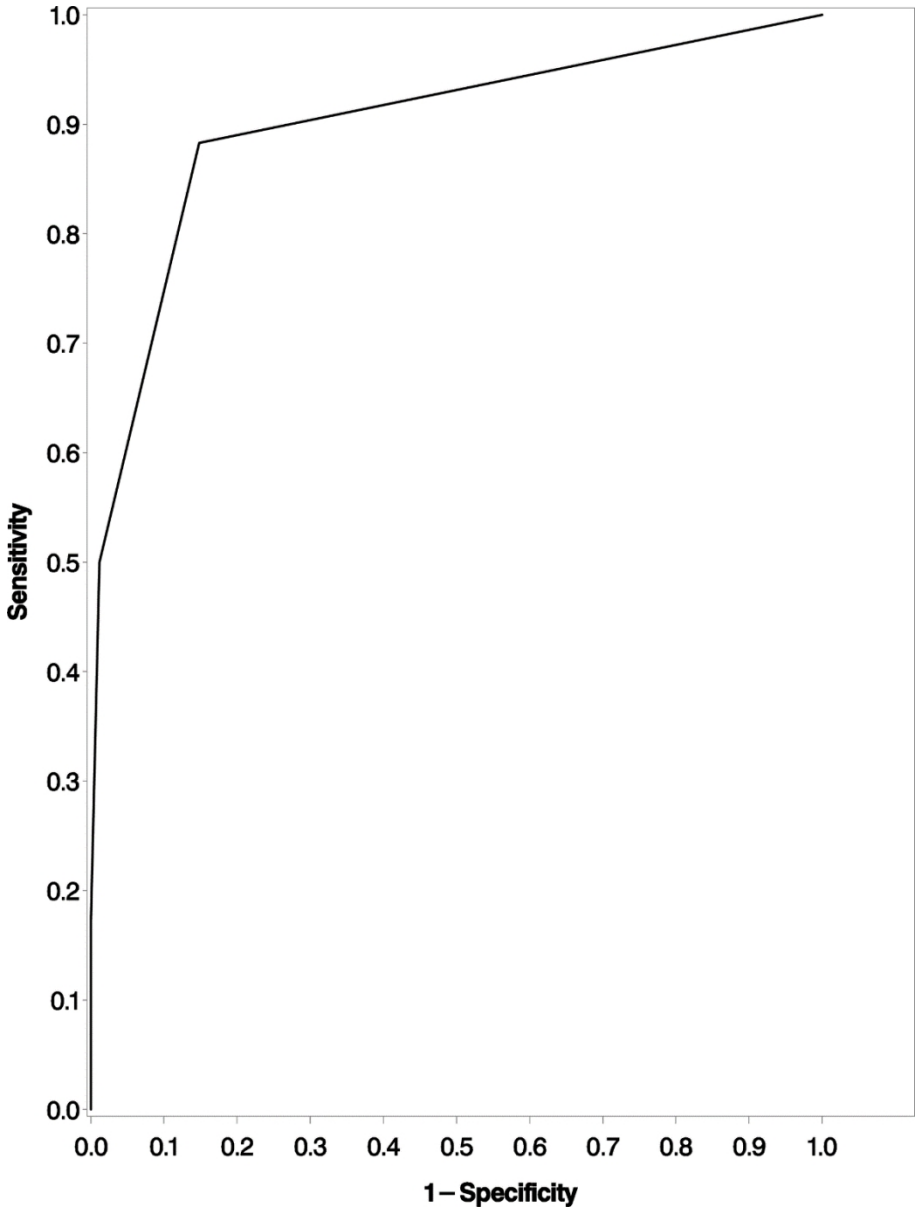
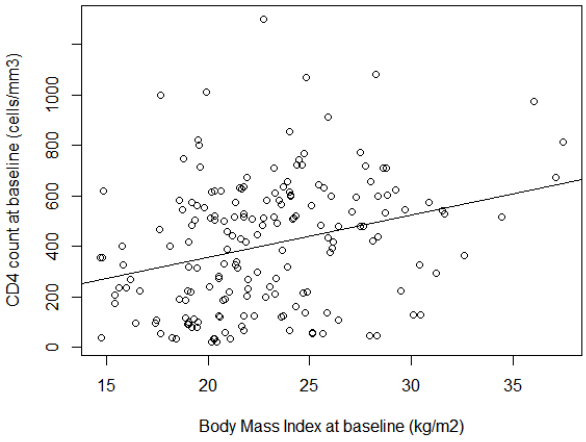


