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CD4 gain percentile curves for monitoring response to antiretroviral therapy in HIV-infected adults

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Abstract

Objectives—We constructed CD4 count gain percentile distributions standardized by baseline CD4 count and assessed the association between poor CD4 count gain and subsequent death and virological failure on antiretroviral treatment (ART).

Design—Secondary analysis of ten years of clinical data from a cohort of adults initiated on ART at the Themba Lethu clinic in Johannesburg, South Africa.

Methods—The generalized additive model for location, scale and shape was used to construct percentile curves for CD4 count gain standardized by baseline CD4 count in the first 28 months of ART. Cox proportional models were used to assess the association between lower percentiles (<50th) of CD4 count gain and subsequent death and virological failure.

Results—Among 9640 non-pregnant adults 7406, with available CD4 count results for CD4 gain calculation at 4months of ART, 843 (8.7%) died subsequently and 1101 (11.4%) experienced virologic failure, respectively. For CD4 gains below the 3rd percentile, the adjusted hazard ratios at different time points ranged between 2.72 and 5.73 for death and between 1.48 to 6.93 for virologic failure. The CD4 percentile curves revealed a gradient of increasing risk of subsequent death and virological failure with lower CD4 gain percentiles and increasing time on ART, and were more informative than the WHO criteria for immunological failure or current CD4 count.

Conclusions—Percentile curves of CD4 count gain provide a simple tool for health care workers in low resource settings to monitor response to ART with improved information regarding risk of death and virological failure compared to current WHO criteria for immunological failure.

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CONTRIBUTORS

MY and AVR designed the study. MM helped with data collection. MY analyzed the data and wrote the first draft of the report. All authors contributed to the interpretation of the data and read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

Keywords

ART monitoring; immunological failure; resource limited countries

INTRODUCTION

Access to antiretroviral therapy (ART) in low- and middle-income countries has dramatically improved in recent years. At the end of 2012, 9.7 million people living with HIV were receiving ART in low- and middle-income countries [1]. Monitoring individuals receiving ART is important to ensure successful treatment, identify adherence problems and individuals at high risk of poor treatment outcomes, and to determine whether antiretroviral regimens should be switched in case of treatment failure [2].

Clinical trials have shown that viral load monitoring is a more sensitive and early indicator of treatment failure than CD4 monitoring [3–5]. In the United States [6], monitoring viral load is therefore recommended every 4–8 weeks until viral suppression is achieved and every 3 to 4 months thereafter during the first 2 years of ART. In addition, CD4 count is monitored every 3 to 6 months. The measurement of viral load requires expensive and sophisticated technologies that are not always feasible or affordable, particularly in resource-poor settings where the burden of disease is greatest. Where viral load is not routinely available, the 2013 WHO antiretroviral guidelines recommend regular CD4 count monitoring [2].

Despite highly specific and widely accepted definitions of viral failure, i.e. viral load ≥ 1000 copies/ml (WHO) or ≥ 200 copies/ml (US) on two consecutive viral load measurements 3 months apart [2, 6], there is no consensus definition for suboptimal immunologic response. Studies have used multiple definitions, most commonly failure to increase CD4 counts above a specific threshold over a specific period of time (e.g., >200 cells/mm³ by 6 months on ART) or an increase in CD4 counts above baseline levels by a certain threshold over a given time period (e.g., <0 , 0–49, 50–99 and ≥ 200 cells/mm³ by 6 months on ART) [7]. The WHO defines immunological failure as a CD4 count at or below baseline level or persistence of CD4 count below 100 cells/mm³ [2]. All these definitions ignore the fact that changes in CD4 count are not homogeneous across baseline levels of CD4 count. Furthermore, studies assessing the predictive value of the WHO definition have shown low sensitivity and low positive predictive values for predicting virological failure, particularly in patients starting ART at higher (>350) CD4 count levels [8].

Defining cut-points for indicators that are continuous in nature (e.g. CD4 count) and for which changes are strongly correlated to values of another continuous indicator (e.g. time on ART) is not uncommon in medicine. One example is that of monitoring changes in weight among children while accounting for variation with age. This is usually addressed by standardizing the distribution of the indicator of interest (e.g. CD4 count) by the continuous cofactor (e.g. time on ART) and using percentile distributions or cut-offs such as minus two standard deviations [9–14].

