

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-059193
Article Type:	Original research
Date Submitted by the Author:	11-Nov-2021
Complete List of Authors:	Munseri, Patricia; Muhimbili University of Health and Allied Sciences School of Medicine, Jassely, Lazaro; Muhimbili University of Health and Allied Sciences School of Medicine, Internal Medicine Tumaini, Basil; Muhimbili University of Health and Allied Sciences, Internal Medicine Hertzmark, Ellen; Harvard University T H Chan School of Public Health, Global Health
Keywords:	INTERNAL MEDICINE, INFECTIOUS DISEASES, HIV & AIDS < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3 1 **Body mass index, proteinuria and total lymphocyte counts in predicting treatment**
4
5 2 **responses among HIV-infected individuals initiated on antiretroviral treatment in Dar es**
6
7 3 **Salaam, Tanzania, 2019: a cohort study**
8
9

10 4
11
12
13 5 Patricia Munseri^{1*}§, Lazaro Jassely^{1*}, Basil Tumaini¹, Ellen Hertzmark²
14
15
16 6

17
18
19 7 ¹ Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es
20
21 8 Salaam, Tanzania

22
23
24 9 ² Department of Global Health and Population, Harvard University T H Chan School of Public
25
26 10 Health, Boston, MA

27
28
29
30 11
31
32 12 § Corresponding author

33
34
35 13 Email: patricia.munseri@gmail.com (PM)
36
37
38 14

39
40
41
42 15 *Shared first Author
43
44
45 16

46
47
48 17 Word count abstract: 291
49

50
51 18 Word count manuscript: 3204
52
53
54
55
56
57

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

For peer review only

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)},

1
2
3 43 lymphopaenia at six months, {adjusted RR = 4.54, 95% CI (2.19-9.39)}, and proteinuria at six
4
5 44 months {adjusted RR = 2.63, 95% CI (1.25-5.54)}.

7
8 45 **Conclusions:** Changes in body mass index, total lymphocyte count, and presence of proteinuria
9
10 46 can monitor and predict ART response and may be particularly helpful in settings when CD4
11
12 47 counts and viral load monitoring are unavailable.

13
14
15
16 48

For peer review only

1
2
3 **49 Article Summary**
4
5

6 **50 Strengths and limitations of this study**
7

- 8
9 **51** ➤ We had complete data on 98% of the originally enrolled participants.
10
11 **52** ➤ In resource-constrained situations, when viral load and CD4 testing are not easily available,
12
13 models such as ours with locally determined easily computable prediction cut-offs can be
14
15 utilized by clinicians to make clinical decisions.
16
17 **53** ➤ Our findings require validation in a study with larger sample size.
18
19 **54** ➤ Local (and time-varying) conditions and treatment standards may influence some of the
20
21 patterns we observed, both in prevalence and in effect.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58

59 Introduction

60 In 2019 about 38 million people were living with HIV globally, with 1.7 million in Tanzania
61 accounting for an estimated prevalence of 4.8% in the Tanzanian population aged 15-49 years (1).

62 Viral load testing is the recommended method for monitoring HIV treatment response (2).

63 However, viral load testing in resource-constrained settings is challenged by limited access, high
64 costs, unavailability at district levels, and in areas where available, a shortage of reagents,
65 compounded by challenges with equipment maintenance (3), as happened during the COVID-19
66 pandemic.

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

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

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

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

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

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

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

103 **Methods**

104 **Study design and population**

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

112 **Sample size estimation**

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

122 **Data collection**

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

1
2
3 125 education attained, marital status, and clinically assessed the participant's WHO HIV clinical
4
5 126 stage. A participant's baseline weight was measured using a SECA weighing scale recording to
6
7 127 the nearest 0.5 kg and height using a wall stadiometer recording to the nearest 0.5 cm. Body mass
8
9 128 index was then computed by dividing the weight in kg by the height in meters squared, the
10
11 129 interpretation of which was adapted from WHO (18).

12
13
14
15 130 About 10ml of venous blood was collected aseptically from each participant; 5ml for CD4 cell
16
17 131 counts, analysed using BD FACSCount™ (Becton Dickenson, USA) and 5ml for complete blood
18
19 132 count to obtain the total lymphocyte counts, analyzed by an auto-analyzer (Cell DNY1800 from
20
21 133 Abbott Diagnostics Division, USA). TLC was categorized as lymphopaenia ($<1 \times 10^9/L$), normal
22
23 134 lymphocyte ($1 \times 10^9/L$ to $4 \times 10^9/L$), and lymphocytosis ($>4.0 \times 10^9/L$). We assessed for proteinuria
24
25 135 by collecting about 15ml of a fresh urine sample from each participant into an empty, clean, dry
26
27 136 container and tested using CYBOW™ strips (DFI Co. Ltd, Korea). Proteinuria was categorized as
28
29 137 negative proteinuria (no proteinuria), trace or 1+ proteinuria (equivalent to 30 to 100 mg/dl), 2+
30
31 138 proteinuria (equivalent to 100 to 300 mg/dl), 3+ proteinuria (equivalent to 300 to 1000 mg/dl), and
32
33 139 4+ proteinuria (equivalent to greater than 1000 mg/dl).

34
35
36
37
38
39 140 At three and six months after ART initiation, a repeat assessment of participants was done for CD4
40
41 141 cell counts, TLC, proteinuria, and weight. At six months, an additional 5ml of blood was collected
42
43 142 from each study participant for viral load measurement using the Abbott RealTime HIV-1 assay.
44
45 143 Participants were classified as virally suppressed at six months after ART initiation if their HIV
46
47 144 viral load was <1000 copies/ml, according to Tanzania HIV treatment guidelines. Levels and
48
49 145 changes in BMI, lymphocytes, and proteinuria were compared between participants who were HIV
50
51 146 suppressed and that of HIV not suppressed.

1
2
3 147 BMI was considered to have changed between one time point and another if it increased or
4
5 148 decreased by over 5%. BMI changes from ART initiation to six months were categorized into three
6
7
8 149 groups as (i) loss of more than 5%, (ii) stable (change between -5% and 5%, or (iii) gain of more
9
10 150 than 5%. The TLC were categorized as (i) lymphopaenia $< 1 \times 10^9$ cells/L, (ii) normal lymphocyte
11
12 151 count $1-4 \times 10^9$ cells/L (iii) Lymphocytosis $> 4 \times 10^9$ cells/L. The TLC pattern change was
13
14 152 categorized as (i) lymphopaenic at 6 months, regardless of the status at baseline or three months;
15
16 153 (ii) increased from lymphopaenic to normal or higher; (iii) stayed normal or higher (no
17
18 154 lymphopaenia seen). Urinary protein patterns were categorized as (i) proteinuria at six months
19
20 155 regardless of baseline proteinuria status; (ii) proteinuria at baseline and/or third month but not six
21
22 156 months; and (iii) no proteinuria seen.
23
24
25

26 157 **Patient and public involvement**

27
28
29 158 Patients or members of the public were not involved in the design, or conduct, or reporting, or
30
31 159 dissemination plans of the research.
32
33
34

35 160 **Statistical methods**

36
37
38 161 Data were analysed using IBM® SPSS® Statistics version 26, R, and SAS version 9.4 (Cary, NC).
39
40 162 Categorical variables such as age group, sex, marital status, level of education, occupation,
41
42 163 categories of BMI, CD4 count, proteinuria, TLC, BMI change, TLC change, and proteinuria
43
44 164 change were summarized as frequencies and proportions. Continuous variables such as age, BMI,
45
46 165 and CD4 count were summarized as means and standard deviations. When necessary, small groups
47
48 166 were combined for analysis. To determine the association between BMI, TLC or urine protein to
49
50 167 CD4 count, we used correlation.
51
52
53
54
55
56
57
58
59
60

1
2
3 168 To determine the relationships between individual predictors and viral non-suppression at six
4
5 169 months, we first used modified Poisson regression for univariable analysis, to determine which
6
7
8 170 variables to include in the multivariable model. For multivariable prediction, all predictors in the
9
10 171 univariable model with a p-value of <0.2 and age, a known confounder, were entered into the
11
12 172 modified Poisson regression model. The results of the Poisson regression model were presented
13
14 173 as relative risk and 95% confidence interval (RR; 95% CI). To determine the test characteristics
15
16 174 (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)) of a
17
18
19 175 score based on the multivariable model, we used two cut-off levels, based on the first quartile and
20
21 176 median of the score among the non-suppressed. The score was the sum of the rounded coefficients
22
23 177 for the variables for which the confidence intervals did not include 1 in a model containing only
24
25 178 these variables. Since these all rounded to 1, this is equivalent to simply counting the number of
26
27
28 179 these characteristics.

30
31 180 Based on practices in low resourced clinics, communication with the patient and the decision to
32
33 181 change the ART regimen depends on the patient's virological status at six months. CD4 cell counts
34
35 182 depend on a blood sample collected at the six-month visit and are therefore unavailable for
36
37
38 183 immediate decision making. We, therefore, excluded all CD4 variables from the model and used
39
40 184 parameters available at the time of the six-month visit to predict viral non-suppression.
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57

185

Results

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

191

192

193 **Figure 1. Consort diagram.**

194

195

196 **Baseline characteristics of study participants**

197 Of the 215 participants analysed, the mean age (SD) was 37.1 ± 11.5 years, 146 (68%) were female,
 198 113 (53%) were never married, 45 (21%) had secondary education or higher, and 117 (54%) were
 199 unemployed (Table 1). Most participants, 59.5%, had normal BMI, while 27% were overweight,
 200 and 13% were underweight. Most participants, 113 (62%), were in WHO HIV clinical stage I, and
 201 only eight (4%) were in stage IV, though 93 (43%) had CD4 counts of 350 cells/ml or below; 83
 202 (39%) were lymphopaenic, and 111 (52%) had proteinuria at baseline.

203

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

Characteristic	n (%)	Mean \pm 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/m²)			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/mm³)			401 ± 253
	<200	55 (25.6%)	
	200-350	38 (17.7%)	
	351-500	39 (18.1%)	
	>500	83 (38.6%)	
Lymphocyte counts (x10⁹cells/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

207

208

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

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

212 Univariable and multivariable analysis by modified Poisson regression.

213

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

217

218 **Predictors of viral non-suppression at six months among HIV-infected participants** 219 **initiated on ART**

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

1
2
3 227 among participants with proteinuria at six months {RR = 2.63; 95% CI (1.25-5.54)}, Table 2. The
4
5 228 area under the Receiver Operating Characteristic (ROC) curve for the multivariable model was
6
7
8 229 0.895 (Fig 2). Baseline HIV stage was not related to HIV non-suppression at six months, {adjusted
9
10 230 RR = 1.14, 95% CI (0.63-2.08)} for baseline WHO HIV clinical stage II and {adjusted RR = 0.82,
11
12 231 95% CI (0.51-1.31)} for advanced baseline WHO HIV clinical stages (III and IV)}.

13
14
15 232 Using the rounded coefficients of the three variables in a model containing only these variables,
16
17 233 which all rounded to 1, we made a “prediction score” with values 0 (n=154, of which 10 were non-
18
19 234 suppressed), 1 (n=36, 13 non-suppressed), 2 (n=17, 15 non-suppressed), and 3 (n=8, all non-
20
21 235 suppressed). The median value of this score among the non-suppressed was 1.5 and the first
22
23 236 quartile value was 1. Thus, having any 2 of the risk factors would be a conservative predictor of
24
25 237 non-suppression, and having any one would be less conservative.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 238 **Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease,**
4
5 239 **proteinuria, and total lymphocyte counts to predict viral non-suppression among HIV-**
6
7 240 **infected individuals initiated on ART in Dar es Salaam, Tanzania, 2019**
8
9
10
11 241
12
13
14 242

15
16
17 243 Using the median score among the non-suppressed as a cut-off (equivalent to having any two of
18
19 244 the components), the sensitivity predicting non-suppression was 0.50, and the specificity was 0.99.
20
21 245 Only 12% of the study population met this criterion. When we lowered the cut-off scores to the
22
23 246 first quartile value among the non-suppressed (i.e. any one of the components), the sensitivity was
24
25 247 0.78 and the specificity was 0.85, with 28% of the study population meeting this criterion.
26
27
28
29
30 248

249 Discussion

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

259 Baseline WHO HIV clinical stage did not help predict HIV non-suppression at six months,
260 possibly because under the current "Test and Treat" strategy (19), most individuals initiating ART
261 are in WHO HIV clinical stages I and II (76% of our sample). Current therapy is highly effective
262 except for a few patients whose disease is so advanced that they die before the medication can
263 improve their immune status (2 patients in this study). Symptomatic individuals with advanced
264 HIV will likely adhere to treatment to alleviate their symptoms. Perhaps patients with advanced
265 disease fear death and therefore are motivated to adhere to ART, with eventual viral suppression.
266 Advanced HIV disease has been shown to be linked with ART adherence (20). Some studies,
267 however, indicate that early HIV stages are linked with high ART adherence and viral suppression
268 (21).

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

1
2
3 272 HIV non-suppression irrespective of a participant's lymphocyte count (normal or low) before ART
4
5 273 initiation. CD4 cells are a subset of lymphocytes and therefore lymphopaenia could be indicative
6
7 274 of a decline in CD4 cells, as a result of high HIV replication with high viral load signifying viral
8
9 275 non-suppression with consequent CD4 cell destruction. Lymphopaenia was significantly
10
11 276 associated with CD4 <500 cells/mm³ at all time points. In this study, an increase in total
12
13 277 lymphocyte cell count from lymphopaenia to normal lymphocyte counts from baseline to six
14
15 278 months was significantly associated with an increase in CD4 cell count (Additional file 1). Total
16
17 279 lymphocyte count is sensitive and specific in predicting CD4 cell counts (16,23) though there have
18
19 280 been contradictory reports (23). The assessment of total lymphocyte counts among patients on
20
21 281 ART, therefore, could serve as an alternative, especially in settings with limited availability of
22
23 282 CD4 cell count measurement. A drop in total lymphocyte count in such a setting can alert a
24
25 283 clinician to the likelihood of immunological failure.

26
27 284 Weight loss with consequent BMI drop of 5% or more at six months while on ART from weight
28
29 285 prior to ART initiation, predicts HIV non-suppression, though HIV non-suppression was not
30
31 286 associated with being underweight prior to ART initiation, perhaps because of the low prevalence
32
33 287 of underweight leading to low power. In this study, sustained weight gain was significantly
34
35 288 associated with viral suppression and sustained weight loss was associated with viral non-
36
37 289 suppression at six months of ART. An increase in weight and hence BMI may be a sign of immune
38
39 290 status improvement signalling a return to health (24) and improved survival (25), while a decrease
40
41 291 in weight and hence BMI may be a sign of HIV progression or decline in CD4 cell counts (5,11,27).
42
43 292 Weight loss may be due to HIV itself, opportunistic infections, or HIV-associated tumours. Weight
44
45 293 loss in both ART naïve and exposed patients has been associated with increased morbidity and
46
47 294 mortality (28,29,30). A study in England observed that each log₁₀ increase in HIV viral load was

1
2
3 295 associated with a 0.92 kg decrease in body weight. However, a decrease in viral load was not
4
5 296 significantly associated with weight gain, contrary to our study (30). Since weight changes
6
7
8 297 correlate with the virological response, losing weight should be viewed as an alarming sign of
9
10 298 virological failure. Monitoring of weight and body mass index prior to ART initiation and during
11
12 299 follow up is a valuable inexpensive way of identifying individuals with possible treatment failure.
13
14
15 300 Half of the ART-naïve study participants had proteinuria, similar to a report from Zimbabwe (31).
16
17 301 The presence of proteinuria was associated with HIV non-suppression. Proteinuria at six months
18
19 302 was a strong predictor for HIV non-suppression. Proteinuria in HIV-infected individuals is
20
21 303 attributed to a direct effect of HIV on the glomerular and tubular epithelial cells with the
22
23 304 progression of HIV disease to AIDS, nephrotoxicity from ART, the progression of chronic kidney
24
25 305 disease and death (14,33). The higher the viral load, the greater the damage to the kidney (33). We
26
27 306 observed a significant proportion of participants with proteinuria of 2+ or above in WHO HIV
28
29 307 clinical stage IV. This underscores the fact that, as HIV disease advances, the greater the damage
30
31 308 to the kidneys as evidenced by increasing proteinuria. Therefore, routine screening for renal
32
33 309 disease may serve not only as a follow-up of renal disease progression but also for HIV treatment
34
35 310 response monitoring.
36
37
38
39
40
41
42
43

312 **Conclusion**

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

1
2
3 317 **List of abbreviations**
4
5

6 318 AIDS: Acquired immunodeficiency syndrome
7

8
9 319 ART: Antiretroviral therapy
10

11
12 320 BMI: Body mass index
13

14
15 321 CD4: Cluster of differentiation 4
16

17
18 322 HIV: Human immunodeficiency virus
19

20
21 323 TLC: Total lymphocyte counts
22

23
24 324 WHO: World Health Organization
25

26
27 325
28

29
30 326
31

32
33 327 **Acknowledgements**
34

35
36 328 We are grateful to the participants for their willingness to take part in this study and to the health
37

38 329 workers from Temeke regional referral hospital, Mbagala Rangi-tatu district hospital, and
39

40 330 Mbagala Kizuiani dispensary for their assistance in participant recruitment and data collection.
41

42
43 331 **Author Contributions**
44

45
46 332 Study design: LJ and PM; data collection: LJ and PM; Data analysis and interpretation: LJ, PM,
47

48 333 BT and EH; Manuscript writing: LJ, PM, BT and EH. All the authors gave final approval of the
49

50
51 334 manuscript.
52

53
54 335
55
56
57

1
2
3 336 **Funding**
4
5

6 337 This research received no specific grant from any funding agency in the public, commercial or
7
8 338 not-for-profit sectors.
9

10
11 339 **Competing interests**
12
13

14 340 None declared.
15

16
17 341 **Patient consent for publication**
18

19
20 342 Not applicable.
21
22

23 343 **Ethics approval**
24

25
26 344 Ethical approval was obtained from the Research and Publications Committee of Muhimbili
27
28 345 University of Health and Allied Sciences (Ref. No. DA.287/298/01A). Permission to conduct the
29
30 346 study was obtained from Temeke Municipal Hospital administration. Participants were enrolled
31
32 347 after providing written informed consent. The confidentiality of patient information was ensured.
33
34 348 Participants without viral suppression at the 6th month of follow up were managed according to
35
36 349 Tanzania National Guidelines for management of HIV and AIDS.
37
38
39

40
41 350 **Data availability statement**
42

43
44 351 The dataset analysed during the current study is available upon reasonable request to the
45
46 352 corresponding author.
47
48

49 353 **ORCID iDs**
50

51
52 354 Basil Tumaini: <https://orcid.org/0000-0002-2894-1684>
53

54 355 Ellen Hertzmark: <https://orcid.org/0000-0003-0148-2761>
55
56
57

1
2
3 356 **Ethics Statement**
4

5
6 357 Muhimbili University of Health and Allied Sciences Institutional Review Board with reference
7
8 358 number DA287/298/01A/
9