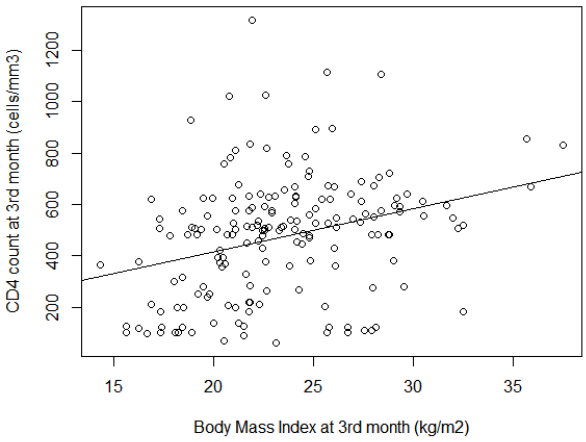

Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among HIV-infected individuals initiated on ART in Dar es Salaam, Tanzania, 2019

137x181mm (220 x 220 DPI)

**Supplementary Figure 1. Scatter plots of BMI and CD4 counts**



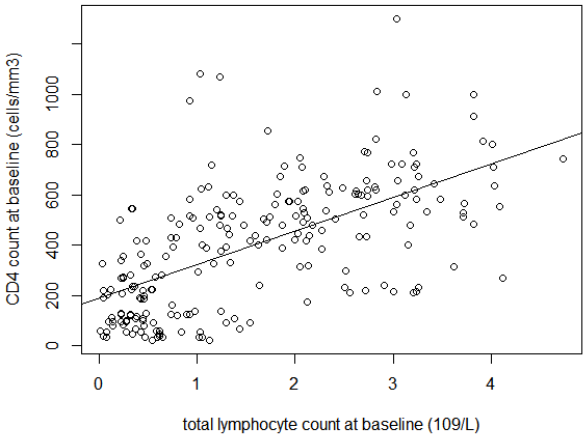

rho 0.287, p <0.0001



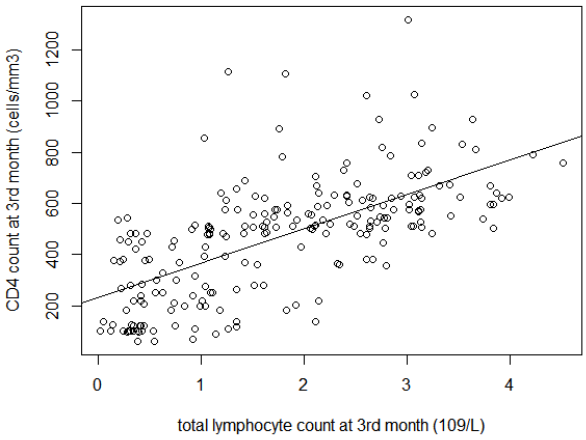

rho 0.305, p <0.0001


rho 0.373, p <0.0001

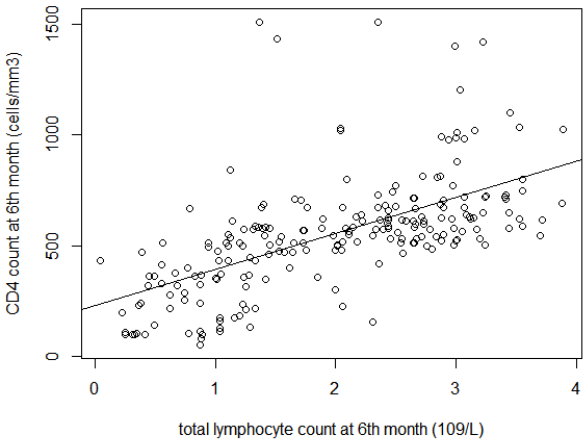
**Supplementary Figure 2. Scatter plots of total lymphocyte count and CD4 count**