The aim of this study was to construct distributions of cumulative CD4 cells gains from ART initiation to 4, 10, 16, 22, and 28 months post-ART initiation, standardized by baseline CD4 count. We also assess the correlation of lower centiles (3rd, 10th, 25th, 33rd, and 50th) of the distributions with subsequent mortality and virological failure and the accuracy of those centiles for early identification of patient at risk of virological failure and death.

METHODS

Data and data source

The data was collected from the Themba Lethu Clinic Cohort, an open cohort of HIV-infected adults (> 18 years) who receive ART at a public ART clinic in Johannesburg, South Africa [15, 16]. At baseline, ART eligibility is assessed clinically and by CD4 count. In line with national guidelines, viral load is not determined at baseline. Patients initiated on ART are monitored by CD4 count and viral load at 4 months post ART initiation and every 6 months thereafter, and at any other time if clinically needed. Patients who fail to achieve viral suppression by month 4 are scheduled for monthly visits until viral suppression is achieved. Patients who miss a clinic visit are actively traced by telephone. Lost to follow-up is defined as being four months late for last scheduled clinic visit. Mortality is ascertained via family, hospital report, and linkage with the South African National Vital Registration Infrastructure Initiative, a system estimated to have 90% sensitivity for adults [17].

Study population

All non-pregnant adults with baseline CD4 count measure < 500 cell/mm³ who initiated ART between April 1, 2004 and April 1, 2014 and had at least one follow-up CD4 count result were included in the analysis.

Definitions

Baseline CD4 was defined as the closest CD4 count measurement collected 6 months before to seven days after ART initiation.

The change in CD4 count at each time point was determined using the closest measure that was collected within ± 6 weeks of the time points of interest (4, 10, 16, 22, and 28 months of ART) and calculated by subtracting the baseline value from the value at the time point of interest. Assuming a linear increase in CD4 count over time within each time interval, we then estimated the CD4 count gain at the time point of interest by dividing the observed change in CD4 count by the real time interval in months and multiplying this by 4 for the 4 month time point or by 6 for all other time points.

Viral failure was defined as the first of two consecutive viral loads >1000 RNA copies/ml after initial suppression or as not achieving viral suppression after at least 12 months on ART.

Construction of baseline-standardized distributions of CD4 cells gain at each time point

To obtain the distribution of CD4 gain at each time point of interest, methods similar to those used by the WHO to construct international growth curves in children [11] were

adapted and used as described previously [14]. The changes in CD4 cells count for each time point was regressed on baseline values using the generalized additive model for location, scale and shape, a method that requires a parametric distribution assumption for the response variable (CD4 gain over time) while allowing the modeling of the distribution parameters as nonparametric (smooth) functions of the explanatory variable (baseline CD4 count) [18]. For CD4 cell gain at each time point, we assumed a Box-Cox power exponential distribution with four parameters relating to location (μ , median), scale (σ , coefficient of variation), skewness (ν , transformation for symmetry), and kurtosis (τ , power exponential parameter), respectively [19]. The number of degrees of freedom (df) was determined by a step-down procedure in a training set which consisted of a random sub-sample of 60% of the total data set. We started with the simplest model that included baseline CD4 count and the fitting of μ and σ curves while keeping $df(\nu)$ and $df(\tau)$ fixed at zero. We searched for the $df(\mu)$ and $df(\sigma)$ that minimized the global deviance as indicated by the generalized Akaike Information Criterion (with penalty 3 for each df used). In the next step, using the selected $df(\mu)$ and $df(\sigma)$, we sequentially searched for the $df(\nu)$ and $df(\tau)$ that minimized the global deviance. In the last step, Q statistic [20] and worm plots [21] were used to fine tune the selected $df(\mu)$, $df(\sigma)$, $df(\nu)$, and $df(\tau)$ [19]. To facilitate the convergence of the models and obtain smoother curves [12, 18, 22], extreme values of CD4 count, i.e. values that looked far apart on visual inspection, were set to missing during the construction of the standardized distributions.

The performance of the fitted models was assessed using a validation set. The proportion of sample that fell below selected centile was used to compare the fit of the training and validated model (see supplemental material, Table 6) [23].

