CD4 Lymphocyte Percentage Predicts Disease Progression in HIV-Infected Patients Initiating Highly Active Antiretroviral Therapy with CD4 Lymphocyte Counts >350 Lymphocytes/mm³

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(See the editorial commentary by Goicoechea and Haubrich, on pages 945–7.)

Background. The optimal timing of highly active antiretroviral therapy (HAART) in human immunodeficiency virus (HIV)–infected patients with ≥200 absolute CD4 lymphocytes/mm³ is unknown. CD4 lymphocyte percentage could add prognostic information.

Methods. Persons who initiated HAART between 1 January 1998 and 1 January 2003, received ≥30 days of therapy, and had baseline CD4 lymphocyte data available were included in the study. The log-rank test for time to event and Cox proportional hazards models were used to determine predictors of a new acquired immunodeficiency syndrome–defining illness or death.

Results. A total of 788 patients met the inclusion criteria. At baseline, subjects had a median of 225 CD4 lymphocytes/mm³ and 17% CD4 lymphocytes. Subjects with <17% CD4 lymphocytes had earlier disease progression, compared with subjects with ≥17%, both in the entire cohort (P < .0001) and of those subjects with >350 absolute CD4 lymphocytes/mm³ at baseline (P = .03). CD4 lymphocyte percentage <17% was the strongest predictor of disease progression among subjects in this latter group (hazard ratio, 3.57; P = .045).

Conclusions. In this cohort, CD4 lymphocyte percentage predicted disease progression in HIV-infected subjects who initiated therapy with >350 CD4 lymphocytes/mm³. This information may help identify persons who will derive the greatest benefit from initiation of HAART.

Highly active antiretroviral therapy (HAART) has dramatically decreased morbidity and mortality due to HIV infection and AIDS [1, 2]. The timing of initiation of HAART is critical; however, clinicians and patients must weigh the benefits and risks of therapy. Benefits include suppression of viral replication, preservation of immune function, prolongation of disease-free survival, and possible decrease in viral transmission. Even with complete virologic suppression, however, eradication of HIV-1 infection cannot be achieved with the current therapy [3, 4]. The risks of therapy include development of drug resistance (which limits future treatment options) and drug toxicity, including lipodystrophy, peripheral neuropathy, dyslipidemias, and, possibly, premature cardiovascular disease [5–7].