10 359

11
12 360 **References**
13

- 14
15 361 1. UNAIDS. UNAIDS data 2020 | UNAIDS [Internet]. 2020 [cited 2020 Aug 19].
16
17 362 <https://www.unaids.org/en/resources/documents/2020/unaids-data>
18
19
20 363 2. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for
21
22 364 treating and preventing HIV infection: recommendations for a public health approach.
23
24 365 World Health Organization; 2016.
25
26 366 https://apps.who.int/iris/bitstream/handle/10665/208825/9789241549684_eng.pdf
27
28 367 3. Roberts T, Bygrave H, Fajardo E, Ford N. Challenges and opportunities for the
29
30 368 implementation of virological testing in resource-limited settings. *Journal of the*
31
32 369 *International AIDS Society.* 2012;15(2):17324. <https://doi.org/10.7448/IAS.15.2.17324>
33
34 370 PMID: 23078767
35
36 371 4. Sepkowitz KA. AIDS - the first 20 years. *New England Journal of Medicine.*
37
38 372 2001;344(23):1764-72. <https://www.nejm.org/doi/full/10.1056/NEJM200106073442306>
39
40 373 PMID: 11396444
41
42 374 5. Mangili A, Murman DH, Zampini AM, Wanke CA, Mayer KH. Nutrition and HIV
43
44 375 infection: review of weight loss and wasting in the era of highly active antiretroviral
45
46 376 therapy from the nutrition for healthy living cohort. *Clinical Infectious Diseases.*
47
48 377 2006;42(6):836-42. <https://doi.org/10.1086/500398> PMID: 16477562
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 378 6. Koethe JR, Lukusa A, Giganti MJ, Chi BH, Nyirenda CK, Limbada MI, et al. Association
4
5 379 between weight gain and clinical outcomes among malnourished adults initiating
6
7 380 antiretroviral therapy in Lusaka, Zambia. *Journal of Acquired Immune Deficiency*
8
9 381 *Syndromes*. 2010;53(4):507-13.
10
11 382 [https://journals.lww.com/jaids/Fulltext/2010/04010/Association_Between_Weight_Gain_](https://journals.lww.com/jaids/Fulltext/2010/04010/Association_Between_Weight_Gain_and_Clinical.12.aspx)
12
13 383 [and_Clinical.12.aspx](https://journals.lww.com/jaids/Fulltext/2010/04010/Association_Between_Weight_Gain_and_Clinical.12.aspx) PMID: 19730111
14
15
16
17 384 7. Mannheimer S, Friedland G, Matts J, Child C, Chesney M. The consistency of adherence
18
19 385 to antiretroviral therapy predicts biologic outcomes for Human Immunodeficiency Virus -
20
21 386 infected persons in clinical trials. *Clinical Infectious Diseases*. 2002;34(8):1115-21.
22
23 387 <https://doi.org/10.1086/339074> PMID: 11915001
24
25
26
27 388 8. Paton NI, Sangeetha S, Earnest A, Bellamy R. The impact of malnutrition on survival and
28
29 389 the CD4 count response in HIV-infected patients starting antiretroviral therapy. *HIV*
30
31 390 *medicine*. 2006;7(5):323-30. <https://doi.org/10.1111/j.1468-1293.2006.00383.x> PMID:
32
33 391 16945078
34
35
36
37 392 9. Wittkop L, Arsandaux J, Trevino A, Schim van der Loeff M, Anderson J, van Sighem A,
38
39 393 et al. CD4 cell count response to first-line combination ART in HIV-2+ patients compared
40
41 394 with HIV-1+ patients: a multinational, multicohort European study. *Journal of*
42
43 395 *Antimicrobial Chemotherapy*. 2017;72(10):2869-78. <https://doi.org/10.1093/jac/dkx210>
44
45 396 PMID: 29091198
46
47
48
49 397 10. Kanya MR, Mayanja-Kizza H, Kambugu A, Bakeera-Kitaka S, Semitala F, Mwebaze-
50
51 398 Songa P, et al. Predictors of long-term viral failure among Ugandan children and adults
52
53 399 treated with antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes*.

- 1
2
3 400 2007;46(2):187-93. PMID: 17693883
4
5
6 401 11. Olaleye AO, Owhonda G, Daramola O, Adejo I, Olayiwola H, Inyang JI, et al. Factors
7
8 402 associated with weight gain among adult patients initiating antiretroviral therapy in Port
9
10 403 Harcourt, Nigeria: a retrospective cohort study. *Infectious Diseases*. 2017;49(8):635-8.
11
12 404 <https://doi.org/10.1080/23744235.2017.1306102> PMID: 28335659
13
14
15
16 405 12. Liu E, Spiegelman D, Semu H, Hawkins C, Chalamilla G, Aveika A, et al. Nutritional
17
18 406 status and mortality among HIV-infected patients receiving antiretroviral therapy in
19
20 407 Tanzania. *Journal of Infectious Diseases*. 2011;204(2):282-90.
21
22 408 <https://doi.org/10.1093/infdis/jir246> PMID: 21673040
23
24
25
26 409 13. Husain NE, Ahmed MH, Almobarak AO, Noor SK, Elmadhoun WM, Awadalla H, et al.
27
28 410 HIV-associated nephropathy in Africa: pathology, clinical presentation and strategy for
29
30 411 prevention. *Journal of Clinical Medicine Research*. 2018;10(1):1-8.
31
32 412 <https://doi.org/10.14740/jocmr3235w> PMID: 29238427
33
34
35
36 413 14. Szczech LA, Hoover DR, Feldman JG, Cohen MH, Gange SJ, Goozé L, et al. Association
37
38 414 between renal disease and outcomes among HIV-infected women receiving or not
39
40 415 receiving antiretroviral therapy. *Clinical Infectious Diseases*. 2004;39(8):1199-206.
41
42 416 <https://doi.org/10.1086/424013> PMID: 15486845
43
44
45
46 417 15. Imlach S, McBreen S, Shirafuji T, Leen C, Bell JE, Simmonds P. Activated peripheral
47
48 418 CD8 lymphocytes express CD4 in vivo and are targets for infection by Human
49
50 419 Immunodeficiency Virus Type 1. *Journal of Virology*. 2001;75(23):11555-64.
51
52 420 <https://doi.org/10.1128/JVI.75.23.11555-11564.2001> PMID: 11689637
53
54
55
56 421 16. Obirikorang C, Quaye L, Acheampong I. Total lymphocyte count as a surrogate marker

- 1
2
3 422 for CD4 count in resource-limited settings. BMC Infectious Diseases. 2012;12:128.
4
5 423 <https://doi.org/10.1186/1471-2334-12-128> PMID: 22676809
6
7
8 424 17. The United Republic of Tanzania; Ministry of Health, Community Development, Gender,
9
10 425 Elderly and Children; National AIDS Control Programme. National guidelines for the
11
12 426 management of HIV and AIDS. 6th Ed, 2017. Available from:
13
14 427 [https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj5](https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/Tanzania_NATIONAL_GUIDELINES_FOR_MANAGEMENT_OF_HIV_AND_AIDS_6TH_EDITION_2017.pdf)
15
16 428 [8iR37uA/File/Tanzania_NATIONAL GUIDELINES FOR MANAGEMENT OF HIV](https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/Tanzania_NATIONAL_GUIDELINES_FOR_MANAGEMENT_OF_HIV_AND_AIDS_6TH_EDITION_2017.pdf)
17
18 429 [AND AIDS 6TH EDITION 2017.pdf](https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/Tanzania_NATIONAL_GUIDELINES_FOR_MANAGEMENT_OF_HIV_AND_AIDS_6TH_EDITION_2017.pdf)
19
20
21
22
23 430 18. World Health Organization. Obesity: preventing and managing the global epidemic. WHO
24
25 431 Technical Report Series. 2000(894).
26
27 432 https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/
28
29
30 433 19. WHO. The use of antiretroviral drugs for treating and preventing HIV infection guidelines
31
32 434 HIV/AIDS Programme. 2016 [cited 2021 Jan 19].
33
34 435 http://apps.who.int/iris/bitstream/10665/85322/1/WHO_HIV_2013.7_eng.pdf
35
36
37
38 436 20. Biressaw S, Abegaz WE, Abebe M, Taye WA, Belay M. Adherence to Antiretroviral
39
40 437 Therapy and associated factors among HIV infected children in Ethiopia: Unannounced
41
42 438 home-based pill count versus caregivers' report. BMC Pediatrics. 2013;13:132.
43
44 439 <https://doi.org/10.1186/1471-2431-13-132> PMID: 24229394
45
46
47
48 440 21. Haberer JE, Bwana BM, Orrell C, Asiimwe S, Amanyire G, Musinguzi N, et al. ART
49
50 441 adherence and viral suppression are high among most non-pregnant individuals with
51
52 442 early-stage, asymptomatic HIV infection: an observational study from Uganda and South
53
54 443 Africa. Journal of the International AIDS Society. 2019;22(2):e25232.
55
56
57

- 1
2
3 444 <https://doi.org/10.1002/jia2.25232> PMID: 30746898
4
5
6 445 22. Kwantwi LB, Tunu BK, Boateng D, Quansah DY. Body Mass Index, Haemoglobin, and
7
8 446 Total Lymphocyte Count as a Surrogate for CD4 Count in Resource Limited Settings. *J*
9
10
11 447 *Biomarkers*. 2017; 2017: 7907352.
12
13 448 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5412137/> PMID: 28484663
14
15
16 449 23. Akinola NO, Olasode O, Adediran IA, Onayemi O, Murainah A, Irinoye O, et al. The
17
18 450 search for a predictor of CD4 cell count continues: Total lymphocyte count is not a
19
20 451 substitute for CD4 cell count in the management of HIV-infected individuals in a
21
22 452 resource-limited setting. *Clinical Infectious Diseases*. 2004;39(4):579–81.
23
24
25 453 <https://doi.org/10.1086/422722> PMID: 15356826
26
27
28 454 24. Koethe JR, Jenkins CA, Lau B, Shepherd BE, Wester W, Rebeiro PF, et al. Higher time-
29
30 455 updated body mass index: association with improved CD4+ cell recovery on HIV
31
32 456 treatment. *Journal of Acquired Immune Deficiency Syndromes*. 2016;73(2):197-204.
33
34 457 <https://dx.doi.org/10.1097%2FQAI.0000000000001035> PMID: 27116044
35
36
37
38 458 25. Biadgilign S, Reda AA, Digaffe T. Predictors of mortality among HIV infected patients
39
40 459 taking antiretroviral treatment in Ethiopia: a retrospective cohort study. *AIDS research*
41
42 460 *and therapy*. 2012;9:15. <https://doi.org/10.1186/1742-6405-9-15> PMID: 22606951
43
44
45 461 26. Reda AA, Biadgilign S, Deribew A, Gebre B, Deribe K. Predictors of Change in CD4
46
47 462 Lymphocyte Count and Weight among HIV Infected Patients on Anti-Retroviral
48
49 463 Treatment in Ethiopia: A Retrospective Longitudinal Study. Ensoli B, editor. *PLoS One*.
50
51 464 2013;8(4):e58595. <https://doi.org/10.1371/journal.pone.0058595> PMID: 23573191
52
53
54
55 465 27. Naidoo K, Yende-Zuma N, Augustine S. A retrospective cohort study of body mass index
56
57

- 1
2
3 466 and survival in HIV infected patients with and without TB co-infection. Infectious
4
5 467 Diseases of Poverty. 2018;7:35. <https://doi.org/10.1186/s40249-018-0418-3> PMID:
6
7 468 29690932
8
9
10
11 469 28. Van der Sande MA, van der Loeff MF, Aveika AA, Sabally S, Togun T, Sarge-Njie R, et
12
13 470 al. Body mass index at time of HIV diagnosis: a strong and independent predictor of
14
15 471 survival. *Journal of Acquired Immune Deficiency Syndromes*. 2004;37(2):1288-94.
16
17 472 <https://doi.org/10.1097/01.qai.0000122708.59121.03> PMID: 15385737
18
19
20
21 473 29. Grinspoon S, Mulligan K. Weight loss and wasting in patients infected with Human
22
23 474 Immunodeficiency Virus. *Clinical Infectious Diseases*. 2003;36(Supplement_2):S69-78.
24
25 475 <https://doi.org/10.1086/367561> PMID: 12652374
26
27
28 476 30. Mwamburi DM, Wilson IB, Jacobson DL, Spiegelman D, Gorbach SL, Knox TA, et al.
29
30 477 Understanding the role of HIV load in determining weight change in the era of highly
31
32 478 active antiretroviral therapy. *Clinical Infectious Diseases*. 2005;40(1):167-73.
33
34 479 <https://doi.org/10.1086/426591> PMID: 15614708
35
36
37
38 480 31. Fana GT, Ndhlovu CE. Renal dysfunction among anti-retroviral therapy naïve HIV
39
40 481 infected patients in Zimbabwe. *The Central African Journal of Medicine*. 2011;57(1-4):1-
41
42 482 5. <https://www.ajol.info/index.php/cajm/article/view/73724> PMID: 24968654
43
44
45
46 483 32. Gupta SK, Smurzynski M, Franceschini N, Bosch RJ, Szczech LA, Kalayjian RC. The
47
48 484 effects of HIV-1 viral suppression and non-viral factors on quantitative proteinuria in the
49
50 485 HAART era. *Antiviral Therapy*. 2009;14(4): 543–549. PMID: 19578239
51
52
53
54 486 33. Rednor SJ, Ross MJ. Molecular mechanisms of injury in HIV-associated nephropathy.
55
56 487 *Frontiers in Medicine*. 2018;5:177. <https://doi.org/10.3389/fmed.2018.00177> PMID:

1
2
3 488 29930940
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

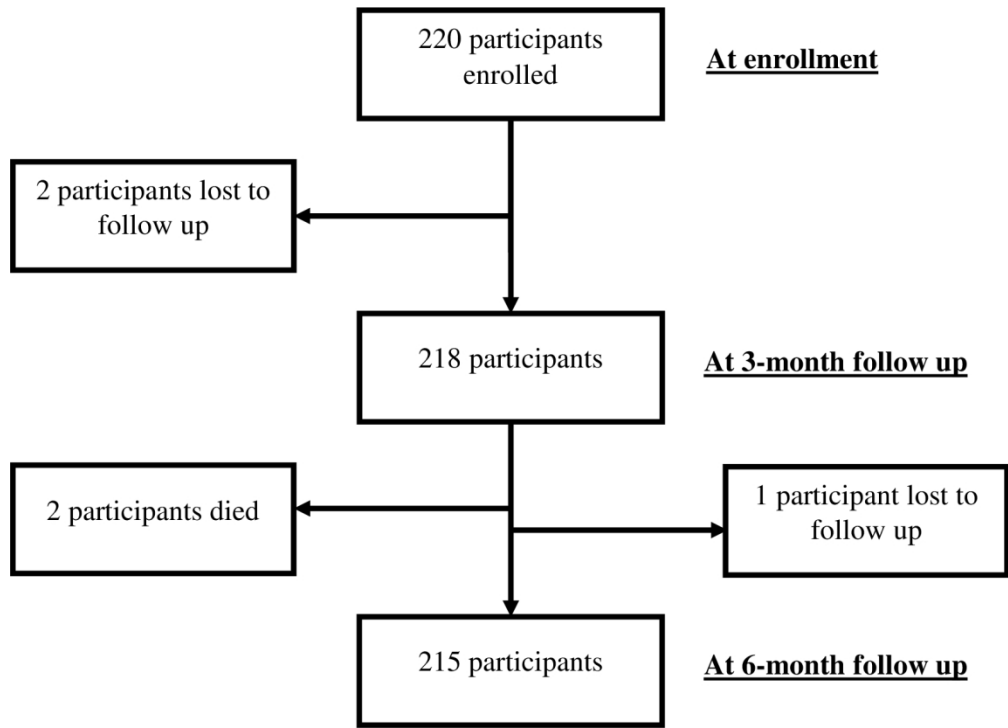


Figure 1. Consort diagram.
146x105mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

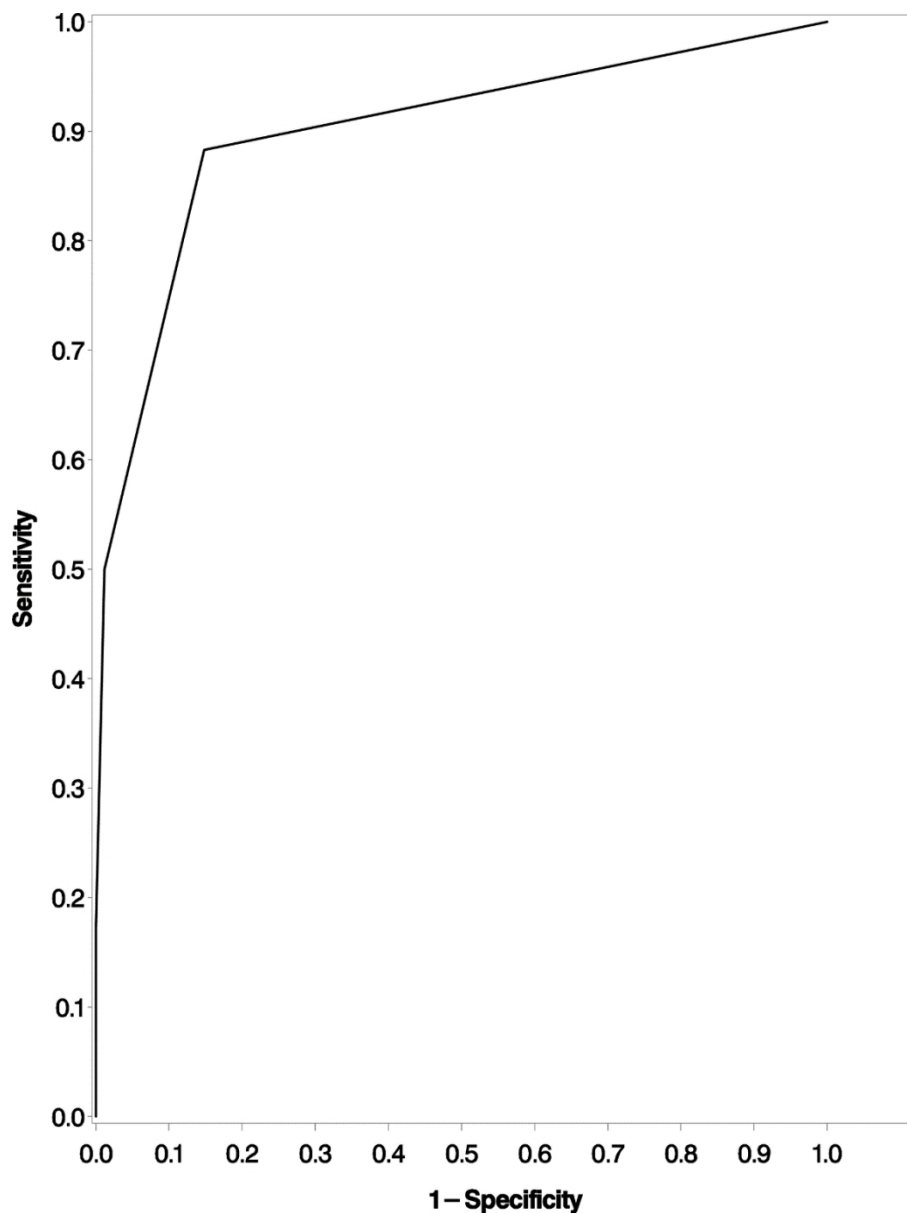
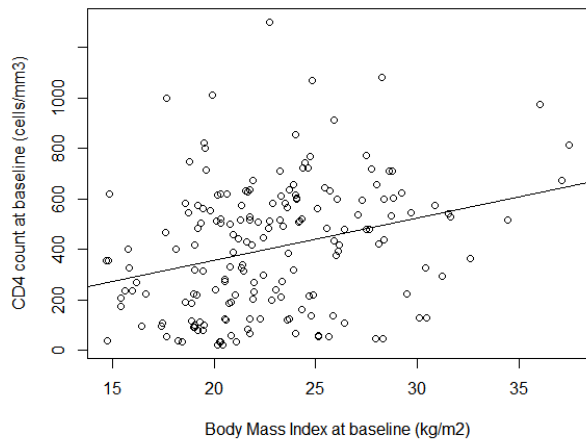


