Evaluation of the World Health Organization staging system for HIV infection and disease in Ethiopia: association between clinical stages and laboratory markers

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Objective: To study the association between the clinical axis of the World Health Organization (WHO) staging system of HIV infection and disease and laboratory markers in HIV-infected Ethiopians.

Design: Cross-sectional study.

Methods: Clinical manifestations and stage of HIV-positive individuals participating in a cohort study of HIV infection progression, and of HIV-positive patients hospitalized with suspicion of AIDS, were compared to CD4+ T-cell count and viral load.

Results: Of the 86 HIV-positive participants of the cohort study, 53 (62%), 16 (19%), 16 (19%), and one (1.2%) were in stage 1, 2, 3 and 4, respectively. Minor weight loss (n = 15) and pulmonary tuberculosis (n = 9) were the most commonly diagnosed conditions among the 38 (44%) symptomatic HIV-positive individuals. Although 23 (27%) HIV-positive participants had CD4+ T-cell counts less than 200×10^6/l, only one was in clinical stage 4. Among 79 hospitalized HIV-positive patients, 15 (19%) and 64 (81%) were in stage 3 and 4, respectively. The majority (83.5%) had CD4+ T-cell counts $< 200 \times 10^6$/l. Individuals at stage 3 had lower CD4+ T-cell counts and higher viral loads when seen in hospital as compared to cohort participants ($P = 0.06$ and 0.008, respectively). When grouping the two study populations, the median CD4+ T-cell count decreased (337, 262, 225, 126, and 78×10^6/l, $P < 0.01$), and the median viral load increased (4.08, 3.89, 4.47, 5.65, and 5.65 $\log_{10}$ copies/ml, $P < 0.01$), with increasing clinical stage of HIV infection (1, 2, 3 cohort, 3 hospital, and 4, respectively). Median CD4+ T-cell counts were remarkably low in HIV-negative participants (749×10^6/l), and in HIV-positive participants at stage 1 and 2 (337 and 262×10^6/l, respectively).

Conclusions: There was a good correlation between WHO clinical stages and biological markers. CD4+ T-cell counts were low in Ethiopians, particularly during early stages of HIV-1 infection, and preliminary reference values at different stages of HIV-1 infection were determined. In HIV-infected Ethiopians, lymphocyte counts less than $1{,}000 \times 10^6$/l in non-hospitalized individuals, and less than $2{,}000 \times 10^6$/l in

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hospitalized patients, had high positive predictive value, but low sensitivity, in identifying subjects with low CD4+ T-cell counts ($< 200 \times 10^6$/l) who would benefit from chemoprophylaxis of opportunistic infections. The on-going longitudinal study will be useful to confirm the prognostic value of the WHO staging system.

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Key words: HIV infection, WHO staging, CD4+ T-cell counts, viral load, Ethiopia, Africa

Introduction

Infection with HIV ultimately results in profound immunodeficiency in which patients may present with various AIDS-defining clinical conditions [1]. However, time to AIDS varies considerably between individuals [2], and identification of clinical and biological markers of progression to AIDS is desirable for the clinical management and proper counseling of HIV-infected patients. In 1990, an interim proposal for a World Health Organization (WHO) staging system for HIV infection and disease was published [3], and revised subsequently [4]. This staging system is based on a combination of clinical and biological parameters, and is meant to be used worldwide. It includes a clinical axis made of 32 conditions (revised version) divided in four stages (stage 4 being equivalent to clinical AIDS), and a laboratory axis with three categories of CD4+ T-cell counts (replaced by lymphocyte counts when CD4+ T-cell counts are not available). Several studies were carried out to validate this staging system. In cross-sectional studies, CD4+ T-cell counts were used as a surrogate marker for survival and found to correlate well with the four clinical stages [4–6]. In longitudinal studies, the best approach for validation purpose, survival was compared between the four stages, and found to decrease with increasing stages [7–8].

However, validation studies need to be repeated, since incubation period of HIV infection and its predictors may depend on many factors including, among others, HIV transmission route [9], HIV variants [10], host immune system [11], nutritional status [12], host genotype [13–14], environmental pathogens [15], and access to healthcare [8]. In this paper, we present a validation study done in Ethiopia where the epidemic is predominantly heterosexual [16–17], the circulating subtype is C [18], and where host immune system, nutritional status, environmental pathogens and access to healthcare may be comparable to those of other developing countries in Africa. We also report on the correlation between the clinical stages and viral load, a more recently described risk factor for HIV infection progression [19].