For persons with HIV-1 infection, absolute CD4 lymphocyte count predicts development of opportunistic illnesses and death due to AIDS [8–11]. Treatment guidelines regarding initiation of HAART have generally been based on absolute CD4 lymphocyte counts. The current treatment guidelines are based on a combination of absolute CD4 lymphocyte counts and HIV-1 RNA levels in plasma [11]. HAART is currently recommended for all symptomatic persons and for those...
Table 1. Clinical and demographic characteristics of the total study cohort and by CD4 lymphocyte categories.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total cohort (n = 788)</th>
<th>Baseline absolute CD4 lymphocyte count</th>
<th>Baseline CD4 lymphocyte percentage</th>
<th>Baseline HIV-1 RNA level, median (IQR), log_{10} copies/mL</th>
<th>Follow-up, median (IQR), weeks</th>
<th>Total events, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 360)</td>
<td>(n = 428)</td>
<td></td>
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<tr>
<td>Age, median (range), years</td>
<td>37 (16–71)</td>
<td>38 (19–65)</td>
<td>36 (16–71) a</td>
<td>37 (17–61)</td>
<td>36 (16–71) a</td>
<td>103 (51–180)</td>
</tr>
<tr>
<td>Female sex, no. (%) of subjects</td>
<td>196 (24.9)</td>
<td>65 (18.1)</td>
<td>131 (30.6) b</td>
<td>68 (17.3)</td>
<td>128 (32.4) b</td>
<td>4.9 (4.2–5.4)</td>
</tr>
<tr>
<td>Race, no. (%) of subjects</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>African American</td>
<td>322 (40.8)</td>
<td>156 (43.3)</td>
<td>166 (38.8)</td>
<td>171 (43.5)</td>
<td>151 (38.2)</td>
<td>247 (34.9)</td>
</tr>
<tr>
<td>White</td>
<td>434 (55.1)</td>
<td>191 (53.1)</td>
<td>243 (56.8)</td>
<td>205 (52.2)</td>
<td>229 (58.0)</td>
<td>7 (2.2)</td>
</tr>
<tr>
<td>Other b</td>
<td>32 (4.1)</td>
<td>13 (3.6)</td>
<td>19 (4.4)</td>
<td>17 (4.3)</td>
<td>15 (3.8)</td>
<td>10 (1.4)</td>
</tr>
<tr>
<td>HIV risk factor, c no. (%) of subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDU</td>
<td>94 (13.3)</td>
<td>47 (15.0)</td>
<td>47 (11.9)</td>
<td>50 (14.6)</td>
<td>44 (12.1)</td>
<td>4.9 (4.2–5.4)</td>
</tr>
<tr>
<td>MSM</td>
<td>314 (44.4)</td>
<td>129 (41.2)</td>
<td>185 (46.8)</td>
<td>144 (42.0)</td>
<td>170 (46.6)</td>
<td>247 (34.9)</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>247 (34.9)</td>
<td>109 (34.8)</td>
<td>138 (34.9)</td>
<td>116 (33.8)</td>
<td>131 (35.9)</td>
<td>7 (2.2)</td>
</tr>
<tr>
<td>Other d</td>
<td>10 (1.4)</td>
<td>7 (2.2)</td>
<td>3 (0.8)</td>
<td>6 (1.8)</td>
<td>4 (1.1)</td>
<td>10 (1.4)</td>
</tr>
<tr>
<td>Baseline HIV-1 RNA level, median (IQR), log_{10} copies/mL</td>
<td>4.9 (4.2–5.4)</td>
<td>5.3 (4.8–5.6)</td>
<td>4.5 (3.8–5.0) a</td>
<td>5.3 (4.7–5.6)</td>
<td>4.5 (3.8–5.0) a</td>
<td>103 (51–180)</td>
</tr>
<tr>
<td>Follow-up, median (IQR), weeks</td>
<td>103 (51–180)</td>
<td>92 (39–162)</td>
<td>110 (65–201) a</td>
<td>96 (42–165)</td>
<td>108 (60–200) a</td>
<td>103 (51–180)</td>
</tr>
<tr>
<td>Total events, no. (%)</td>
<td>140 (17.8)</td>
<td>99 (27.5)</td>
<td>41 (9.6) a</td>
<td>99 (25.2)</td>
<td>41 (10.4) a</td>
<td>140 (17.8)</td>
</tr>
</tbody>
</table>

NOTE. IDU, injection drug use; IQR, interquartile range; MSM, men who have sex with men.

a P<.05, vs. lower CD4 lymphocyte percentage or absolute CD4 lymphocyte count categories, by Fisher’s exact test or the Wilcoxon rank sum test.

b Includes Hispanic, Asian, and Asian–Pacific Islander.

c Data available on 708 subjects.

d Includes bisexual contact, both IDU and MSM, and transfusion associated.

e Data available on 769 subjects.
with an absolute CD4 lymphocyte count <200 lymphocytes/mm³, regardless of symptoms. Treatment should be considered when the absolute CD4 lymphocyte count is 200–350 lymphocytes/mm³, but there are insufficient data to make specific recommendations regarding asymptomatic persons with an absolute CD4 lymphocyte count ≥200 lymphocytes/mm³ [11, 12].

CD4 lymphocyte percentage could potentially add prognostic information and assist in deciding when to initiate antiretroviral therapy. Studies from the pre-HAART era found that CD4 lymphocyte percentage was more stable [13] and had greater prognostic significance than absolute CD4 lymphocyte count [13, 14]. This has not yet been fully assessed in persons receiving HAART. Gebo et al. reported that CD4 lymphocyte percentage did not provide additional prognostic information, compared with absolute CD4 lymphocyte count, for prediction of short-term (median, 90 days) development of new opportunistic infections [15]. However, that study was limited to persons with CD4 lymphocyte counts ≤350 lymphocytes/mm³ and included persons who were not receiving HAART. To assess the longer-term prognostic utility of CD4 lymphocyte percentage, we performed an observational cohort study to determine the contribution of baseline CD4 lymphocyte percentage at the time of initiation of HAART to prediction of subsequent progression to AIDS-defining illness or death.

SUBJECTS AND METHODS

Study cohort. The cohort was composed of patients receiving HIV care at the Comprehensive Care Center in Nashville, Tennessee. Clinical data were entered directly into an electronic medical record, either by medical providers at the time of the patient encounter, by automated data upload (e.g., laboratory results), or through entry by clinic personnel (e.g., deaths). Missing data were obtained by review of medical records via standardized abstraction. The study was approved by the Vanderbilt Institutional Review Board.

