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Targeting CD4 testing to a clinical subgroup of patients could limit unnecessary CD4 measurements, premature antiretroviral treatment and costs in Thyolo District, Malawi

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Summary Malawi offers antiretroviral treatment (ART) to all HIV-positive adults who are clinically classified as being in WHO clinical stage III or IV without 'universal' CD4 testing. This study was conducted among such adults attending a rural district hospital HIV/AIDS clinic (a) to determine the proportion who have CD4 counts $\geq$350 cells/μl, (b) to identify risk factors associated with such CD4 counts and (c) to assess the validity and predictive values of possible clinical markers for CD4 counts $\geq$350 cells/μl. A CD4 count $\geq$350 cells/μl was found in 36 (9%) of 401 individuals who are thus at risk of being placed prematurely on ART. A body mass index (BMI) >22 kg/m^2, the absence of an active WHO indicator disease at the time of presentation for ART, and a total lymphocyte count >1200 cells/μl were significantly associated with such a CD4 count. The first two of these variables could serve as clinical markers for selecting subgroups of patients who should undergo CD4 testing. In a resource-limited district setting, assessing the BMI and checking for active opportunistic infections are routine clinical procedures that could be used to target CD4 measurements, thereby minimising unnecessary CD4 measurements, unnecessary (too early) treatment and costs.

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1. Introduction

Malawi is an impoverished country with an estimated 900 000 people living with HIV and AIDS. Despite the fact that an estimated 170 000 people are in immediate need of antiretroviral therapy (ART) (MOH, 2003a), as of March 2004 barely 5000 people were receiving ART. There is thus a desperate need to scale-up treatment and Malawi has laid out ambitious plans to place 80 000 people on ART by 2005 (MOH, 2003b).

Initiation of ART is usually based on measurement of the CD4 T-cell lymphocyte count, a laboratory test that acts as a 'proxy' for the state of the immune system. However, this test is still expensive, the technology is rather sophisticated, and in Malawi the number of CD4 testing facilities is limited. To avoid CD4 testing acting as a barrier to scaling-up treatment, Malawi has decided to offer ART to all HIV-positive adults who are classified as being in WHO clinical stages III and IV without universal CD4 testing (MOH, 2003b; WHO, 2004).

However, we are concerned that in our setting, a certain proportion of individuals who are clinically classified as being in WHO stage III or IV might have CD4 counts $\geq$350 cells/μl (a CD4 count <350 cells/μl being the surrogate marker or 'proxy' for the state of immunity that is considered the correct starting point for ART in this group of patients in Malawi).

Although this proportion of individuals might be relatively small, there are a number of implications. First, individuals might be at risk of being given treatment too early in the course of their HIV-related illness, with the consequent discomforts associated with daily pill intake, possible adverse side effects and regular follow-up visits. Second, resistance to first-line drugs is likely to develop with time under programme conditions (Coetzee et al., 2004; Richard et al., 2004; Vergne et al., 2002) and starting ART too early might unnecessarily undermine the potential benefits to be gained at a later stage when treatment would be most required. Third, ART is still expensive and the cost of unnecessarily placing individuals on treatment on a country-wide scale might be considerable.

Although universal CD4 testing would be ideal in guiding decisions on ART eligibility, Malawi has neither the resources nor the capacity to embark on such a venture on a country-wide basis. An alternative strategy might be to introduce a system of initial screening to identify clinical subgroups of patients who are more likely to have CD4 counts $\geq$350 cells/μl and subsequently to target CD4 measurements to such subgroups.

Thus, this study was conducted among HIV-positive adults classified in WHO stage III or IV (and attending a district hospital HIV/AIDS clinic) (a) to determine the proportion who have CD4 counts of $\geq$350 cells/μl, (b) to identify risk factors associated with such CD4 counts and (c) to assess the validity (sensitivity, specificity) and predictive values of possible clinical markers of CD4 counts $\geq$350 cells/μl that could be used for targeted CD4 measurements.