Materials and Methods

Study subjects

Study subjects originated from two different population groups: individuals at all stages of HIV-1 infection, including early stages, were recruited among factory workers participating in an on-going cohort study on HIV-1 infection progression; and individuals at advanced stages of HIV-1 infection were recruited among hospitalized patients who were suspected clinically of having AIDS.

The cohort study on HIV-1 infection progression was established in a factory in the suburbs of Addis Ababa, the capital city of Ethiopia, in early 1997. All factory workers aged 18–44 years were invited to participate in the cohort study and those willing to participate underwent the following procedures: pre-test counseling and obtaining of informed consent for HIV testing and study participation; interview on socio-demographic characteristics, medical history, and sexual behavior; clinical examination, including systematic searching for any clinical condition included in the revised WHO clinical staging of HIV infection and disease (see Appendix); and blood drawing in EDTA vacutainer tubes (Becton and Dickinson, San Jose, California, USA) for serological analysis of HIV-1 and syphilis antibodies. All participants enrolled between February 1997 and April 1998 were included in this study.

Patients with advanced stages of HIV-1 infection were recruited from a nearby central referral hospital of Addis Ababa, Saint Paul hospital, during delivery of diagnostic services by our laboratory to this hospital. Blood samples from patients with clinical suspicion of AIDS or advanced HIV infection were sent to the Ethiopian–Netherlands AIDS Research Project (ENARP) laboratory at the Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa, for HIV-1 antibody testing and CD4+ T-cell count assessment. The referral form accompanying the blood specimens was filled in by the physician in charge, who was asked to mention any condition of the revised WHO clinical staging of HIV-1 infection [4]. All patients were offered pre- and post-test counseling whilst undergoing HIV testing. All consecutive HIV-

positive patients from Saint Paul hospital diagnosed between November 1996 and April 1997 were enrolled in this study.

For all study participants, physicians were not aware of the HIV serological status of the individuals at the time they were filling in the clinical forms. Clinicians working at the cohort site and at the hospital used the same guidelines for diagnosis of each stage-specific HIV-related condition. These guidelines were developed by two of the investigators (E.K. & A.L.F.), taking into account the diagnostic facilities available both at the cohort site and at the hospital. Many diagnoses were presumptive, particularly for stage 4-defining conditions, since laboratory facilities at the study clinic and at the hospital were limited. Weight loss was estimated based on the participants' recollection of their own weight, since the study was cross-sectional. HIV wasting syndrome was not considered in patients with pulmonary or extra-pulmonary tuberculosis.

Laboratory methods

Screening for plasma antibodies against HIV-1 was performed by an Enzyme Linked Immunosorbent Assay (ELISA) using Organon Vironostika Kits (Organon Teknika BV, Boxtel, the Netherlands) in all cohort study participants, plus Western blot (Genelabs Diagnostics, Leuven, Belgium) confirmation for those with positive ELISA, and by HIVSPOT (Genelabs Diagnostics) plus ELISA for hospital patients. Total lymphocyte counts were determined by Coulter counter (Coulter Electronics Ltd, Miami, Florida, USA), and lymphocyte subsets by flow cytometry using a FACScan (Becton Dickinson, San Jose, California, USA) [20]. Viral load was determined by quantifying the amount of HIV RNA in plasma samples using Nucleic Acid Sequence Based Amplification (NASBA)/NUCLISENS kits (M.G. and S.J., Organon Teknika BV, Boxtel, The Netherlands). NASBA for hospitalized patients was performed at the Human Retrovirology Laboratory at the Academic Medical Center, Amsterdam, The Netherlands, and NUCLISENS for cohort study participants was performed at the ENARP laboratory. Lower detection limit (LDL) for NASBA was 3.0 $\log_{10}$ copies/ml and for NUCLISENS 2.6 $\log_{10}$ copies/ml. Total lymphocyte, CD4+ T-cell counts, and CD8+ T-cell counts were determined in all HIV-positive and a random selection (20%) of HIV-negative participants, and viral load in 50% of all HIV-positive study participants (randomly selected within each clinical stage, with at least ten samples from each stage).

