ORIGINAL RESEARCH ARTICLE

Screening HIV-infected adults in Malawi for anaemia: impact on eligibility for antiretroviral therapy

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Summary: Clinical staging determines antiretroviral therapy (ART) eligibility when CD4 count is not available. Haemoglobin (Hb) $\leq$8 g/dL is an indication for the treatment. We measured Hb in HIV-positive Malawian adults undergoing clinical assessment for ART eligibility and calculated the percentage of patients with CD4 $\leq$ 350 cells/μL deemed eligible for ART by clinical staging with and without Hb measurement, using the existing threshold and an alternative proposed after comparing Hb values to CD4 counts. Three hundred and thirty-eight patients had CD4 counts measured and 226 (67%) had CD4 $\leq$ 350 cells/μL. Thirty-six (16%) patients with low CD4 count were eligible for ART by clinical assessment alone, 48 (21%) when Hb was also measured with a threshold of $\leq$8 g/dL and 74 (34%) with a threshold of $\leq$10 g/dL. Measuring Hb alongside clinical assessment could increase the number of patients with CD4 $\leq$ 350 cells/μL starting ART by 33% using a threshold of Hb $\leq$ 8 g/dL or 114% with a threshold of $\leq$10g/dL.

Keywords: HIV, AIDS, treatment, antiretroviral therapy, treatment threshold, CD4 count, clinical staging, haemoglobin, anaemia, resource-poor areas, Malawi, eligibility, Africa

INTRODUCTION

Antiretroviral therapy (ART) in sub-Saharan Africa has expanded to many areas with limited or no access to laboratory facilities. When used in these settings ART can be very effective with an acceptable side-effect profile.[1] The World Health Organization (WHO) recommends that ART is commenced when the patient is in WHO clinical stages III or IV or when the CD4 count falls below 350 cells/μL. Unfortunately, CD4 count testing is not widely available in low-income countries. Point-of-care CD4 count tests are being developed,[2] but are not yet available for routine use.

In settings where CD4 count testing is not available, clinical assessment for symptoms and signs of HIV-associated conditions, as defined by the WHO clinical staging system, determine eligibility for ART. This leads to delaying ART until HIV-associated pathology has occurred, which is associated with increased mortality.[3] In addition, the recently widely introduced strategy to start ART at an earlier stage of the HIV infection, by changing the CD4 threshold to 350 cells/μl, will obviously have no effect in these settings, leading to further inequities in health-care provision. A cheap, reliable field test, able to identify asymptomatic patients likely to meet CD4 count criteria for starting ART could be of great value in HIV care in resource-poor areas.

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Haemoglobin (Hb) measurement is a candidate for such a role. An Hb value $\leq$8.0 g/dL is the current recommended threshold to indicate WHO clinical stage III disease and anaemia has been associated with low CD4 count in some African settings.[4,5] The combination of low Hb and low total lymphocyte count has been used to predict low CD4 count in pregnant Zambian women.[6] The utility of Hb alone as a predictor of low CD4 count in adult HIV population in Africa has not been assessed.

It is likely that the majority of cases of anaemia in HIV-infected adults are directly or indirectly caused by HIV. Previous investigation[7] of the underlying cause of anaemia in 83 adult HIV-infected patients with severe anaemia (Hb $\leq$ 7 g/dL) admitted to our hospital found non-HIV-associated conditions in only 10% of cases. These consisted mostly of 'parasitic cause of anaemia' and vitamin B_{12} deficiency. HIV-related conditions were found in two-thirds, consisting of bacteraemia in 24% and tuberculosis in 43%.

Prevalence rates of anaemia recorded in HIV-infected African adults have varied widely from 0.7% to 80%.[8–12] These surveys used different definitions of anaemia and none were conducted in outpatients being assessed for ART eligibility. The prevalence of Hb $\leq$ 10.0 g/dL in Malawian adults commencing ART as part of a trial of antiretroviral efficacy was 17%,[13] but these patients had been prescreened for trial entry and all had CD4 $\leq$ 300 cells/μL. These results may not therefore be representative of the general outpatient HIV population.

