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CD4 Variability in Malawi: Implications for Use of a CD4 Threshold of 500 Cells/mm^3 Versus Universal Eligibility for Antiretroviral Therapy

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Background. Given the uncertainty about the ability of a single CD4 count to accurately classify a patient as antiretroviral therapy (ART) eligible, we sought to understand the extent to which CD4 variability results in misclassification at a CD4 threshold of 500 cells/mm^3.

Methods. We performed a prospective study of CD4 variability in Malawian human immunodeficiency virus-infected, ART-naive, World Health Organization (WHO) stage 1 or 2, nonpregnant adults. CD4 counts were performed daily for 8 days. We fit a Bayesian linear mixed-effects model of log-transformed CD4 cell counts to the data. We used Monte Carlo approximations to estimate misclassification rates for different observed values of CD4. The misclassification rate was calculated based on the conditional probability of true CD4 given the geometric mean of observed CD4 measurements.

Results. Fifty patients were enrolled from 2 sites. The median age was 33.5 years (interquartile range, 27.5–40.0) and 34 (68%) were female. Misclassification rates were <1% when the observed CD4 counts were ≤250 or ≥750 cells/mm^3. Rates of misclassification were high at observed CD4 counts between 350 and 650 cells/mm^3, particularly when a single measurement was used (up to 46.7%).

Conclusions. Our data show that ART eligibility based on a single CD4 count results in highest risk of misclassification when observed CD4 counts are in the range of 350–650 cells/mm^3. Given the benefits of early ART, countries should weigh the costs and complexity of CD4 testing using a 500 cell/mm^3 threshold against the cost savings and public health benefits of universal eligibility.

Keywords. antiretroviral eligibility; antiretroviral therapy; CD4 cell count measurement.

The use of CD4 for determining degree of immunosuppression, risk for opportunistic infections, and eligibility for antiretroviral therapy (ART) is well established [1–4]. CD4 thresholds for ART initiation have increased over time based on both randomized and observational data showing benefits of earlier initiation of therapy [1–5]. Most recently, 2 large randomized studies have been published showing the benefits of ART at CD4 thresholds above 350–500 cells/mm^3 [1, 6], and in September 2015, the World Health Organization (WHO) updated its guidelines to recommend universal eligibility [7]. The Malawi National Guidelines currently recommend CD4 testing every 3 months for human immunodeficiency virus (HIV)-infected individuals, with initiation of treatment at a CD4 count of ≤500 cells/mm^3;

however, in 2016, the guidelines are expected to change to universal eligibility without baseline CD4 testing (A. Jahn, personal communication, 23 February 2016, Boston, Massachusetts).

Many resource-constrained countries do not have the capacity to rapidly increase the number of people on ART and may choose to continue to use a CD4 threshold of 500 cells/mm^3 to triage those most in need of treatment. Certain countries, such as Mozambique [8], have continued to use a threshold of 350 cells/mm^3 due to limited capacity to expand care. In these settings, eligibility has typically been based on a single CD4 count, and instrument and/or physiologic variability has the potential to misclassify patients, resulting in initiation of ART in patients with true CD4 counts above the eligibility threshold or missing an opportunity for starting ART in patients with a true CD4 count below the eligibility threshold [9, 10]. Prior studies have shown that physiologic variability exists in CD4 counts due to instrument error and biologic factors such as smoking [11–13], menstruation [14], physical exercise [15–17], time of day [18], and concurrent illness [19, 20]. In high-resource settings, before recent recommendations recommending universal ART, CD4 cell counts were frequently repeated to establish a baseline, particularly when CD4 values were near a treatment threshold level.

CD4 programs have high financial and opportunity costs for resource-limited countries, including laboratory instruments, procurement of reagents and other supplies, and human resources [21]. Investment in CD4 infrastructure can result in decreased availability of funds for other HIV- and non-HIV-related programs. Given the uncertainty about the ability of a single CD4 count to accurately classify a patient as ART eligible and the cost of maintaining a CD4 program in a resource-limited setting such as Malawi, we sought to understand misclassification risk using a single CD4 value in the setting of a treatment threshold of 500 cells/mm^3. We hypothesized that, due to the natural physiologic variability of CD4 counts, use of a single CD4 count for ART eligibility results in high rates of misclassification for patients with observed CD4 counts near the 500 threshold (±100 cells/mm^3), and that this finding may have implications for Malawi and other countries making decisions about continuing CD4 threshold-driven eligibility versus moving to universal eligibility without baseline CD4 measurement.