Statistical analysis

Data were analysed using Stata statistical package (Stata Statistical Software, Stata Corporation, College Station, Texas, USA). Proportions were compared between groups using Chi-square or Fischer's exact test where appropriate ($P < 0.05$ was considered statistically significant). Distributions of continuous variables were compared between groups using a non-parametric method (Mann–Whitney test). Tests for trend were done using the Cuzick non-parametric test for trends across ordered groups. Correlation between CD4+ T-cell count and viral load was analyzed by the Spearman's rank correlation test.

Results

Between February 1997 and April 1998, 750 individuals were enrolled in the cohort study of HIV infection progression, representing 70.8% of the eligible study population (n = 1,060). The following were excluded from further analysis: 13 HIV-negative individuals with no clinical data; two HIV-positive individuals with no CD4+ T-cell counts; and one study participant with discordant HIV serological assay results and indeterminate Western blot. Data are presented on 734 cohort participants, of which 398 (54%) were males (mean age: 37 years), and 336 (46%) were females (mean age: 34 years). HIV prevalence was 46/398 (11.6%) and 40/336 (11.9%) for males and females respectively ($P > 0.05$), and was higher in younger participants (16.5% for age < 35 years, and 8.7% for age ≥ 35 years, $P = 0.001$). Table 1 displays the clinical manifestations and CD4+ T-cell counts of the HIV-positive study subjects. The majority of them (48/86 = 55%) were asymptomatic, whereas a total of 50 clinical conditions were recorded in the remaining 38 subjects. Using the clinical axis of the WHO staging system of HIV infection and disease, we categorized 53 (61.6%) HIV-positive participants in stage 1, 16 (18.6%) in stage 2, 16 (18.6%) in stage 3, and one (1.2%) in stage 4. This distribution differed significantly ($P = 0.001$) from that of HIV-negative participants (78.6%, 13.3%, 8.0%, and 0.2% for stages 1, 2, 3, and 4 respectively). Without using the performance scale (see appendix), one HIV-positive participant would have been categorized at stage 2 instead of stage 3. The most common manifestation listed in the WHO staging system observed in HIV-positive participants was minor weight loss (n = 15), followed by pulmonary tuberculosis (n = 9). Although 23/86 (27%) HIV-positive participants had CD4+ T-cell counts $< 200 \times 10^6$/l, only one was in clinical stage 4 (chronic herpes simplex infection). Interestingly, CD4+ T-cell counts were remarkably low in HIV-negative participants (median = 758×10^6/l respectively), and in HIV-positive participants at stage 1 and 2 (median = 337 and 262×10^6/l respectively). Also, the CD4+ T-cell counts were lower in males when compared to females (median = 713 versus 806×10^6/l respectively in HIV-negatives, $P = 0.07$, and 253 versus 354×10^6/l respectively in HIV-positives, $P = 0.01$). Median hemoglobin level was lower in HIV-positive individuals when

Table 1. Clinical manifestations and corresponding median CD4+ T-cell counts in 86 HIV-positive factory workers participating in a cohort study on HIV infection progression in Ethiopia, 1997–1998.

Clinical condition	n	median CD4+ T-cell count* $\times 10^6$/l (range)
Asymptomatic	48	324 (89–966)
Minor weight loss	15	270 (79–504)
Pulmonary tuberculosis	9	140 (45–436)
Persistant generalized lymphadenopathy	8	375 (286–798)
Oral candidiasis	6	268 (94–436)
Major weight loss	5	317 (151–490)
Herpes Zoster	4	144 (92–270)
Minor mucocutaneous lesions	2	140 & 317
Herpes simplex infection > 1 month	1	198

*Some patients had more than one condition. For any condition, the median CD4+ T-cell count was calculated based on individuals who had no other condition at a higher stage.

compared to HIV-negatives (14.3 g/dl versus 15.3 g/dl respectively, $P < 0.0001$), and was higher in males when compared to females (16.1 g/dl versus 14.3 g/dl respectively in HIV-negatives, $P < 0.0001$, and 15 g/dl versus 13.2 g/dl respectively in HIV-positives, $P < 0.0001$).