Point-of-care Hb measurement is possible using the HemoCue system. This is a portable device, which can be used with minimal training. It has been validated for use in district hospitals[14] and has since been widely used in Malawi. Haemoglobin is currently not routinely measured as part of the ART eligibility assessment in Malawi.

We aimed to investigate the impact of point-of-care Hb measurement on ART eligibility, by determining the number of patients with WHO clinical stage III anaemia (i.e. Hb $\leq$ 8 g/dL) among those who were in WHO clinical stages I or II after history and physical examination alone. We compared this with the number of patients eligible for ART as a result of a low CD4 count (i.e. $\leq$350 cells/μL).

The WHO CD4 threshold for ART eligibility was changed from 250 to 350 cells/μL in 2010.[15] We considered whether the Hb threshold for ART eligibility might also need adaptation in light of this move to commence ART earlier. We therefore considered whether a higher Hb value than 8.0 g/dL would be more effective at correctly identifying patients eligible for ART by the 350 cells/μL CD4 cut-off.

METHODS

The study took place in the outpatient adult ART clinic of Queen Elizabeth Central Hospital, Blantyre, Malawi (QECH) in February 2011. All patients who had a documented positive HIV test and had never previously received ART were offered enrolment in the study. Patients with known malignancy or pregnancy were excluded. A sample size calculation could not be performed due to the absence of previously published data on anaemia rates in this patient group and the variation of rates of anaemia documented in other HIV-positive groups. A recruitment target of 500 patients was set based on the number of new patients expected to attend the clinic during the predetermined study period.

Participants underwent a structured history and physical examination and the WHO clinical stage was thereby determined. Capillary blood was obtained by finger prick and Hb was measured by HemoCue (HemoCue AB, Ängelholm, Sweden). A venous blood sample was drawn for CD4 count (Epics XL/MCL Flow Cytometer, Beckman Coulter, Johannesburg, RSA with formal internal and external quality control procedures) at the QECH laboratory as part of standard care. Study staff were not aware of CD4 count results when conducting the clinical assessments and Hb tests. Patients were not tested for additional underlying causes of anaemia as part of the study, but any established diagnosis was noted.

Data were entered into a Filemaker Pro Database. PASW 18.0.3 statistics package (IBM, New York, USA) and R version 2.13.2 (R Foundation for Statistical Computing, Vienna, Austria) were used for analysis. We calculated the prevalence of WHO clinical stage III anaemia (Hb $\leq$ 8.0 g/dL) as well as the numbers of patients with mild (Hb 10.1–12 g/dL in men or 10.1–11 g/dL in women), moderate (Hb 7.0–10 g/dL) and severe anaemia (Hb $<$ 7.0 g/dL) according to WHO definitions. We noted the number of patients who were eligible for ART after clinical assessment and the number of extra patients eligible following Hb measurement using the Hb $\leq$ 8 g/dL threshold and after CD4 counting, with binomial confidence intervals.

The relationship between CD4 count and Hb was examined via a scatter plot and linear model. Positive and negative predictive values for the use of the Hb test to identify CD4 $\leq$ 350 cells/μL were obtained for each whole number cut-off for Hb between 8.0 and 12 g/dL. We considered that an alternative Hb threshold could be appropriate if it had a $>$90% positive predictive value for CD4 $\leq$ 350 cells/μL with a low number of false-positives. If an alternative threshold met these criteria, the number of patients subsequently eligible for ART under this new limit would be obtained.

Ethical approval was granted by the College of Medicine Research and Ethics Committee and the Liverpool School of Tropical Medicine.

RESULTS

We assessed 500 patients. The median age was 33 (range 16–81) years and 340 (68%) patients were women. Clinical assessment revealed signs of a WHO stage III or IV condition in 59 (12%) patients.