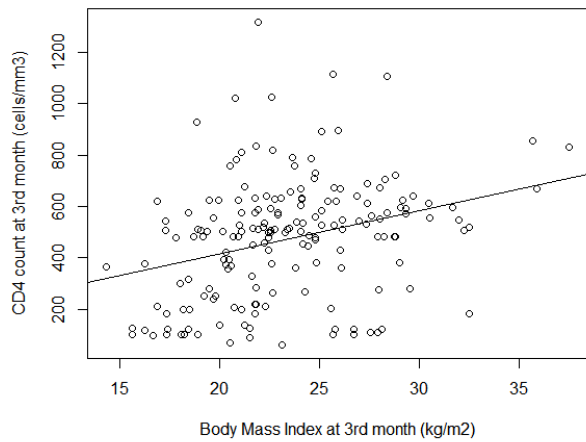
Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among HIV-infected individuals initiated on ART in Dar es Salaam, Tanzania, 2019

137x181mm (220 x 220 DPI)

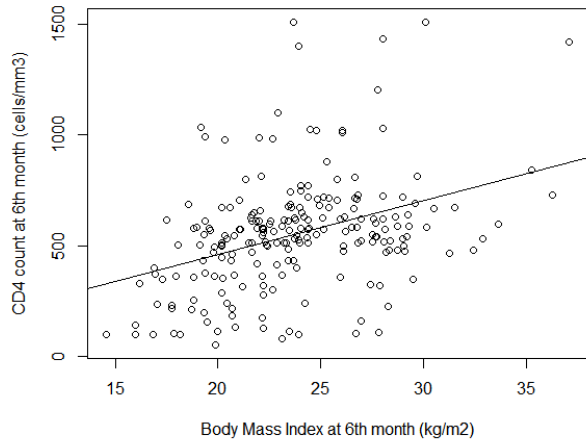
Supplementary Figure 1. Scatter plots of BMI and CD4 counts



rho 0.287, p <0.0001



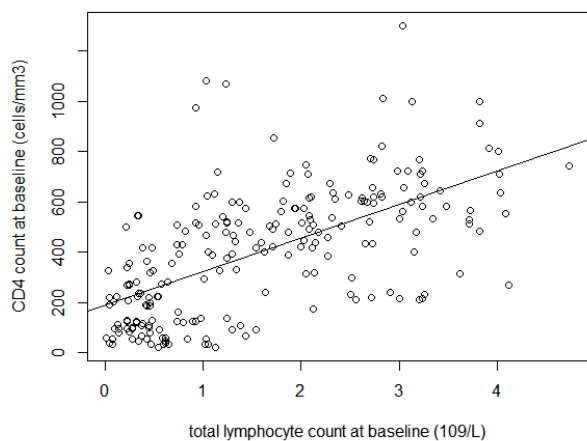
rho 0.305, p <0.0001



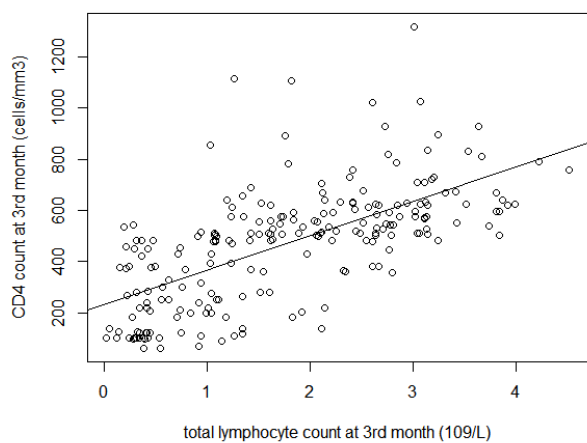
rho 0.373, p <0.0001

view only

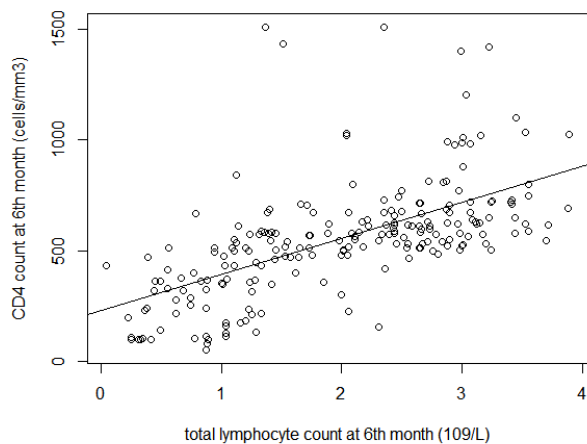
Supplementary Figure 2. Scatter plots of total lymphocyte count and CD4 count



rho 0.613, p <0.0001



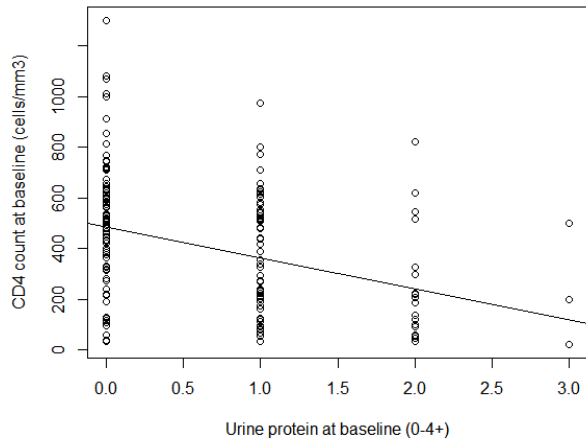
rho 0.650, p <0.0001



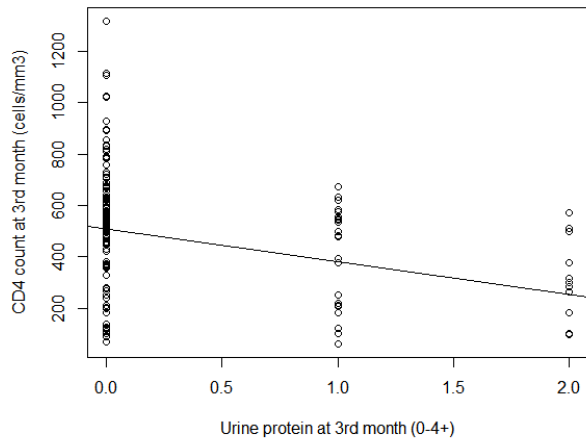
rho 0.602, p <0.0001

view only

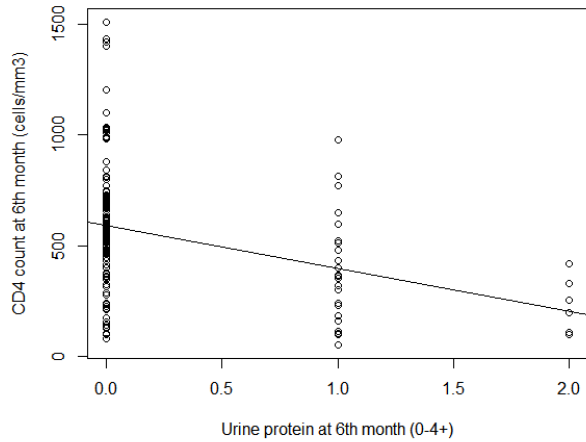
Supplementary Figure 3. Scatter plots of urine protein and CD4 count



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 cohort reporting guidelines, and cite them as:

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

			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

1	Bias	#9	Describe any efforts to address potential sources of bias	
2				
3				
4	Study size	#10	Explain how the study size was arrived at	8
5				
6				
7	Quantitative	#11	Explain how quantitative variables were handled in the	9-11
8	variables		analyses. If applicable, describe which groupings were chosen,	
9			and why	
10				
11				
12				
13				
14				
15	Statistical	#12a	Describe all statistical methods, including those used to control	
16	methods		for confounding	
17				
18				
19				
20	10,11			
21				
22				
23	Statistical	#12b	Describe any methods used to examine subgroups and	10, 11
24	methods		interactions	
25				
26				
27				
28				
29	Statistical	#12c	Explain how missing data were addressed	12
30	methods			
31				
32				
33				
34	Statistical	#12d	If applicable, explain how loss to follow-up was addressed	-
35	methods			
36				
37				
38				
39	Statistical	#12e	Describe any sensitivity analyses	
40	methods			
41				
42				
43				
44				
45	11			
46				
47				
48	Results			
49				
50				
51	Participants	#13a	Report numbers of individuals at each stage of study—eg	12
52			numbers potentially eligible, examined for eligibility, confirmed	(figure 1)
53			eligible, included in the study, completing follow-up, and	
54			analysed. Give information separately for for exposed and	
55				
56				
57				
58				
59				
60				

unexposed groups if applicable.

1			
2			
3			
4	Participants	#13b	Give reasons for non-participation at each stage
5			
6			12
7			(figure 1)
8			
9	Participants	#13c	Consider use of a flow diagram
10			
11			
12	12 (figure 1)		
13			
14			
15	Descriptive data	#14a	Give characteristics of study participants (eg demographic,
16			clinical, social) and information on exposures and potential
17			confounders. Give information separately for exposed and
18			unexposed groups if applicable.
19			
20			
21			
22			
23			
24			
25	Descriptive data	#14b	Indicate number of participants with missing data for each
26			variable of interest
27			
28			
29			
30	See 12		
31			
32			
33	Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)
34			
35			
36	12		
37			
38			
39			
40	Outcome data	#15	Report numbers of outcome events or summary measures
41			over time. Give information separately for exposed and
42			unexposed groups if applicable.
43			
44			
45			
46			
47	14		
48			
49			
50	Main results	#16a	Give unadjusted estimates and, if applicable, confounder-
51			adjusted estimates and their precision (eg, 95% confidence
52			interval). Make clear which confounders were adjusted for and
53			why they were included
54			
55			
56			
57			
58			
59			
60			

1	Main results	#16b	Report category boundaries when continuous variables were	12-15
2			categorized	
3				
4				
5				
6	Main results	#16c	If relevant, consider translating estimates of relative risk into	
7			absolute risk for a meaningful time period	
8				
9				
10				
11				
12	-			
13				
14				
15	Other analyses	#17	Report other analyses done—eg analyses of subgroups and	16
16			interactions, and sensitivity analyses	
17				
18				
19				
20	Discussion			
21				
22				
23	Key results	#18	Summarise key results with reference to study objectives	20
24				
25				
26	Limitations	#19	Discuss limitations of the study, taking into account sources of	5
27			potential bias or imprecision. Discuss both direction and	
28			magnitude of any potential bias.	
29				
30				
31				
32				
33				
34	Interpretation	#20	Give a cautious overall interpretation considering objectives,	18-20
35			limitations, multiplicity of analyses, results from similar studies,	
36			and other relevant evidence.	
37				
38				
39				
40				
41				
42	Generalisability	#21	Discuss the generalisability (external validity) of the study	20
43			results	
44				
45				
46				
47	Other Information			
48				
49				
50	Funding	#22	Give the source of funding and the role of the funders for the	22
51			present study and, if applicable, for the original study on which	
52			the present article is based	
53				
54				
55				
56				
57				
58				
59				
60				

1 None The STROBE checklist is distributed under the terms of the Creative Commons Attribution
2 License CC-BY. This checklist can be completed online using <https://www.goodreports.org/>, a tool
3 made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-059193.R1
Article Type:	Original research
Date Submitted by the Author:	02-Apr-2022
Complete List of Authors:	Munseri, Patricia; Muhimbili University of Health and Allied Sciences School of Medicine, Jassely, Lazaro; Muhimbili University of Health and Allied Sciences School of Medicine, Internal Medicine Tumaini, Basil; Muhimbili University of Health and Allied Sciences, Internal Medicine Hertzmark, Ellen; Harvard University T H Chan School of Public Health, Global Health
Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	HIV/AIDS
Keywords:	INTERNAL MEDICINE, INFECTIOUS DISEASES, HIV & AIDS < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3 **1 Body mass index, proteinuria and total lymphocyte counts in predicting treatment**
4
5 **2 responses among ART naïve individuals with HIV initiated on antiretroviral treatment in**
6
7 **3 Dar es Salaam, Tanzania, 2019: a cohort study**
8
9
10
11
12
13

14 5 Patricia Munseri^{1*}§, Lazaro Jassely^{1*}, Basil Tumaini¹, Ellen Hertzmark²
15
16
17 6

18
19
20 7 ¹ Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es
21
22 8 Salaam, Tanzania

23
24
25 9 ² Department of Global Health and Population, Harvard University T H Chan School of Public
26
27 10 Health, Boston, MA

28
29
30
31
32
33 12 § Corresponding author

34
35
36 13 Email: patricia.munseri@gmail.com (PM)
37
38
39 14

40
41
42 15 *Shared first Author
43
44
45 16

46
47
48 17 Word count abstract: 294
49

50
51 18 Word count manuscript: 3563
52
53
54
55
56
57

1
2
3 19 **Keywords:** monitoring ART treatment; HIV/AIDS; CD4 count; BMI in HIV; proteinuria in
4
5 20 HIV; viral suppression
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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)},

1
2
3 43 lymphopaenia at six months, {adjusted RR = 4.54, 95% CI (2.19-9.39)}, and proteinuria at six
4
5 44 months {adjusted RR = 2.63, 95% CI (1.25-5.54)}.

6
7
8 45 **Conclusions:** Change in body mass index, total lymphocyte count, and presence of proteinuria can
9
10 46 monitor and predict ART response and may be particularly helpful in settings when CD4 counts
11
12
13 47 and viral load monitoring are unavailable.

14
15
16 48

17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

49 Article Summary

50 Strengths and limitations of this study

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

58

59 Introduction

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

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

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

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

85 Weight gain following ART initiation may reflect slowed resting energy expenditure resulting
86 from viral suppression and a decrease in HIV enteropathy [5]. Weight gain, especially among
87 individuals with low BMI, is associated with improved survival and decreased risk of clinical
88 failure [6]. ART responses depend on adherence [7], nutritional status at baseline [8], HIV subtype
89 [9], and ART combination regimen [10]. In Port Harcourt, Nigeria, among 318 participants with
90 HIV infection aged ≥ 18 years initiated on ART, almost 70% and 55% of participants gained at
91 least 1 kg weight in the first six months and one year of treatment, respectively [11]. Previous
92 studies in Tanzania have shown that a decrease in nutrition status within the first three months of
93 ART initiation was associated with mortality [12].

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

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

For peer review only

105 **Methods**

106 **Study design and population**

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

116 **Sample size estimation**

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

126

127 **Data collection**

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

135 About 10ml of venous blood was collected aseptically from each participant; 5ml for CD4 cell
136 counts, analysed using BD FACSCount™ (Becton Dickenson, USA) and 5ml for complete blood
137 count to obtain the total lymphocyte counts, analysed by an auto-analyser (Cell DNY1800 from
138 Abbott Diagnostics Division, USA). TLC was categorized as lymphopaenia ($<1 \times 10^9/L$), normal
139 lymphocyte ($1 \times 10^9/L$ to $4 \times 10^9/L$), and lymphocytosis ($>4.0 \times 10^9/L$). We assessed for proteinuria
140 by collecting about 15ml of a fresh urine sample from each participant into an empty, clean, dry
141 container and tested using CYBOW™ strips (DFI Co. Ltd, Korea). Proteinuria was categorized as
142 negative proteinuria (no proteinuria), trace or 1+ proteinuria (equivalent to 30 to 100 mg/dl), 2+
143 proteinuria (equivalent to 100 to 300 mg/dl), 3+ proteinuria (equivalent to 300 to 1000 mg/dl), and
144 4+ proteinuria (equivalent to greater than 1000 mg/dl).

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

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

152 BMI was considered to have changed between one time point and another if it increased or
153 decreased by over 5%. BMI changes from ART initiation to six months were categorized into three
154 groups as (i) loss of more than 5%, (ii) stable (change between -5% and 5%, or (iii) gain of more
155 than 5%. The TLC were categorized as (i) lymphopaenia < 1×10^9 cells/L, (ii) normal lymphocyte
156 count $1-4 \times 10^9$ cells/L (iii) Lymphocytosis > 4×10^9 cells/L. The TLC pattern change was
157 categorized as (i) lymphopaenic at 6 months, regardless of the status at baseline or three months;
158 (ii) increased from lymphopaenic to normal or higher; (iii) stayed normal or higher (no
159 lymphopaenia seen). Urinary protein patterns were categorized as (i) proteinuria at six months
160 regardless of baseline proteinuria status; (ii) proteinuria at baseline and/or third month but not six
161 months; and (iii) no proteinuria seen.

162 **Patient and public involvement**

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

165 **Statistical methods**

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

1
2
3 171 were combined for analysis. To determine the association between BMI, TLC or urine protein to
4
5 172 CD4 count, we used correlation.
6
7

8 173 To determine the relationships between individual predictors and viral non-suppression at six
9
10 174 months, we first used modified Poisson regression for univariable analysis with an assumption that
11
12 175 viral non suppression is a non-rare outcome (more than 10%), to determine which variables to
13
14 176 include in the multivariable model [19,20]. For multivariable prediction, all predictors in the
15
16 177 univariable model with a p-value of <0.2 and age, a known confounder, were entered into the
17
18 178 modified Poisson regression model. The results of the Poisson regression model were presented
19
20 179 as relative risk (RR) and 95% confidence interval (RR; 95% CI). To determine the test
21
22 180 characteristics (sensitivity, specificity, positive predictive value (PPV), negative predictive value
23
24 181 (NPV)) of a score based on the multivariable model, we used two cut-off levels, based on the first
25
26 182 quartile and median of the score among the non-suppressed. The score was the sum of the rounded
27
28 183 coefficients for the variables for which the confidence intervals did not include 1 in a model
29
30 184 containing only these variables. Since these all rounded to 1, this is equivalent to simply counting
31
32 185 the number of these characteristics.
33
34
35
36
37
38

39 186 Based on practices in low resourced clinics, communication with the patient and the decision to
40
41 187 change the ART regimen depends on the patient's virological status at six months. CD4 cell counts
42
43 188 depend on a blood sample collected at the six-month visit and are therefore unavailable for
44
45 189 immediate decision making. We, therefore, excluded all CD4 variables from the model and used
46
47 190 parameters available at the time of the six-month visit to predict viral non-suppression.
48
49
50
51
52
53
54
55
56
57
58
59
60

191 **Results**

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

198
199
200 **Figure 1. Consort diagram.**

201
202
203 **Baseline characteristics of study participants**

204 Of the 215 participants analysed, the mean age (SD) was 37.1 ± 11.5 years, 146 (68%) were female,
 205 113 (53%) were never married, 45 (21%) had secondary education or higher, and 117 (54%) were
 206 unemployed (Table 1). Most participants, 59.5%, had normal BMI, while 27% were overweight,
 207 and 13% were underweight. Most participants, 113 (62%), were in WHO HIV clinical stage I, and
 208 only eight (4%) were in stage IV, though 93 (43%) had CD4 counts of 350 cells/ml or below; 83
 209 (39%) were lymphopaenic, and 111 (52%) had proteinuria at baseline.