Between November 1996 and April 1997, 170 blood samples were sent to our laboratory from Saint Paul hospital for HIV testing and CD4+ T-cell count analysis. Of these, 81 tested positive for HIV-1 antibodies. Clinical data were complete for 79 (98%) HIV-positive patients. Forty-two (53%) were males (mean age = 36 years), and 37 (47%) were females (mean age = 33 years). Table 2 displays their clinical manifestations and CD4+ T-cell counts. A total of 228 clinical conditions were recorded in 79 subjects, including 20 of the 32 (62%) clinical conditions listed in the WHO staging system. Fifteen (19%) patients were in stage 3, and 64 (81%) were in stage 4. Without the performance scale, 23 in stage 4 would have been categorized at stage 3. The most common manifestation of the WHO clinical staging observed was oral candidiasis (n = 40), followed by pulmonary tuberculosis (n = 34) and HIV wasting syndrome (n = 27). The vast majority of patients (66/79 = 84%) had CD4+ T-cell counts $< 200 \times 10^6$/l, and 28/79 (35%) had CD4+ T-cell counts $< 50 \times 10^6$/l with a minimum of 4×10^6/l. The median CD4+ T-cell count was lower in this group of hospitalized patients (83×10^6/l) when compared to HIV-positive participants (310×10^6/l) of the cohort study ($P < 0.0001$).

Table 2. Clinical manifestations and CD4+ T-cell counts in 79 HIV-positive patients hospitalized with suspicion of AIDS in Ethiopia, 1996–1997.

Clinical condition	n	median CD4+ T-cell count* $\times 10^6$/l (range)
Oral candidiasis	40	126 (5–352)
Pulmonary tuberculosis	34	174 (12–332)
HIV wasting syndrome	27	48 (4–726)
Minor weight loss	24	NA
Recurrent upper respiratory tract infections	16	NA
Prolonged fever	16	150 (5–352)
Minor monocutaneous lesions	14	NA
Severe/recurrent bacterial infections	10	NA
Herpes zoster	9	NA
Esophageal candidiasis	8	70 (7–137)
Extra-pulmonary tuberculosis	6	143 (7–232)
HIV encephalopathy	6	36 (7–88)
Brain toxoplasmosis	5	88 (25–402)
Persistent generalized lymphadenopathy	4	NA
Oral hairy leukoplakia	2	186
Vaginal candidiasis	2	NA
Herpes simplex infection > 1 month	2	26 & 60
Major weight loss	1	NA
Chronic diarrhea	1	NA
Pneumocystis pneumonia	1	235

*Some patients had more than one condition. For any condition, the median CD4+ T-cell count was calculated based on individuals who had no other condition at a higher stage. NA, Not available – denoting a condition that was always associated with another condition of higher stage.

Figure 1a and Table 3 show the distribution of CD4+ T-cell counts and CD4/CD8 ratios among HIV-negative and HIV-positive study subjects categorized by WHO clinical stage. The median CD4+ T-cell count was higher for HIV-negative when compared to HIV-positive individuals (758 versus 191×10^6/l, $P < 0.0001$), and decreased with higher clinical stages among HIV-positive study subjects (test for trend, $P < 0.01$). We separated patients at stage 3 depending on whether they were seen in hospital or at the cohort site, since median CD4+ T-cell counts were lower in the former (126 versus 225, $P = 0.06$). CD8+ T-cell

Table 3. CD4+ T-cell count distribution, and median CD4:CD8 ratio in HIV-negative and HIV-positive individuals at various stages of the WHO staging system of HIV infection and disease in Ethiopia, 1996–1998.

Clinical stage (S)	n	median CD4+ T-cell count $\times 10^6$/l (range)	CD4 count (%n)				CD4:CD8 ratio
			< 50	50–199	200–499	≥ 500	
HIV negative	157	758 (298–1789)	0	0	12	88	1.17
HIV positive S-1	53	337 (89–966)	0	17	60	23	0.34
HIV positive S-2	16	262 (79–530)	0	37	37	25	0.25
HIV positive S-3							
Cohort	16	225 (45–490)	6	38	56	0	0.27
Hospital	15	126 (5–352)	12	69	19	0	0.24
HIV positive S-4	65	78 (4–726)*	40	45	12	3	0.14*

*$P < 0.01$; test for trend comparing values in HIV-positive individuals at different WHO clinical stages.

