

# Loss of correlation between HIV viral load and CD4+ T-cell counts in HIV/HTLV-1 co-infection in treatment naïve Mozambican patients

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**Summary:** Seven hundred and four HIV-1/2-positive, antiretroviral therapy (ART) naïve patients were screened for HTLV-1 infection. Antibodies to HTLV-1 were found in 32/704 (4.5%) of the patients. Each co-infected individual was matched with two HIV mono-infected patients according to World Health Organization clinical stage, age  $\pm$  5 years and gender. Key clinical and laboratory characteristics were compared between the two groups. Mono-infected and co-infected patients displayed similar clinical characteristics. However, co-infected patients had higher absolute CD4+ T-cell counts ( $P = 0.001$ ), higher percentage CD4+ T-cell counts ( $P < 0.001$ ) and higher CD4/CD8 ratios ( $P < 0.001$ ). Although HIV plasma RNA viral loads were inversely correlated with CD4+ T-cell-counts in mono-infected patients ( $P < 0.0001$ ), a correlation was not found in co-infected individuals ( $P = 0.11$ ). Patients with untreated HIV and HTLV-1 co-infection show a dissociation between immunological and HIV virological markers. Current recommendations for initiating ART and chemoprophylaxis against opportunistic infections in resource-poor settings rely on more readily available CD4+ T-cell counts without viral load parameters. These guidelines are not appropriate for co-infected individuals in whom high CD4+ T-cell counts persist despite high HIV viral load states. Thus, for co-infected patients, even in resource-poor settings, HIV viral loads are likely to contribute information crucial for the appropriate timing of ART introduction.

**Keywords:** HIV, HTLV-1, CD4+ T lymphocytes, lymphocytosis, Mozambique

## INTRODUCTION

Human T-cell lymphotropic virus type 1 (HTLV-1) and human immunodeficiency virus type 1/2 (HIV-1/2) are genetically and functionally distinct retroviruses that target CD4+ T lymphocytes through different surface receptors. HTLV-1 is frequently associated with lymphocytosis, although malignant transformation of infected cells, producing acute or chronic T-cell leukaemia/lymphoma, is much less common.<sup>1</sup> In contrast, HIV infection causes shortened survival time and impaired production of CD4+ T-cells, with a progressive decline of this cell population in the peripheral blood.<sup>2</sup>

More than 50% of the global HIV-infected population, and around 72% (or 4.7 million) of individuals in need of antiretroviral (ARV) therapy live in sub-Saharan Africa, a region that also bears a significant burden of HTLV-1 infection.<sup>3,4</sup> In 2007, only one in four people in need of treatment in this region was on ARV therapy.<sup>3</sup> In Mozambique, a country with 19 million inhabitants, the prevalence of HIV infection in

adults is estimated to be 16.0%. In October 2008, an estimated 489,000 patients were being followed in HIV outpatient clinics in Mozambique. Of the 430,000 people who were in need of ARV drugs throughout the country, only about 121,000 were initiated on this therapy by October 2008 (*Source of Information: Medical Assistance Department, Ministry of Health, Mozambique*). ARV therapy initiation in Mozambique is primarily guided by the CD4+ T-cell count and by the HIV clinical stage on the basis that CD4+ T-cell count is considered to be a reliable and available parameter to guide decision-making during management of HIV infection,<sup>5,6</sup> whereas HIV viral load monitoring is generally unavailable throughout the country.

The basis of the heterogeneous geographical and demographic distribution of HTLV-1 in Africa is not well understood. In southern and central Africa, the prevalence of HTLV-1 infection has been reported to range between 0.5% and 7.1%,<sup>7-13</sup> peaking in groups reporting high rates of sexual activity and in HIV-infected individuals,<sup>14-19</sup> and in this population sexual transmission of HTLV-1 is thought to be a dominant mode of acquisition. With the HIV/AIDS epidemic on the rise, it is foreseeable that individuals co-infected with HIV and HTLV-1 will be frequently seen at HIV treatment and care centres.

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Despite the high prevalence of both viruses in sub-Saharan Africa, little data are available about the clinical presentation of co-infected individuals in the region. Studies in other regions, including South American, show that in co-infected patients clinical progression of HIV infection occurs despite normal or high CD4+ T-cell counts.<sup>20-22</sup> These apparently paradoxical findings are of a even greater relevance in the context of the scaling-up of ARV therapy programmes throughout sub-Saharan Africa as CD4 cell levels are the dominant criterion for timing institution of antiretroviral therapy (ART). Against this background, we investigated the clinical and laboratory characteristics of HIV-infected patients with HTLV-1 co-infection in Maputo, Mozambique.