2. Methods

2.1. Study setting and population

The study was conducted between April 2003 and January 2004 in Thyolo District, a rural region in southern Malawi. The district has a population of 450 000 inhabitants. All consecutive HIV-positive individuals aged over 13 years classified in WHO clinical stages III and IV and presenting to the HIV/AIDS clinic in the main district public hospital (Thyolo Hospital) were involved in the study.

Thyolo Hospital is one of the pioneer facilities in Malawi that started offering voluntary counselling and HIV testing (VCT) and HIV-related care activities. Since 1999, this hospital offers VCT to patients on the wards, mothers attending antenatal care, patients with tuberculosis and all those who wish to know their HIV status. HIV testing is conducted using rapid whole blood test kits according to the WHO strategy II for HIV antibody testing (UNAIDS/WHO, 1997). All HIV-positive individuals undergo a complete medical assessment for HIV-related diseases and are subsequently categorised into different WHO stages on the basis of clinical findings. Individuals are managed for their opportunistic infections and those in WHO stage III or IV are offered co-trimoxazole prophylaxis at a dose of 960 mg daily, provided there are no contraindications.

Although VCT and HIV/AIDS-related care were available in Thyolo Hospital since 1999, ART only became available in the same facility from April 2003 (4 years later). All individuals in WHO stage III or IV who presented to this clinic from April 2003 were considered eligible for ART.

The WHO staging system is non-regressive, and thus all HIV-positive individuals once classified as being in WHO stage III or IV from previous visits for episodes of opportunistic infections (between 1999 and April 2003) maintain their staging on subsequent follow-up visits, even if they have been treated and cured of their active opportunistic

infection and had no active opportunistic infection at the time of being considered for ART. Some individuals in WHO stage III may indeed have progressed with time to WHO stage IV. Thus, individuals presenting to the HIV/AIDS clinic from April 2003 onwards included two groups of individuals; those 'with' active episodes of HIV-related disease and those 'without' active HIV-related disease. The latter included those keeping to scheduled follow-up visits at the HIV/AIDS clinic after having been treated for a previous episode(s) of active opportunistic infection(s).

For the purposes of this study, an active HIV-related disease refers to any clinically apparent WHO stage III or IV defining condition that occurred at the time of being considered for ART (from April 2003).

ART (a fixed-dose combination of stavudine, lamivudine and nevirapine) is offered once active HIV-related disease has been stabilised (MOH, 2003c).

2.2. Laboratory measurements and body mass index

CD4 counts were performed in all patients classified in WHO stage III or IV who were being considered for ART. This was initially measured using a Coulter Cyto-Sphere CD4 Kit (Coulter Corporation, Miami, FL, USA) at the Blantyre Diagnostic Laboratories. From August 2003, measurement of CD4 lymphocyte counts was carried out using FacsCount® (Immunocytometry Systems; Becton Dickinson, Franklin Lakes, NJ, USA) at the John Hopkins Research Laboratory, Blantyre. Both laboratories are considered reference laboratories in the country and they have strict quality control standards. The two techniques used for CD4 estimation are internationally acceptable, robust and known to have a low average coefficient of variation (Johnson et al., 1995; Sherman et al., 1999).

Total lymphocyte count (TLC) and haemoglobin (Hb) were determined by automated blood analyser (ABX micros 60; ABX Diagnostics, Montpellier, France). For TLC, a cut-off of 1200 cells/μl was used, as this is recommended as a substitute for CD4 counts when the latter is unavailable and HIV-related symptoms exist (Johnson et al., 1995).

Height and weight measurements are carried out on a routine basis and were used to determine the body mass index (BMI; weight in kg divided by height in m^2). A normal BMI is defined as 18.5—24.9 kg/m^2. An arbitrary middle value cut-off of the normal BMI (rounded at BMI of 22 kg/m^2) was used to categorise those with a BMI 18.5—22 kg/m^2 as being in the lower range of normal and those with a BMI > 22 kg/m^2 as being either in the upper range of normal (BMI 22—24.9 kg/m^2), overweight (BMI 25—29.9 kg/m^2) or obese (BMI $\geq$ 30 kg/m^2).