Association of lower (3rd, 10th, 25th, 33rd, and 50th) centiles of CD4 cells gain, the WHO criteria for immunological criteria, and current CD4 count values at months 4, 10, 16, 22, and 28 on ART with subsequent mortality and virological failure

For the outcome of death, person-time started at start of ART (baseline) and was censored at the earliest of 1) recorded date of death or 2) at the date of last visit to the clinic. For the outcome of virological failure, follow-up time started at ART initiation (baseline) with follow-up censored at the earliest of 1) first viral load >1000 RNA copies/ml, 2) date of death or 3) date of last visit to the clinic. Cox proportional hazard models were used to estimate the crude and adjusted hazard ratios (HR) and their 95% confidence intervals (CI) for the association between the lower centiles of CD4 cells count gain at each time point with each of the two outcomes considered. A Cox proportional model was also used to estimate the strength of the association between WHO definition for immunological failure and subsequent mortality and virological failure at each time point. The proportional hazard assumption was formally evaluated for all baseline covariates (BMI, WHO clinical stage, hemoglobin, CD4 count and age) using the Kolmogorov-type supremum test [24]. Using a stepwise backward selection procedure and Wald test, all covariates that did not contribute significantly to the fit of each model were dropped. Analyses were done using SAS 9.3 (SAS Institute, Cary, NC). All tests were conducted using a 2-sided 0.05 significance level, without correction for multiple comparisons.

Sensitivity, specificity, negative and positive predictive values of the WHO definition for immunologic failure and CD4 count gain percentile for subsequent mortality and virological failure

We used standard definitions [25] to compute the sensitivity, specificity, positive and negative predictive values of the WHO definition for immunological failure and the 3rd, 10th, 25th, 33rd, and 50th percentiles of CD4 count gain for predicting subsequent death and virological failure.

RESULTS

Characteristics of the cohort at ART initiation

Of the 21921 non-pregnant adults with baseline CD4 count ≥ 500 cells/mm³ initiating ART between April 1, 2004 and April 1, 2014, 1022 died, 745 were lost to follow up or transferred out, 391 were administratively censored before reaching 4 months of ART. Of the 19980 completing ART for at least 4 months, 14194 had at least one follow-up CD4 count collected by the seventh month of ART including 9640 at 4 months ± 6 weeks post ART initiation. A CD4 count result was available at 10, 16, 22, and 28 months ± 6 weeks of ART for 7406, 5577, 4804, and 3446 individuals, respectively.

Among the 9640 included in the analysis of 4 months CD4 gain, 40.6% were male, median age was 37.2 [inter quartile range (IQR) 32.0 – 43.5] years, 39.4% were in WHO clinical stage III or IV, and almost half (49.7%) had a baseline CD4 count below 100 cells/mm³ (Table 1). Baseline characteristics of those excluded did not differ substantially from those included in the analysis (Table 4, supplemental material).

Cumulative CD4 gains at months 4, 10, 16, 22, and 28 months of ART and their baseline-standardized distributions

The median cumulative gain in CD4 count increased from four to 28 months on ART, with the greatest gain in patients with lowest baseline CD4 counts: from 118 and 36 cells/mm³ at 4 months to 278 and 148 cells/mm³ at 28 months for those with baseline CD4 count of 50 and 450 cells/mm³, respectively (Figure 1).

Association between the 3rd, 10th, 25th, 33rd, and 50th percentile of cumulative CD4 gain and subsequent time to death

Among the 9640 patients initiated on ART who were retained in the cohort at 4 months of follow up and have both baseline and the 4 months CD4 count measurements, 843 (8.7%) died after 4 months of ART, with most (48.3%) deaths occurring early (between 4 and 10 months), 436 deaths occurring after 10 months, 244 after 16 months, 203 after 22 months, and 131 after 28 months of ART. Among patients who died, about half had a CD4 gain below the 33rd percentile: 46.5% at 4 months, 49.8% at 10 months, 50.0% at 16 months, 53.7% at 22 months, and 49.6% at 28 months. Low CD4 count gain at any time point was associated with subsequent death, with strength of association increasing with decreasing centiles of CD4 gain and increasing length of follow-up (Table 2). For example, the HR (95% CI) for subsequent death among patients whose CD4 gain at 4, 10, 16, 22, and 28

months was below the 3rd percentile, was 2.72 (2.03, 3.65), 3.65 (2.50, 5.33), 4.24 (2.72, 6.61), 4.89 (2.98, 7.96), and 5.73 (3.26, 10.06), respectively.