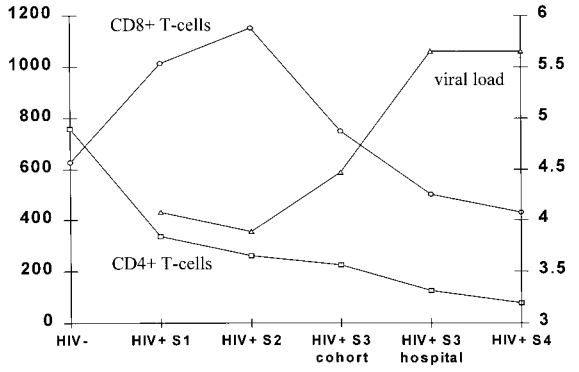

Fig. 1. Median CD4+ T-cell count (□, $*10^6$/l on the left axis, n = 157 for HIV- and n = 165 for HIV+), CD8+ T-cell count (o, $*10^6$/l on the left axis, n = 157 for HIV- and n = 165 for HIV+), and viral load (Δ, $*\log_{10}$ copies/ml on the right axis, n = 90), by clinical stage (HIV- and HIV+ S1 to S4) of the WHO staging system of HIV infection and disease.

counts increased with early HIV infection (stages 1 and 2), and then decreased towards progression to AIDS (Fig. 1). The CD4/CD8 ratio was 1.2 in HIV-negative participants, and decreased with higher clinical stages among HIV-positives (test for trend, $P < 0.01$).

Decreasing lymphocyte counts were also associated with increasing clinical stages in HIV-positives (test for trend, $P < 0.01$). However, the three lymphocyte count categories (> 2000, 1000–2000, $< 1000 \times 10^6$/l) proposed to replace the three CD4+ T-cell count categories (> 500, 200–500, $< 200 \times 10^6$/l) in countries with limited laboratory facilities [4] did not operate well in HIV-positives, except for the category with the highest counts. The sensitivity and specificity of each lymphocyte count category in use to classify individuals according to the WHO laboratory axis based on CD4+ T-cell count categories (gold standard) were respectively: 15/18 (83%, 95% CI = 59%–96%) and 118/147 (80%, 95% CI = 73%–86%) for the highest category (lymphocyte counts $> 2000 \times 10^6$/l and CD4+ T-cell counts $> 500 \times 10^6$/l); 37/58 (64%, 95% CI = 50%–76%) and 71/107 (66%, 95% CI = 57%–75%) for the intermediate category (lymphocyte counts $1000–2000 \times 10^6$/l and CD4+ T-cell counts $200–500 \times 10^6$/l); and 46/89 (52%, 95% CI = 41%–62%) and 74/76 (97%, 95% CI = 91%–100%) for the lowest category (lymphocyte counts $< 1000 \times 10^6$/l and CD4+ T-cell counts $< 200 \times 10^6$/l).

Individuals with CD4+ T-cell counts $< 200 \times 10^6$/l may rapidly progress to AIDS, and/or benefit from chemoprophylaxis for opportunistic infections. However, CD4+ T-cell counts are not available in most developing country laboratories, and predictors of low CD4+ T-cell counts should be identified. In Table 4, we show the positive predictive value of a combination of lymphocyte counts and clinical stage for having low CD4+ T-cell counts ($< 200 \times 10^6$/l). At stage 1, 9/53 (17%) of the HIV-positive study participants had CD4+ T-cells $< 200 \times 10^6$/l. However, those with lymphocyte count $< 2{,}000 \times 10^6$/l had a higher probability of low CD4+ T-cell counts (23%, 38%, and 67% for lymphocyte counts < 2,000, < 1,500, and $< 1{,}000 \times 10^6$/l respectively). The same applies for HIV-infected participants at stage 2, for whom the overall probability of low CD4+ T-cell counts was 38%, but was higher than 70% for any lymphocyte counts $< 2{,}000 \times 10^6$/l. At stage 3 and 4, the probability of low CD4+ T-cell counts was high in hospitalized patients (81% and 84% respectively), and only lymphocyte counts $< 1{,}000 \times 10^6$/l modified substantially this figure by increasing it to 100% and 97% respectively. However, sensitivity of lymphocyte counts $< 1{,}000 \times 10^6$/l in detecting low CD4+ T-cell counts was low (around 50% for most clinical stages).