Persons who were at least 16 years of age at the time of initiation of their first HAART regimen, had demographic data available, and had received their first HAART regimen for at least 30 days between 1 January 1998 and 31 January 2003 were included in the study. Subjects were required to have a baseline absolute CD4 lymphocyte count and CD4 lymphocyte percentage available within 180 days before or 45 days after initiation of HAART. Subjects were stratified according to their baseline absolute CD4 lymphocyte count and CD4 lymphocyte percentage as follows: absolute CD4 lymphocyte count <200, 200–350, 351–500, and >500 lymphocytes/mm³; and CD4 lymphocyte percentage <14%, 14%–20%, 21%–28%, and >28%. These strata were derived from the 1993 Centers for Disease Control and Prevention (CDC) recommendations [16]. In addition, the median CD4 lymphocyte percentage of the study population was used for analyses. Baseline HIV RNA level was the test result obtained closest to initiation of HAART (within 180 days before initiation).

For the present study, the first HAART regimen was defined as the first regimen that contained an HIV-1 protease inhibitor (PI) and/or a nonnucleoside reverse-transcriptase inhibitor (NNRTI) in combination with nucleoside reverse-transcriptase inhibitors (NRTIs) or as the first regimen that contained 3 NRTIs (zidovudine, lamivudine, and abacavir). Thus, the first HAART regimen was categorized as PI based, NNRTI based, containing both, or containing >2 NRTIs. Data on prior ART were collected, and subjects were categorized as having received no prior ART (naïve), having received prior ART (but not at the time of initiation of their first HAART regimen), or receiving 1 or 2 NRTIs immediately before initiation of the first HAART regimen.

Definition of study events. A study event was considered to be any new opportunistic infection, other AIDS-defining illnesses (e.g., Kaposi sarcoma and wasting), or death after initiation of the first HAART regimen. Diagnoses of AIDS-defining illnesses were based on the 1993 CDC classification criteria [16]; diagnoses based on absolute CD4 lymphocyte counts <200 lymphocytes/mm³ were excluded. These events were ascertained from the database by use of standardized queries with codes from the International Classification of Diseases, Ninth Edition.
Revision. All events were confirmed by chart review; diagnoses of abnormal weight loss or wasting were confirmed by use of the CDC criteria for HIV wasting syndrome [16]. Uncertain events were clarified by review and agreement of the authors. Subjects who did not have a clinical event were followed in the cohort until their last clinic appointment date (last date of service) or until 31 January 2003, if the last date of service was after 31 January 2003.

Statistical analysis. Continuous variables were compared by use of the Wilcoxon rank sum (Mann-Whitney U) test. Categorical variables were compared by use of the χ² test and Fisher's exact test. The log-rank test for time to event was used for comparisons of Kaplan-Meier survival analyses. Cox proportional hazards models were used to determine predictors of events while adjusting for covariates. Stata SE (version 8.2; Stata Corporation) was used for data analyses.

RESULTS

Of the 788 patients who met the inclusion criteria, 25% were female, and 41% were African American; the median age at initiation of HAART was 37 years (table 1). The median (interquartile range [IQR]) absolute CD4 lymphocyte count and CD4 lymphocyte percentage at initiation of the first HAART regimen were 225 lymphocytes/mm³ (83–369 lymphocytes/mm³) and 17% (9%–25%), respectively. Baseline absolute CD4 lymphocyte count and CD4 lymphocyte percentage values were obtained after initiation of HAART (median [IQR], 27 [9–34] days) for 19 subjects (2%). The median (IQR) length of follow-up was 103
Figure 2. A, Kaplan-Meier survival curve of progression to an AIDS-defining illness or death among subjects with absolute CD4 lymphocyte counts >200 lymphocytes/mm$^3$, by CD4 lymphocyte percentage category (<17% [broken line] or ≥17% [solid line]). $P = .08$, log-rank test. B, Kaplan-Meier survival curve of progression to an AIDS-defining illness or death among subjects with absolute CD4 lymphocyte counts >350 lymphocytes/mm$^3$, by CD4 lymphocyte percentage category (<17% [broken line] or ≥17% [solid line]). $P = .03$, log-rank test.

weeks (51–180 weeks). One hundred forty subjects (18%) developed an AIDS-defining illness or died during follow-up.