For the purpose of analysis, BMI was thus categorised into three groups as follows: malnutrition, BMI < 18.5 kg/m^2; lower range of normal, BMI = 18.5—22 kg/m^2; upper range of normal, overweight or obese, BMI > 22 kg/m^2. The personnel conducting weight and height measurements had been trained and were supervised during the entire course of the study.

2.3. Statistical analysis

Data analysis was done using Epi Info software (CDC, Atlanta, GA, USA) and Stata 8.2 software (Stata Corp., College Station, TX, USA).

In the analysis to determine the proportion of individuals with a CD4 count $\geq$350 cells/μl, all individuals in WHO clinical stage III or IV were included. This was justified for the following reasons. (a) In Malawi, ART is being offered (without CD4 counting) to a 'group' of individuals that includes all those in WHO clinical stages III and IV. We were thus interested to know what proportion of this 'same group' have CD4 counts $\geq$350 cells/μl, irrespective of current WHO guidelines recommending ART without CD4 testing for only those in WHO stage IV (WHO, 2004). (b) In our setting, laboratory facilities are often non-existent or at best limited to very basic tests and WHO staging is often based on pure clinical acumen. As there are several conditions that could mimic WHO stage III and stage IV disease, there is a certain risk that patients might be wrongly classified. The designated WHO stage (even if this is stage IV) may thus not necessarily be a 'proxy' of the real immune status of the patient. (c) As discussed earlier, a considerable proportion of patients in our study cohort were on routine follow-up visits. These individuals had no active opportunistic infection because they had been treated during previous visits. Moreover, the fact that they were on co-trimoxazole prophylaxis might have an effect on the evolution of CD4 counts (Mermin et al., 2004).

A CD4 count $\geq$350 cells/μl was designated as the dependent variable for identifying potential risk associations. The measures of risk were determined by crude odds ratios (OR) and adjusted odds ratios. OR were adjusted using multivariate logistic regression and all related *P*-values are based on Wald's test. The χ^2 test and χ^2 for linear trend were used to test for differences in proportions and for linear

trends, respectively. The validity of markers was assessed using the CD4 count as the gold standard. The level of significance was set at $P = 0.05$ or less and 95% CI was used throughout.

3. Results

3.1. Characteristics of the study population

There were 422 HIV-positive adults in WHO stage III or IV who presented to the ART clinic during the study period. Of these, 21 patients were excluded from the study: height was not measured in 16 individuals who were too ill to stand, and blood samples for 5 patients were insufficient for performing TLC and Hb analysis. Among the 401 individuals in whom data were complete, there were 116 (29%) men and 285 (71%) women, with a median age of 34 years (range, 15—60 years). The median CD4 count for all individuals was 138 cells/μl (interquartile range (IQR), 67—217 cells/μl). The median follow-up period of patients by the HIV/AIDS clinic was 167 days (range, 7 days to 3.6 years; IQR, 71 days to 3.2 years).

The mean BMI on presentation was 19.8 kg/m^2 (range, 11.4—36.5 kg/m^2). Of all individuals, 129 (32%) were malnourished (BMI < 18.5 kg/m^2), 184 (46%) were within the lower range of normal (BMI, 18.5—22 kg/m^2) and 88 (22%) had a BMI either in the upper range of normal or were obese (BMI, 22—24.9 kg/m^2 or above). The median Hb was 11 g/dl (range, 5.1—15 g/dl) and the mean TLC was 1800 cells/μl (range, 250—6500 cells/μl). All individuals in the study were receiving co-trimoxazole prophylaxis.

3.2. Proportion of patients 'with' and 'without' an active WHO defining disease

Of the 401 consecutive individuals who presented to the HIV/AIDS clinic during the study period, 293 (73%) individuals were classified in WHO stage III and 108 (27%) in stage IV. Of these individuals, 295 (74%) had an active WHO defining disease at the time of being considered for ART. The remaining 106 (26%) individuals either presented as part of a scheduled follow-up visit (11%) or had other conditions that could not be classified as an active WHO defining indicator disease (15%). Table 1 shows the proportion of individuals presenting with and without active WHO defining diseases, stratified by CD4 counts (<350 cells/μl or ≥350 cells/μl).