rho 0.613, p <0.0001

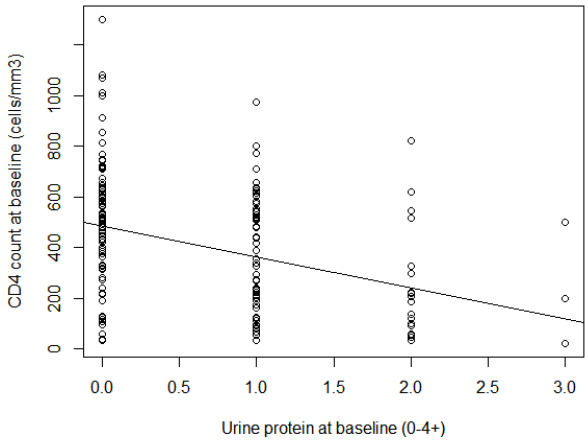



rho 0.650, p <0.0001

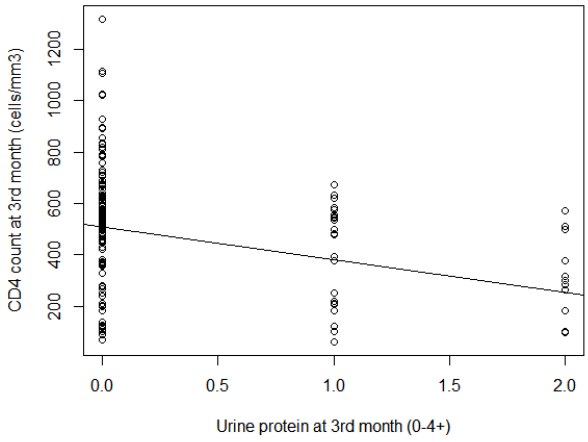



rho 0.602, p <0.0001

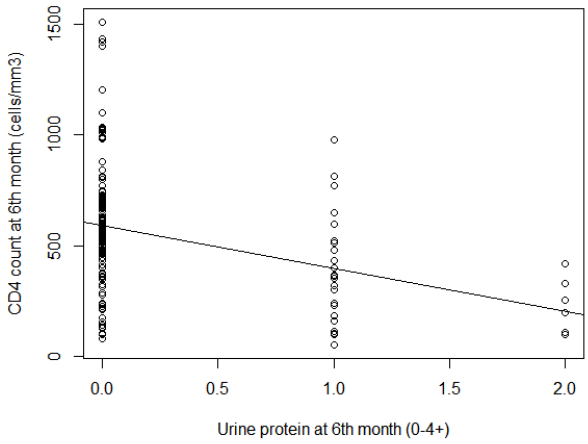
**Supplementary Figure 3. Scatter plots of urine protein and CD4 count**




rho -0.364, p <0.0001




rho -0.334, p <0.0001




rho -0.372, p <0.0001

# Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.



| | | Reporting Item | Page Number |
|---|---|---|---|
| **Title and abstract** | | | |
| Title | #1a | Indicate the study's design with a commonly used term in the title or the abstract | 1 |
| Abstract | #1b | Provide in the abstract an informative and balanced summary | 3 |

| | | | |
|---|---|---|---|
| | | of what was done and what was found | |
| **Introduction** | | | |
| Background / rationale | #2 | Explain the scientific background and rationale for the investigation being reported | 6 |
| Objectives | #3 | State specific objectives, including any prespecified hypotheses | 7 |
| **Methods** | | | |
| Study design | #4 | Present key elements of study design early in the paper | 8 |
| Setting | #5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 8-10 |
| Eligibility criteria | #6a | Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. | 8-10 |
| Eligibility criteria | #6b | For matched studies, give matching criteria and number of exposed and unexposed | - |
| Variables | #7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 9,10 |
| Data sources / measurement | #8 | For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable. | 8-10 |

| | | | |
|---|---|---|---|
| Bias | #9 | Describe any efforts to address potential sources of bias | |
| Study size | #10 | Explain how the study size was arrived at | 8 |
| Quantitative variables | #11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why | 9-11 |
| Statistical methods | #12a | Describe all statistical methods, including those used to control for confounding | 10,11 |
| Statistical methods | #12b | Describe any methods used to examine subgroups and interactions | 10, 11 |
| Statistical methods | #12c | Explain how missing data were addressed | 12 |
| Statistical methods | #12d | If applicable, explain how loss to follow-up was addressed | - |
| Statistical methods | #12e | Describe any sensitivity analyses | 11 |
| **Results** | | | |
| Participants | #13a | Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and | 12 (figure 1) |

| | | | |
|---|---|---|---|
| | | unexposed groups if applicable. | |
| Participants | #13b | Give reasons for non-participation at each stage | 12 (figure 1) |
| Participants | #13c | Consider use of a flow diagram | 12 (figure 1) |
| Descriptive data | #14a | Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. | 12,13 |
| Descriptive data | #14b | Indicate number of participants with missing data for each variable of interest | See 12 |
| Descriptive data | #14c | Summarise follow-up time (eg, average and total amount) | 12 |
| Outcome data | #15 | Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable. | 14 |
| Main results | #16a | Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 14-16 |

| | | | |
|---|---|---|---|
| Main results | #16b | Report category boundaries when continuous variables were categorized | 12-15 |
| Main results | #16c | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| - | | | |
| Other analyses | #17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 16 |
| **Discussion** | | | |
| Key results | #18 | Summarise key results with reference to study objectives | 20 |
| Limitations | #19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. | 5 |
| Interpretation | #20 | Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. | 18-20 |
| Generalisability | #21 | Discuss the generalisability (external validity) of the study results | 20 |
| **Other Information** | | | |
| Funding | #22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 22 |