METHODS

We performed a prospective study of CD4 variability at 2 hospital-based ART clinics in the Central Region of Malawi. Subjects were eligible if they were HIV-infected, ART-naive, 18 years and older, and were staged by a clinician as WHO stage 1 or 2. Individuals were excluded if they were younger than 18 years of age, pregnant, had been on ART at any time in the past (except for short course prevention of mother-to-child transmission regimens), if they had WHO stage 3 or 4 conditions, or if they had any concurrent illness. Convenience sampling was used to enroll 25 subjects from each of the 2 clinical sites. All subjects approached consented to enrollment. At the time of presentation for WHO staging, patients were invited to participate in the study and were enrolled after written informed consent was obtained. The study was approved by the Malawi College of Medicine Research Committee (COMREC approval P.08/14/1613) and was given a nonhuman subjects designation by the University of California, Los Angeles.

At enrollment, a baseline questionnaire was completed to collect demographic and clinical information and blood was drawn for CD4 cell count determination. All participants were asked to return to the clinic for CD4 count determination for an additional 7 consecutive weekdays (a total of 8 CD4 cell counts per participant). Collection of blood samples was alternated between morning and afternoon to allow for assessment of diurnal variation of CD4 cell counts. Subjects were interviewed before each sample collection to ask about new symptoms of illness and to document travel time (physical exertion), tobacco use, and, for female participants, menstruation.

Samples were tested on a BD FACSCount instrument (BD Biosciences) located at each of the 2 hospitals. Controls were performed daily and recorded. Every 10th specimen was repeated to determine instrument precision. The laboratory is enrolled in an external quality assurance program through the United Kingdom National External Quality Assurance Service [22].

Statistical Methods

Data were entered into a log located at each laboratory, and weekly data were entered into Microsoft Excel. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Distributions of continuous variables were summarized using means, standard deviations (SDs), and quartiles, whereas distributions of categorical variables were summarized using frequencies and percentages. Within-subject CD4 variability was also summarized by within-subject SD. Variability was summarized separately for low (<350 cells/mm^3), medium (350–650 cells/mm^3), and high mean CD4 count (>650 cells/mm^3) patients.

We fit a Bayesian linear mixed-effects model of log-transformed CD4 cell counts to the data. Using samples from the posterior distribution, we used Monte Carlo approximations to estimate misclassification rates for different observed CD4 values. The misclassification rate is defined as (1) the conditional probability the true CD4 count is below 500 cells/mm^3 given a geometric mean of N measurements is above 500 cells/mm^3 (upward misclassification whereby an individual who should be ART eligible is deemed ineligible) or (2) the true CD4 count is above 500 cells/mm^3 given the geometric mean of N measurements is below 500 cells/mm^3 (downward misclassification whereby an individual who would not be ART eligible is deemed eligible). Estimated misclassification rates are reported for a single CD4 count and up to 4 additional repeat measures based on observed CD4 counts from 50 to 950 cells/mm^3 by intervals of 100 cells/mm^3.

We used the same model to determine the accuracy rate of a single versus repeated CD4 measures for a range of hypothesized true CD4 counts. The accuracy rate is defined as the conditional probability (1) that a geometric mean of N measurements is below 500 cells/mm^3 given that the true CD4 is above 500 cells/mm^3 or (2) that a geometric mean of N measurements is above 500 cells/mm^3 given that the true CD4 is below 500 cells/mm^3. Estimated accuracy rates are reported for a single CD4 count and up to 4 additional repeat measures based on true CD4 counts from 50 to 950 cells/mm^3 by intervals of 100 cells/mm^3. For all analyses, estimated rates are summarized in terms of posterior means and 95% credible intervals.

To evaluate the appropriateness of the assumption of CD4 log-normality, model residuals were computed from the fitted models of log CD4, and a Quantile-Quantile (QQ) plot was constructed (Supplementary Figure 1). The QQ plot provided reasonable support for assuming that log CD4 is normally distributed within subjects. A precision analysis was performed using a Bayesian linear mixed-effects model to estimate the

proportion of within-subject variation in log CD4 explained by the instrument.