The mean Hb was 12.0 g/dL (SD 2.5). Table 1 shows that 134 (27%) of patients were anaemic, but only 13 (3%) had severe anaemia. Hb $\leq$ 8.0 g/dL was noted in 31 (6%) patients, who thus met the WHO definition of clinical stage III and eligibility for ART. The study took place during the wet season when risk of malaria transmission and associated anaemia is relatively high. We did not test for malaria or underlying causes of anaemia, but recorded any diagnosis already documented. An underlying cause of anaemia was noted in 13 (7%) of the anaemic patients. In eight of these the underlying cause was another WHO stage III/IV condition, with tuberculosis found in four cases and recent severe bacterial infection in another four. Only five cases had a non-HIV-related potential cause. These consisted of three cases of malaria and two of recent miscarriage. CD4 count was requested on all 500 patients and a result was received in 338 (68%) cases, of whom 226 (67%) had CD4 $\leq$ 350 cells/μL. The missing results were mostly due to break down of the CD4 machine during the last part of the study period.

Haemoglobin and CD4 count were found to have a moderately positive correlation (0.37) with a strongly significant

Table 1 **Anaemia prevalence by WHO definitions**

Anaemia classification	Range (g/dL)	Number of patients (%)	Confidence interval (%)
Mild	Men: 10.1–12 Female: 10.1–11	86 (17.2)	14.0–20.8
Moderate	7.1–10	86 (17.2)	14.0–20.8
Severe	$\leq$7.0	13 (2.6)	1.4–4.4
WHO stage III disease	$\leq$8.0	31 (6.2)	4.3–8.7

WHO, World Health Organization

Table 2 **Predictive values of different Hb levels for the identification of patients with CD4 count $\leq$350 cells/μL**

Hb cut-off levels (g/dL)	Positive predictive value (%) (n = 226)	Negative predictive value (%) (n = 112)
$\leq$8 (n = 22)	100.0	35.4
$\leq$9 (n = 44)	93.2	37.1
$\leq$10 (n = 66)	90.9	39.0
$\leq$11 (n = 111)	82.9	41.0
$\leq$12 (n = 167)	76.6	42.7

Hb, haemoglobin

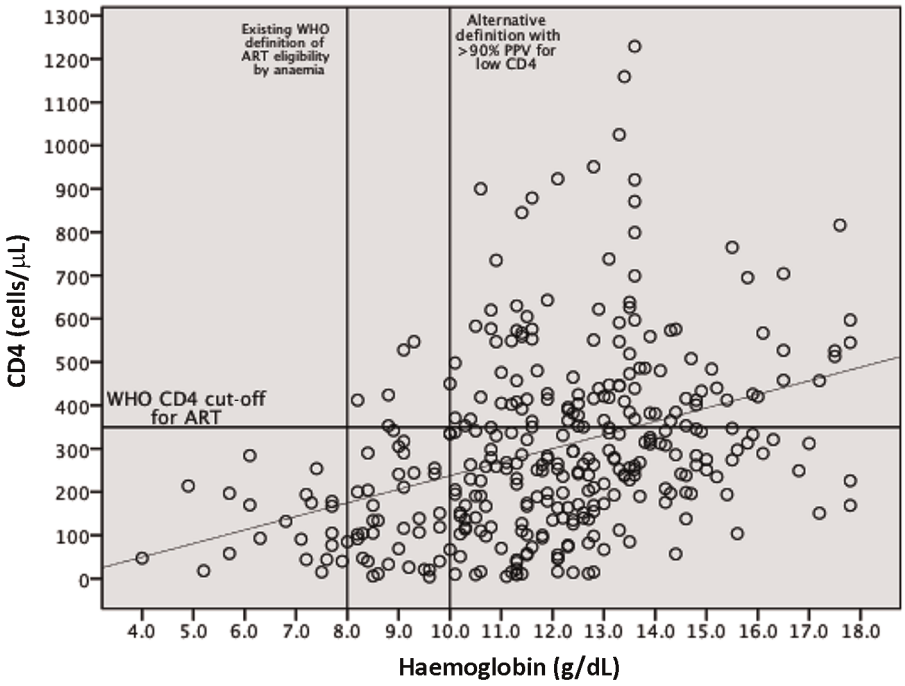

Figure 1 **Scatter plot of haemoglobin against CD4 count for patients who had results for both tests, with a linear line of best fit ($n = 338$)**