Association between the 3rd, 10th, 25th, 33rd, and 50th percentile of cumulative CD4 gain and subsequent time to virological failure

In total, 1101 (11.4%) patients experienced virological failure after 4 months, 812 after 10 months, 662 after 16 months, 633 after 22 months, and 500 after 28 months of ART. Of patients who experienced virological failure, 30.5%, 36.1%, 43.8%, 48.9%, and 50.4% had a CD4 gain below the 33rd percentile at 4, 10, 16, 22, and 28 months, respectively. As with mortality, lower percentiles of CD4 gain at any time point were associated with subsequent virological failure with the strength of association increasing with decreasing centiles and increasing time on ART (Table 2). For example, the HR (95% CI) for subsequent virological failure for patients whose CD4 gain at 4, 10, 16, 22, and 28 months was below the 3rd percentile for instance, was 1.48 (1.01, 2.15), 2.69 (1.91, 3.78), 4.00 (2.91, 5.50), 6.93 (5.17, 9.30), and 4.49 (3.14, 6.43), respectively.

Association between WHO-defined immunologic failure and subsequent mortality and virological failure

The proportion of participants meeting the WHO criteria for immunological failure decreased with time on ART, from 19.8% at 4 months, to 9.8% at 10 months, 6.7% at 16 months, 5.2% at 22 months and 4.1% at 28 months. There was a U shape relationship between the WHO-defined immunologic failure and baseline CD4 levels with higher proportion of patients with lowest and highest baseline CD4 count being identified as immunological failure, particularly at earlier follow-up time points (Figure 2). The strength of the association between WHO defined immunological failure and subsequent mortality or virological failure increased with increasing time on ART (Table 2). The aHR (95% CI) for subsequent virological failure was 0.84 (0.71, 1.00) at 4 months and increased to 1.78 (1.44, 2.20) at 10 months, 3.30 (2.59, 4.21) at 16 months, 5.54 (4.29, 7.15) at 22 months, and 4.78 (3.46, 6.61) at 28 months. Similarly, the aHR (95% CI) for subsequent death increased from 1.74 (1.48, 2.04) at 4 months to 5.30 (3.07, 9.16) at 28 months.

Association between current CD4 count and subsequent mortality and virological failure

The median (IQR) current CD4 count at 4, 10, 16, 22, and 28 months was 211 (135, 302), 256 (177, 356), 307 (218, 416), 340 (244, 460), and 370 (266, 493) cells/mm³, respectively. The aHR (95% CI) for subsequent death for every 50 cells/mm³ decrease in the current CD4 count was similar at all time points, ranging between 1.16 (1.12, 1.20) at 4 months and 1.23 (1.15, 1.32) at 28 months (Table 2). The aHR (95% CI) for subsequent virological failure ranged from 0.97 (0.94, 1.00) at 4 months to 1.16 (1.12, 1.20) at 28 months.

Sensitivity, specificity, and predictive values of lower centiles of CD4 gain and WHO-defined immunological failure as early indicators of virological failure and death

As expected, sensitivity decreased and specificity increased with lower CD4 gain centiles (Table 3). The negative predictive values (NPV) for mortality were high (> 90%) at each time point and for all CD4 gain centiles below 50. The positive predictive value (PPV) was

low and decreased with increasing time on ART. The NPV for virological failure was also high (> 83%) for any centiles but constant over time. The PPV for virological failure tended to increase with decreasing centiles and with increasing time on ART. The PPV for virological failure was higher than that for mortality, especially at later time points and lower centiles. The NPV of the WHO definition for immunological failure was also high, > 90% for death and > 85% for virological failure. Similarly to low CD4 gain centiles, the PPV was low for death (<15%) at all time points. The PPV of the WHO definition for immunological failure for subsequent virological failure was lower than that of the third percentile of the CD4 gain distribution, especially early after ART initiation.

DISCUSSION

Using data from a large cohort of patients receiving care at an ART clinic in South Africa, we constructed percentile curves for CD4 count gain on ART, standardized by baseline CD4 count. Associations with subsequent death and virological failure showed an increasing strength of association with lower centiles and longer time on ART. The use of CD4 gain centiles allowed a graded risk assessment of predicted death and virological failure compared to the binary yes/no risk profile associated with the WHO definition for immunological failure and demonstrated stronger associations with subsequent death and virological failure compared to current CD4 count. The positive predictive value however remained low, especially for risk of death.