RESULTS

Twenty-five patients were enrolled at each of 2 sites for a total enrollment of 50 individuals. The median age was 33.5 years (IQR, 27.5–40.0) and 34 were female (68%). A total of 387 blood samples were analyzed for CD4 cell count with a mean of 7.7 samples per patient (range, 6–9). A total of 228 samples were collected in the morning (59.2%) and 157 (40.8%) in the afternoon, and no patients reported smoking on the day of sample collection. Among women, 25 samples were collected during menses (9.7% of all samples). The majority of the participants traveled to clinic in less than 30 minutes (N = 289, 78.3%) or 30–60 minutes (N = 58, 15.7%). The mean CD4 count across all participants' samples was 596 cells/mm^3 (SD = 289; range, 63–1687). There was a significant difference in the geometric mean CD4 count between specimens collected in the morning versus afternoon (477 cells/mm^3 in the morning vs 565 cells/mm^3 in the afternoon, $P < .001$). Participant and visit characteristics are summarized in Table 1.

The average within-subject SD for all individuals was 89 cells/mm^3 (SD = 54; range, 10–273). The lowest average variability was observed among patients with mean CD4 counts <350 cells/mm^3 (average SD = 34 cells/mm^3), whereas the highest

was among patients with CD4 counts >650 cells/mm^3 (average SD = 127 cells/mm^3). Within-subject SDs, grouped by within-subject CD4 mean, are summarized in Table 2.

Using CD4 data from our sample, we modeled the relationship between observed CD4 counts and true CD4 counts on the log scale using a Bayesian linear mixed-effects model, and we estimated misclassification rates based on a single versus repeated measures (up to 5 CD4 measures). Misclassification rates were less than 1% when the observed CD4 count was ≤250 or ≥750 cells/mm^3. Rates of misclassification were highest at observed CD4 counts between 350 and 650 cells/mm^3, particularly when a single measurement was used. In these CD4 strata, repeated measures did result in marked decreases in misclassification rates. At 450 cells/mm^3, misclassification decreased from 46.7% with one measurement to 34.6% with a second measurement and 26.9% with a third measurement. At 550 cells/mm^3, misclassification was reduced from 16.8% with one measurement to 13.6% with a second and 11.5% with a third test. At 650 cells/mm^3, there was benefit from performing a second CD4 count with misclassification reduced from 4.1% to 1.2%, but there was diminishing benefit with >2 repeat tests. Results are summarized in Table 3.

Table 4 describes the accuracy of a single versus repeated CD4 measures based on the hypothesized true CD4 count. For true CD4 counts below 500 cells/mm^3, the rates define

Table 1. Patient and Visit Characteristics

Patient Characteristics	Total (N = 50)	Partners in Hope (N = 25)	Madisi (N = 25)
Age			
Mean (SD)	35.5 (10.5)	34.2 (8.9)	36.6 (11.9)
Median (Q1–Q3)	33.5 (27.5–40.0)	35.0 (29.0–38.0)	33.0 (27.0–41.0)
Min-Max	20.0–63.0	20.0–55.0	22.0–63.0
Missing	2	2	0
Sex, n (%)			
Male	16 (32.0)	5 (20.0)	11 (44.0)
Female	34 (68.0)	20 (80.0)	14 (56.0)
Visit Characteristics	(N = 387)	(N = 193)	(N = 194)
Time collected, n (%)			
AM	228 (59.2)	124 (64.9)	104 (53.6)
PM	157 (40.8)	67 (35.1)	90 (46.4)
Missing	2	2	0
Did you smoke today? n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Missing	1	1	0
If female, are you menstruating? n (%)	25 (9.7)	22 (11.8)	3 (1.5)
NA/missing	6	6	0
Time taken to reach the hospital, n (%)			
<30 min	289 (78.3)	133 (72.2)	156 (84.3)
30–60 min	58 (15.7)	41 (22.3)	17 (9.2)
1–2 h	12 (3.3)	1 (0.1)	11 (5.9)
>2 h	10 (2.7)	9 (4.9)	1 (0.1)
NA/missing	18	9	9

Abbreviations: Max, maximum; Min, minimum; NA, not applicable; SD, standard deviation.