## METHODS

### Study design

A nested case-control study was designed to enrol HIV positive patients presenting to an HIV Outpatient Clinic (HOP) for their clinical assessment after an HIV screening test. HIV-positive patients co-infected with HTLV-1 (co-infected patients) were defined as cases and HIV-positive patients without co-infection with HTLV-1 (mono-infected patients) were considered to be controls allowing assessment of the impact of HTLV-1 superimposed on HIV-1 infection.

This study was approved by the National Health Bioethics Committee in Mozambique and by the Sydney University Ethics Committee, Australia. The Sydney South West Area Health Service Ethics Research Committee (RPAH Zone) was also notified and endorsed the study.

### Subjects

All subjects for the study were recruited from an ongoing cohort of HIV-infected individuals at the HOP within the Alto Maé Health Centre in Maputo City, Mozambique. Patients are referred to this HOP from a voluntary counselling and testing (VCT) service located within the same Health Centre. This VCT service is one of many institutions offering free of charge HIV diagnosis for the public in Maputo City.

Informed consent to participate in the study was requested from all consecutive patients coming to the HOP for routine HIV-related clinical care visits in the period between 13 March and 2 June 2006. Exclusion criteria were present or past history of ARV therapy, age less than 18 years, current pregnancy, and those who declined to participate or were unable to give informed consent.

### Demographic, clinical and laboratory data

All consenting patients eligible for the study gave 5 mL of venous blood at the first visit. This specimen was used to determine HTLV-1 status. Two weeks after the first visit, patients returned to collect their HTLV-1 test result. At this time, each patient with a positive HTLV-1 result (case) was matched with two patients with negative HTLV-1 results (controls) for further study. The matching was performed based on World Health Organization (WHO) clinical staging system for HIV, age ( $\pm 5$  years) and gender. For cases for which there were more than two possible controls, the two closest in age to the case were selected. The selection of controls was made

without prior knowledge of full blood counts or CD4+ T-cell counts results.

The researcher using a structured questionnaire then interviewed cases and controls. The questionnaire included: sociodemographic data, sexual/reproductive history and clinical data. General physical and neurological examination was performed in all patients enrolled to assess patient status by WHO clinical staging system for HIV and to stage HTLV-1 related diseases, specifically the presence of neurological manifestations<sup>23</sup> and skin lesions. To minimize the risk of recall bias caused by the clinical researcher's knowledge of HTLV-1 status, another medical doctor who was blinded to HTLV-1 status confirmed all neurological signs and symptoms or skin lesions. Agreement between the two doctors was taken as the definitive finding. Finally, an additional 7 mL of blood was drawn from both cases and controls to perform T-cell subsets and HIV-RNA viral load.

### Laboratory assays

#### HIV serology

All patients enrolled in this study were tested for HIV 1/2 infection at the VCT services of the Health Centre. The national algorithm for HIV diagnosis using the sequential testing with two rapid assays was followed. All individuals were first screened using the Determine HIV-1/2 test (Abbott Laboratories, Tokyo, Japan). Individuals whose specimens were reactive on the screening assay were further tested using the Uni-Gold HIV test (Trinity Biotech, Ireland). We did not perform laboratory assays to discriminate infections with HIV-1 from those with HIV-2 as HIV-1 is known to cause more than 99% of HIV infections in Mozambique (*Source of information: Ministry of Health, Mozambique*).

#### HTLV serology

Blood samples were screened for HTLV-1 using an immunoenzymatic qualitative test, Murex HTLV-1 + 2 (Murex Biotech Limited, Dartford, UK). Samples with reactive results were further tested using a Western blot, HTLV BLOT 2.4 (Genelabs<sup>®</sup> Diagnostics, Geneva, Switzerland). All patients reactive to antigens codified by the GAG gene (p19 with or without p24) and to two antigens encoded by the ENV gene (GD21 and rgp46-I) were considered as positive for HTLV-1.

#### T-cell subsets immunophenotyping

Immunophenotyping was performed on fresh whole blood using a FACSCalibur<sup>™</sup> flow cytometer (Becton Dickinson, USA). MultiTEST reagents, TruCOUNT tubes and MultiTEST software (all from Becton Dickinson, California, USA) were used in a lyse-no wash protocol to determine absolute and percentage values for T-cell subsets (CD4+ T-cells, CD8+ T-cells and CD4+ T/CD8+ T ratio).