Although virologic monitoring is the gold standard for ART monitoring, randomized control trials [5, 26, 27] found routine monitoring of CD4 count to perform similar to virologic monitoring in term of prediction of disease progression and mortality. The WHO based the preference of viral load over CD4 count monitoring on the poor accuracy of routine CD4 count monitoring for early detection of treatment failure when using the WHO definition for immunological failure [28]. As we demonstrated in this analysis, the poor accuracy may, at least in part, be attributable to the high proportion of patients at the extremes of baseline CD4 count that are misclassified as immunological failure when using the WHO criteria to define immunological failure (Figure 2). The proposed CD4 gain percentiles standardized to baseline CD4 count correct this problem and slightly improve the accuracy for early detection of treatment failure. While the use of CD4 count gain percentile curve as an indicator of subsequent risk of treatment failure still suffered from low positive predictive value, especially in the first year of treatment, the CD4 centile curves offer health care workers a tool to grade the risk of subsequent death and virological failure in their patients. As the CD4 gain drops from the 50th to 3rd centile, the risk of subsequent death increases from a 1.6 to 2.7 fold hazard at 4 months of ART and from a 1.9 to 5.7 fold hazard at 28 months of ART. Similarly, as CD4 count gain drops from the 50th to 3rd centile, the hazard of subsequent virological failure increases from a 1.1 to 2.7 fold hazard at 10 months of ART and from a 1.8 to 5.6 fold hazard at 22 months of ART. This insight in increasing risk could allow health care workers to target interventions such as more frequent clinical follow-up, intensified adherence counseling and intensified screening for opportunistic infections to those patients at highest risk or patients with increasing risk profiles over time.

Our analysis has many strengths, including a large sample allowing the use of a training and validation dataset, long follow-up (median follow-up time on ART of 54 months), and regular availability of viral load measurement. The exclusion of patients initiating ART due to lack of regular CD4 counts, transfer out or loss to follow up is a limitation, but the bias introduced may be minimal as baseline characteristics of those excluded from the analysis did not differ from those included. The restriction to patients receiving ART at a single center is another limitation that may limit generalizability. Although, the baseline characteristics of patients in our cohort were similar to patients receiving ART in the region [16] and the median of current CD4 count at each time point were similar to that reported from an analysis of data from 22 sites in Africa, two in Asia, and three in Latin America [29], confirmation of the results using data from other facilities in the region or multiple regions would strengthen our findings.

In conclusion, while viral load is the gold standard for monitoring the response to antiretroviral treatment, [2] viral load assays are not yet routinely available in most settings with high burden of HIV. In those setting the WHO recommends that CD4 count be used. The percentile curves for monitoring CD4 gain may be a simple tool enabling health care workers in resource limited settings to identify patients at increased risk of virological failure and death and target interventions at those with highest or increasing risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. CD4 gain centile curves at month at month 4, 10, 16, 22, and 28 of ART among non-pregnant HIV-infected adults

Curves were obtained using Box Cox power exponential distribution (BCPE) and the generalized additive model for location, scale, and shape.

Model for centile curves at four months (A): BCPE (baseline CD4 count, $df(\mu) = 12.9$, $df(\sigma) = 10.5$, $df(\nu) = 8$, $df(\tau) = 3$). Model for centile curves at ten months (B): BCPE (baseline CD4 count, $df(\mu) = 3.2$, $df(\sigma) = 2.2$, $df(\nu) = 0.5$, $df(\tau) = 0$). Model for centile curves at sixteen months (C): BCPE (baseline CD4 count, $df(\mu) = 0$, $df(\sigma) = 2.7$, $df(\nu) = 0.5$, $df(\tau) = 0$). Model for centile curves at twenty-two months (D): BCPE (baseline CD4 count, $df(\mu) = 3.9$,

$df(\sigma) = 2.0$, $df(\nu) = 3.7$, $df(\tau)=0.5$). Model for centile curves at twenty-eight months (D):
BCPE (baseline CD4 count, $df(\mu) = 2.0$, $df(\sigma) = 0.7$, $df(\nu) = 1.3$, $df(\tau)=2.0$).

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Figure 2. Proportion of patients with WHO-defined immunological failure by time on ART and by baseline CD4 count

Table 1

Characteristics at ART initiation in 9640 HIV-infected adults included in the analysis of 4-month CD4 count gain

	number**	Percentage
Sex		
Male	3915	40.61
Female	5725	59.39
WHO Clinical stage §		
I	5681	58.93
II	159	1.65
III	3130	32.47
IV	670	6.95
CD4 count: median (IQR) *	101	(40, 172)
0 – 100	4792	49.71
101 – 200	3434	35.62
201 – 350	1260	13.07
351 – 500	154	1.6
Hemoglobin: median (IQR)	11.8	(10.20, 13.20)
< 10 g/dl	7148	79.09
>= 10 g/dl	1890	20.91
BMI: median (IQR) §	21.67	(19.23, 24.89)
< 18.5	1541	17.86
18.5–24.9	4999	57.93
25 – 29.9	1427	16.54
30 and up	663	7.68
Age in years: median (IQR)	37.20	(32.00, 43.50)
<25	345	3.58
25–29	1232	12.78
30–34	2193	22.75
35–39	2230	23.13
40–44	1613	16.73
45–49	1036	10.75
50–55	578	6
>55	413	4.28

¶ BMI = Body mass index (kg/cm²), IQR = interquartile range.