3.3. Proportion of individuals with a CD4 count ≥350 cells/μl

Of all individuals classified in WHO stage III or IV, there were 36 (9%) who had a CD4 count of ≥350 cells/μl, whilst 365 (91%) had CD4 counts <350 cells/μl.

Table 1 Number (and %) of individuals presenting with and without active WHO defining diseases, stratified by CD4 counts

	CD4 <350 cells/μl	CD4 ≥350 cells/μl	Total
Active WHO defining disease ($n = 295$)			
Oesophageal candidiasis	33 (100)	0	33
Kaposi's sarcoma	9 (82)	2 (18)	11
Cryptococcal meningitis	12 (100)	0	12
Oral candidiasis	86 (97)	3 (3)	89
Chronic diarrhoea >1 month	31 (100)	0	31
Prolonged fever >1 month	7 (70)	3 (30)	10
Wasting syndrome	7 (88)	1 (13)	8
Pneumocystis carinii pneumonia	3 (100)	0	3
Severe bacterial pneumonia	29 (94)	2 (6)	31
Pulmonary tuberculosis	51 (98)	1 (2)	52
Extrapulmonary tuberculosis	14 (93)	1 (7)	15
No active WHO defining disease ($n = 106$)			
Patients presenting with other conditions[a]	58 (94)	4 (6)	62
Patients on routine follow-up visits	25 (57)	19 (43)	44
Total	365 (91)	36 (9)	401

[a] Other conditions included presumed malaria, vaginal mycosis, skin mycosis, scabies, bacterial pneumonia, otitis media and skin rashes.

Table 2 Risk factors associated with CD4 counts ≥350 cells/μl

Variable	Median CD4 counts (IQR)	No. with CD4 count ≥350 cells/μl (%)	OR	Adjusted OR (95% CI)[a]	*P*-value
Gender					
Men	131 (556—203)	7/116 (6)	1	1	
Women	140 (73—221)	29/285 (10.2)	1.7	1.8 (0.7—4.6)	0.21
WHO stage					
Stage III	145 (79—221)	25/293 (8.5)	1	1	
Stage IV	95 (30—204)	11/108 (10.2)	1.2	2.1 (0.9—5.0)	0.09
BMI on presentation (kg/m^2)					
<22	128 (58—202)	18/313 (5.8)	1	1	
>22	175 (111—295)	18/88 (20.5)	4.2	4.2 (1.9—9.2)	<0.001
Hb (g/dl)					
<10	85 (31—162)	6/132 (4.5)	1	1	
>10	166 (88—236)	30/269 (11.2)	2.6	2.0 (0.7—5.3)	0.2
TLC (cells/mm^3)					
<1200	69 (30—144)	3/104 (2.9)	1	1	
>1200	153 (88—236)	33/297 (11.1)	4.2	3.4 (1.0—11.9)	0.05
Active TB (all types)					
Present	131 (54—192)	2/67 (3.0)	1	1	
Absent	140 (69—221)	34/334 (10.2)	3.7	1.8 (0.4—8.9)	0.46
Active HIV-related disease[b]					
Present	125 (58—200)	13/295 (4.4)	1	1	
Absent	185 (101—292)	23/106 (21.7)	6.0	6.8 (3.0—15.5)	<0.001

IQR: interquartile range; OR: odds ratio; BMI: body mass index; Hb: haemoglobin; TLC: total lymphocyte count; TB: tuberculosis.
[a] Adjusted for gender, WHO clinical stage, BMI, Hb, TLC, active TB and active WHO defining disease on presentation.
[b] Any active WHO defining indicator disease at the time of presentation for antiretroviral treatment (this includes individuals who have been on routine follow-up visits and who had been successfully treated for an active opportunistic infection(s) during previous visit(s)).

3.4. Risk factors associated with a CD4 count ≥350 cells/μl

Significant risk factors associated with a CD4 count of ≥350 cells/μl included a BMI > 22 kg/m^2, the absence of an active HIV-related disease on presentation and a TLC > 1200 cells/μl (Table 2).