210
211 **Table 1. Characteristics of 215 study participants at ART initiation, Dar es Salaam,**
 212 **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/m²)		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/mm³)		401 ± 253
<200	55 (25.6%)	
200-350	38 (17.7%)	
351-500	39 (18.1%)	
>500	83 (38.6%)	
Lymphocyte counts (x10⁹cells/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%)

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

214

215

216 **Table 2. Predictors of HIV viral load non-suppression at six months among 215 ART naïve**
 217 **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

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

219 Univariable and multivariable analysis by modified Poisson regression.

220

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

224

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

226 **ART**

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

1
2
3 232 a drop in body mass index at six months of 5% or more from baseline, 80% were non-suppressed
4
5 233 {RR = 2.73; 95% CI (1.36-5.47)}. In an alternative analysis, we considered BMI changes of 10%,
6
7
8 234 but only 9 of the non-suppressed participants (20%) had such large decreases. The risk of HIV
9
10 235 non-suppression at six months was higher among participants with proteinuria at six months {RR
11
12 236 = 2.63; 95% CI (1.25-5.54)}, Table 2. The area under the Receiver Operating Characteristic (ROC)
13
14 237 curve for the multivariable model was 0.895 (Fig 2). Baseline HIV stage was not related to HIV
15
16 238 non-suppression at six months, {adjusted RR = 1.14, 95% CI (0.63-2.08)} for baseline WHO HIV
17
18 239 clinical stage II and {adjusted RR = 0.82, 95% CI (0.51-1.31)} for advanced baseline WHO HIV
19
20 240 clinical stages (III and IV)}.

21
22
23
24 241 Using the rounded coefficients of the three variables in a model containing only these variables,
25
26 242 which all rounded to 1, we made a “prediction score” with values 0 (n=154, of which 10 were non-
27
28 243 suppressed), 1 (n=36, 13 non-suppressed), 2 (n=17, 15 non-suppressed), and 3 (n=8, all non-
29
30 244 suppressed). The median value of this score among the non-suppressed was 1.5 and the first
31
32 245 quartile value was 1. Thus, having any 2 of the risk factors would be a conservative predictor of
33
34 246 non-suppression, and having any one would be less conservative.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 247 **Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease,**
4
5 248 **proteinuria, and total lymphocyte counts to predict viral non-suppression among ART naïve**
6
7 249 **individuals with HIV initiated on ART in Dar es Salaam, Tanzania, 2019**
8
9

10
11 250

12
13
14 251

15
16
17 252 Using the median score among the non-suppressed as a cut-off (equivalent to having any two of
18
19 253 the components), the sensitivity predicting non-suppression was 0.50, and the specificity was 0.99.

20
21 254 Only 12% of the study population met this criterion. When we lowered the cut-off scores to the
22
23 255 first quartile value among the non-suppressed (i.e. any one of the components), the sensitivity was

24
25
26 256 0.78 and the specificity was 0.85, with 28% of the study population meeting this criterion.
27
28

29
30 257
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

258 Discussion

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

268 Baseline WHO HIV clinical stage did not help predict HIV non-suppression at six months,
269 possibly because under the current "Test and Treat" strategy [21], most individuals initiating ART
270 are in WHO HIV clinical stages I and II (76% of our sample). Current therapy is highly effective
271 except for a few patients whose disease is so advanced that they die before the medication can
272 improve their immune status (2 patients in this study). Symptomatic individuals with advanced
273 HIV will likely adhere to treatment to alleviate their symptoms. Perhaps patients with advanced
274 disease fear death and therefore are motivated to adhere to ART, with eventual viral suppression.
275 Advanced HIV disease has been shown to be linked with ART adherence [22]. Some studies,
276 however, indicate that early HIV stages are linked with high ART adherence and viral suppression
277 [23].

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

1
2
3 281 HIV non-suppression irrespective of a participant's lymphocyte count (normal or low) before ART
4
5 282 initiation. CD4 cells are a subset of lymphocytes and therefore lymphopaenia could be indicative
6
7 283 of a decline in CD4 cells, as a result of high HIV replication with high viral load signifying viral
8
9 284 non-suppression with consequent CD4 cell destruction. Lymphopaenia was significantly
10
11 285 associated with CD4 <500 cells/mm³ at all time points. In this study, an increase in total
12
13 286 lymphocyte cell count from lymphopaenia to normal lymphocyte counts from baseline to six
14
15 287 months was significantly associated with an increase in CD4 cell count (Additional file 1). Total
16
17 288 lymphocyte count is sensitive and specific in predicting CD4 cell counts [16][24] though there
18
19 289 have been contradictory reports [25]. The assessment of total lymphocyte counts among patients
20
21
22 290 on ART, therefore, could serve as an alternative, especially in settings with limited availability of
23
24 291 CD4 cell count measurement. A drop in total lymphocyte count in such a setting can alert a
25
26 292 clinician to the likelihood of immunological failure. A drop in lymphocytes could also signal the
27
28 293 possibility of immunological non responders, who will need primary and secondary prophylaxis
29
30
31 294 for opportunistic infection.

32
33
34
35
36 295 Weight loss with consequent BMI drop of 5% or more at six months while on ART from weight
37
38 296 prior to ART initiation, predicts HIV non-suppression, though HIV non-suppression was not
39
40 297 associated with being underweight prior to ART initiation, perhaps because of the low prevalence
41
42 298 of underweight leading to low power. In this study, sustained weight gain was significantly
43
44 299 associated with viral suppression and sustained weight loss was associated with viral non-
45
46 300 suppression at six months of ART. An increase in weight and hence BMI may be a sign of immune
47
48 301 status improvement signalling a return to health [26] [27]and improved survival [28], while a
49
50 302 decrease in weight and hence BMI may be a sign of HIV progression or decline in CD4 cell counts
51
52 303 [5][11][29]. Weight loss may be due to HIV itself, opportunistic infections, or HIV-associated

1
2
3 304 tumours. Failure to gain weight has been associated with efavirenz toxicity over time as was
4
5 305 observed in a study in South Africa [30]. Weight loss in both ART naïve and exposed patients has
6
7
8 306 been associated with increased morbidity and mortality [31][32]. A study in England observed that
9
10 307 each log₁₀ increase in HIV viral load was associated with a 0.92 kg decrease in body weight.
11
12 308 However, a decrease in viral load was not significantly associated with weight gain, contrary to
13
14 309 our study [33]. Since weight changes correlate with the virological response, losing weight should
15
16 310 be viewed as an alarming sign of virological failure. Monitoring of weight and body mass index
17
18 311 prior to ART initiation and during follow up is a valuable inexpensive way of identifying
19
20 312 individuals with possible treatment failure. In an alternative analysis, we considered BMI changes
21
22 313 of 10%, but only 9 of the non-suppressed participants (20%) had such large decreases, making the
23
24 314 10% decrease not useful as a cut-off in our situation.

25
26
27
28
29 315 Half of the ART-naïve study participants had proteinuria, similar to a report from Zimbabwe [34].
30
31 316 The presence of proteinuria was associated with HIV non-suppression. Proteinuria at six months
32
33 317 was a strong predictor of HIV non-suppression. Proteinuria in individuals with HIV is attributed
34
35 318 to a direct effect of HIV on the glomerular and tubular epithelial cells with the progression of HIV
36
37 319 disease to AIDS, nephrotoxicity from ART, the progression of chronic kidney disease and death
38
39 320 [14][35]. The higher the viral load, the greater the damage to the kidney [36]. We observed a
40
41 321 significant proportion of participants with proteinuria of 2+ or above in WHO HIV clinical stage
42
43 322 IV. This underscores the fact that, as HIV disease advances, the greater the damage to the kidneys
44
45 323 as evidenced by increasing proteinuria. Therefore, routine screening for renal disease may serve
46
47 324 not only as a follow-up of renal disease progression but also for HIV treatment response
48
49 325 monitoring.

1
2
3 326 Our findings require validation in a study with a larger sample size. Our small sample may have
4
5 327 constrained some predictors of viral non-suppression. Similar studies conducted in different
6
7 328 locations are also needed since local conditions and treatment standards may influence some
8
9
10 329 observed patterns, both in prevalence and effect. Furthermore, use of new antiviral drugs and
11
12 330 changes in patient characteristics at presentation may change our estimates, and possibly the
13
14 331 important predictor variables. We recommend further studies to examine the relationship between
15
16 332 virological response and anaemia as well as opportunistic infections and AIDS associated
17
18 333 malignancies especially now that ART is initiated early.

21
22 334 One strength of our study is the cohort design with complete follow up data at three and six months
23
24 335 for 98% of the enrolled participants. Although our scoring system is crude, it is easy to compute
25
26 336 and is likely to be valid for a wide variety of situations, whereas a score based on more precise
27
28 337 computations would at best work only in our location.

32 338

35 339 **Conclusion**

38 340 A drop in BMI by more than 5%, persistent lymphopaenia or decrease in total lymphocyte count
39
40 341 to lymphopaenia, and proteinuria at six months are associated with HIV non-suppression at 6
41
42 342 months after ART initiation. Scores based on these parameters are easy to use and can serve as
43
44 343 alternatives to CD4 cell counts and viral load assessment in facilities with scarcity.

48 344 **List of abbreviations**

51 345 AIDS: Acquired immunodeficiency syndrome

54 346 ART: Antiretroviral therapy

1
2
3 347 BMI: Body mass index
4

5
6 348 CD4: Cluster of differentiation 4
7

8
9 349 HIV: Human immunodeficiency virus
10

11
12 350 TLC: Total lymphocyte counts
13

14
15 351 WHO: World Health Organization
16

17
18 352
19

20
21 353
22

23 24 354 **Acknowledgements** 25

26
27 355 We are grateful to the participants for their willingness to take part in this study and to the health
28
29 356 workers from Temeke regional referral hospital, Mbagala Rangi-tatu district hospital, and
30
31 357 Mbagala Kizuiani dispensary for their assistance in participant recruitment and data collection.
32
33

34 35 358 **Author Contributions** 36

37 359 Study design: LJ and PM; data collection: LJ and PM; Data analysis and interpretation: LJ, PM,
38
39 360 BT and EH; Manuscript writing: LJ, PM, BT and EH. All the authors gave final approval of the
40
41 361 manuscript.
42
43

44
45 362
46

47 48 363 **Funding** 49

50
51 364 This research received no specific grant from any funding agency in the public, commercial or
52
53 365 not-for-profit sectors.
54
55

1
2
3 366 **Competing interests**
4

5
6 367 None declared.
7
8

9 368 **Patient consent for publication**
10

11
12 369 Not applicable.
13
14

15 370 **Ethics approval**
16

17
18 371 Ethical approval was obtained from the Research and Publications Committee of Muhimbili
19
20 372 University of Health and Allied Sciences (Ref. No. DA.287/298/01A). Permission to conduct the
21
22 373 study was obtained from Temeke Municipal Hospital administration. Participants were enrolled
23
24 374 after providing written informed consent. The confidentiality of patient information was ensured.
25
26 375 Participants without viral suppression at the 6th month of follow up were managed according to
27
28 376 Tanzania National Guidelines for management of HIV and AIDS.
29
30

31
32 377 **Data availability statement**
33

34
35 378 The dataset analysed during the current study is available upon reasonable request to the
36
37 379 corresponding author.
38
39

40 380 **ORCID iDs**
41

42
43 381 Basil Tumaini: <https://orcid.org/0000-0002-2894-1684>
44

45
46 382 Ellen Hertzmark: <https://orcid.org/0000-0003-0148-2761>
47

48 383 **Ethics Statement**
49

50
51 384 Muhimbili University of Health and Allied Sciences Institutional Review Board with reference
52
53 385 number DA287/298/01A/
54

55
56 386
57

1
2
3 387 **References**
4
5

- 6 388 1. UNAIDS. UNAIDS data 2020 | UNAIDS [Internet]. 2020 [cited 2020 Aug 19].
7
8 389 <https://www.unaids.org/en/resources/documents/2020/unaids-data>
9
10
11 390 2. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for
12
13 391 treating and preventing HIV infection: recommendations for a public health approach.
14
15 World Health Organization; 2016.
16 392
17 https://apps.who.int/iris/bitstream/handle/10665/208825/9789241549684_eng.pdf
18 393
19
20
21 394 3. Roberts T, Bygrave H, Fajardo E, Ford N. Challenges and opportunities for the
22
23 395 implementation of virological testing in resource-limited settings. *Journal of the*
24
25 *International AIDS Society*. 2012;15(2):17324. <https://doi.org/10.7448/IAS.15.2.17324>
26 396
27 PMID: 23078767
28 397
29
30
31 398 4. Sepkowitz KA. AIDS - the first 20 years. *New England Journal of Medicine*.
32
33 399 2001;344(23):1764-72. <https://www.nejm.org/doi/full/10.1056/NEJM200106073442306>
34
35 PMID: 11396444
36 400
37
38
39 401 5. Mangili A, Murman DH, Zampini AM, Wanke CA, Mayer KH. Nutrition and HIV
40
41 402 infection: review of weight loss and wasting in the era of highly active antiretroviral
42
43 403 therapy from the nutrition for healthy living cohort. *Clinical Infectious Diseases*.
44
45 404 2006;42(6):836-42. <https://doi.org/10.1086/500398> PMID: 16477562
46
47
48
49 405 6. Koethe JR, Lukusa A, Giganti MJ, Chi BH, Nyirenda CK, Limbada MI, et al. Association
50
51 406 between weight gain and clinical outcomes among malnourished adults initiating
52
53 407 antiretroviral therapy in Lusaka, Zambia. *Journal of Acquired Immune Deficiency*
54
55 408 *Syndromes*. 2010;53(4):507-13.
56
57

- 1
2
3 409 [https://journals.lww.com/jaids/Fulltext/2010/04010/Association_Between_Weight_Gain_](https://journals.lww.com/jaids/Fulltext/2010/04010/Association_Between_Weight_Gain_and_Clinical.12.aspx)
4
5 and_Clinical.12.aspx PMID: 19730111
6 410
7
8 411 7. Mannheimer S, Friedland G, Matts J, Child C, Chesney M. The consistency of adherence
9
10 to antiretroviral therapy predicts biologic outcomes for Human Immunodeficiency Virus -
11 412
12 infected persons in clinical trials. *Clinical Infectious Diseases*. 2002;34(8):1115-21.
13 413
14 <https://doi.org/10.1086/339074> PMID: 11915001
15 414
16
17 415 8. Paton NI, Sangeetha S, Earnest A, Bellamy R. The impact of malnutrition on survival and
18
19 the CD4 count response in HIV-infected patients starting antiretroviral therapy. *HIV*
20 416
21 *medicine*. 2006;7(5):323-30. <https://doi.org/10.1111/j.1468-1293.2006.00383.x> PMID:
22 417
23 16945078
24 418
25
26 419 9. Wittkop L, Arsandaux J, Trevino A, Schim van der Loeff M, Anderson J, van Sighem A,
27
28 et al. CD4 cell count response to first-line combination ART in HIV-2+ patients compared
29 420
30 with HIV-1+ patients: a multinational, multicohort European study. *Journal of*
31 421
32 *Antimicrobial Chemotherapy*. 2017;72(10):2869-78. <https://doi.org/10.1093/jac/dkx210>
33 422
34 PMID: 29091198
35 423
36
37 424 10. Kanya MR, Mayanja-Kizza H, Kambugu A, Bakeera-Kitaka S, Semitala F, Mwebaze-
38
39 Songa P, et al. Predictors of long-term viral failure among Ugandan children and adults
40 425
41 treated with antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes*.
42 426
43 2007;46(2):187-93. PMID: 17693883
44 427
45
46 428 11. Olaleye AO, Owhonda G, Daramola O, Adejo I, Olayiwola H, Inyang JI, et al. Factors
47
48 associated with weight gain among adult patients initiating antiretroviral therapy in Port
49 429
50 Harcourt, Nigeria: a retrospective cohort study. *Infectious Diseases*. 2017;49(8):635-8.
51 430
52
53
54
55
56
57
58
59
60

- 1
2
3 431 <https://doi.org/10.1080/23744235.2017.1306102> PMID: 28335659
4
5
6 432 12. Liu E, Spiegelman D, Semu H, Hawkins C, Chalamilla G, Aveika A, et al. Nutritional
7
8 433 status and mortality among HIV-infected patients receiving antiretroviral therapy in
9
10 434 Tanzania. *Journal of Infectious Diseases*. 2011;204(2):282-90.
11
12 435 <https://doi.org/10.1093/infdis/jir246> PMID: 21673040
13
14
15
16 436 13. Husain NE, Ahmed MH, Almobarak AO, Noor SK, Elmadhoun WM, Awadalla H, et al.
17
18 437 HIV-associated nephropathy in Africa: pathology, clinical presentation and strategy for
19
20 438 prevention. *Journal of Clinical Medicine Research*. 2018;10(1):1-8.
21
22 439 <https://doi.org/10.14740/jocmr3235w> PMID: 29238427
23
24
25
26 440 14. Szczech LA, Hoover DR, Feldman JG, Cohen MH, Gange SJ, Goozé L, et al. Association
27
28 441 between renal disease and outcomes among HIV-infected women receiving or not
29
30 442 receiving antiretroviral therapy. *Clinical Infectious Diseases*. 2004;39(8):1199-206.
31
32 443 <https://doi.org/10.1086/424013> PMID: 15486845
33
34
35
36 444 15. Imlach S, McBreen S, Shirafuji T, Leen C, Bell JE, Simmonds P. Activated peripheral
37
38 445 CD8 lymphocytes express CD4 in vivo and are targets for infection by Human
39
40 446 Immunodeficiency Virus Type 1. *Journal of Virology*. 2001;75(23):11555-64.
41
42 447 <https://doi.org/10.1128/JVI.75.23.11555-11564.2001> PMID: 11689637
43
44
45
46 448 16. Obirikorang C, Quaye L, Acheampong I. Total lymphocyte count as a surrogate marker
47
48 449 for CD4 count in resource-limited settings. *BMC Infectious Diseases*. 2012;12:128.
49
50 450 <https://doi.org/10.1186/1471-2334-12-128> PMID: 22676809
51
52
53 451 17. The United Republic of Tanzania; Ministry of Health, Community Development, Gender,
54
55 452 Elderly and Children; National AIDS Control Programme. National guidelines for the