Table 3 **Eligibility for ART following clinical assessment and extra patients identified using two alternative Hb thresholds**

	Patients eligible for ART after clinical staging alone		Extra patients eligible for ART using Hb ≤ 8.0 g/dL	Extra patients eligible for ART using Hb ≤ 10.0 g/dL
	Yes	No		
Patients with CD4 counts				
CD4 ≤ 350 cells/μL ($N = 226$)	36 (15.9%) (11.4–21.4)	190 (84.1%) (78.6–88.6)	12 (5.3%) (2.8–9.1%)	41 (18.1%) (13.3–23.8%)
CD4 > 350 cells/μL ($N = 112$)	8 (7.1%) (3.1–13.6)	104 (92.9%) (86.4–96.9)	0 (0.0%) (0.0–3.2%)	4 (3.6%) (1.0–8.9%)
All patients $N = 500$	59 (11.8%) (9.1–15.0)	441 (88.2%) (85.0–90.9)	19 (3.8%) (2.3–5.9%)	70 (14.0%) (11.1–17.4%)

ART, antiretroviral therapy; Hb, haemoglobin

P value ($P < 0.001$). We calculated the positive (PPV) and negative predictive values (NPV) of different Hb thresholds for the identification of patients with CD4 count ≤350 cells/μL (Table 2). A threshold of 10 g/dL had a PPV of 91% and would identify 60 of 226 patients (29%) with a CD4 ≤ 350 cells/μL, whereas the existing WHO stage III threshold of 8.0 g/dL had a PPV of 100% but only identified 22 (10%) with CD4 ≤ 350 cells/μL. The negative predictive values were low for all possible cut-offs, ranging between 36% and 43%.

The scatter plot (Figure 1), shows a wide range of CD4 values, and many of the low CD4 values are not reflected by correspondingly low Hb values, which is shown by the low negative predictive values. Many patients with low CD4 have normal or near-normal Hb, but all patients with Hb ≤ 8.0 g/dL had low CD4 count. Many more patients had an Hb of ≤10.0 g/dL, but only six patients would be considered as 'false-positives' with Hb ≤ 10.0 g/dL had CD4 > 350 cells/μL. We therefore used Hb ≤ 10.0 g/dL as an alternative threshold in the following analysis as it was the highest whole number cut-off which met our predetermined criteria of PPV >90% and also had a low number of false-positive results.

We calculated the number of patients eligible for ART after clinical assessment alone. We then examined the number of additional patients who would be eligible for treatment using the two alternative Hb thresholds (Table 3).

Clinical assessment alone only identified 15.9% of patients with CD4 ≤ 350 cells/μL. The measurement of Hb identified an additional 5.3% of this group as eligible for ART using the current WHO threshold of 8 g/dL. An additional 18.1% were identified using the alternative threshold of 10 g/dL. Hb measurement using the 10 mg/dL threshold more than doubled the number of patients with CD4 ≤ 350 cells/μL who would be identified as eligible for treatment in areas where CD4 count is not available. Measuring Hb did not result in any patients with CD4 > 350 cells/μL commencing ART using the current WHO threshold of 8 g/dL. Using the alternative threshold of 10 g/dL resulted in six (3.6%) of these patients commencing ART. This included two patients with additional WHO stage III/IV conditions for whom ART would be indicated regardless of CD4 count.

