CD4 Counts and Viral Loads of Newly Diagnosed HIV-Infected Individuals: Implications for Treatment as Prevention

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Abstract

Objective: To report the viral load and CD4 count in HIV-infected, antiretroviral naive, first-time HIV-testers, not immediately eligible for treatment initiation by current South Africa treatment guidelines.

Design: This was a cross-sectional study in a high-volume, free-of-charge HIV testing centre in Soweto, South Africa.

Methods: We enrolled first time HIV testers and collected demographic and risk-behaviour data and measured CD4 count and viral load.

Results: Between March and October 2011, a total of 4,793 adults attended VCT and 1,062 (22%) tested positive. Of the 1,062, 799 (75%) were ART naive and 348/799 (44%) were first-time HIV testers. Of this group of 348, 225 (65%) were female. Overall their median age, CD4 count and viral load was 34 years (IQR: 28-41), 364 (IQR: 238-542) cells/mm3 and 13,000 (IQR: 2050-98171) copies/ml, respectively. Female first time HIV testers had higher CD4 counts (419 IQR: 262-582 vs. 303 IQR: 199-418 cells/mm3) and lower viral loads (9,100 vs. 34,000 copies/ml) compared to males. Of 183 participants with CD4 count >350 cells/mm3, 62 (34%) had viral loads >10,000 copies/ml.

Conclusions: A large proportion of HIV infected adults not qualifying for immediate ART at the CD4 count threshold of 350 cells/mm3 have high viral loads. HIV-infected men at their first HIV diagnosis are more likely to have lower CD4 counts and higher viral loads than women.

Introduction

Anti-retroviral therapy (ART) initiation has been shown to dramatically reduce HIV transmission in discordant heterosexual couples prompting revisions to treatment eligibility criteria. [1–3] Responding to this, new guidelines recommend starting ART either at HIV diagnosis, or at CD4 counts of ≤500 cells/mm3. [4,5] Current South African (SA) treatment guidelines for ART include recommendations for treatment initiation at a CD4 threshold of ≤ 350 cells/mm3 in non-pregnant, well adolescents and adults.[4,5].

ART initiation is traditionally based on CD4 counts. In conjunction with viral loads, they allow prognostication. [6] Moreover, viral loads provide a measure of infectivity; individuals with low or suppressed viral loads have markedly lower transmission rates. [7,8]

This study reports the distribution of CD4 counts and viral loads in ART-naive, first-time HIV testers and relates CD4 counts to current South African ART initiation thresholds, and the proportion of participants not qualifying for immediate initiation of ART by the CD4 criterion but who have high viral loads and high potential of onward HIV transmission.

Materials and Methods

We analysed data collected at the ZAZI clinic which is a free-of-charge, voluntary counselling and testing (VCT) facility on the campus of the Chris Hani Baragwanath Academic Hospital in Soweto, South Africa. The clinic is connected by a pedestrian bridge to a busy taxi rank. ZAZI tested 15,824 walk-in patients from October 2010 to September 2011. Participants included in this analysis had to be ART-naive, self-report being HIV tested for the first time, and have no clinical evidence of an AIDS defining condition (WHO Stage 1 or 2 diseases).
Study design

This was a cross-sectional study. All consenting participants were sequentially enrolled, had demographic and behavioural profiles captured and were symptom-screened for tuberculosis and other opportunistic infections. Behavioural profile questions related to number of sexual partners, condom and alcohol usage in the month prior to testing were collected using face-to-face interviews in routine VCT counselling procedure by counsellors. Blood draws for CD4 count and viral load were taken at the time of HIV diagnosis. Those eligible for ART (with CD4 $\geq 350$ cells/mm$^3$ as per South African HIV treatment guidelines [4]) were referred to treatment facilities whereas the remaining were followed up at ZAZI at six month intervals. Study data collection was conducted between March 2011 and October 2011.

HIV testing was by double-rapid test algorithm. Those testing negative on SD Bioline HIV 1/2 3.0 rapid test (Standard Diagnostics Inc, Gyeonggi-do, Republic of Korea) were not retested. Positive rapid tests were confirmed with a second rapid test (First Response, Premier Medical Corporation Limited, Daman, India). Discordant rapid test results were resolved with a laboratory HIV ELISA test. Blood was drawn and stored in tubes in room temperature where laboratory staff would collect them daily at scheduled times. No blood stayed overnight in the clinic. HIV-infected clients received CD4 and viral load results within two days of testing and those eligible for treatment and care were referred to local clinics.

Laboratory testing

Viral Load testing was conducted using the Nuclisens Nucleic Acid Sequence Based Amplification (NASBA, bioMerieux, Inc. Durham, NC) test platform for most tests (the detection limit for HIV viral load was $< 400$ copies/ml) and the Real Time HIV-1 Amplification Polymerase Chain reaction (PCR) (ABBOTT Molecular, Inc, Des Plaines, IL) platform in a few instances. CD4 cells were enumerated using the dual platform method. [9].