#### HIV-RNA viral load

Plasma samples were stored at  $-70^{\circ}\text{C}$  until viral load was assayed on a COBAS<sup>®</sup> TaqMan 48 analyser (Roche Diagnostics, Penzberg, Germany) for automated amplification and detection of nucleic acid. HIV-1 viral load was determined using the COBAS<sup>®</sup> TaqMan<sup>™</sup> HIV-1 test, for use with the High Pure System (all from Roche Diagnostics, Germany). This assay can quantify HIV-1 RNA over a range of 40–10,000,000 copies/mL.

## Statistical analysis

Data were double entered in a Microsoft Office Access 2003 database by two independent data monitors. Accuracy of data was compared using Epi Info Version 3.3.2. All data were exported to be analysed in Stata software (StataCorp 2005. Stata Statistical Software: Release 9.0, College Station, TX, USA).

Descriptive statistics was used to describe demographics and medical history. To test significance of the difference between cases and controls with respect to baseline demographic characteristics, sexual/reproductive history, clinical and laboratory parameters, Pearson's  $\chi^2$ , Fisher's exact test and Mann-Whitney U test were used, as appropriate. Correlation between CD4+ T-cell counts and HIV-RNA viral loads as well as correlation between body mass index (BMI) and CD4+ T-cell count among HTLV-1 subgroups in different HIV clinical stages were tested with Spearman's rank correlation coefficient. The level of statistical significance was set at 0.05.

## RESULTS

### Demographic characteristics and clinical history

From 724 HIV 1/2- infected patients invited to participate, 704 (97.4%) consented to be screened for HTLV-1 infection. HTLV-1 infection was identified in 32/704 patients (4.5%, 95% CI 3.0–6.0). Three patients with a positive HTLV-1 antibody test did not return to collect their result and were excluded from the study. Sexual history, clinical presentation and laboratory findings were compared between 29 HIV/HTLV-1 dually infected patients and 59 matched patients who were HIV-positive but HTLV-1 negative.

Co-infected and mono-infected patients were comparable with respect to demographic characteristics as well as sexual and reproductive history (Table 1). The median age was 40 years (intraquartile range [IQR] 34.0–48.0) versus 41 years (IQR 32.0–47.0) for co-infected and mono-infected patients, respectively. No patient had a past history of injecting intravenous drugs. However, a previous blood transfusion was reported by 6/29 (20.7%, 95% CI 0.059–0.354) co-infected patients and by 14/59 (23.7%, 95% CI 0.128–0.346) mono-infected patients ( $P = 0.75$ ). History of scarification was reported by 22/29 (75.9%, 95% CI 0.603–0.915) and by 41/59 (69.5%, 95% CI 0.578–0.812) individuals in the co-infected and mono-infected group, respectively ( $P = 0.54$ ). Clinical episodes of sexually transmitted infections (STIs) were reported by 12/29 (41.4%, 95% CI 0.235–0.593) of co-infected patients and by 27/59 (45.8%, 95% CI 0.331–0.585) of mono-infected patients ( $P = 0.70$ ).

### Clinical manifestations and HIV disease staging

Twenty-four (83.8%) patients with co-infection and 47 (79.7%) with mono-infection were in stage I and II of HIV infection. No patient had an AIDS defining condition (Table 1). Median BMI decreased in both groups with clinical progression of HIV infection and it tended to be lower in co-infected patients (21.79 kg/m<sup>2</sup>, 95% CI 18.64–26.05) compared with mono-infected patients (23.95 kg/m<sup>2</sup>, 95% CI 21.34–27.97). However, this difference was not statistically significant ( $P = 0.06$ ).

The most common neurological manifestation in both groups was sensory symptoms (tingling, pins, needles and burning

Table 1 Demographics, sexual history and clinical characteristics of patients with HIV infection only and those with HIV and HTLV-1

	HIV–HTLV-1 co-infected patients (n = 29)	HIV mono-infected patients (n = 59)	P values
<b>Gender</b>			
Men	4 (14%)	8 (14%)	
Women	25 (86%)	51 (86%)	0.98
<b>Age (years)</b>			
Median	40.0	41.0	0.99
IQR	34.0–48.0	32.0–47.0	
Range	20.0–65.0	18.0–60.0	
<b>Marital status</b>			0.17
Single	5 (17%)	9 (15%)	
Married	9 (31%)	31 (53%)	
Widow	6 (21%)	11 (19%)	
Divorced	9 (31%)	8 (14%)	
Received blood transfusion	6 (21%)	14 (24%)	0.75
History of scarification	22 (76%)	41 (70%)	0.54
History of sexually transmitted history	12 (41%)	27 (46%)	0.70
<b>Age of first sexual contact (years)</b>			0.43
Median	17.0	17.0	
IQR	15.5–19.0	16.0–19.0	
Range	14.0–25.0	14.0–25.0	
<b>Number of sexual partners, median (IQR)</b>			
Last month	0 (0–1)	1 (0–1)	0.33
Last 3 months	0 (0–1)	1 (0–1)	0.98
Last 12 months	1 (0–1)	1 (0–1)	0.81
Lifetime	3 (2–4)	3 (2–4)	0.86
<b>Number of pregnancies</b>			0.41
Median (IQR)	4 (2–7)	5 (3–7)	
<b>Number of children</b>			0.45
Median (IQR)	3 (1–4)	3 (1–5)	
<b>Number of miscarriages</b>			0.39
Median (IQR)	2 (1–3)	2 (1–2)	
<b>Body mass index (kg/m<sup>2</sup>)</b>			0.06
Median	21.79	23.95	
IQR	18.64–26.05	21.34–27.97	
Range	15.24–33.98	14.60–36.91	
<b>HIV clinical stage (WHO)</b>			0.94
I	7 (24%)	14 (23%)	
II	17 (59%)	33 (56%)	
III	5 (17%)	12 (21%)	
IV	0	0	