DISCUSSION

We have performed a prevalence survey of anaemia in HIV-positive African adults being assessed for ART eligibility.

We have confirmed that anaemia is common and that low Hb levels are associated with low CD4 counts. As in previous studies,[7] few anaemic HIV-positive adults had an underlying cause of anaemia not related to HIV.

Clinical assessment identified a small minority (15.9%) of patients who need ART using CD4 count criteria and Hb measurement only added 5.3% of these patients using the existing WHO clinical stage III definition of Hb $\leq$8.0 g/dL. Exploration of the relationship between Hb and CD4 count suggests that an Hb threshold of 10 g/dL (the WHO definition of moderate anaemia) corresponded better with the CD4 count threshold of 350 cells/μL, identifying an extra 18.1% of the patients entitled to ART by CD4 count criteria, compared with clinical assessment alone.

Although using the higher Hb value for ART eligibility would include a small number of patients with CD4 counts >350 cells/μL, anaemia has been directly associated with increased mortality in many studies of HIV-positive patients.[1,16–18] It is therefore likely that this small group of patients would benefit from early ART initiation. We therefore argue that an Hb threshold of 10 g/dL may be a more appropriate cut-off for starting ART in circumstances where CD4 counting is not available, as the global move to start ART earlier would otherwise be meaningless in these places.

The limitations of our study include the fact that it was conducted over a short period, which precluded determination of seasonal variations in the anaemia prevalence, for instance due to malaria. Recruitment was limited to non-pregnant adults and took place in an urban clinic. Results might be different in other groups. We did not perform a thorough investigation of underlying causes of anaemia since this would not be possible in settings where CD4 counts are unavailable.

We did not record the number of patients taking co-trimoxazole prophylaxis. This might affect the rate of anaemia due to its antimalarial activity.[19] We suspect the impact of this is likely to be minimal, as malaria is an uncommon cause of anaemia in HIV-positive patient in Malawi[7] and the normal practice at our clinic was to assess the need to commence co-trimoxazole after CD4 count was measured. As we measured Hb before CD4 count had been measured very few patients would have been receiving this drug. There was also an interruption in the supply of co-trimoxazole during the study that would have further reduced the numbers on this drug.

We relied on measurement of CD4 count performed during routine care, and only 68% of patients had a test result. We do not think this biased our results, however, as the cause was an interruption of the CD4 count service during the last part of the study. We examined the impact of our proposed new Hb threshold in the same population that we used to define that threshold and recognize the importance of repeating the study in a different population.

Measuring Hb by HemoCue currently costs around US$0.75 per test compared with around US$4.50 for CBC and around US$8.50 for CD4 count. Point-of-care CD4 counts have a projected cost of US$6–8 per test. Even cheaper methods of assessing Hb exist (e.g. WHO Color Scale) but may not be accurate enough to use for this purpose.[14] If this cost differential persists there may be a role for routine HemoCue Hb screening in resource-poor areas prior to CD4 count measurement. Those who are eligible by clinical staging or Hb might not require an expensive CD4 count before starting ARVs. A formal study of the cost-effectiveness of different protocols in the context of assessing ART eligibility may be warranted.

The ideal method of ensuring all patients with CD4 $\leq$ 350 cells/μL commence ART would be universal access to CD4 counts. This could be achieved with point-of-care CD4 tests. Until these become widely available, practical difficulties in delivering CD4 counts are likely to continue restricting access to treatment for many persons living in areas where HIV testing and ART are available. In the meantime, measuring Hb with an existing point-of-care test such as HemoCue and using a new ART eligibility threshold of $\leq$10 g/dL could provide a substantial improvement on the current standard of care in settings without CD4 measurement facilities. We have shown that in settings where CD4 counts are not universally available, incorporating Hb measurement into the clinical assessment of HIV-positive patients can more than double the number of patients with low CD4 counts deemed eligible to start ART.

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Conflict of interest: None declared.

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