** Totals vary by baseline characteristics due to missing data.

§ The closest value available from 30 days before to seven days after ART initiation.

* The closest CD4 count measurement from 6 months before to seven days post-ART initiation

Table 2

Association of lower (3rd, 10th, 25th, 33rd, and 50th) centiles of CD4 cells gain at months 4, 10, 16, 22, and 28 post-ART and WHO immunological failure with subsequent mortality and virological failure

Time to Death after 4 months					Time to Viral failure [£]						
Total		events		HR (95 Confidence Interval)		Total		events		HR (95 Confidence Interval)	
N	n(%)	crude	Adjusted*	N	n(%)	crude	adjusted [‡]				
CD4 gain at 4 month after ART initiation											
3rd	273	56 (20.51)	3.06 (2.34, 4.02)	264	34 (12.88)	1.61 (1.14, 2.26)	1.48 (1.01, 2.15)				
10th	916	153 (16.70)	2.43 (2.04, 2.89)	2386	245 (10.27)	1.09 (0.88, 1.35)	1.09 (0.87, 1.38)				
25th	2431	313 (12.88)	1.88 (1.63, 2.16)	3196	330 (10.33)	0.92 (0.80, 1.06)	0.92 (0.79, 1.08)				
33rd	3250	392 (12.06)	1.80 (1.58, 2.07)	3196	330 (10.33)	0.90 (0.79, 1.02)	0.90 (0.78, 1.04)				
50th	4837	514 (10.63)	1.63 (1.42, 1.87)	4767	502 (10.53)	0.88 (0.78, 0.99)	0.87 (0.76, 0.99)				
CD4 gain at 10 month after ART initiation											
3rd	185	29 (15.68)	3.21 (2.20, 4.68)	176	43 (24.43)	2.72 (1.99, 3.71)	2.69 (1.91, 3.78)				
10th	641	79 (12.32)	2.43 (1.91, 3.10)	627	115 (18.34)	1.77 (1.45, 2.15)	1.82 (1.47, 2.26)				
25th	1739	160 (9.20)	1.89 (1.55, 2.29)	1719	252 (14.66)	1.40 (1.21, 1.62)	1.47 (1.26, 1.75)				
33rd	2295	202 (8.80)	1.85 (1.53, 2.24)	2274	315 (13.85)	1.27 (1.11, 1.46)	1.35 (1.16, 1.58)				
50th	3494	278 (7.96)	1.83 (1.50, 2.22)	3472	449 (12.93)	1.14 (1.00, 1.31)	1.16 (1.00, 1.34)				
CD4 gain at 16 month after ART initiation											
3rd	153	25 (16.34)	4.67 (3.09, 7.07)	148	49 (33.11)	3.83 (2.86, 5.13)	4.00 (2.91, 5.50)				
10th	496	54 (10.89)	3.07 (2.27, 4.15)	491	116 (23.63)	2.52 (2.07, 3.09)	2.62 (2.10, 3.27)				
25th	1292	106 (8.20)	2.51 (1.95, 3.23)	1287	237 (18.41)	1.92 (1.64, 2.26)	2.01 (1.67, 2.40)				
33rd	1727	122 (7.06)	2.14 (1.66, 2.75)	1722	290 (16.84)	1.74 (1.49, 2.03)	1.80 (1.51, 2.14)				
50th	2570	165 (3.22)	2.24 (1.71, 2.93)	2563	390 (15.22)	1.60 (1.37, 1.87)	1.69 (1.42, 2.01)				
CD4 gain at 22 month after ART initiation											
3rd	123	19 (15.45)	4.36 (2.72, 7.00)	120	59 (49.17)	5.67 (4.33, 7.42)	6.93 (5.17, 9.30)				