Viral load was measured in 90 HIV-positive individuals. Fig. 1b displays the viral load results according to the WHO clinical stages. Viral load increased with WHO clinical stages: median (range) was 4.08 (< LDL –5.45) $\log_{10}$ copies/ml at stage 1 (n = 12); 3.89 (< LDL –5.46) at stage 2 (n = 16); 4.47 (< LDL –5.73) at stage 3 for cohort participants (n = 14), 5.65 (4.26 –6) at stage 3 for hospitalized patients (n = 8); and 5.65 (3–6.72) at stage 4 (n = 40), ($P < 0.01$). As for CD4+ T-cell counts, viral load was significantly different between patients at stage 3, when seen at the cohort site and in hospital (P = 0.008). Viral load was negatively correlated with CD4+ T-cell counts (Spearman correlation coefficient, r = –0.55).

Discussion

Clinical stages correlated well with CD4+ T-cell counts in Ethiopian HIV-infected individuals, as has been reported in other studies elsewhere [4–6]. Using CD4+ T-cell counts as a proxy for survival, we may infer that the clinical axis of the WHO staging system will predict survival in HIV-positive Ethiopians. These findings were obtained despite the limited facilities available for the diagnosis of HIV-related conditions at both study sites, suggesting that the clinical axis of the WHO staging system remains useful in developing countries. Also, some simple clinical information, such as weight loss, should be viewed cautiously when based on participant's own recollection of their past weight. It is likely that estimation of weight loss based on successive measurements on the same weighing machine would be more valid. We found it helpful to separate ambulatory (cohort site) and hospitalized stage 3 HIV-infected subjects, since laboratory markers indicated more advanced disease in the latter category. This simple distinction (ambulatory versus hospitalized) may be

Table 4. Positive predictive value (PPV in %) and sensitivity (%) of different lymphocyte counts in identifying HIV-positive individuals with CD4+ T-cell counts < 200×10^6/l by HIV clinical stage (n = 165).

	Lymphocyte count $\times 10^6$/l					
	< 1000		< 1500		< 2000	
	PPV	Sensitivity	PPV	sensitivity	PPV	sensitivity
Stage 1	2/3 (67)	2/9 (22)	5/13 (38)	5/9 (56)	8/35 (23)	8/9 (89)
Stage 2	3/3 (100)	3/6 (50)	5/7 (71)	5/6 (83)	5/7 (71)	5/6 (83)
Stage 3						
Cohort	5/5 (100)	5/7 (71)	5/9 (56)	5/7 (71)	6/13 (46)	6/7 (86)
Hospital	7/7 (100)	7/13 (54)	13/14 (93)	13/13 (100)	13/14 (93)	13/13 (100)
Stage 4*	29/30 (97)	29/54 (54)	38/39 (97)	38/54 (70)	47/52 (90)	47/54 (87)

*Stage 4 study participants used in this analysis were all hospitalized patients. The only study participant at stage 4 from the cohort study was not included.

a useful addition to the existing WHO staging system. A prospective study is continuing among the factory workers, in order to confirm these early findings by estimating the time to death associated with each clinical stage and laboratory marker.

One striking result of this study was the low CD4+ T-cell counts observed in HIV-positive Ethiopian individuals, particularly in people at work: 23/86 (27%) of the HIV-positive workers had CD4+ T-cell counts less than 200×10^6/l, a count that defines having AIDS in the 1993 revised CDC classification [21]. We do not believe that our sample of factory workers was biased towards sick individuals, since recruitment was based on systematic invitation of all workers, participation in the study was high (70.8%), and all participants were healthy enough to perform their activities at the factory. Also, the majority (80%) of HIV-positive study participants were in early clinical stages of HIV infection (stage 1 or 2). Misclassification of participants already at stage 3 into stage 1 or 2 because of lack of diagnostic facilities is unlikely, since most diagnoses of the first three stages of the WHO system rely on simple clinical investigations. Rather, we believe that CD4+ T-cell counts are low in Ethiopian HIV-positive individuals, as shown by the median CD4+ T-cell counts at stage 1 and 2 (317 and 252×10^6/l respectively). By comparison, the median CD4+ T-cell counts at stage 1 and 2 were > 500 and > 400×10^6/l respectively in the WHO international study of the WHO staging system [4]. Moreover, CD4+ T-cell counts were also low in HIV-negative Ethiopians (mean = 786 compared to 949×10^6/l in the WHO international study of the WHO staging system), a finding already reported elsewhere [22,23]. In a separate paper, we have looked at this issue in more detail and found that the low CD4+ T-cell values are related to both low absolute lymphocyte counts and low relative CD4+ T-cell subsets [24]. Reference values for Ethiopians at different stages of HIV-1 infection were therefore determined in this study and are available in Table 3. These preliminary values need to be confirmed by validation on a larger number of individuals. Whether low CD4+ T-cell counts in HIV-negatives and in HIV-positives at early stages of HIV infection result in more rapid progression to AIDS will be determined as part of our on-going cohort study.