Fifty-three percent of first HAART regimens included an HIV-1 PI, 23% were NNRTI based, 4% included both a PI and an NNRTI, and 20% were composed of 3 NRTIs. Fifty-nine percent of subjects were treatment naive, whereas 34% had received prior NRTIs but were not receiving ART at the time when they initiated their first HAART regimen. The remainder (7%) intensified existing NRTI therapy to HAART. There was no difference in outcome between those who received prior ART and those who were treatment naive at initiation of their first HAART regimen (data not shown).

The distribution of CD4 lymphocyte percentage, according to absolute CD4 lymphocyte count strata, is presented in table 2. Although most subjects with CD4 lymphocyte counts <200 lymphocytes/mm$^3$ also had CD4 lymphocyte percentages <14%, 26% of such subjects had CD4 lymphocyte percentages ≥14%. Among subjects with CD4 lymphocyte counts ≥200 lymphocytes/mm$^3$, there was a broad distribution of CD4 lymphocyte percentages across all strata. Thirty-six (16%) of 223 subjects with absolute CD4 lymphocyte counts >350 lymphocytes/mm$^3$ had CD4 lymphocyte percentages ≤20%. Because of the discordant disease progression seen with preliminary Kaplan-Meier survival curves and to maximize the number of subjects in the comparison groups, we used the median CD4 lymphocyte percentage (17%) to compare disease progression within absolute CD4 lymphocyte count strata.

Progression to AIDS-defining illness or death was significantly more likely among subjects with baseline absolute CD4 lymphocyte counts <200 lymphocytes/mm$^3$ than among those with absolute CD4 lymphocyte counts ≥200 lymphocytes/mm$^3$ ($P < .0001$) (figure 1A) and was more likely among those with
baseline CD4 lymphocyte percentages <17% than among those with baseline CD4 lymphocyte percentages ≥17% (P < .0001) (figure 1B). Among subjects who initiated HAART with absolute CD4 lymphocyte counts ≥200 lymphocytes/mm³, there was no difference in time to event among the different absolute CD4 lymphocyte count strata (P = .18). However, among this same group, there tended to be an increased event rate in subjects with a CD4 lymphocyte percentage <17% (P = .08) (figure 2A). Among subjects with an absolute CD4 lymphocyte count ≥350 lymphocytes/mm³, those with a CD4 lymphocyte percentage <17% had faster progression to AIDS-defining illness or death than did subjects with a CD4 lymphocyte percentage ≥17% (P = .03) (figure 2B).

Cox proportional hazards models were used to assess the effect of baseline characteristics on subsequent progression to AIDS-defining illness or death (table 3). We assessed the effects of age, race (white vs. nonwhite), baseline HIV-1 RNA level, prior ART (none vs. any), and CD4 lymphocyte percentage (<17% vs. ≥17%) with our models. Race was included in the models because nonwhite race was associated with disease progression by a univariate log-rank test (P = .003), likely because of lower absolute CD4 lymphocyte counts and CD4 lymphocyte percentages at the time of initiation of HAART. Among all study participants, regardless of baseline absolute CD4 lymphocyte count, CD4 lymphocyte percentage <17%, HIV-1 RNA level, and nonwhite race were independent predictors of subsequent disease progression or death. Among subjects with baseline absolute CD4 lymphocyte counts <200 lymphocytes/mm³, nonwhite race was associated with disease progression, and HIV-1 RNA level tended to be associated. Among subjects with baseline absolute CD4 lymphocyte counts ≥200 lymphocytes/mm³, only baseline HIV-1 RNA level was an independent predictor of disease progression (P = .005). Among subjects with baseline absolute CD4 lymphocyte counts ≥350 lymphocytes/mm³, CD4 lymphocyte percentage <17% predicted disease progression (hazard ratio [HR], 3.57 [95% confidence interval, 1.03–12.34]) (P = .045). Older age was associated with disease progression in this subject group, but it was not statistically significant in multivariate models. The results of the Kaplan-Meier and Cox proportional hazards models did not change when only subjects with CD4 data available from before initiation of HAART were included (data not shown).

**DISCUSSION**

There may be persons with absolute CD4 lymphocyte counts ≥200 lymphocytes/mm³ who could derive greater benefit from earlier initiation of HAART, compared with others. Identifying such persons would be extremely important and would help to clarify the optimal timing of initiation of HAART. CD4 lymphocyte percentage could potentially assist in the identification of these persons and, therefore, in treatment decisions.