Table 2. Within-Subject Sample Standard Deviation of CD4[a]

Within-Subject Sample Mean of CD4	<350 Cells/mm³ (N = 10)	350–650 Cells/mm³ (N = 20)	>650 Cells/mm³ (N = 20)
Mean (SD)	34 (13)	78 (42)	127 (49)
Median (IQR)	37 (27–40)	71 (54–88)	127 (94–148)
Range	14–60	10–213	42–273

Abbreviations: IQR, interquartile range; SD, standard deviation.

[a] Categories are defined based on the within-subject mean CD4.

the probability that the observed CD4 count will be below 500 cells/mm³, and for true CD4 counts above 500 cells/mm³, the rates define probability that the observed CD4 count will be above 500 cells/mm³. The accuracy is highest at ≤250 and ≥850 cells/mm³ and lowest (50%) when the true CD4 counts is at the cutoff threshold of 500 cells/mm³. In these scenarios, repeating CD4 measures has little to no benefit on improving accuracy. Accuracy is lowest at true CD4 counts of 450 and 550 cells/mm³ (71.6% and 69.8%, respectively), and performing 2 measurements results in marked improvements to 79.1% at 450 cells/mm³ to 76.8% at 550 cells/mm³. At these CD4 counts, there is additional improvement with a third CD4 sample and diminishing benefits with the fourth and fifth tests.

To characterize the contribution of instrument variability to the overall CD4 variability, we repeated every tenth sample on the same instrument. Based on these tests (N = 31), the coefficient of variation for the instrument was 4.6% (confidence interval, 3.2%–5.6%), with 7.4% of within-subject variability explained by instrument imprecision.

DISCUSSION

Our study demonstrates that the highest risk of misclassification occurs when eligibility for ART is based on a single CD4 value for patients with observed CD4 counts in the range of 350–650 cells/mm³. A single CD4 count performs well at both ends of the CD4 strata (for observed CD4 counts of ≤250 or ≥750

cells/mm³), with <1% risk of misclassification. Our data show that risk of misclassification can be decreased by increasing the number of CD4 tests performed. Of note, based on our modeling data, most misclassification is downward, with individuals more likely to be misclassified as ART eligible rather than ART ineligible.

The recently published START study showed decreases in acquired immune deficiency syndrome (AIDS) and non-AIDS events with early initiation of ART regardless of CD4 count or geographic location [6]. Likewise, the TEMPRANO study showed a decrease in severe morbidity with early ART, particularly tuberculosis and invasive bacterial infections [1]. As a result of these studies, the 2015 WHO guidelines recommended universal eligibility for ART [7]. Despite these recommendations, many resource-constrained countries may continue to use CD4 thresholds given barriers related to cost and feasibility of expansion of ART to all HIV-infected individuals.

Our study was prompted by discussions with the Ministry of Health regarding Malawi's decision to switch from a CD4-guided eligibility approach (using 500 cells/mm³) to universal eligibility. Malawi implemented Option B+ in 2011 and has other universally eligible groups, such as children under 5 years of age (A. Jahn, personal communication, 2 October 2014). The goal of the study was to understand the accuracy of a single CD4 count to determine eligibility for the remaining groups of HIV-infected individuals not covered under the National Guidelines and to weigh these findings against the cost and complexity of ongoing CD4 determination for ART eligibility. In Malawi, CD4 infrastructure has been challenging due to intermittent stock outs of blood draw supplies and reagents, issues with instrument maintenance including point-of-care (POC) machines, systems barriers related to sample transport and results reporting, and the overall costs of the program. Given the low accuracy of a single CD4 count for individuals with observed CD4 counts of 350–650 cells/mm³, mounting evidence for the benefits of early ART, and challenges and costs of

Table 3. Misclassification Rates (%) by Observed CD4 Based on the Geometric Mean of N Measurements