A CD4 count ≥350 cells/μl was found in 7 (5.4%) of 130 individuals with a BMI < 18.5 kg/m^2, in 20 (9.7%) of 206 individuals with a BMI 18.5—22 kg/m^2 and in 9 (13.8%) of 65 individuals with a BMI > 22 kg/m^2 (χ^2 for linear trend = 4.1; $P = 0.04$).

3.5. Validity and predictive values of BMI, HIV-related disease and TLC for targeted CD4 testing

The sensitivity, specificity and predictive values of using BMI > 22 kg/m^2, the absence of an active WHO defining disease, the combination of the two, and TLC are shown in Table 3.

Screening using a combination of BMI > 22 kg/m^2 and the absence of an active WHO defining indicator disease (at the time of presentation for ART) would require 24 (6%) of 401 individuals to undergo CD4 testing of whom 10 (42%) would have a CD4 count ≥350 cells/μl. Thus, 1 in every 2.4 individuals in this clinical subgroup will have a CD4 count ≥350 cells/μl. Running CD4 measurements on all individuals with a BMI > 22 kg/m^2 would single out 88 (22%) of 401 individuals for CD4 counts and would increase the number detected with a CD4 count ≥350 cells/μl to 18 (or 50% of a total of 36 individuals with CD4 counts ≥350 cells/μl).

4. Discussion

This study shows that if ART was initiated on the basis of WHO clinical staging, approximately one out of every 10 individuals presenting in WHO clinical stage III or IV will have a CD4 count of ≥350 cells/μl and is at risk of being placed prematurely on ART. A BMI > 22 kg/m^2, the absence of an active WHO indicator disease and a TLC > 1200 cells/μl are associated with such CD4 counts.

Table 3 Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy of specific variables for detecting individuals with a CD4 count ≥350 cells/μl ($n = 401$)

	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy[a]
BMI > 22 kg/m^2	18/36 (50%)	295/365 (81%)	18/88 (20%)	295/313 (94%)	313/401 (78%)
Active HIV disease absent[b]	23/36 (64%)	282/365 (77%)	23/106 (22%)	282/295 (96%)	295/401 (74%)
Combination BMI > 22 g/m^2 and disease absent	10/36 (28%)	351/365 (96%)	10/24 (42%)	351/377 (93%)	361/401 (90%)
TLC > 1200 cells/mm^3	33/36 (92%)	101/365 (28%)	33/297 (11%)	101/104 (97%)	134/401 (33%)

BMI: body mass index; TLC: total lymphocyte count.
[a] Sum of the correctly identified individuals with CD4 counts >350 cells/μl and ≤350 cells/μl divided by all those screened.
[b] Absence of an active WHO defining indicator disease.

Whilst it is reassuring that the great majority (91%) of HIV-positive individuals classified in WHO stage III or IV have CD4 counts <350 cells/μl and are deserving of ART, there is still the concern about the proportion of individuals with a CD4 count ≥350 cells/μl (9% in our study) that might be started too early on ART.

Our study may provide a way forward in trying to limit this problem. Assessing the BMI (Zachariah et al., 2002) and examining patients for active WHO defining diseases constitute part of routine clinical procedures in the Thyolo clinic, as in other HIV/AIDS clinics in Malawi. For clinic staff, this would mean that there will be no additional workload related to identifying these two subgroups, which is an important operational consideration for such clinics that are often overloaded and understaffed (Kober and Van Damme, 2004; MOH, 1999, 2003d). Two possible options could be considered for targeting CD4 measurements. The first option would be to select individuals in the subgroup with a BMI > 22 kg/m^2 and who have no active WHO indicator disease. Targeting CD4 measurements in this subgroup of 24 (6%) individuals out of a total of 401 would help save 10 unnecessary (too early) treatments. The second option would be simply to run CD4 measurements on all individuals with a BMI > 22 kg/m^2. This in turn would single out 88 (22%) of 401 individuals for CD4 measurements and would save 18 unnecessary treatments (or 50% of the total of 36 individuals with CD4 counts ≥350 cells/μl).