This large proportion of HIV-positive individuals with low CD4+ T-cell counts (< 200×10^6/l) and few clinical manifestations would benefit from chemoprophylaxis of opportunistic infections. Examples of diseases which may be prevented by chemoprophylaxis in HIV-positive individuals are pulmonary tuberculosis and *Toxoplasma* encephalitis [25–27], both highly prevalent in the study population: pulmonary tuberculosis was the most common opportunistic infection documented among the factory workers during this cross-sectional survey, and an earlier study had documented a prevalence of 80% of anti-*Toxoplasma* antibodies among the same factory workers [28]. Implementation of tuberculosis chemoprophylaxis has certain requirements (sputum examination and chest X-ray to rule out active pulmonary tuberculosis, close supervision and follow-up) which are available in the context of this cohort study. In HIV-positive subjects with low CD4+ T-cell counts, *Toxoplasma* encephalitis, as well as *Pneumocystis carinii* pneumonia, *I. belli* enteritis, and common bacterial infections may be prevented by cotrimoxazole [27], a drug that is economical enough to be administered in Ethiopia. However, there may be concerns regarding the safety of cotrimoxazole [29], which will need to be investigated in Ethiopian populations.

Among hospitalized patients, oral candidiasis, pulmonary tuberculosis, and HIV wasting syndrome were the three most commonly diagnosed manifestations. The absence of elaborate laboratory diagnostic facilities may have resulted in the high proportion of patients diagnosed with HIV wasting syndrome (27/79 = 34.2%), when compared to a similar study performed among hospitalized patients in Abidjan in which HIV wasting syndromes were only 16% of all diagnoses [30]. Median CD4+ T-cell counts were similar in patients with AIDS in our study (78×10^6/l), in patients hospi-

talized with HIV infection in Abidjan (84×10^6/l) [30], in African AIDS patients in London (47×10^6/l) [31], and in AIDS patients included in the WHO international study of the WHO staging system (between 50 and 100×10^6/l) [4]. Thus, opportunistic infections most likely occur at the same level of CD4+ T-cell counts throughout the world, regardless of the status of other immune functions, of the virulence of pathogens, or of the provision of chemoprophylactic or antiretroviral regimens. However, comparisons between hospitalized patients worldwide may be biased by differences in referral patterns, admission criteria, and patients management, and should therefore be made with caution.

Lymphocyte count categories could not be substituted for CD4+ T-cell count categories in the laboratory axis of the WHO staging, except for the highest category where sensitivity and specificity of high lymphocyte counts ($> 2000 \times 10^6$/l) for use in diagnosing high CD4+ T-cell counts ($> 500 \times 10^6$/l) were above 80%. For other categories, risk of misclassification would be high: in the intermediate category (lymphocyte counts between 1000 and 2000×10^6/l replacing CD4+ T-cell counts between 200 and 500×10^6/l), both sensitivity and specificity were low (64% and 66% respectively), and 36% of observations would have been misclassified. In the lowest category, low lymphocyte counts ($< 1000 \times 10^6$/l) were highly specific (97%) for low CD4+ T-cell counts ($< 200 \times 10^6$/l), but had low sensitivity (52%).

Predictors of CD4+ T-cell counts $< 200 \times 10^6$/l would be useful to identify individuals whose risk of progression to AIDS is high, and who would benefit from measures such as chemoprophylaxis of opportunistic infections. As can be seen from Table 4, cohort participants with lymphocytes counts $< 1000 \times 10^6$/l, or hospitalized patients with lymphocytes counts $< 2000 \times 10^6$/l, had a high probability of having CD4+ T-cell counts $< 200 \times 10^6$/l (> 90%, except for stage 1 where only three individuals could be investigated). However, the sensitivity of lymphocytes counts $< 1000 \times 10^6$/l for use in detecting CD4+ T-cell counts $< 200 \times 10^6$/l was low, and about half of HIV-infected subjects with low CD4+ T-cell counts would have been missed using this criteria.