Written consent was obtained from each participant for their information to be stored in the clinic database and used for research purposes. The study was approved by the University of the Witwatersrand Human Research Ethics Committee.

Statistical analysis

Median and interquartile ranges were determined for age, CD4 count and viral loads. Continuous measures were compared using the Kruskall-Wallis non-parametric test. CD4 count was categorised dichotomously based on the South African treatment guidelines while viral loads were classified using $10,000$ copies/ml as probable high infectivity, based on prior HIV transmission related studies.[8,10,11] Overall frequencies and their percentages were determined for categorical variables and by

![Figure 1. Participant Disposition Flow Diagram.](doi:10.1371/journal.pone.0090754.g001)
The viral loads between males and females were compared while adjusting for CD4 count and age using analysis of covariance. Behavioural variables stratified by CD4 count and viral load categories were compared using the chi-square test. We determined the correlation between log10 viral load versus CD4 count, and included the least squares line by gender on a scattergram. All hypothesis testing used two-sided tests at 5% significance level using SAS 9.3 (SAS Institute, Cary, NC) statistical software.

### Results

A total of 4,793 adults attended VCT between March and October 2011, of whom 1062 (22%) tested HIV-positive (Figure 1). Of the 1062 HIV-infected adults, 799 (75%) were ART naïve, of whom 348 (44%) were first time testers. The leading reasons for testing are presented in Table 1.

#### Table 1. Characteristics of all asymptomatic, first time HIV testers at enrolment.

| Variable                              | Overall (N = 348) | Male (N = 123) | Female (N = 225) | p-value |
|---------------------------------------|-------------------|----------------|------------------|---------|
| Median Age at enrolment (IQR)         | 34 (28–41)        | 36 (30–42)     | 33 (27–40)       | 0.0086  |
| Median CD4 Count (IQR)                | 364 (238–542)     | 303 (199–418)  | 419 (262–582)    | 0.0001  |
| CD4 Count Categories                  |                   |                |                  |         |
| ≤ 350 (%)                             | 165 (47.4)        | 74 (60.2)      | 91 (40.4)        | 0.0004  |
| > 350 (%)                             | 183 (52.6)        | 49 (39.8)      | 134 (59.6)       |         |
| Median Viral Load (IQR)               | 13000             | 34000          | 9100             | 0.0005  |
| Viral Load Categories                 |                   |                |                  |         |
| ≤ 10,000 c/ml (%)                     | 161 (46.3)        | 44 (35.8)      | 117 (52.0)       | 0.0037  |
| > 10,000 c/ml (%)                     | 187 (53.7)        | 79 (64.2)      | 108 (48.0)       |         |
| No. of partners                       |                   |                |                  |         |
| 1 (%)                                 | 286 (86.0)        | 97 (82.2)      | 189 (95.9)       | < 0.0001|
| > 1 (%)                               | 29 (9.2)          | 21 (17.8)      | 8 (4.1)          |         |
| Condom usage*                         |                   |                |                  |         |
| No (%)                                | 113 (36.0)        | 43 (36.8)      | 70 (35.5)        | -       |
| Not sexually active (%)                | 7 (2.2)           | 1 (0.9)        | 6 (3.1)          |         |
| Sometimes (%)                         | 15 (4.6)          | 7 (6.0)        | 8 (4.1)          |         |
| Yes (%)                               | 179 (57.0)        | 66 (56.4)      | 113 (57.4)       |         |
| Alcohol usage*                        |                   |                |                  |         |
| Yes (%)                               | 159 (50.5)        | 84 (71.2)      | 75 (38.1)        | < 0.0001|
| No (%)                                | 156 (49.5)        | 34 (28.8)      | 122 (61.9)       |         |
| Reason for testing                    |                   |                |                  |         |
| General check-up (%)                  | 272 (78.4)        | 110 (90.2)     | 162 (72.0)       | -       |
| Illness (%)                           | 13 (3.8)          | 6 (4.9)        | 7 (3.1)          |         |
| Pregnancy (%)                         | 13 (3.8)          | N/A            | 13 (5.8)         |         |
| Unknown (%)                           | 33 (9.5)          | 5 (4.1)        | 28 (12.4)        |         |
| Other (%)                             | 16 (4.6)          | 1 (0.8)        | 15 (6.7)         |         |

* In the last month prior to the study.

* P-values for condom usage and reason for testing not presented due to small numbers within group.