There are a number of considerations and potential advantages of targeting CD4 measurements to a selected clinical subgroup of patients. First, as the algorithms fall short of detecting all individuals with a CD4 count ≥350 cells/μl, a proportion of individuals (respectively, 18/36 for BMI alone and 26/36 for the combination of BMI and opportunistic disease) still risk being placed prematurely on ART. Nevertheless, the overall diagnostic accuracy (the ability to detect correctly all those who require and do not require ART) of the current national strategy of offering ART on the simple basis of WHO staging would increase from 91% to 93.5% using the combination of BMI and an active WHO indicator disease. Using BMI alone, this would increase from 91% to 95.5%. In the absence of being able to offer universal CD4 testing, there is thus still an improvement (albeit to a modest extent) in overall decisions that guide ART eligibility.

Second, in terms of numbers of unnecessary treatments that are saved and the related costs, there might be tangible financial benefits for an impoverished country such as Malawi. Using the current national target of placing 80 000 people on ART by 2005 (MOH, 2003a, 2003b), there are an estimated 7200 (9% of the 80 000) individuals who might be prematurely placed on ART. Targeted CD4 measurements as discussed above could save 1991 and 3600 unnecessary (too early) treatments, respectively. Using an average estimated yearly cost of US$600 for drugs alone, this would amount to between US$1 194 600 and US$2 160 000, respectively, in cost savings per year. This calculation is under limits of the assumption that in all patients ART would be deferred for an average period of 1 year.

Third, the pre-selection procedure will substantially reduce the number of individuals who will need to be sent for CD4 measurements compared with a strategy of universal testing. In this study this comprised only 24 (6%) of 401 patients when using a combination of BMI and the absence of an active WHO defining opportunistic disease and 88 (22%) if BMI was used alone. With an average cost of approximately US$14 per CD4 measurement using a FacsCount machine, this too translates into substantial cost savings compared with universal CD4 testing.

The relatively lower numbers of CD4 tests that would be requested would also allow the important possibility of introducing alternative 'manual' CD4 techniques for ART delivery sites. These techniques are less sophisticated and far less expensive than current automated techniques such as the FacsCount. The Dynabeads (Dynal Biotech, Oslo, Norway) technique, for instance, is a manual technique that is known to be robust and low cost for resource-limited settings (Diagbouga et al., 2003). It can also be implemented using a normal light microscope in district laboratories where neither the infrastructure nor the resources to install and run automated CD4 counting exists.

Despite the potential advantages, there are a number of limitations and unanswered questions related to this study and its findings. First, the proposed algorithms are based on the results of a model that has not been validated on a different data set or setting. Further research in this respect is thus required. Second, we used an arbitrary cut-off for BMI that was rounded at 22 kg/m^2 and subsequently applied for both men and women in our study. This was a choice of the authors who preferred to have a standardised parameter for the sake of simplicity of implementation. It must be kept in mind that different BMI cut-offs may apply in other settings where anthropometric values may be different (e.g. in Asia).

Although we have used a CD4 threshold of 350 cells/μl as we considered this to be the correct surrogate marker for starting ART in Malawi, studies in Europe suggest that this threshold might be too high to start ART in view of costs, side effects and deteriorating motivation for life-long treatment and suggest a cut-off 250—300 cells/μl. Different cut-offs might thus be applicable to other settings.

Finally, all the individuals under follow-up in our study were on daily co-trimoxazole prophylaxis and this is likely to have had a beneficial effect on the incidence of certain opportunistic infections as well the evolution of CD4 counts and viral load (Mermin et al., 2004). However, we are unable to substantiate the extent to which this intervention itself might have contributed to reducing morbidity and the particular effect it would have had on CD4 counts in our cohort.

Malawi is desperately trying to place thousands of people on ART on a fast track basis and is currently using only WHO clinical staging to identify those who are in need of ART. Our study describes how CD4 measurements could be targeted to a clinically selected subgroup of patients, with the potential advantages of limiting unnecessary CD4 measurements, unnecessary (too early) treatments and costs.

Conflicts of interest statement
The authors have no conflicts of interest concerning the work reported in this paper.

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