Since the WHO staging system was proposed and evaluated in many countries, viral load has been established as a new prognostic marker of progression to AIDS [19, 32]. Few studies have attempted to confirm these findings among HIV-infected individuals living in developing countries, where HIV-1 subtypes other than B circulate. In this study, in individuals most likely infected with HIV-1 subtype C [18], viral load increased with clinical stage, and was negatively correlated with CD4+ T-cell counts. A higher viral load in hospitalized patients compared to cohort participants may be consistent with the higher prevalence of opportunistic infections observed in the former. Viral loads, although similar at early stage to loads observed in HIV-infected homosexual men in Amsterdam [33], were higher at AIDS stage in Ethiopians (5.65 versus < 5 $\log_{10}$ copies/ml). This difference might be partially explained by the absence of anti-retroviral drug treatment in Ethiopians, whereas 55% of the Dutch patients used zidovudine at some point of their disease [33]. Viral load determination is expensive, and still restricted to sophisticated laboratories, limiting its use in developing countries. The inclusion of this important laboratory marker in the WHO staging system of HIV infection and disease will depend on the development of simpler and cheaper techniques.

In conclusion, this study shows that the clinical stages of the WHO staging system of HIV infection and disease correlated well with laboratory markers of HIV infection progression. CD4+ T-cell counts were low in Ethiopians, particularly during early stages of HIV-1 infection, and preliminary reference values at different stages of HIV-1 infection were determined. In HIV-infected Ethiopians, lymphocyte counts of less than $1{,}000 \times 10^6$/l in non-hospitalized individuals, and less than $2{,}000 \times 10^6$/l in hospitalized patients, had high positive predictive value, but low sensitivity, in identifying subjects with low CD4+ T-cell counts ($< 200 \times 10^6$/l) who would benefit from chemoprophylaxis against opportunistic infections. The on-going longitudinal study will be useful for confirming these progression markers, that can be used by medical personnel and counselors taking care of Ethiopian HIV-infected individuals.

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Appendix

List of clinical conditions by clinical stage

Clinical stage 1

1. Asymptomatic infection
2. Persistent generalized lymphadenopathy
3. Acute retroviral infection

Performance scale 1: asymptomatic, normal activity.

Clinical stage 2

4. Unintentional weight loss, < 10% of body weight
5. Minor mucocutaneous manifestations (e.g. seborrheic dermatitis, prurigo, fungal nail infections, oropharyngeal ulcerations, angular cheilitis)
6. Herpes zoster, within the previous 5 years
7. Recurrent upper respiratory tract infections (e.g. bacterial sinusitis)

and/or performance scale 2: symptoms, but nearly fully ambulatory.

Clinical stage 3

8. Unintentional weight loss, > 10% of body weight
9. Chronic diarrhoea, > 1 month
10. Prolonged fever (intermittent or constant) > 1 month

11. Oral candidiasis (erythematous or pseudomembranous)
12. Oral hairy leukoplakia
13. Pulmonary tuberculosis (typical or atypical), within the previous year
14. Severe bacterial infections (e.g. pneumonia, pyomyositis)
15. Vulvovaginal candidiasis, chronic (> 1 month) or poorly responsive to therapy

and/or performance scale 3: in bed < 50% of normal daytime, but > normal, during previous month.

Clinical stage 4

16. HIV wasting syndrome
17. *Pneumocystis carinii* pneumonia
18. Toxoplasmosis of the brain
19. Cryptosporidiosis with diarrhoea, > 1 month
20. Isosporiasis with diarrhoea, > 1 month
21. Cryptococcosis, extrapulmonary
22. Cytomegalovirus disease of an organ other than liver, spleen or lymph node
23. Herpes simplex virus infection, mucocutaneous (> 1 month) or visceral (any duration)
24. Progressive multifocal leukoencephalopathy
25. Any disseminated endemic mycosis (e.g. histoplasmosis, coccidioidomycosis)
26. Candidiasis of the oesophagus, trachea, bronchi or lungs
27. Atypical mycobacteriosis, disseminated
28. Non-typhoid *Salmonella* septicaemia
29. Extrapulmonary tuberculosis
30. Lymphoma
31. Kaposi's sarcoma
32. HIV encephalopathy

and/or performance scale 4: in bed > 50% of normal daytime during previous month.