Time to Death after 4 months					Time to Viral failure [£]				
Total		events		HR (95 Confidence Interval)	Total		events		HR (95 Confidence Interval)
N	n(%)	crude	Adjusted*		N	n(%)	crude	adjusted [£]	
10th	414	44 (10.63)	2.98 (2.13, 4.16)	3.32 (2.34, 2.34)	410	139 (33.90)	3.54 (2.94, 4.27)	4.09 (3.31, 5.05)	
25th	1096	85 (7.76)	2.31 (1.74, 3.05)	2.37 (1.74, 3.21)	1092	257 (23.53)	2.35 (2.01, 2.76)	2.45 (2.05, 2.94)	
33rd	1446	109 (7.54)	2.49 (1.89, 3.28)	2.48 (1.83, 3.36)	1442	309 (21.43)	2.18 (1.86, 2.55)	2.27 (1.90, 2.71)	
50th	2192	137 (6.25)	2.19 (1.63, 2.94)	2.01 (1.46, 2.77)	2188	393 (17.96)	1.81 (1.54, 2.13)	1.85 (1.54, 2.22)	
CD4 gain at 28 month after ART initiation									
3rd	110	15 (13.64)	3.40 (3.20, 9.39)	5.73 (3.26, 10.06)	108	39 (36.11)	3.54 (2.54, 4.93)	4.49 (3.14, 6.43)	
10th	316	31 (9.81)	3.67 (2.45, 5.49)	3.83 (3.26, 5.92)	313	93 (29.71)	2.90 (2.31, 3.64)	3.28 (2.54, 4.25)	
25th	868	60 (6.91)	2.68 (1.90, 3.78)	2.63 (1.80, 3.86)	865	206 (23.82)	2.37 (1.98, 2.84)	2.46 (2.00, 3.03)	
33rd	1139	65 (5.71)	2.09 (1.49, 2.95)	2.01 (1.37, 2.95)	1136	252 (22.18)	2.28 (1.91, 2.72)	2.43 (1.98, 2.99)	
50th	1735	87 (5.01)	2.04 (1.42, 2.93)	1.95 (1.30, 2.93)	1732	316 (18.24)	1.83 (1.53, 2.20)	1.93 (1.56, 2.39)	
WHO immunological Failure									
4	1990	272 (13.67)	1.94 (1.68, 2.24)	1.74 (1.48, 2.04)	1959	212 (10.82)	0.96 (0.84, 1.13)	0.84 (0.71, 1.00)	
10	704	84 (11.93)	2.34 (1.84, 2.96)	2.34 (2.34, 3.02)	689	122 (17.71)	1.71 (1.41, 2.08)	1.78 (1.44, 2.20)	
16	370	50 (13.51)	3.92 (2.88, 5.35)	3.74 (2.65, 5.27)	364	101 (27.75)	3.10 (2.50, 3.83)	3.30 (2.59, 4.21)	
22	244	35 (14.34)	4.08 (2.84, 5.88)	5.37 (3.64, 7.95)	240	96 (40.00)	4.11 (3.30, 5.12)	5.54 (4.29, 7.15)	
28	158	17 (10.76)	4.11 (2.47, 6.84)	5.30 (3.07, 9.16)	156	55 (35.26)	3.43 (2.59, 4.56)	4.78 (3.46, 6.61)	
Current CD4 count [§]									
4	9640	843 (8.74)	1.18 (1.15, 1.22)	1.16 (1.12, 1.20)	9512	1096 (11.52)	1.02 (0.99, 1.04)	0.97 (0.94, 1.00)	
10	6903	436 (6.32)	1.19 (1.14, 1.24)	1.16 (1.11, 1.21)	6873	868 (12.63)	1.06 (1.03, 1.09)	1.07 (1.04, 1.10)	
16	5131	244 (4.76)	1.21 (1.15, 1.27)	1.18 (1.11, 1.24)	5120	659 (12.87)	1.11 (1.08, 1.14)	1.15 (1.11, 1.20)	
22	4359	203 (4.66)	1.21 (1.15, 1.27)	1.20 (1.13, 1.27)	4351	629 (14.46)	1.15 (1.12, 1.18)	1.17 (1.13, 1.21)	
28	3428	131 (3.82)	1.21 (1.14, 1.29)	1.23 (1.15, 1.32)	3422	497 (14.52)	1.13 (1.10, 1.17)	1.16 (1.12, 1.20)	

*Time from ART initiation to death or last clinic visit whichever occurred first, censored at 59 months.

£Time from ART initiation to the date of first two consecutive viral loads >1 000 RNA copies/ml after at least 12 month of ART or last clinic visit after 12 months of ART, whichever occurred first.