| Observed CD4 (Cells/mm³) | Misclassification Rates % (95% Credible Interval) (N = number of CD4 Samples) | | | | |
	N = 1	N = 2	N = 3	N = 4	N = 5
50	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)
150	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)
250	<0.1 (<0.1 to 0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)
350	5.8 (2.3 to 10.7)	0.6 (0.1 to 1.7)	0.1 (<0.1 to 0.3)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)
450	46.7 (31.3 to 63.5)	34.6 (16.2 to 57.2)	26.9 (8.7 to 52.0)	21.5 (4.7 to 46.9)	17.5 (2.6 to 41.9)
550	16.8 (7.3 to 26.8)	13.6 (3.4 to 25.1)	11.5 (1.8 to 23.2)	9.9 (0.9 to 21.9)	8.5 (0.5 to 20.4)
650	4.1 (1.1 to 7.7)	1.2 (0.1 to 2.9)	0.4 (<0.1 to 1.1)	0.1 (<0.1 to 0.4)	<0.1 (<0.1 to 0.2)
750	0.9 (0.2 to 1.9)	0.1 (<0.1 to 0.2)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)
850	0.2 (<0.1 to 0.4)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)
950	<0.1 (<0.1 to 0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)	<0.1 (<0.1 to <0.1)

Table 4. Accuracy Rates (%) by True CD4 Based on the Geometric Mean of N Measurements

True CD4 (cells/mm³)	Accuracy Rates % (95% Credible Interval) (N = number of CD4 samples)				
	N = 1	N = 2	N = 3	N = 4	N = 5
50	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)
150	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)
250	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)
350	97.3 (96.4 to 98.1)	99.7 (99.4 to 99.8)	>99.9 (99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)
450	71.6 (70.2 to 73.1)	79.1 (77.3 to 80.8)	83.9 (82.1 to 85.7)	87.4 (85.5 to 89.1)	89.9 (88.2 to 91.6)
500	50.0 (50.0 to 50.0)	50.0 (50.0 to 50.0)	50.0 (50.0 to 50.0)	50.0 (50.0 to 50.0)	50.0 (50.0 to 50.0)
550	69.8 (68.4 to 71.1)	76.8 (75.1 to 78.4)	81.5 (79.7 to 83.3)	85.0 (83.1 to 86.7)	87.6 (85.8 to 89.3)
650	92.3 (90.7 to 93.7)	97.8 (96.9 to 98.5)	99.3 (98.9 to 99.6)	99.8 (99.6 to 99.9)	99.9 (99.8 to >99.9)
750	98.6 (97.9 to 99.1)	99.9 (99.8 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)
850	99.8 (99.6 to 99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)
950	>99.9 (99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)	>99.9 (>99.9 to >99.9)

supporting CD4 infrastructure, in early to mid 2016, Malawi plans to move to universal eligibility and baseline CD4 counts will no longer be performed.

We excluded a major contribution of instrument variability in our study by evaluating instrument precision. Variability from the CD4 instrument can be an important real-world factor affecting accuracy, with a resultant increase in misclassification rates, particularly when treatment decisions are based on a single CD4 value. Several recent studies have shown that, in certain settings, instrument variability is higher with POC instruments [9, 23]. Given the widespread use of POC CD4 testing, continuous quality control around use of these instruments is critical in order to minimize misclassification due to instrument error. Country programs that retain CD4 cell counts to determine eligibility should consider performing early repeat CD4 counts for those with an initial observed value between 350 and 650 cells/mm³ to improve accuracy and staging. However, additional CD4 counts will increase cost and complexity of programs and may result in delays in ART initiation. Data from pre-ART programs suggest delays in initiation result in loss to follow-up [24–26], and these data should be considered as countries weigh factors about the use of 1 or more CD4 counts to determine eligibility for therapy.

Limitations

Our findings are based on data from a random sample population in Malawi. We excluded those with WHO stage 3 and 4 disease and focused on the short-term variability of CD4 cell counts (over approximately 1 week). Differences in baseline CD4 counts or potential differences in CD4 variability in other populations could lead to an increased or decreased impact of CD4 variability on misclassification risk and the accuracy of a single CD4 for ART eligibility. Although we collected data on factors known to affect CD4 cell count, there may be additional sources of variability not captured by our analysis. This analysis focused on the use of CD4 for ART eligibility. Availability of CD4 as part of clinical care remains an important tool, and consideration should be given for continued support of infrastructure for testing, particularly at district hospitals and other large referral centers.

CONCLUSIONS

Our data show that ART eligibility based on a single CD4 count results in risk of misclassification, which is highest in those with observed CD4 counts in the range of 350–650 cells/mm³. Given evidence of the benefits of earlier ART, including improved health outcomes and prevention of HIV transmission, countries should weigh the costs and complexity of CD4 testing using a 500 cell/mm³ threshold against the cost savings and public health benefits of universal eligibility.

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