- 1
2
3 453 management of HIV and AIDS. 6th Ed, 2017. Available from:
4
5 454 <https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj5>
6
7 455 8iR37uA/File/Tanzania_NATIONAL GUIDELINES FOR MANAGEMENT OF HIV
8
9 456 AND AIDS 6TH EDITION 2017.pdf
10
11
12
13 457 18. World Health Organization. Obesity: preventing and managing the global epidemic. WHO
14
15 458 Technical Report Series. 2000(894).
16
17 459 https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/
18
19
20 460 https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/
21
22
23 461 19. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and
24
25 462 differences. *American Journal of Epidemiology*. 2005; 162(3):199-200.
26
27 463 <https://doi.org/10.1093/aje/kwi188> PMID: 15987728
28
29
30
31 464 20. Dwivedi AK, Mallawaarachchi I, Lee S, Tarwater P. Methods for estimating relative risk
32
33 465 in studies of common binary outcomes. *Journal of Applied Statistics*. 2014; 41(3):484-
34
35 466 500. <https://doi.org/10.1080/02664763.2013.840772>
36
37
38
39 467 21. WHO. The use of antiretroviral drugs for treating and preventing HIV infection guidelines
40
41 468 HIV/AIDS Programme. 2016 [cited 2021 Jan 19].
42
43 469 http://apps.who.int/iris/bitstream/10665/85322/1/WHO_HIV_2013.7_eng.pdf
44
45
46 470 22. Biressaw S, Abegaz WE, Abebe M, Taye WA, Belay M. Adherence to Antiretroviral
47
48 471 Therapy and associated factors among HIV infected children in Ethiopia: Unannounced
49
50 472 home-based pill count versus caregivers' report. *BMC Pediatrics*. 2013;13:132.
51
52 473 <https://doi.org/10.1186/1471-2431-13-132> PMID: 24229394
53
54
55
56
57
58
59
60

- 1
2
3 474 23. Haberer JE, Bwana BM, Orrell C, Asiimwe S, Amanyire G, Musinguzi N, et al. ART
4
5 475 adherence and viral suppression are high among most non-pregnant individuals with
6
7 476 early-stage, asymptomatic HIV infection: an observational study from Uganda and South
8
9 477 Africa. *Journal of the International AIDS Society*. 2019;22(2):e25232.
10
11 478 <https://doi.org/10.1002/jia2.25232> PMID: 30746898
12
13
14
15 479 24. Kwantwi LB, Tunu BK, Boateng D, Quansah DY. Body Mass Index, Haemoglobin, and
16
17 480 Total Lymphocyte Count as a Surrogate for CD4 Count in Resource Limited Settings. *J*
18
19 481 *Biomarkers*. 2017; 2017: 7907352.
20
21 482 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5412137/> PMID: 28484663
22
23
24
25 483 25. Akinola NO, Olasode O, Adediran IA, Onayemi O, Murainah A, Irinoye O, et al. The
26
27 484 search for a predictor of CD4 cell count continues: Total lymphocyte count is not a
28
29 485 substitute for CD4 cell count in the management of HIV-infected individuals in a
30
31 486 resource-limited setting. *Clinical Infectious Diseases*. 2004;39(4):579–81.
32
33 487 <https://doi.org/10.1086/422722> PMID: 15356826
34
35
36
37 488 26. Koethe JR, Jenkins CA, Lau B, Shepherd BE, Wester W, Rebeiro PF, et al. Higher time-
38
39 489 updated body mass index: association with improved CD4+ cell recovery on HIV
40
41 490 treatment. *Journal of Acquired Immune Deficiency Syndromes*. 2016;73(2):197-204.
42
43 491 <https://dx.doi.org/10.1097%2FQAI.0000000000001035> PMID: 27116044
44
45
46
47 492 27. Biadgilign S, Reda AA, Digaffe T. Predictors of mortality among HIV infected patients
48
49 493 taking antiretroviral treatment in Ethiopia: a retrospective cohort study. *AIDS research*
50
51 494 *and therapy*. 2012;9:15. <https://doi.org/10.1186/1742-6405-9-15> PMID: 22606951
52
53
54
55 495 28. Reda AA, Biadgilign S, Deribew A, Gebre B, Deribe K. Predictors of Change in CD4
56
57
58
59

- 1
2
3 496 Lymphocyte Count and Weight among HIV Infected Patients on Anti-Retroviral
4
5 497 Treatment in Ethiopia: A Retrospective Longitudinal Study. Ensoli B, editor. PLoS One.
6
7 498 2013;8(4):e58595. <https://doi.org/10.1371/journal.pone.0058595> PMID: 23573191
9
10
11 499 29. Griesel R, Kawuma AN, Wasmann R, Sokhela S, Akpomiemie G, Venter WF, et al.
12
13 500 Concentration-response relationships of dolutegravir and efavirenz with weight change
14
15 501 after starting antiretroviral therapy. *British Journal of Clinical Pharmacology*. 2022; 88(3):
16
17 502 883- 893. <https://doi.org/10.1111/bcp.15177> PMID: 34954840
18
19
20
21 503 30. Naidoo K, Yende-Zuma N, Augustine S. A retrospective cohort study of body mass index
22
23 504 and survival in HIV infected patients with and without TB co-infection. *Infectious*
24
25 505 *Diseases of Poverty*. 2018;7:35. <https://doi.org/10.1186/s40249-018-0418-3> PMID:
26
27 506 29690932
28
29
30
31 507 31. Van der Sande MA, van der Loeff MF, Aveika AA, Sabally S, Togun T, Sarge-Njie R, et
32
33 508 al. Body mass index at time of HIV diagnosis: a strong and independent predictor of
34
35 509 survival. *Journal of Acquired Immune Deficiency Syndromes*. 2004;37(2):1288-94.
36
37 510 <https://doi.org/10.1097/01.qai.0000122708.59121.03> PMID: 15385737
38
39
40
41 511 32. Grinspoon S, Mulligan K. Weight loss and wasting in patients infected with Human
42
43 512 Immunodeficiency Virus. *Clinical Infectious Diseases*. 2003;36(Supplement_2):S69-78.
44
45 513 <https://doi.org/10.1086/367561> PMID: 12652374
46
47
48 514 33. Mwamburi DM, Wilson IB, Jacobson DL, Spiegelman D, Gorbach SL, Knox TA, et al.
49
50 515 Understanding the role of HIV load in determining weight change in the era of highly
51
52 516 active antiretroviral therapy. *Clinical Infectious Diseases*. 2005;40(1):167-73.
53
54 517 <https://doi.org/10.1086/426591> PMID: 15614708
55
56
57

- 1
2
3 518 34. Fana GT, Ndhlovu CE. Renal dysfunction among anti-retroviral therapy naïve HIV
4
5 519 infected patients in Zimbabwe. *The Central African Journal of Medicine*. 2011;57(1-4):1-
6
7 520 5. <https://www.ajol.info/index.php/cajm/article/view/73724> PMID: 24968654
8
9
10
11 521 35. Gupta SK, Smurzynski M, Franceschini N, Bosch RJ, Szczech LA, Kalayjian RC. The
12
13 522 effects of HIV-1 viral suppression and non-viral factors on quantitative proteinuria in the
14
15 523 HAART era. *Antiviral Therapy*. 2009;14(4): 543–549. PMID: 19578239
16
17
18 524 36. Rednor SJ, Ross MJ. Molecular mechanisms of injury in HIV-associated nephropathy.
19
20 525 *Frontiers in Medicine*. 2018;5:177. <https://doi.org/10.3389/fmed.2018.00177> PMID:
21
22 29930940
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

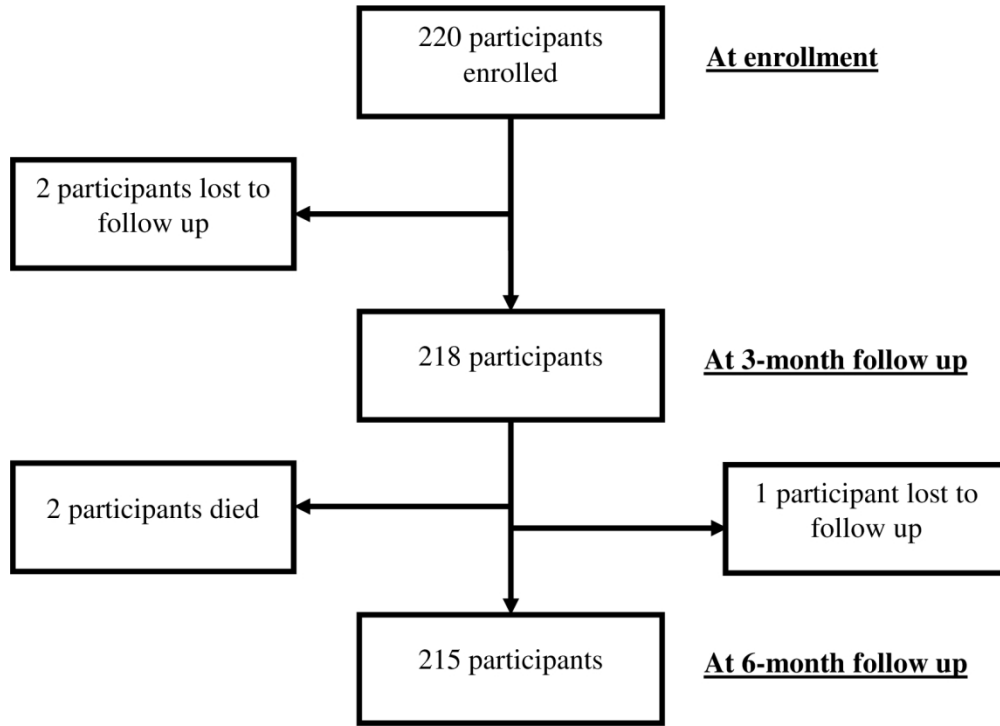


Figure 1. Consort diagram.

146x105mm (300 x 300 DPI)

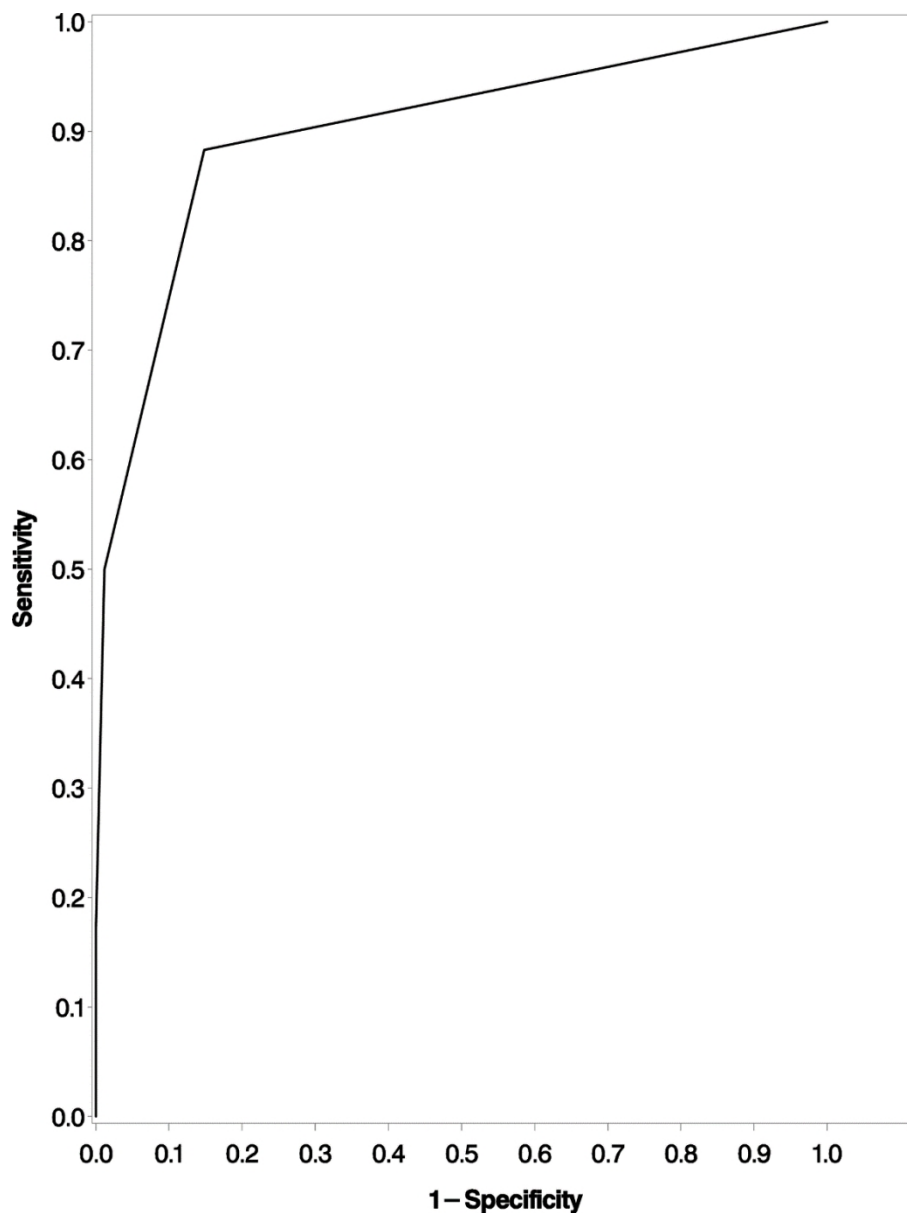
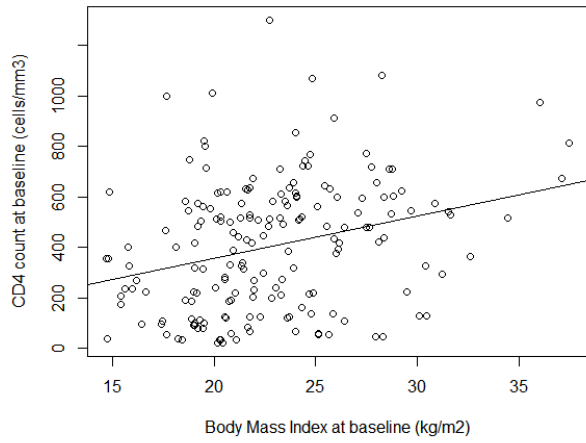


Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among HIV-infected individuals initiated on ART in Dar es Salaam, Tanzania, 2019

137x181mm (220 x 220 DPI)

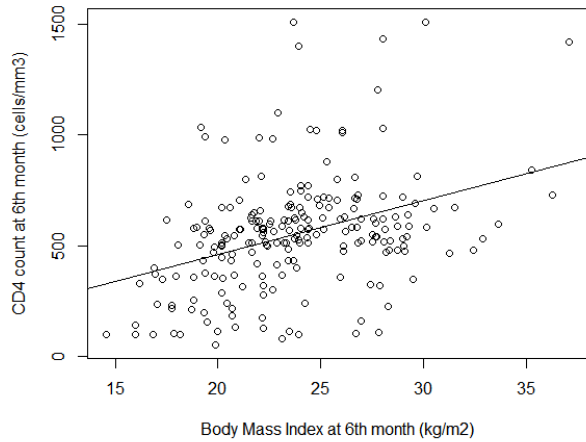
Supplementary Figure 1. Scatter plots of BMI and CD4 counts



rho 0.287, p <0.0001

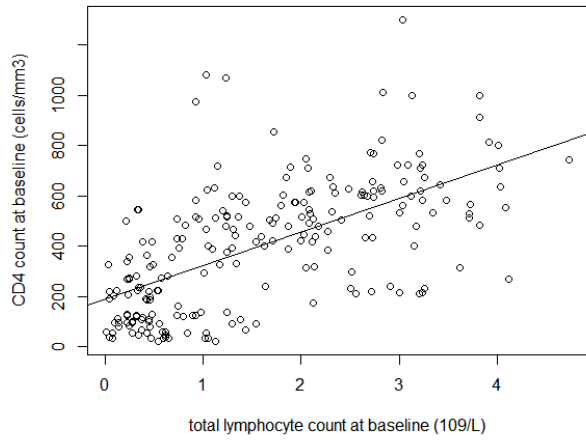


rho 0.305, p <0.0001

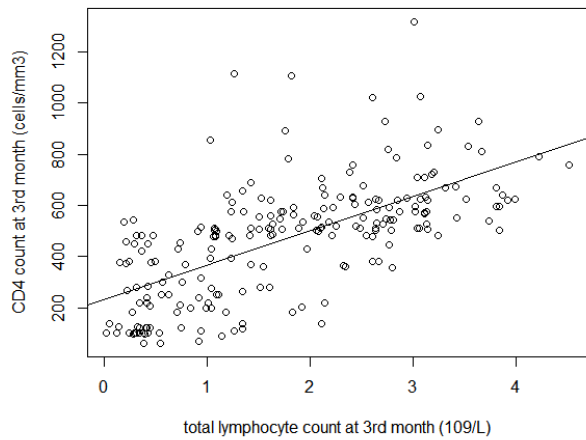


rho 0.373, p <0.0001

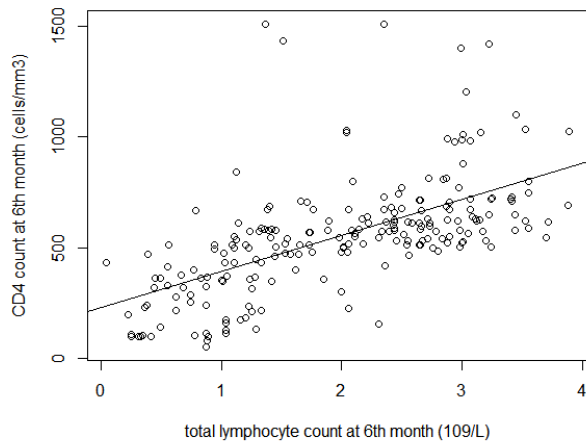
Supplementary Figure 2. Scatter plots of total lymphocyte count and CD4 count



rho 0.613, p <0.0001

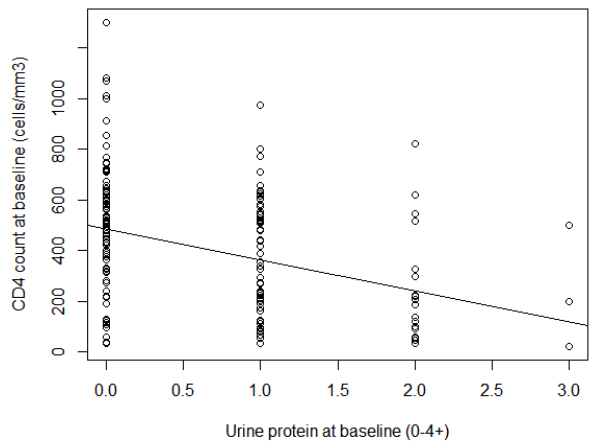


rho 0.650, p <0.0001

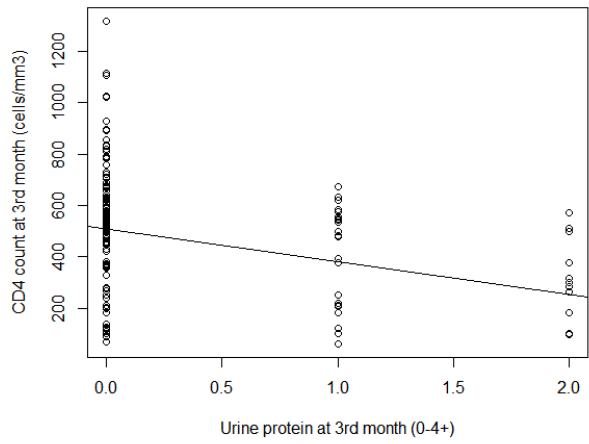


rho 0.602, p <0.0001

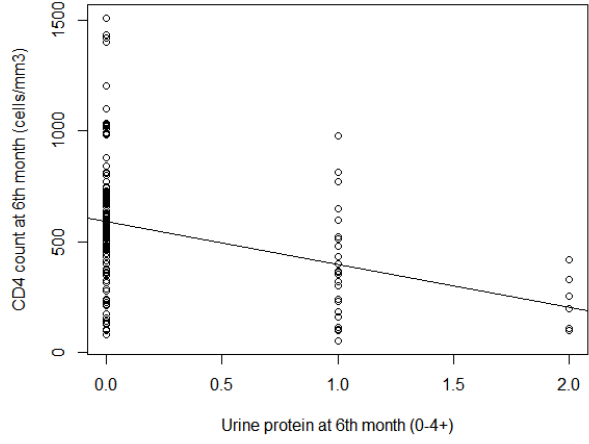
Supplementary Figure 3. Scatter plots of urine protein and CD4 count