HTLV-1= human T-cell lymphotropic virus; HIV= human immunodeficiency virus; IQR = intraquartile range; WHO = World Health Organization

sensations), which was reported in 5/29 (17.2%, 95% CI 0.034–0.309) patients with co-infection and in 8/59 (13.6%, 95% CI 0.049–0.223) patients with mono-infection ( $P = 0.65$ ). Two (6.9%, 95% CI –0.023–0.161) patients with HTLV-1 were found to have hyperreflexia of lower limbs associated with positive Babinski sign and clonus ( $P = 0.11$ ). Further neurological investigation of these cases was not undertaken.

Skin lesions observed in co-infected and mono-infected patients, respectively, included 6/29 (20.7%, 95% CI 0.059–0.354) versus 20/59 (33.9%, 95% CI 0.218–0.460) with papular pruritic eruption ( $P = 0.20$ ), 6/29 (20.7%, 95% CI 0.060–0.354) versus 11/59 (18.6%, 95% CI 0.087–0.285) with

Table 2 Laboratory characteristics of patients with HIV infection only and those with HIV and HTLV-1

	HIV-HTLV-1 co-infected patients (n = 29)	HIV mono-infected patients (n = 59)	P values
<b>Total leukocytes (10<sup>3</sup>/mm<sup>3</sup>)</b>			0.04
Median	4.4	3.9	
IQR	3.9–5.7	3.1–4.9	
Range	2.69–12.98	2.03–8.14	
<b>Total lymphocytes (10<sup>3</sup> cells/mm<sup>3</sup>)</b>			0.15
Median	1.78	1.54	
IQR	1.28–1.94	1.38–2.26	
Range	0.64–4.52	0.62–3.95	
<b>CD4+ T-cell count (cells/mm<sup>3</sup>)</b>			0.001
Median	525	274	
IQR	310–827	183–436	
Range	62–3363	35–986	
<b>CD4+ T-cell count (%)</b>			<0.001
Median	24.86	15.86	
IQR	18.97–32.73	9.37–21.03	
Range	7.57–65.42	2.41–38.00	
<b>CD8+ T-cell count (cells/mm<sup>3</sup>)</b>			0.71
Median	1002	937	
IQR	649–1090	666–1358	
Range	325–2876	234–2851	
<b>CD8+ T-cell count (%)</b>			0.01
Median	46.83	54.17	
IQR	36.23–53	42.71–61.34	
Range	21.21–70.78	28.83–85.70	
<b>CD4+ T/CD8 + T ratio</b>			<0.001
Median	0.48	0.31	
IQR	0.34–0.82	0.16–0.43	
Range	0.12–3.09	1.22–0.03	
<b>HIV-RNA viral load (copies/mL)</b>			0.36
Median	56,385	37,573	
IQR	14,749–277,557	11,322–176,837	
Range	0–743,253	78–4,805,932	

HTLV-1= human T-cell lymphotropic virus; HIV= human immunodeficiency virus; IQR = intraquartile range

dermatophytosis ( $P = 0.82$ ) and 4/29 (13.8%, 95% CI 0.0125–0.264) versus 6/59 (10.2%, 95% CI 0.025–0.179) with evidence of past herpes zoster ( $P = 0.62$ ). None of the co-infected patients presented with erythema palmare and cutaneous candidiasis, while 1/29 (3.4%) case of each occurred in mono-infected patients ( $P = 0.67$ ).