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[†] Adjusted for baseline hemoglobin and age for 4 months, age and BMI for 10 months, age, BMI, hemoglobin, and WHO clinical stage at 16 and 22 months, no covariate met the inclusion criteria for 28 months model.

[‡] Adjusted for baseline CD4 count and age for 4 and 10 months, age, CD4 count, and WHO clinical stage a 16 and 22 months, and age alone at 28 months.

[§] Hazard ratio are for every 50 cells/mm³ increase in current CD4 count value.

Table 3

Accuracy of CD4 count gains and WHO immunological failure by interval time for early identification of patients with subsequent virological failure or deaths.

	Deaths				Virological Failure			
	4 months post ART initiation				4 months post ART initiation			
centiles	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV
3rd	6.6%	97.5%	20.5%	91.6%	3.1%	97.3%	12.9%	88.5%
10th	18.1%	91.3%	16.7%	92.1%	22.3%	78.4%	10.3%	90.1%
25th	37.1%	75.9%	12.9%	92.6%	30.0%	65.9%	10.3%	87.8%
33th	46.5%	67.5%	12.1%	92.9%	30.0%	58.1%	10.3%	83.8%
50th	61.0%	50.9%	10.6%	93.2%	45.6%	49.3%	10.5%	87.4%

10 months post ART initiation					10 months post ART initiation				
3rd	6.7%	97.6%	15.7%	93.9%	4.9%	97.8%	24.4%	87.6%	
10th	18.1%	91.3%	12.3%	94.3%	13.2%	91.5%	18.3%	87.9%	
25th	36.7%	75.6%	9.2%	94.7%	28.9%	75.6%	14.7%	88.0%	
33th	46.3%	67.6%	8.8%	94.9%	36.1%	67.4%	13.9%	87.9%	
50th	63.8%	50.3%	8.0%	95.4%	51.5%	49.7%	12.9%	87.6%	

16 months post ART initiation					16 months post ART initiation				
3rd	10.2%	97.4%	16.3%	95.6%	7.4%	97.8%	33.1%	87.7%	
10th	22.1%	91.0%	10.9%	95.9%	17.5%	91.6%	23.6%	88.2%	
25th	43.4%	75.7%	8.2%	96.4%	35.8%	76.5%	18.4%	88.9%	
33th	50.0%	67.2%	7.1%	96.4%	43.8%	67.9%	16.8%	89.1%	
50th	67.6%	50.8%	6.4%	96.9%	58.9%	51.3%	15.2%	89.4%	

22 months post ART initiation					22 months post ART initiation				
3rd	9.4%	97.5%	15.4%	95.7%	9.3%	98.4%	49.2%	86.4%	
10th	21.7%	91.1%	10.6%	96.0%	22.0%	92.7%	33.9%	87.5%	
25th	41.9%	75.7%	7.8%	96.4%	40.6%	77.6%	23.5%	88.5%	
33th	53.7%	67.8%	7.5%	96.8%	48.8%	69.6%	21.4%	88.9%	
50th	67.5%	50.6%	6.3%	97.0%	62.1%	51.8%	18.0%	88.9%	

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centiles	Deaths				Virological Failure			
	4 months post ART initiation				4 months post ART initiation			
	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV
	28 months post ART initiation				28 months post ART initiation			
3rd	11.5%	97.1%	13.6%	96.5%	7.4%	97.8%	33.1%	87.7%
10th	23.7%	91.4%	9.8%	96.8%	17.5%	91.6%	23.6%	88.2%
25th	45.8%	75.5%	6.9%	97.2%	35.8%	76.5%	18.4%	88.9%
33th	49.6%	67.4%	5.7%	97.1%	43.8%	67.9%	16.8%	89.1%
50th	66.4%	50.0%	5.0%	97.4%	58.9%	51.3%	15.2%	89.4%

WHO Immunological Failure							
Months	Sens	Spec	PPV	NPV	Sens	Spec	NPV
4	32.0%	80.6%	13.7%	92.5%	19.1%	79.4%	88.2%
10	19.0%	90.5%	11.9%	94.3%	13.9%	90.6%	87.9%
16	20.2%	93.5%	13.5%	95.9%	15.1%	94.1%	88.1%
22	17.0%	95.0%	14.3%	95.9%	15.0%	96.1%	86.8%
28	12.9%	95.7%	10.8%	96.5%	10.8%	96.6%	86.3%

Abbreviation: Sens = Sensitivity; Spec = Specificity; PPV = Positive Predictive Value, NPV = Negative Predictive Value