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 cohort reporting guidelines, and cite them as:

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

			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

1	Bias	#9	Describe any efforts to address potential sources of bias	
2				
3				
4	Study size	#10	Explain how the study size was arrived at	8
5				
6				
7	Quantitative	#11	Explain how quantitative variables were handled in the	9-11
8	variables		analyses. If applicable, describe which groupings were chosen,	
9			and why	
10				
11				
12				
13				
14				
15	Statistical	#12a	Describe all statistical methods, including those used to control	
16	methods		for confounding	
17				
18				
19				
20	10,11			
21				
22				
23	Statistical	#12b	Describe any methods used to examine subgroups and	10, 11
24	methods		interactions	
25				
26				
27				
28				
29	Statistical	#12c	Explain how missing data were addressed	12
30	methods			
31				
32				
33				
34	Statistical	#12d	If applicable, explain how loss to follow-up was addressed	-
35	methods			
36				
37				
38				
39	Statistical	#12e	Describe any sensitivity analyses	
40	methods			
41				
42				
43				
44				
45	11			
46				
47				
48	Results			
49				
50				
51	Participants	#13a	Report numbers of individuals at each stage of study—eg	12
52			numbers potentially eligible, examined for eligibility, confirmed	(figure 1)
53			eligible, included in the study, completing follow-up, and	
54			analysed. Give information separately for for exposed and	
55				
56				
57				
58				
59				
60				

unexposed groups if applicable.

1			
2			
3			
4	Participants	#13b	Give reasons for non-participation at each stage
5			
6			12
7			(figure 1)
8			
9	Participants	#13c	Consider use of a flow diagram
10			
11			
12	12 (figure 1)		
13			
14			
15	Descriptive data	#14a	Give characteristics of study participants (eg demographic,
16			clinical, social) and information on exposures and potential
17			confounders. Give information separately for exposed and
18			unexposed groups if applicable.
19			
20			
21			
22			
23			
24			
25	Descriptive data	#14b	Indicate number of participants with missing data for each
26			variable of interest
27			
28			
29			
30	See 12		
31			
32			
33	Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)
34			
35			
36	12		
37			
38			
39			
40	Outcome data	#15	Report numbers of outcome events or summary measures
41			over time. Give information separately for exposed and
42			unexposed groups if applicable.
43			
44			
45			
46			
47	14		
48			
49			
50	Main results	#16a	Give unadjusted estimates and, if applicable, confounder-
51			adjusted estimates and their precision (eg, 95% confidence
52			interval). Make clear which confounders were adjusted for and
53			why they were included
54			
55			
56			
57			
58			
59			
60			

1	Main results	#16b	Report category boundaries when continuous variables were	12-15
2			categorized	
3				
4				
5				
6	Main results	#16c	If relevant, consider translating estimates of relative risk into	
7			absolute risk for a meaningful time period	
8				
9				
10				
11				
12	-			
13				
14				
15	Other analyses	#17	Report other analyses done—eg analyses of subgroups and	16
16			interactions, and sensitivity analyses	
17				
18				
19				
20	Discussion			
21				
22				
23	Key results	#18	Summarise key results with reference to study objectives	20
24				
25				
26	Limitations	#19	Discuss limitations of the study, taking into account sources of	5
27			potential bias or imprecision. Discuss both direction and	
28			magnitude of any potential bias.	
29				
30				
31				
32				
33				
34	Interpretation	#20	Give a cautious overall interpretation considering objectives,	18-20
35			limitations, multiplicity of analyses, results from similar studies,	
36			and other relevant evidence.	
37				
38				
39				
40				
41	Generalisability	#21	Discuss the generalisability (external validity) of the study	20
42			results	
43				
44				
45				
46				
47	Other Information			
48				
49				
50	Funding	#22	Give the source of funding and the role of the funders for the	22
51			present study and, if applicable, for the original study on which	
52			the present article is based	
53				
54				
55				
56				
57				
58				
59				
60				

1 None The STROBE checklist is distributed under the terms of the Creative Commons Attribution
2 License CC-BY. This checklist can be completed online using <https://www.goodreports.org/>, a tool
3 made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-059193.R2
Article Type:	Original research
Date Submitted by the Author:	12-May-2022
Complete List of Authors:	Munseri, Patricia; Muhimbili University of Health and Allied Sciences School of Medicine, Jassely, Lazaro; Muhimbili University of Health and Allied Sciences School of Medicine, Internal Medicine Tumaini, Basil; Muhimbili University of Health and Allied Sciences, Internal Medicine Hertzmark, Ellen; Harvard University T H Chan School of Public Health, Global Health
Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	HIV/AIDS
Keywords:	INTERNAL MEDICINE, INFECTIOUS DISEASES, HIV & AIDS < INFECTIOUS DISEASES

SCHOLARONE™
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

1
2
3 **1 Body mass index, proteinuria and total lymphocyte counts in predicting treatment**
4
5 **2 responses among ART naïve individuals with HIV initiated on antiretroviral treatment in**
6
7 **3 Dar es Salaam, Tanzania, 2019: a cohort study**
8
9
10
11
12
13

14 5 Patricia Munseri^{1*}§, Lazaro Jassely^{1*}, Basil Tumaini¹, Ellen Hertzmark²
15
16
17 6

18
19
20 7 ¹ Department of Internal Medicine, Muhimbili University of Health and Allied Sciences, Dar es
21
22 8 Salaam, Tanzania

23
24
25 9 ² Department of Global Health and Population, Harvard University T H Chan School of Public
26
27 10 Health, Boston, MA

28
29
30
31
32
33 12 § Corresponding author
34
35

36 13 Email: patricia.munseri@gmail.com (PM)
37
38
39 14

40
41
42 15 *Shared first Author
43
44
45 16

46
47
48 17 Word count abstract: 294
49
50

51 18 Word count manuscript: 4122
52
53
54
55
56
57

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

For peer review only

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)},

1
2
3 43 lymphopaenia at six months, {adjusted RR = 4.54, 95% CI (2.19-9.39)}, and proteinuria at six
4
5 44 months {adjusted RR = 2.63, 95% CI (1.25-5.54)}.

7
8 45 **Conclusions:** Change in body mass index, total lymphocyte count, and presence of proteinuria can
9
10 46 monitor and predict ART response and may be particularly helpful in settings when CD4 counts
11
12 47 and viral load monitoring are unavailable.

13
14
15
16 48

For peer review only

49 Article Summary

50 Strengths and limitations of this study

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

58

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

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

85 Weight gain following ART initiation may reflect slowed resting energy expenditure resulting
86 from viral suppression and a decrease in HIV enteropathy [5]. Weight gain, especially among
87 individuals with low BMI, is associated with improved survival and decreased risk of clinical
88 failure [6]. ART responses depend on adherence [7], nutritional status at baseline [8], HIV subtype
89 [9], and ART combination regimen [10]. In Port Harcourt, Nigeria, among 318 participants with
90 HIV infection aged ≥ 18 years initiated on ART, almost 70% and 55% of participants gained at
91 least 1 kg weight in the first six months and one year of treatment, respectively [11]. Previous
92 studies in Tanzania have shown that a decrease in nutrition status within the first three months of
93 ART initiation was associated with mortality [12].

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

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

1
2
3 102 This study aimed at assessing the following routinely accessible parameters: body mass index,
4
5 103 proteinuria, and total lymphocyte counts (TLC), and their patterns of change in monitoring HIV
6
7
8 104 treatment responses at six months following ART initiation.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

105 **Methods**

106 **Study design and population**

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

116 **Sample size estimation**

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

126

127 **Data collection**

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

135 About 10ml of venous blood was collected aseptically from each participant; 5ml for CD4 cell
136 counts, analysed using BD FACSCount™ (Becton Dickenson, USA) and 5ml for complete blood
137 count to obtain the total lymphocyte counts, analysed by an auto-analyser (Cell DNY1800 from
138 Abbott Diagnostics Division, USA). TLC was categorized as lymphopaenia ($<1 \times 10^9/L$), normal
139 lymphocyte ($1 \times 10^9/L$ to $4 \times 10^9/L$), and lymphocytosis ($>4.0 \times 10^9/L$). We assessed for proteinuria
140 by collecting about 15ml of a fresh urine sample from each participant into an empty, clean, dry
141 container and tested using CYBOW™ strips (DFI Co. Ltd, Korea). Proteinuria was categorized as
142 negative proteinuria (no proteinuria), trace or 1+ proteinuria (equivalent to 30 to 100 mg/dl), 2+
143 proteinuria (equivalent to 100 to 300 mg/dl), 3+ proteinuria (equivalent to 300 to 1000 mg/dl), and
144 4+ proteinuria (equivalent to greater than 1000 mg/dl).

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

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

152 BMI was considered to have changed between one time point and another if it increased or
153 decreased by over 5%. BMI changes from ART initiation to six months were categorized into three
154 groups as (i) loss of more than 5%, (ii) stable (change between -5% and 5%, or (iii) gain of more
155 than 5%. The TLC were categorized as (i) lymphopaenia < 1×10^9 cells/L, (ii) normal lymphocyte
156 count $1-4 \times 10^9$ cells/L (iii) Lymphocytosis > 4×10^9 cells/L. The TLC pattern change was
157 categorized as (i) lymphopaenic at 6 months, regardless of the status at baseline or three months;
158 (ii) increased from lymphopaenic to normal or higher; (iii) stayed normal or higher (no
159 lymphopaenia seen). Urinary protein patterns were categorized as (i) proteinuria at six months
160 regardless of baseline proteinuria status; (ii) proteinuria at baseline and/or third month but not six
161 months; and (iii) no proteinuria seen.

162 **Patient and public involvement**

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

165 **Statistical methods**

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

1
2
3 171 were combined for analysis. To determine the association between BMI, TLC or urine protein to
4
5 172 CD4 count, we used correlation.
6
7

8 173 To determine the relationships between individual predictors and viral non-suppression at six
9
10 174 months, we first used modified Poisson regression for univariable analysis with an assumption that
11
12 175 viral non suppression is a non-rare outcome (more than 10%), to determine which variables to
13
14 176 include in the multivariable model [19,20]. For multivariable prediction, all predictors in the
15
16 177 univariable model with a p-value of <0.2 and age, a known confounder, were entered into the
17
18 178 modified Poisson regression model. The results of the Poisson regression model were presented
19
20 179 as relative risk (RR) and 95% confidence interval (RR; 95% CI). To determine the test
21
22 180 characteristics (sensitivity, specificity, positive predictive value (PPV), negative predictive value
23
24 181 (NPV)) of a score based on the multivariable model, we used two cut-off levels, based on the first
25
26 182 quartile and median of the score among the non-suppressed. The score was the sum of the rounded
27
28 183 coefficients for the variables for which the confidence intervals did not include 1 in a model
29
30 184 containing only these variables. Since these all rounded to 1, this is equivalent to simply counting
31
32 185 the number of these characteristics.
33
34
35
36
37
38

39 186 Based on practices in low resourced clinics, communication with the patient and the decision to
40
41 187 change the ART regimen depends on the patient's virological status at six months. CD4 cell counts
42
43 188 depend on a blood sample collected at the six-month visit and are therefore unavailable for
44
45 189 immediate decision making. We, therefore, excluded all CD4 variables from the model and used
46
47 190 parameters available at the time of the six-month visit to predict viral non-suppression.
48
49
50
51
52
53
54
55
56
57

191

Results

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

198

199

200 **Figure 1. Consort diagram.**

201

202

203 Baseline characteristics of study participants

204 Of the 215 participants analysed, the mean age (SD) was 37.1 ± 11.5 years, 146 (68%) were female,
 205 113 (53%) were never married, 45 (21%) had secondary education or higher, and 117 (54%) were
 206 unemployed (Table 1). Most participants, 59.5%, had normal BMI, while 27% were overweight,
 207 and 13% were underweight. Most participants, 113 (62%), were in WHO HIV clinical stage I, and
 208 only eight (4%) were in stage IV, though 93 (43%) had CD4 counts of 350 cells/ml or below; 83
 209 (39%) were lymphopaenic, and 111 (52%) had proteinuria at baseline.

210

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

Characteristic	n (%)	Mean \pm 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/m²)		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/mm³)		401 ± 253
<200	55 (25.6%)	
200-350	38 (17.7%)	
351-500	39 (18.1%)	
>500	83 (38.6%)	
Lymphocyte counts (x10⁹cells/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%)

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

214

215

216 **Table 2. Predictors of HIV viral load non-suppression at six months among 215 ART naïve**
 217 **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

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

219 Univariable and multivariable analysis by modified Poisson regression.

220

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

224

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

226 **ART**

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

232 a drop in body mass index at six months of 5% or more from baseline, 80% were non-suppressed
233 {RR = 2.73; 95% CI (1.36-5.47)}. In an alternative analysis, we considered BMI changes of 10%,
234 but only 9 of the non-suppressed participants (20%) had such large decreases. The risk of HIV
235 non-suppression at six months was higher among participants with proteinuria at six months {RR
236 = 2.63; 95% CI (1.25-5.54)}, Table 2. The area under the Receiver Operating Characteristic (ROC)
237 curve for the multivariable model was 0.895 (Fig 2). Baseline HIV stage was not related to HIV
238 non-suppression at six months, {adjusted RR = 1.14, 95% CI (0.63-2.08)} for baseline WHO HIV
239 clinical stage II and {adjusted RR = 0.82, 95% CI (0.51-1.31)} for advanced baseline WHO HIV
240 clinical stages (III and IV)}.

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

1
2
3 247 **Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease,**
4
5 248 **proteinuria, and total lymphocyte counts to predict viral non-suppression among ART naïve**
6
7 249 **individuals with HIV initiated on ART in Dar es Salaam, Tanzania, 2019**
8
9

10 250
11
12
13 251
14
15
16
17 252 Using the median score among the non-suppressed as a cut-off (equivalent to having any two of
18
19 253 the components), the sensitivity predicting non-suppression was 0.50, and the specificity was 0.99.
20
21 254 Only 12% of the study population met this criterion. When we lowered the cut-off scores to the
22
23 255 first quartile value among the non-suppressed (i.e. any one of the components), the sensitivity was
24
25 256 0.78 and the specificity was 0.85, with 28% of the study population meeting this criterion.
26
27
28
29
30 257

258 Discussion

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

268 Baseline WHO HIV clinical stage did not help predict HIV non-suppression at six months,
269 possibly because under the current "Test and Treat" strategy [21], most individuals initiating ART
270 are in WHO HIV clinical stages I and II (76% of our sample). Current therapy is highly effective
271 except for a few patients whose disease is so advanced that they die before the medication can
272 improve their immune status (2 patients in this study). Symptomatic individuals with advanced
273 HIV will likely adhere to treatment to alleviate their symptoms. Perhaps patients with advanced
274 disease fear death and therefore are motivated to adhere to ART, with eventual viral suppression.
275 Advanced HIV disease has been shown to be linked with ART adherence [22]. Some studies,
276 however, indicate that early HIV stages are linked with high ART adherence and viral suppression
277 [23].

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

1
2
3 281 HIV non-suppression irrespective of a participant's lymphocyte count (normal or low) before ART
4
5 282 initiation. CD4 cells are a subset of lymphocytes and therefore lymphopaenia could be indicative
6
7 283 of a decline in CD4 cells, as a result of high HIV replication with high viral load signifying viral
8
9 284 non-suppression with consequent CD4 cell destruction. Lymphopaenia was significantly
10
11 285 associated with CD4 <500 cells/mm³ at all time points. In this study, an increase in total
12
13 286 lymphocyte cell count from lymphopaenia to normal lymphocyte counts from baseline to six
14
15 287 months was significantly associated with an increase in CD4 cell count (Additional file 1). Total
16
17 288 lymphocyte count is sensitive and specific in predicting CD4 cell counts [16,24] though there have
18
19 289 been contradictory reports [25]. The assessment of total lymphocyte counts among patients on
20
21 290 ART, therefore, could serve as an alternative, especially in settings with limited availability of
22
23 291 CD4 cell count measurement. A drop in total lymphocyte count in such a setting can alert a
24
25 292 clinician to the likelihood of immunological failure. A drop in lymphocytes could also signal the
26
27 293 possibility of immunological non responders, who will need primary and secondary prophylaxis
28
29 294 for opportunistic infection.