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#### Table 2. Distribution of viral loads by CD4 count categories above 350 cells/mm.

| CD4 Count Category | Median (IQR) | ≤ 10,000 copies/ml (N = 121) | > 10,000 copies/ml (N = 62) | p-value* |
|--------------------|--------------|------------------------------|-----------------------------|----------|
| 350–399            | 8,400 (2,000–36,000) | 15 (12%)                     | 14 (23%)                    | 0.0742   |
| 400–449            | 3,000 (280–14,000)   | 24 (20%)                     | 9 (15%)                     | 0.3758   |
| 450–499            | 5,700 (1,100–30,000) | 13 (11%)                     | 6 (10%)                     | 0.8229   |
| ≥ 500              | 2,800 (360–15,000)   | 69 (57%)                     | 33 (52%)                    | 0.6243   |

*p-value represents the comparison of proportions with viral loads below and above 10,000 copies/ml.

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exclusion of ineligible participants were: being previously tested (n = 451) and not being ART naïve (n = 263). The median age of all ineligible participants was 35 years (IQR: 30-40), their median CD4 count and viral load was 304 cells/mm³ (IQR: 188-446) and 26,000 copies/ml (IQR: 4,100-140,000), respectively. Ineligible participants were excluded from further analyses.

The median age of the ART-naïve, first time testers was 34 years (IQR: 28-41). Males presented at older age than females (36 vs. 33; p = 0.0086). The overall median CD4 count was 364 cells/mm³ (IQR: 238.0-541.5); median CD4 count in females was higher than males (419 cells/mm³ vs. 303 cells/mm³; p = 0.0001). A total of 183 (53%) participants had CD4 count >350 cells/μL.

Table 3. Distribution of behavioural characteristics by CD4 and viral load.

| Variable                  | CD4 ≤ 350 | CD4 > 350 | p-value* | Viral Load ≤ 10,000 | Viral Load > 10,000 | p-value* |
|---------------------------|-----------|-----------|----------|---------------------|---------------------|----------|
| Number of partners        |           |           |          |                     |                     |          |
| >1 (%)                    | 19/165 (13.0) | 10/183 (5.9) | 0.0414   | 10/161 (6.8)       | 19/187 (11.2)      | 0.1838   |
| Condom usage              |           |           |          |                     |                     |          |
| Yes (%)                   | 82/165 (56.2) | 97/183 (57.7) | 0.5375   | 86/161 (59.3)      | 93/187 (55.0)      | 0.4930   |
| Alcohol usage             |           |           |          |                     |                     |          |
| Yes (%)                   | 79/165 (54.1) | 80/183 (47.3) | 0.4363   | 69/161 (47.3)      | 90/187 (53.3)      | 0.3250   |
| Reason for testing        |           |           |          |                     |                     |          |
| General check-up (%)      | 131/165 (79.4) | 141/183 (77.5) | 0.5971   | 121/161 (75.2)     | 151/187 (81.2)     | 0.2079   |

*p-value represents the comparison of proportions between CD4 count and viral load categories.

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Figure 2. Correlation plot of log₁₀ viral load and CD4 count taken at the same visit, stratified by gender with least-squares line for males and females.

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The proportion of males and females with CD4 count >350 cells/mm³ and with viral loads >10,000 copies/ml were similar (males 19/49 [39%] vs. females 43/134 [32%]; p = 0.3975). Those with a CD4 count ≤350 cells/mm³ were more likely to have more than one partner than participants with CD4 >350 (p = 0.0414). Condom use, alcohol use and reason for HIV testing were similar between the two CD4 strata of ≤350 and >350 cells/mm³ (Table 3). There was a negative moderate Pearson correlation of -0.504 between log₁₀ viral load and CD4 count (Figure 2) for all participants.

Discussion

We report CD4 counts and viral loads in adult first-time HIV testers who were well, ART naive, and diagnosed with HIV at an easily accessible, high volume, free-of-charge VCT centre. A large proportion of HIV infected adults not qualifying for immediate ART at the CD4 count threshold of 350 cells/mm³ had high viral loads. Of the ART-naïve first time testers whose CD4 count was above the CD4 threshold for ART initiation as per South African guidelines, 34% had a VL > 10,000 copies/ml suggesting that CD4 count at the time of HIV diagnosis is a poor proxy for HIV transmission risk. Consideration should be given to replacing CD4 count and age, females had a significantly lower HIV viral load compared to males (p = 0.0067).

Of those with CD4 > 350 cells/mm³ and VL > 10,000 copies/ml, males were more likely to report alcohol use (15/19 (79%) vs. 15/39 (33%); p = 0.0011). Those with a CD4 ≤ 350 cells/mm³ were more likely to have more than one partner than participants with CD4 > 350 (p = 0.0414). Condom use, alcohol use and reason for HIV testing were similar between the two CD4 strata of ≤350 and >350 cells/mm³ (Table 3). There was a negative moderate Pearson correlation of -0.504 between log₁₀ viral load and CD4 count (Figure 2) for all participants.

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