## Lymphocyte profiles

Co-infected patients had higher median of total leukocytes count, higher CD4+ T-cell (absolute/percent) count, lower CD8+ T-cell percent count and higher CD4+ T/CD8+ T ratio (Table 2). Among co-infected patients, the median CD4+ T-cell absolute count was 525 cells/mm<sup>3</sup> (IQR 310–827) compared with 274 cells/mm<sup>3</sup> (IQR 183–436) in mono-infected patients ( $P = 0.001$ ). Furthermore, 25/29 (86.2%, 95% CI 0.736–0.988) of co-infected patients had a CD4+ T-cell count greater than 200 cell/mm<sup>3</sup> compared with 41/59 (69.5%,  $v = 0.578$ –0.812) patients with mono-infection (matched OR = 2.74, 95% CI 0.81–9.29). Co-infected patients were 7.2 times (95% CI 1.59–32.51) more likely to have a CD4+ T-cell count above 500 cells/mm<sup>3</sup> (Table 3).

Analysis of the relationship between CD4+ T-cell count and HIV clinical stage (Figure 1) demonstrated that in patients with mono-infection, CD4+ T-cells counts were significantly lower in groups with evidence of clinical progression of HIV infection (95% CI  $-0.59$  to  $-0.15$ ,  $P = 0.0024$ ). In contrast, in patients with co-infection, CD4+ T-cells counts did not decrease with progression of HIV infection (95% CI  $-0.34$  to  $0.39$ ,  $P = 0.8926$ ).

## HIV-1 viral load

The median HIV RNA viral load was 56,385 copies/mL (IQR 14,749–277,557) in co-infected patients compared with 37,573 copies/mL (IQR 11,322–176,837) in mono-infected patients ( $P = 0.36$ ). In both groups, the HIV-RNA load tended to increase with clinical progression of HIV infection (Figure 2). However, a statistically significant increase of HIV-RNA viral load by HIV clinical stage was only observed in patients with co-infection ( $P = 0.04$  versus  $P = 0.10$ ).

As expected, we documented a strong inverse relationship between levels of HIV-RNA and absolute CD4+ T-cell counts in mono-infected patients (95% CI  $-0.73$  to  $-0.38$ ,  $P < 0.0001$ ). However, of importance, a similar relationship was not observed in patients with HIV/HTLV-1 co-infection (95% CI  $-0.60$  to  $0.07$ ,  $P = 0.11$ ).

## DISCUSSION

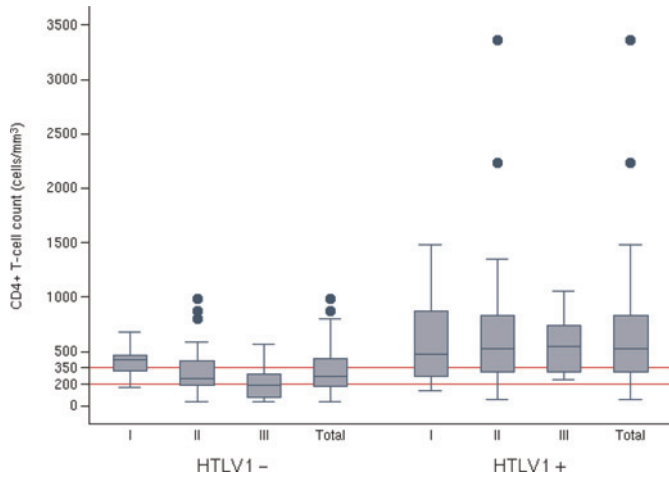
The prevalence of HTLV-1 infection is known to vary from region to region. However, in the same geographical region, it can vary in different population subgroups, probably reflecting the diverse modes of its transmission.<sup>24</sup> The overall prevalence of anti-HTLV-1 antibodies among HIV positive patients found

Table 3 Odds ratio of CD4+ T-cell count strata in patients with HIV infection and those with HIV and HTLV-1

Characteristics	HIV-HTLV-1 co-infected patients (n = 29)	HIV mono-infected patients (n = 59)	Odds ratio (95% CI)	P value
<b>CD4+ T-cell count (cells/mm<sup>3</sup>)</b>				0.001
<200	4 (14%)	18 (31%)	1	
200–500	9 (31%)	31 (52%)	1.31 (0.35–4.9)	
>500	16 (55%)	10 (17%)	7.2 (1.59–32.51)	
<b>CD4+ T-cell count (%)</b>				<0.001
<15	5 (17%)	24 (41%)	1	
15–20	4 (14%)	16 (27%)	1.2 (0.28–5.25)	
>20	20 (69%)	19 (32%)	5.05 (1.47–17.40)	

HTLV-1, human T-cell lymphotropic virus; HIV= human immunodeficiency virus; CI = confidence interval