30
31
32
33
34
35
36 295 Weight loss with consequent BMI drop of 5% or more at six months while on ART from weight
37
38 296 prior to ART initiation, predicts HIV non-suppression, though HIV non-suppression was not
39
40 297 associated with being underweight prior to ART initiation, perhaps because of the low prevalence
41
42 298 of underweight leading to low power. In this study, sustained weight gain was significantly
43
44 299 associated with viral suppression and sustained weight loss was associated with viral non-
45
46 300 suppression at six months of ART. An increase in weight and hence BMI may be a sign of immune
47
48 301 status improvement signalling a return to health [26,27] and improved survival [28], while a
49
50 302 decrease in weight and hence BMI may be a sign of HIV progression or decline in CD4 cell counts
51
52 303 [5,11,29]. Weight loss may be due to HIV itself, opportunistic infections, or HIV-associated

1
2
3 304 tumours. Failure to gain weight has been associated with efavirenz toxicity over time as was
4
5 305 observed in a study in South Africa [30]. Weight loss in both ART naïve and exposed patients has
6
7
8 306 been associated with increased morbidity and mortality [31,32]. A study in England observed that
9
10 307 each log₁₀ increase in HIV viral load was associated with a 0.92 kg decrease in body weight.
11
12 308 However, a decrease in viral load was not significantly associated with weight gain, contrary to
13
14 309 our study [33]. Since weight changes correlate with the virological response, losing weight should
15
16 310 be viewed as an alarming sign of HIV viral non suppression from any cause. Monitoring of weight
17
18 311 and body mass index prior to ART initiation and during follow up is a valuable inexpensive way
19
20 312 of identifying individuals with possible viral non suppression. In an alternative analysis, we
21
22 313 considered BMI changes of 10%, but only 9 of the non-suppressed participants (20%) had such
23
24 314 large decreases, making the 10% decrease not useful as a cut-off in our situation.
25
26
27
28

29 315 Half of the ART-naïve study participants had proteinuria, similar to a report from Zimbabwe [34].
30
31 316 The presence of proteinuria was associated with HIV non-suppression. Proteinuria at six months
32
33 317 was a strong predictor of HIV non-suppression. Proteinuria in individuals with HIV is attributed
34
35 318 to a direct effect of HIV on the glomerular and tubular epithelial cells with the progression of HIV
36
37 319 disease to AIDS, nephrotoxicity from ART, the progression of chronic kidney disease and death
38
39 320 [14,35] The higher the viral load, the greater the damage to the kidney [36]. We observed a
40
41 321 significant proportion of participants with proteinuria of 2+ or above in WHO HIV clinical stage
42
43 322 IV. This underscores the fact that, as HIV disease advances, the greater the damage to the kidneys
44
45 323 as evidenced by increasing proteinuria. Therefore, routine screening for renal disease may serve
46
47 324 not only as a follow-up of renal disease progression but also for HIV treatment response
48
49 325 monitoring.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 326 The presence of proteinuria, lymphopaenia, and a drop in BMI of 5% are relatively simple
4
5 327 parameters to monitor among people living with HIV on ART especially in a setting where viral
6
7
8 328 load monitoring is a challenge. The presence of any of these parameters should alert a clinician on
9
10 329 the possibility of viral non-response and review adherence issues including individualized
11
12 330 enhanced adherence counselling and subsequent treatment options.

13
14
15 331 Our findings require validation in a study with a larger sample size. Our small sample may have
16
17 332 constrained some predictors of viral non-suppression. Similar studies conducted in different
18
19 333 locations are also needed since local conditions and treatment standards may influence some
20
21 334 observed patterns, both in prevalence and effect. Furthermore, use of new antiviral drugs and
22
23 335 changes in patient characteristics at presentation may change our estimates, and possibly the
24
25 336 important predictor variables. We recommend further studies with extended follow up of patients
26
27 337 beyond six months to monitor further change in lymphopaenia, proteinuria and drop in BMI of 5%
28
29 338 or more especially for individuals maintained on the same regimen after enhanced adherence
30
31 339 counselling. We recommend further studies to examine the relationship between virological
32
33 340 response and anaemia as well as opportunistic infections and AIDS associated malignancies
34
35 341 especially now that ART is initiated early.

36
37
38 342 One strength of our study is the cohort design with complete follow up data at three and six months
39
40 343 for 98% of the enrolled participants. Although our scoring system is crude, it is easy to compute
41
42 344 and is likely to be valid for a wide variety of situations, whereas a score based on more precise
43
44 345 computations would at best work only in our location.

45
46
47 346

48
49
50
51
52
53
54 347 **Conclusion**

1
2
3 348 A drop in BMI by more than 5%, persistent lymphopaenia or decrease in total lymphocyte count
4
5 349 to lymphopaenia, and proteinuria at six months are associated with HIV non-suppression at 6
6
7
8 350 months after ART initiation. Scores based on these parameters are easy to use and can serve as
9
10 351 alternatives to CD4 cell counts and viral load assessment in facilities with scarcity.

352 **List of abbreviations**

16 353 AIDS: Acquired immunodeficiency syndrome

19 354 ART: Antiretroviral therapy

22 355 BMI: Body mass index

25 356 CD4: Cluster of differentiation 4

28 357 HIV: Human immunodeficiency virus

31 358 TLC: Total lymphocyte counts

34 359 WHO: World Health Organization

37 360

40 361

43 362 **Acknowledgements**

46 363 We are grateful to the participants for their willingness to take part in this study and to the health
47
48 364 workers from Temeke regional referral hospital, Mbagala Rangi-tatu district hospital, and
49
50 365 Mbagala Kizuiani dispensary for their assistance in participant recruitment and data collection.

53 366 **Author Contributions**

1
2
3 367 Study design: LJ and PM; data collection: LJ and PM; Data analysis and interpretation: LJ, PM,
4
5 368 BT and EH; Manuscript writing: LJ, PM, BT and EH. All the authors gave final approval of the
6
7
8 369 manuscript.
9

10
11 370

12 13 14 371 **Funding**

15
16 372 This research received no specific grant from any funding agency in the public, commercial or
17
18
19 373 not-for-profit sectors.
20

21 22 374 **Competing interests**

23
24
25 375 None declared.
26

27 28 376 **Patient consent for publication**

29
30
31 377 Not applicable.
32

33 34 378 **Ethics approval**

35
36
37 379 Ethical approval was obtained from the Research and Publications Committee of Muhimbili
38
39 380 University of Health and Allied Sciences (Ref. No. DA.287/298/01A). Permission to conduct the
40
41 381 study was obtained from Temeke Municipal Hospital administration. Participants were enrolled
42
43 382 after providing written informed consent. The confidentiality of patient information was ensured.
44
45 383 Participants without viral suppression at the 6th month of follow up were managed according to
46
47
48 384 Tanzania National Guidelines for management of HIV and AIDS.
49

50 51 385 **Data availability statement** 52 53 54 55 56 57

1
2
3 386 The dataset analysed during the current study is available upon reasonable request to the
4
5 387 corresponding author.
6
7

8 388 **ORCID iDs**

9
10
11 389 Basil Tumaini: <https://orcid.org/0000-0002-2894-1684>

12
13 390 Ellen Hertzmark: <https://orcid.org/0000-0003-0148-2761>

14 391 **Ethics Statement**

15
16
17 392 Muhimbili University of Health and Allied Sciences Institutional Review Board with reference
18
19 393 number DA287/298/01A/
20
21
22

23 394

24 395 **References**

- 25
26
27
28 396 1. UNAIDS. UNAIDS data 2020 | UNAIDS [Internet]. 2020 [cited 2020 Aug 19].
29 397 <https://www.unaids.org/en/resources/documents/2020/un aids-data>
30
31
32 398 2. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for
33 399 treating and preventing HIV infection: recommendations for a public health approach.
34 400 World Health Organization; 2016.
35 401 https://apps.who.int/iris/bitstream/handle/10665/208825/9789241549684_eng.pdf
36
37
38 402 3. Roberts T, Bygrave H, Fajardo E, Ford N. Challenges and opportunities for the
39 403 implementation of virological testing in resource-limited settings. *Journal of the*
40 404 *International AIDS Society*. 2012;15(2):17324. <https://doi.org/10.7448/IAS.15.2.17324>
41 405 PMID: 23078767
42
43
44 406 4. Sepkowitz KA. AIDS - the first 20 years. *New England Journal of Medicine*.
45 407 2001;344(23):1764-72. <https://www.nejm.org/doi/full/10.1056/NEJM200106073442306>
46 408 PMID: 11396444
47
48
49 409 5. Mangili A, Murman DH, Zampini AM, Wanke CA, Mayer KH. Nutrition and HIV
50 410 infection: review of weight loss and wasting in the era of highly active antiretroviral
51
52
53
54
55
56
57

- 1
2
3 411 therapy from the nutrition for healthy living cohort. *Clinical Infectious Diseases*.
4 412 2006;42(6):836-42. <https://doi.org/10.1086/500398> PMID: 16477562
5
6
7 413 6. Koethe JR, Lukusa A, Giganti MJ, Chi BH, Nyirenda CK, Limbada MI, et al. Association
8 414 between weight gain and clinical outcomes among malnourished adults initiating
9 415 antiretroviral therapy in Lusaka, Zambia. *Journal of Acquired Immune Deficiency*
10 416 *Syndromes*. 2010;53(4):507-13.
11 417 [https://journals.lww.com/jaids/Fulltext/2010/04010/Association_Between_Weight_Gain_](https://journals.lww.com/jaids/Fulltext/2010/04010/Association_Between_Weight_Gain_and_Clinical.12.aspx)
12 418 [and_Clinical.12.aspx](https://journals.lww.com/jaids/Fulltext/2010/04010/Association_Between_Weight_Gain_and_Clinical.12.aspx) PMID: 19730111
13
14 419 7. Mannheimer S, Friedland G, Matts J, Child C, Chesney M. The consistency of adherence
15 420 to antiretroviral therapy predicts biologic outcomes for Human Immunodeficiency Virus -
16 421 infected persons in clinical trials. *Clinical Infectious Diseases*. 2002;34(8):1115-21.
17 422 <https://doi.org/10.1086/339074> PMID: 11915001
18
19 423 8. Paton NI, Sangeetha S, Earnest A, Bellamy R. The impact of malnutrition on survival and
20 424 the CD4 count response in HIV-infected patients starting antiretroviral therapy. *HIV*
21 425 *medicine*. 2006;7(5):323-30. <https://doi.org/10.1111/j.1468-1293.2006.00383.x> PMID:
22 426 16945078
23
24 427 9. Wittkop L, Arsandaux J, Trevino A, Schim van der Loeff M, Anderson J, van Sighem A,
25 428 et al. CD4 cell count response to first-line combination ART in HIV-2+ patients compared
26 429 with HIV-1+ patients: a multinational, multicohort European study. *Journal of*
27 430 *Antimicrobial Chemotherapy*. 2017;72(10):2869-78. <https://doi.org/10.1093/jac/dkx210>
28 431 PMID: 29091198
29
30 432 10. Kanya MR, Mayanja-Kizza H, Kambugu A, Bakeera-Kitaka S, Semitala F, Mwebaze-
31 433 Songa P, et al. Predictors of long-term viral failure among Ugandan children and adults
32 434 treated with antiretroviral therapy. *Journal of Acquired Immune Deficiency Syndromes*.
33 435 2007;46(2):187-93. PMID: 17693883
34
35 436 11. Olaleye AO, Owhonda G, Daramola O, Adejo I, Olayiwola H, Inyang JI, et al. Factors
36 437 associated with weight gain among adult patients initiating antiretroviral therapy in Port
37 438 Harcourt, Nigeria: a retrospective cohort study. *Infectious Diseases*. 2017;49(8):635-8.
38 439 <https://doi.org/10.1080/23744235.2017.1306102> PMID: 28335659
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 440 12. Liu E, Spiegelman D, Semu H, Hawkins C, Chalamilla G, Aveika A, et al. Nutritional
4 441 status and mortality among HIV-infected patients receiving antiretroviral therapy in
5 442 Tanzania. *Journal of Infectious Diseases*. 2011;204(2):282-90.
6
7 443 <https://doi.org/10.1093/infdis/jir246> PMID: 21673040
8
9
10 444 13. Husain NE, Ahmed MH, Almobarak AO, Noor SK, Elmadhoun WM, Awadalla H, et al.
11 445 HIV-associated nephropathy in Africa: pathology, clinical presentation and strategy for
12 446 prevention. *Journal of Clinical Medicine Research*. 2018;10(1):1-8.
13 447 <https://doi.org/10.14740/jocmr3235w> PMID: 29238427
14
15
16 448 14. Szczech LA, Hoover DR, Feldman JG, Cohen MH, Gange SJ, Goozé L, et al. Association
17 449 between renal disease and outcomes among HIV-infected women receiving or not
18 450 receiving antiretroviral therapy. *Clinical Infectious Diseases*. 2004;39(8):1199-206.
19 451 <https://doi.org/10.1086/424013> PMID: 15486845
20
21
22 452 15. Imlach S, McBreen S, Shirafuji T, Leen C, Bell JE, Simmonds P. Activated peripheral
23 453 CD8 lymphocytes express CD4 in vivo and are targets for infection by Human
24 454 Immunodeficiency Virus Type 1. *Journal of Virology*. 2001;75(23):11555-64.
25 455 <https://doi.org/10.1128/JVI.75.23.11555-11564.2001> PMID: 11689637
26
27
28 456 16. Obirikorang C, Quaye L, Acheampong I. Total lymphocyte count as a surrogate marker
29 457 for CD4 count in resource-limited settings. *BMC Infectious Diseases*. 2012;12:128.
30 458 <https://doi.org/10.1186/1471-2334-12-128> PMID: 22676809
31
32
33 459 17. The United Republic of Tanzania; Ministry of Health, Community Development, Gender,
34 460 Elderly and Children; National AIDS Control Programme. National guidelines for the
35 461 management of HIV and AIDS. 6th Ed, 2017. Available from:
36 462 [https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj5](https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/Tanzania_NATIONAL_GUIDELINES_FOR_MANAGEMENT_OF_HIV_AND_AIDS_6TH_EDITION_2017.pdf)
37 463 [8iR37uA/File/Tanzania_NATIONAL GUIDELINES FOR MANAGEMENT OF HIV](https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/Tanzania_NATIONAL_GUIDELINES_FOR_MANAGEMENT_OF_HIV_AND_AIDS_6TH_EDITION_2017.pdf)
38 464 [AND AIDS 6TH EDITION 2017.pdf](https://www.differentiatedservicedelivery.org/Portals/0/adam/Content/NqQGryocrU2RTj58iR37uA/File/Tanzania_NATIONAL_GUIDELINES_FOR_MANAGEMENT_OF_HIV_AND_AIDS_6TH_EDITION_2017.pdf)
39
40
41 465 18. World Health Organization. Obesity: preventing and managing the global epidemic. WHO
42 466 Technical Report Series. 2000(894).
43 467 https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 468 https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/
4
5
6 469 19. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and
7
8 470 differences. *American Journal of Epidemiology*. 2005; 162(3):199-200.
9
10 471 <https://doi.org/10.1093/aje/kwi188> PMID: 15987728
11
12 472 20. Dwivedi AK, Mallawaarachchi I, Lee S, Tarwater P. Methods for estimating relative risk
13
14 473 in studies of common binary outcomes. *Journal of Applied Statistics*. 2014; 41(3):484-
15
16 474 500. <https://doi.org/10.1080/02664763.2013.840772>
17
18 475 21. WHO. The use of antiretroviral drugs for treating and preventing HIV infection guidelines
19
20 476 HIV/AIDS Programme. 2016 [cited 2021 Jan 19].
21
22 477 http://apps.who.int/iris/bitstream/10665/85322/1/WHO_HIV_2013.7_eng.pdf
23
24 478 22. Biressaw S, Abegaz WE, Abebe M, Taye WA, Belay M. Adherence to Antiretroviral
25
26 479 Therapy and associated factors among HIV infected children in Ethiopia: Unannounced
27
28 480 home-based pill count versus caregivers' report. *BMC Pediatrics*. 2013;13:132.
29
30 481 <https://doi.org/10.1186/1471-2431-13-132> PMID: 24229394
31
32 482 23. Haberer JE, Bwana BM, Orrell C, Asiimwe S, Amanyire G, Musinguzi N, et al. ART
33
34 483 adherence and viral suppression are high among most non-pregnant individuals with
35
36 484 early-stage, asymptomatic HIV infection: an observational study from Uganda and South
37
38 485 Africa. *Journal of the International AIDS Society*. 2019;22(2):e25232.
39
40 486 <https://doi.org/10.1002/jia2.25232> PMID: 30746898
41
42 487 24. Kwantwi LB, Tunu BK, Boateng D, Quansah DY. Body Mass Index, Haemoglobin, and
43
44 488 Total Lymphocyte Count as a Surrogate for CD4 Count in Resource Limited Settings. *J*
45
46 489 *Biomarkers*. 2017; 2017: 7907352.
47
48 490 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5412137/> PMID: 28484663
49
50 491 25. Akinola NO, Olasode O, Adediran IA, Onayemi O, Murainah A, Irinoye O, et al. The
51
52 492 search for a predictor of CD4 cell count continues: Total lymphocyte count is not a
53
54 493 substitute for CD4 cell count in the management of HIV-infected individuals in a
55
56 494 resource-limited setting. *Clinical Infectious Diseases*. 2004;39(4):579–81.
57
58 495 <https://doi.org/10.1086/422722> PMID: 15356826

- 1
2
3 496 26. Koethe JR, Jenkins CA, Lau B, Shepherd BE, Wester W, Rebeiro PF, et al. Higher time-
4 497 updated body mass index: association with improved CD4+ cell recovery on HIV
5 498 treatment. *Journal of Acquired Immune Deficiency Syndromes*. 2016;73(2):197-204.
6 499 <https://dx.doi.org/10.1097%2FQAI.0000000000001035> PMID: 27116044
7
8
9
10 500 27. Biadgilign S, Reda AA, Digaffe T. Predictors of mortality among HIV infected patients
11 501 taking antiretroviral treatment in Ethiopia: a retrospective cohort study. *AIDS research*
12 502 *and therapy*. 2012;9:15. <https://doi.org/10.1186/1742-6405-9-15> PMID: 22606951
13
14 503 28. Reda AA, Biadgilign S, Deribew A, Gebre B, Deribe K. Predictors of Change in CD4
15 504 Lymphocyte Count and Weight among HIV Infected Patients on Anti-Retroviral
16 505 Treatment in Ethiopia: A Retrospective Longitudinal Study. *Ensolu B*, editor. *PLoS One*.
17 506 2013;8(4):e58595. <https://doi.org/10.1371/journal.pone.0058595> PMID: 23573191
18
19
20 507 29. Naidoo K, Yende-Zuma N, Augustine S. A retrospective cohort study of body mass index
21 508 and survival in HIV infected patients with and without TB co-infection. *Infectious*
22 509 *Diseases of Poverty*. 2018;7:35. <https://doi.org/10.1186/s40249-018-0418-3> PMID:
23 510 29690932
24
25
26 511 30. Griesel R, Kawuma AN, Wasmann R, Sokhela S, Akpomiemie G, Venter WF, et al.
27 512 Concentration-response relationships of dolutegravir and efavirenz with weight change
28 513 after starting antiretroviral therapy. *British Journal of Clinical Pharmacology*. 2022; 88(3):
29 514 883- 893. <https://doi.org/10.1111/bcp.15177> PMID: 34954840
30
31
32 515 31. Van der Sande MA, van der Loeff MF, Aveika AA, Sabally S, Togun T, Sarge-Njie R, et
33 516 al. Body mass index at time of HIV diagnosis: a strong and independent predictor of
34 517 survival. *Journal of Acquired Immune Deficiency Syndromes*. 2004;37(2):1288-94
35 518 <https://doi.org/10.1097/01.qai.0000122708.59121.03> PMID: 15385737
36
37
38 519 32. Grinspoon S, Mulligan K. Weight loss and wasting in patients infected with Human
39 520 Immunodeficiency Virus. *Clinical Infectious Diseases*. 2003;36(Supplement_2):S69-78.
40 521 <https://doi.org/10.1086/367561> PMID: 12652374
41
42
43 522 33. Mwamburi DM, Wilson IB, Jacobson DL, Spiegelman D, Gorbach SL, Knox TA, et al.
44 523 Understanding the role of HIV load in determining weight change in the era of highly
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 524 active antiretroviral therapy. *Clinical Infectious Diseases*. 2005;40(1):167-73.
4
5 525 <https://doi.org/10.1086/426591> PMID: 15614708
6
7 526 34. Fana GT, Ndhlovu CE. Renal dysfunction among anti-retroviral therapy naïve HIV
8
9 527 infected patients in Zimbabwe. *The Central African Journal of Medicine*. 2011;57(1-4):1-
10
11 528 5. <https://www.ajol.info/index.php/cajm/article/view/73724> PMID: 24968654
12
13 529 35. Gupta SK, Smurzynski M, Franceschini N, Bosch RJ, Szczech LA, Kalayjian RC. The
14
15 530 effects of HIV-1 viral suppression and non-viral factors on quantitative proteinuria in the
16
17 531 HAART era. *Antiviral Therapy*. 2009;14(4): 543–549. PMID: 19578239
18
19 532 36. Rednor SJ, Ross MJ. Molecular mechanisms of injury in HIV-associated nephropathy.
20
21 533 *Frontiers in Medicine*. 2018;5:177. <https://doi.org/10.3389/fmed.2018.00177> PMID:
22
23 534 29930940
24
25 535