The most notable finding of the present study was that CD4 lymphocyte percentage <17% was associated with an increased risk of clinical disease progression among subjects with baseline absolute CD4 lymphocyte counts ≥200 lymphocytes/mm³. Low CD4 lymphocyte percentage was of borderline statistical significance in Kaplan-Meier survival analyses for subjects with absolute CD4 lymphocyte counts ≥200 lymphocytes/mm³, but it was statistically significant for subjects with absolute CD4 lymphocyte counts ≥350 lymphocytes/mm³. For this subject group, in Cox proportional hazards models, CD4 lymphocyte percentage <17% was the strongest predictor of disease progression (HR, 3.57). Although additional studies with larger populations are needed to confirm these findings, they are consistent with those from studies from the pre-HAART era, which found CD4 lymphocyte percentage to be a better predictor of disease progression than absolute CD4 lymphocyte count [13, 14]. Of note, both of these studies were conducted with persons with relatively high baseline CD4 lymphocyte counts. This may
also explain why our findings differ from those of Gebo et al. [15], who assessed the predictive power of CD4 lymphocyte percentage in the HAART era but limited their assessment to persons with lower CD4 lymphocyte counts (≤350 lymphocytes/mm³). Of note, in our study, CD4 lymphocyte percentage provided no prognostic information for subjects with absolute CD4 lymphocyte counts <200 lymphocytes/mm³. This is consistent with the findings from the study by Gebo et al. and demonstrates the very strong predictive power of absolute CD4 lymphocyte counts <200 lymphocytes/mm³ in determining the risk of disease progression. As noted above, however, it is persons with absolute CD4 lymphocyte counts ≥200 lymphocytes/mm³ for whom additional prognostic information is needed to determine the optimal time to initiate HAART.

Guidelines for the initiation of HAART have fluctuated between the recommendation that therapy be started at higher versus lower absolute CD4 lymphocyte counts. Shortly after the introduction of PIs and HAART, it was recommended to “hit...early and hard” [17]. Subsequent observational studies noted that HAART improved clinical disease progression only among persons with absolute CD4 lymphocyte counts <200 lymphocytes/mm³ [18, 19], prompting guidelines advising that HAART was clearly recommended only when absolute CD4 lymphocyte counts were <200 lymphocytes/mm³. More recently, in studies with longer follow-up, there has been a suggestion that HAART slows disease progression when initiated at CD4 lymphocyte counts of 200–350 lymphocytes/mm³ [20, 21] and possibly at CD4 lymphocyte counts >350 lymphocytes/mm³ [22]. In addition, in a study that compared HIV-infected injection drug users with HIV-seronegative persons in the same cohort, only initiation of HAART at absolute CD4 lymphocyte counts >350 lymphocytes/mm² was associated with survival that was comparable to that seen in HIV-seronegative persons [23]. Thus, there may be a swing back toward earlier initiation of HAART, particularly if the toxicity of such regimens decreases.

Not all subjects included in the present study were antiretroviral naive at the time of initiation of HAART. However, receipt of prior ART was not associated with an increased risk of disease progression in the analysis. During the time of the study, combination ART that included 3 NRTIs was considered to be HAART. It has subsequently been shown that triple-NRTI regimens are not as effective as efavirenz-based HAART [24].

Markers of adherence to ART were not available for this study cohort. Future studies that assess the role that CD4 lymphocyte percentage plays in prediction of disease progression should take into account adherence, a known predictor of survival in persons with CD4 counts of 200–350 lymphocytes/mm³ [25]. Additionally, we were unable to account for non-AIDS-related comorbid conditions in this cohort.

With the above limitations noted, the following conclusions can be drawn from the present study. CD4 lymphocyte percentage <17% before initiation of the first HAART regimen predicted subsequent clinical disease progression in persons with baseline absolute CD4 lymphocyte counts >350 lymphocytes/mm³. Although validation in additional cohorts is needed, these findings suggest that CD4 lymphocyte percentage may assist in identifying persons with higher absolute CD4 lymphocyte counts who would benefit most from early initiation of HAART.

Acknowledgments

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References

15. Gebo KA, Gallant JE, Keruly JC, Moore RD. Absolute CD4 vs. CD4
25. Wood E, Hogg RS, Yip B, Harrigan PR, O’Shaughnessy MV, Montaner JS. Effect of medication adherence on survival of HIV-infected adults who start highly active antiretroviral therapy when the CD4+ cell count is 0.200 to 0.350 × 10^3 cells/L. Ann Intern Med 2003; 139:810–6.