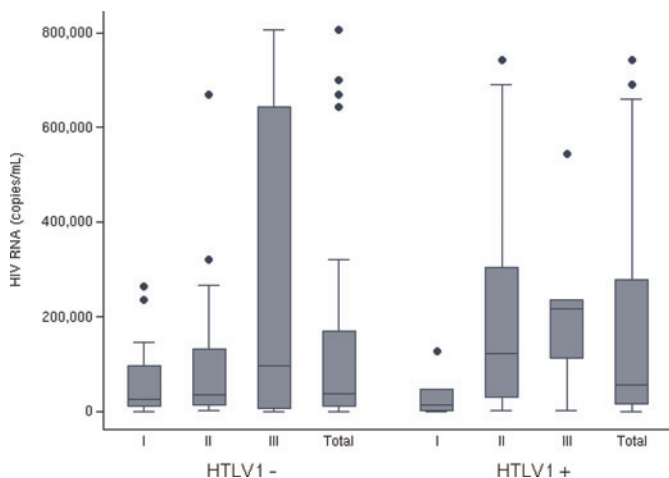




**Figure 1** CD4+ T-cell counts (cell/mm<sup>3</sup>) distribution in HIV mono-infected patients and HIV/HTLV-1 co-infected patients according to HIV clinical stage. HTLV 1<sup>-</sup>, HIV-infected patients; HTLV 1<sup>+</sup>, HIV-infected patients co-infected with HTLV-1. I, HIV clinical stage 1; II, HIV clinical stage 2; III, HIV clinical stage 3. Mono-infection is infection with HIV only; co-infection is infection with HTLV-1 and HIV

in this study is markedly higher than previously reported in African blood donors.<sup>9,25,26</sup> This would be consistent with those subjects with HIV also being at increased behavioral risk for acquiring HTLV-1 infection as both viruses have the same routes of transmission. However, it could equally be consistent with widespread epidemics of HIV and HTLV-1 running in parallel but with different transmissibility parameters.

In some countries with a high prevalence of intravenous drug use (IDU), the prevalence of HTLV-1 infection is considerably higher.<sup>27</sup> In our study, we did not report any case of IDU. It has been estimated that 80–90% of HIV infections in the developing world are due to heterosexual transmission.<sup>28,29</sup> It is most likely that, in our setting, the major form of horizontal transmission of HTLV-1 is through heterosexual intercourse.



**Figure 2** HIV-RNA viral load (copies/mL) distribution in HIV mono-infected patients and HIV/HTLV-1 co-infected patients according to HIV clinical stage. HTLV 1<sup>-</sup>, HIV-infected patients; HTLV 1<sup>+</sup>, HIV-infected patients co-infected with HTLV-1. I, HIV clinical stage 1; II, HIV clinical stage 2; III, HIV clinical stage 3. Mono-infection is infection with HIV only; co-infection is infection with HTLV-1 and HIV

However, it may also be possible that the transmission of retroviruses through needles and surgical instruments at health facilities or at traditional healing practices play a relevant epidemiological role.

As already reported in other geographical regions and diverse populations,<sup>19,20,22,27,30,31</sup> Mozambican patients co-infected with HTLV-1 displayed higher CD4+ T-cell counts when compared with mono-infected patients matched for clinical stage of HIV disease. We could not investigate the relationship between HTLV-1 infection and HIV disease progression in individual patients due to the cross-sectional design of our study.

Nevertheless, it was evident that BMI decreased with clinical progression of HIV infection in all patients. This suggests that HIV disease progression also leads to clinical deterioration of co-infected patients. In fact, BMI was generally lower in co-infected patients, although this was shown not to be statistically significant ( $P = 0.06$ ). We did not find any statistical difference comparing demographic, sexual and reproductive characteristics between cases and controls, which makes it less likely that the high CD4+ T-cell counts found in co-infected patients were biased by the difference in duration of HIV infection. Incidental to the primary goals of this study, we looked at clinical features in major organ systems but could not associate any neurological symptoms or skin lesions with HTLV-1 infection, probably due to the small number of patients enrolled in the study.

In southern Africa, patients showing CD4+ T lymphocyte counts that do not reflect their HIV clinical staging should be suspected as having HTLV-1 infection. In our study, 60.0% (3/5) of co-infected patients with HIV clinical stage III had an absolute CD4+ T-cell count above 350 cells/mm<sup>3</sup>. In co-infected patients, CD4+ T lymphocyte counts are not reliable markers to guide initiation of prophylaxis against opportunistic infections or HAART. The current general recommendations to start HAART in resource poor settings are based on the combined use of HIV clinical staging and the CD4+ T-cells count. An exception is considered for patients with chronic HIV infection who have CD4+ T-cell counts between 201–350 cell/mm<sup>3</sup>, in whom HAART can be started when HIV-RNA viral load levels indicate significant risk of progression.<sup>23</sup>

This study suggests that HIV-RNA viral load increases markedly with clinical progression of HIV-HTLV co-infected patients (Figure 2). Therefore, HIV-RNA viral load might give important additional information about the true risk of early progression to more advanced clinical stages in these patients. Additionally, HIV viral load is also likely to be a much more reliable guide to determining intervention points with ARV in HIV-HTLV co-infected subjects.