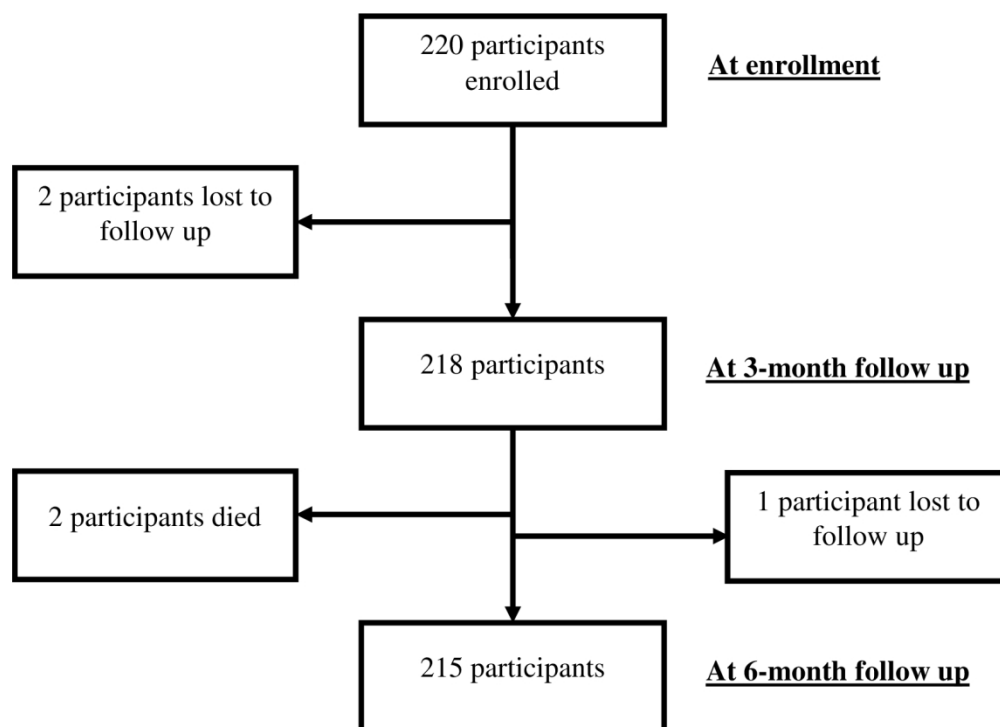


Figure 1. Consort diagram.

146x105mm (300 x 300 DPI)

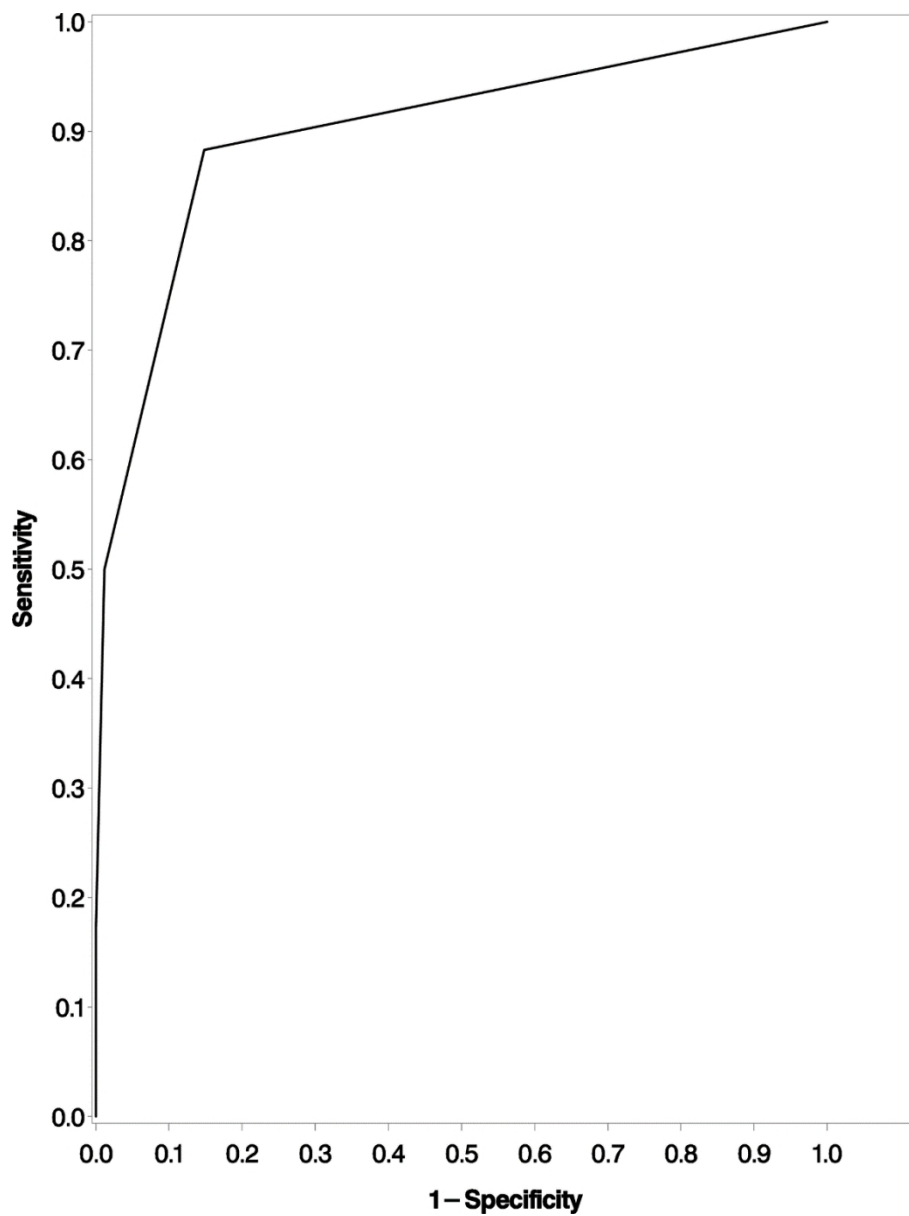
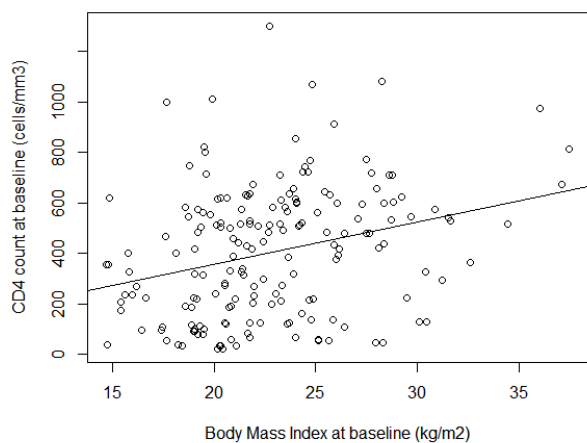


Figure 2. Receiver Operating Characteristic curve assessing a model using BMI decrease, proteinuria, and total lymphocyte counts to predict viral non-suppression among HIV-infected individuals initiated on ART in Dar es Salaam, Tanzania, 2019

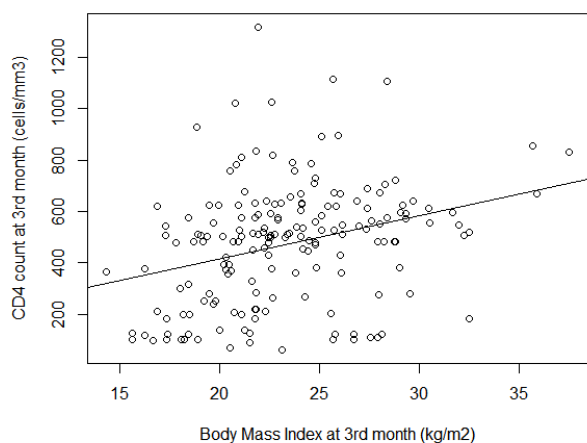
137x181mm (220 x 220 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

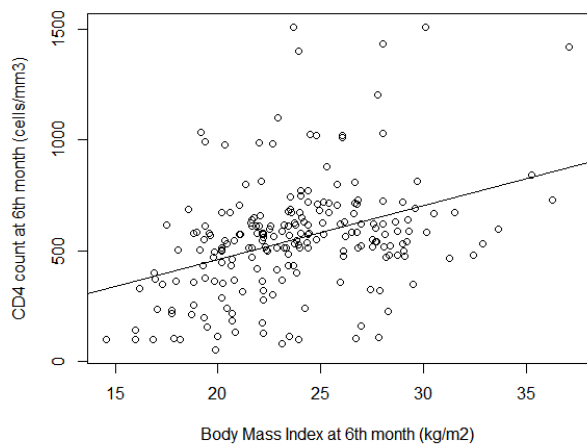
Supplementary Figure 1. Scatter plots of BMI and CD4 counts



rho 0.287, p <0.0001



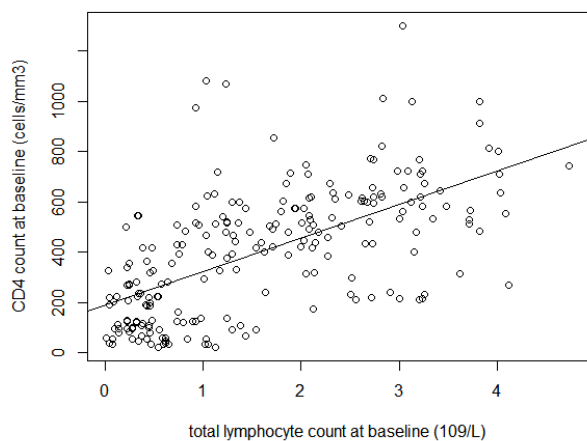
rho 0.305, p <0.0001



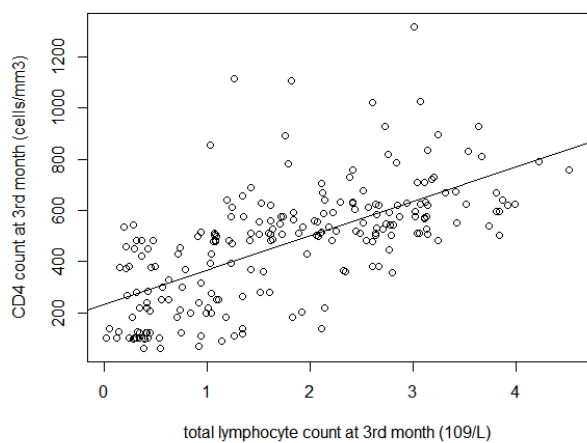
rho 0.373, p <0.0001

view only

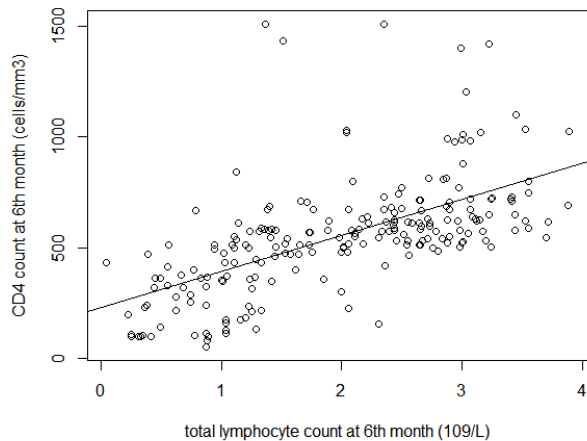
Supplementary Figure 2. Scatter plots of total lymphocyte count and CD4 count



rho 0.613, p <0.0001

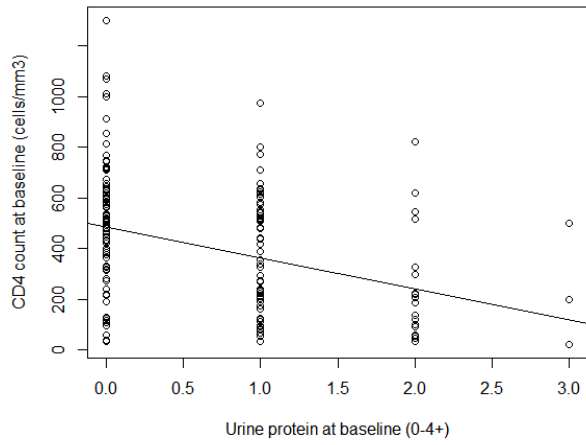


rho 0.650, p <0.0001

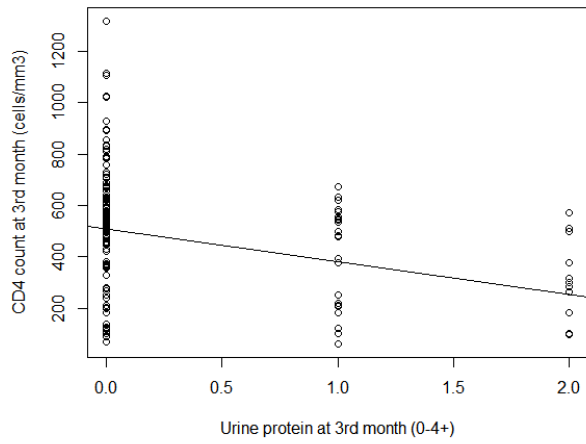


rho 0.602, p <0.0001

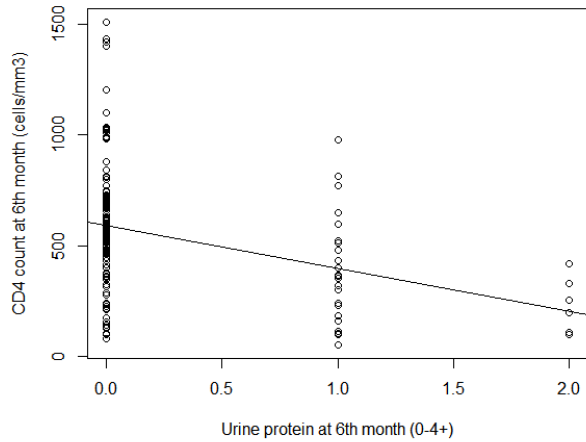
Supplementary Figure 3. Scatter plots of urine protein and CD4 count



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 cohort reporting guidelines, and cite them as:

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

			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

1	Bias	#9	Describe any efforts to address potential sources of bias	
2				
3				
4	Study size	#10	Explain how the study size was arrived at	8
5				
6				
7	Quantitative	#11	Explain how quantitative variables were handled in the	9-11
8	variables		analyses. If applicable, describe which groupings were chosen,	
9			and why	
10				
11				
12				
13				
14				
15	Statistical	#12a	Describe all statistical methods, including those used to control	
16	methods		for confounding	
17				
18				
19				
20	10,11			
21				
22				
23	Statistical	#12b	Describe any methods used to examine subgroups and	10, 11
24	methods		interactions	
25				
26				
27				
28				
29	Statistical	#12c	Explain how missing data were addressed	12
30	methods			
31				
32				
33				
34	Statistical	#12d	If applicable, explain how loss to follow-up was addressed	-
35	methods			
36				
37				
38				
39	Statistical	#12e	Describe any sensitivity analyses	
40	methods			
41				
42				
43				
44				
45	11			
46				
47				
48	Results			
49				
50				
51	Participants	#13a	Report numbers of individuals at each stage of study—eg	12
52			numbers potentially eligible, examined for eligibility, confirmed	(figure 1)
53			eligible, included in the study, completing follow-up, and	
54			analysed. Give information separately for for exposed and	
55				
56				
57				
58				
59				
60				

unexposed groups if applicable.

1			
2			
3			
4	Participants	#13b	Give reasons for non-participation at each stage
5			
6			12
7			(figure 1)
8			
9	Participants	#13c	Consider use of a flow diagram
10			
11			
12	12 (figure 1)		
13			
14			
15	Descriptive data	#14a	Give characteristics of study participants (eg demographic,
16			clinical, social) and information on exposures and potential
17			confounders. Give information separately for exposed and
18			unexposed groups if applicable.
19			
20			
21			
22			
23			
24			
25	Descriptive data	#14b	Indicate number of participants with missing data for each
26			variable of interest
27			
28			
29			
30	See 12		
31			
32			
33	Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)
34			
35			
36	12		
37			
38			
39	Outcome data	#15	Report numbers of outcome events or summary measures
40			over time. Give information separately for exposed and
41			unexposed groups if applicable.
42			
43			
44			
45			
46			
47	14		
48			
49			
50	Main results	#16a	Give unadjusted estimates and, if applicable, confounder-
51			adjusted estimates and their precision (eg, 95% confidence
52			interval). Make clear which confounders were adjusted for and
53			why they were included
54			
55			
56			
57			
58			
59			
60			

1	Main results	#16b	Report category boundaries when continuous variables were	12-15
2			categorized	
3				
4				
5				
6	Main results	#16c	If relevant, consider translating estimates of relative risk into	
7			absolute risk for a meaningful time period	
8				
9				
10				
11				
12	-			
13				
14				
15	Other analyses	#17	Report other analyses done—eg analyses of subgroups and	16
16			interactions, and sensitivity analyses	
17				
18				
19				
20	Discussion			
21				
22				
23	Key results	#18	Summarise key results with reference to study objectives	20
24				
25				
26	Limitations	#19	Discuss limitations of the study, taking into account sources of	5
27			potential bias or imprecision. Discuss both direction and	
28			magnitude of any potential bias.	
29				
30				
31				
32				
33				
34	Interpretation	#20	Give a cautious overall interpretation considering objectives,	18-20
35			limitations, multiplicity of analyses, results from similar studies,	
36			and other relevant evidence.	
37				
38				
39				
40				
41				
42	Generalisability	#21	Discuss the generalisability (external validity) of the study	20
43			results	
44				
45				
46				
47	Other Information			
48				
49				
50	Funding	#22	Give the source of funding and the role of the funders for the	22
51			present study and, if applicable, for the original study on which	
52			the present article is based	
53				
54				
55				
56				
57				
58				
59				
60				

1 None The STROBE checklist is distributed under the terms of the Creative Commons Attribution
2 License CC-BY. This checklist can be completed online using <https://www.goodreports.org/>, a tool
3 made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60