Our results support the hypothesis that, in HIV/HTLV-1 co-infected patients, the dissociation between clinical and immunological markers derive from a loss of the inverse correlation between the HIV viral load and the CD4+ T-cell count as a direct result of HTLV-1 infection. This study did not address the molecular basis of the protection of CD4 T-cells from rapid decline in association with HIV viraemia but it provides a strong rationale for such future studies. Our results are of particular relevance for care/treatment scale-up programmes in sub-Saharan Africa, where CD4+ T lymphocyte counts constitute the main laboratory parameter used for checking HIV disease progression as well as guiding HAART initiation and monitoring. The small number of our sample size, the

absence of severe AIDS defining illnesses in the studied population and the low numbers of patients on WHO stage III of HIV infection constitute limitations of this study. These limitations, together with the cross-sectional nature of the study, did not allow us to define the impact of co-infection on HIV disease progression.

Despite its limitations, this study does not support the use in HTLV-1 co-infected patients of current recommendations for initiation of ART and chemoprophylaxis against opportunistic infections according to CD4+ T-cell counts. Thus, in settings with a high prevalence of HTLV-1 infection among the HIV-1 infected population, serological testing for HTLV-1 should ideally be available so that HIV viral load might be integrated into the treatment initiation algorithm. Defining appropriate thresholds of HIV viral load relevant to intervention points for co-infected patients will require longitudinal studies.

In the short term, it is unlikely that overburdened health systems in sub-Saharan Africa will be able to routinely test for HTLV-1 infection and/or HIV viral load. Therefore, in patients with dissociation between clinical and immunological parameters, the WHO clinical staging system, with minimal weight placed on CD4 T-cell data, should be recommended to monitor progression of HIV infection and guide initiation of therapy.

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#### REFERENCES

- Hollingsworth P. Mechanisms of T-cell activation by human T-cell lymphotropic virus type I. *Microbiol Mol Biol Rev* 1999;**63**:308-33
- Gallo RC. Human retroviruses after 20 years: a perspective from the past and prospects for their future control. *Immunol Rev* 2002;**185**:236-65
- UNAIDS. 2008 Report on the Global AIDS Epidemic, 2008. [http://www.unaids.org/en/knowledgecentre/HIVDATA/globalreport/2008/2008\\_global\\_ASP](http://www.unaids.org/en/knowledgecentre/HIVDATA/globalreport/2008/2008_global_ASP). ISBN 978 929 2737116
- Mills EJ, Nachega JB, Buchan I, et al. Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis. *JAMA* 2006;**296**:679-90
- Gazzard B, Bernard AJ, Boffito M, et al. British HIV Association (BHIVA) guidelines for the treatment of HIV-infected adults with antiretroviral therapy (2006). *HIV Med* 2006;**7**:487-503
- Hammer SM, Saag MS, Schechter M, et al. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society - USA panel. *Top HIV Med* 2006;**14**:827-43
- Armah HB, Narter-Olaga EG, Adjei AA, Asomaning K, Gyasi RK, Tettey Y. Seroprevalence of human T-cell lymphotropic virus type I among pregnant women in Accra, Ghana. *J Med Microbiol* 2006;**55**(Part 6):765-70
- Van Rensburg EJ, Lemmer HR, Joubert JJ. Prevalence of viral infections in Mozambican refugees in Swaziland. *East Afr Med J* 1995;**72**:588-90
- Collenberg E, Ouedraogo T, Ganame J, et al. Seroprevalence of six different viruses among pregnant women and blood donors in rural and urban Burkina Faso: a comparative analysis. *J Med Virol* 2006;**78**:683-92
- Louis JP, Gardon J, Trebucq A, et al. Epidemiological features of retroviral infection by HTLV-1 in central Africa. *Bull Soc Pathol Exot* 1993;**86**:163-8
- Saxinger W, Blattner WA, Levine PH, et al. Human T-cell leukemia virus (HTLV-I) antibodies in Africa. *Science* 1984;**225**:1473-6
- Holmgren B, da Silva Z, Larsen O, Vastrup P, Andersson S, Aaby P. Dual infections with HIV-1, HIV-2 and HTLV-I are more common in older women than in men in Guinea-Bissau. *Aids* 2003;**17**:241-53
- van der Ryst E, Joubert G, Smith MS, Terblanche M, Mollentze F, Pretorius AM. HTLV-I infection in the Free State region of South Africa: a sero-epidemiologic study. *Cent Afr J Med* 1996;**42**:65-8
- Barcellos NT, Fuchs SC, Mondini LG, Murphy EL. Human T lymphotropic virus type I/II infection: prevalence and risk factors in individuals testing for HIV in counseling centers from Southern Brazil. *Sex Transm Dis* 2006;**33**:302-6
- Broutet N, de Queiroz Sousa A, Basilio FP, Sa HL, Simon F, Dabis F. Prevalence of HIV-1, HIV-2 and HTLV antibody, in Fortaleza, Ceara, Brazil, 1993-1994. *Int J STD AIDS* 1996;**7**:365-9
- Carneiro-Proietti AB, Ribas JG, Catalan-Soares BC, et al. Infection and disease caused by the human T-cell lymphotropic viruses type I and II in Brazil. *Rev Soc Bras Med Trop* 2002;**35**:499-508
- Gotuzzo E, Escamilla J, Phillips IA, Sanchez J, Wignall FS, Antogni J. The impact of human T-lymphotropic virus type I/II infection on the prognosis of sexually acquired cases of acquired immunodeficiency syndrome. *Arch Intern Med* 1992;**152**:1429-32
- Morimoto HK, Caterino-De-Araujo A, Morimoto AA, et al. Seroprevalence and risk factors for human T-cell lymphotropic virus type 1 and 2 infection in human immunodeficiency virus-infected patients attending AIDS referral center health units in Londrina and other communities in Parana, Brazil. *AIDS Res Hum Retroviruses* 2005;**21**:256-62
- Scapellato PG, Bottaro EG, Seoane MB, et al. Clinical, epidemiological and immunological study of patients coinfecting with HIV and HTLV-1. *Medicina (B Aires)* 2004;**64**:413-8
- Schechter M, Harrison LH, Halsey NA, et al. Coinfection with human T-cell lymphotropic virus type I and HIV in Brazil. Impact on markers of HIV disease progression. *JAMA* 1994;**271**:353-7
- Fantry L, De Jonge E, Auwaerter PG, Lederman HM. Immunodeficiency and elevated CD4 T lymphocyte counts in two patients coinfecting with human immunodeficiency virus and human lymphotropic virus type I. *Clin Infect Dis* 1995;**21**:1466-8
- Schechter M, Moulton LH, Harrison LH. HIV viral load and CD4+ lymphocyte counts in subjects coinfecting with HTLV-I and HIV-1. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;**15**:308-11
- Hammer SM, Saag MS, Schechter M, et al. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA panel. *JAMA* 2006;**296**:827-43
- Planoulaine S, Buigues RP, Murphy EL, et al. Demographic and familial characteristics of HTLV-1 infection among an isolated, highly endemic population of African origin in French Guiana. *Int J Cancer* 1998;**76**:331-6
- Ampofo W, Nii-Trebi N, Ansah J, et al. Prevalence of blood-borne infectious diseases in blood donors in Ghana. *J Clin Microbiol* 2002;**40**:3523-5
- Analo HI, Akanmu AS, Akinsete I, Njoku OS, Okany CC. Seroprevalence study of HTLV-1 and HIV infection in blood donors and patients with lymphoid malignancies in Lagos, Nigeria. *Cent Afr J Med* 1998;**44**:130-4
- Beilke MA, Theall KP, O'Brien M, et al. Clinical outcomes and disease progression among patients coinfecting with HIV and human T lymphotropic virus types 1 and 2. *Clin Infect Dis* 2004;**39**:256-63
- Gouws E, White PJ, Stover J, Brown T. Short term estimates of adult HIV incidence by mode of transmission: Kenya and Thailand as examples. *Sex Transm Infect* 2006;**82**(Suppl. 3):iii51-5
- Hunter DJ. AIDS in sub-Saharan Africa: the epidemiology of heterosexual transmission and the prospects for prevention. *Epidemiology* 1993;**4**:63-72
- Guerreiro JB, Porto MA, Santos SB, Lacerda L, Ho JL, Carvalho EM. Spontaneous neutrophil activation in HTLV-1 infected patients. *Braz J Infect Dis* 2005;**9**:510-4
- Harrison LH, Schechter M. Coinfection with HTLV-I and HIV: increase in HTLV-I-related outcomes but not accelerated HIV disease progression? *AIDS Patient Care STDS* 1998;**12**:619-23

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