Selection by Indication of Potent Antiretroviral Therapy Use in a Large Cohort of Women Infected with Human Immunodeficiency Virus

L. Ahdieh,1 S. J. Gange,1 R. Greenblatt,2 H. Minkoff,3 K. Anastos,4 M. Young,5 M. Nowicki,6 A. Kovacs,7 M. Cohen,8 and A. Muñoz9

To characterize selection factors related to therapy initiation, the authors investigated the extent to which key markers of human immunodeficiency virus (HIV) disease severity were associated with initiation of potent antiretroviral therapy (ART). Logistic regression was used to determine the effects of CD4+ cell count and HIV RNA level on potent ART initiation during 6-month periods among 2,059 HIV-infected US women enrolled in the Women's Interagency HIV Study. Low CD4+ counts and high HIV RNA levels were significantly (p < 0.05) associated with initiation of potent ART. During all periods between April 1996 and March 1998, CD4+ counts were more strongly associated with potent ART initiation than HIV RNA levels were; however, during the last period, both were associated (odds ratio per 100 CD4+-count decrease = 1.17, p < 0.01; odds ratio per 1 log10 increase in HIV RNA level = 1.48, p < 0.05). For a CD4+ count of 500 cells/ml and an HIV RNA level of 5,000 copies/ml, the probability of potent ART initiation increased from 0.5% to 16.8% between October 1995–March 1996 and October 1997–March 1998, suggesting earlier initiation of potent ART. Given the documented occurrence of confounding by indication, prospectively collected, time-dependent data on markers of disease progression and therapy use should be considered when making population-level comparisons before and after introduction of potent ART. Am J Epidemiol 2000;152:923–33.

CD4 lymphocyte count; confounding factors (epidemiology); HIV; RNA; therapeutics

A common feature of observational studies with ongoing follow-up is that data are collected on the therapeutic choices of cohort participants (1). When the effectiveness of such therapeutic interventions is assessed in the context of a cohort study, it is important to recognize that persons who have the greatest need and/or access to therapies have a greater chance of receiving them. The term “confounding by indication” has been used to describe such situations, in which decisions to treat are dependent on indicators of prognosis (2). Petri et al. used the term “channeling” to describe this selective allocation of medications to persons with pre-existing morbidities or problems (3). More recently, Salas et al. (4) proposed the use of “confounding by severity” as a more descriptive term for interventions administered on the basis of disease severity. Failure to consider the impact of such confounding may lead to misinterpretation of study findings.

Once a therapy is proven to be efficacious in randomized clinical trials, it is introduced to the population at large. A therapy is deemed “effective” if its introduction and use by members of the population are associated with overall declines in adverse clinical outcomes. Observational/cohort studies with ongoing follow-up provide the comprehensive data needed to characterize who is and who is not receiving therapy. Typically, the most ill persons are most likely to receive therapy; thus, effectiveness measures the impact, at the population level, of treating those most in need of intervention.

In the context of acquired immunodeficiency syndrome (AIDS), the decision to initiate potent antiretroviral therapy (ART) is based on the severity of illness, often as indicated by depletion of CD4+ cells and increased plasma human immunodeficiency virus (HIV) RNA levels (5). As such, treatment is predicated by disease progression and is, in effect, confounded by indication or severity. The potential for such confounding is not, however, unique to the treatment of HIV or other infectious diseases. It has been raised.
in the context of evaluating drug treatments for hypertension (6), asthma (7), and the risk of attempted suicide after use of antidepressants (8), among others. Regardless of the disease of interest, to fully analyze the operation of confounding, it is necessary to use time-dependent exposure data that are immediately associated with outcomes of interest and are collected throughout the period of disease progression.

As described recently (9), rigorously conducted cohort studies permit both confirmation of the therapeutic efficacy estimated in clinical trials (10–12) and determination of population-level effectiveness (13–16). The former requires that the nonrandom allocation of interventions be accounted for and that relevant confounders be known and measurable (17). Alternatively, because such observational studies approximate, as closely as possible, the “natural history” of disease progression, they are uniquely suited to evaluation of therapeutic effectiveness and examination of the extent to which efficacious therapies benefit those people who use them (18). However, it is only when such studies involve ongoing ascertainment—of both therapy use and outcomes of interest—that conclusions are not compromised by ecological biases or selection by indication (19, 20).

While epidemiologic studies can rarely adjust fully for this kind of confounding (21), cohort studies offer the information needed to document and quantify the effect of confounding by indication. Furthermore, because cohort studies track persons over periods of time during which available therapies change and, by their design, collect prospective data on markers of disease progression, they allow characterization of the differential effects of therapies with regard to such markers (22). To better appreciate and document the effects of selection by indication, we characterized the immunologic and virologic parameters associated with use and nonuse of potent ART among HIV-infected participants in the Women’s Interagency HIV Study. In addition, we investigated some of the pitfalls of cross-sectional comparisons of treated and untreated persons and the advantages of examining overall changes over calendar time in a large longitudinal cohort study.

MATERIALS AND METHODS

Study population and data collection

This study was based on the Women’s Interagency HIV Study, an ongoing multisite study of the natural history of HIV infection in women (23). During a 13-month recruitment period from October 1994 through November 1995, 2,059 HIV-seropositive and 569 HIV-seronegative women were enrolled at six clinical consortia (Bronx/Manhattan, New York; Brooklyn, New York; Chicago, Illinois; Los Angeles, California; San Francisco Bay Area, California; and Washington, DC). After a baseline visit, participants made follow-up visits every 6 months, consisting of an extensive interviewer-administered questionnaire, a physical examination, and the collection of specimens, including phlebotomy for the determination of CD4\(^+\) cell count and plasma HIV RNA level.

T-cell subsets were determined by immunofluorescence using flow cytometry in laboratories participating in the National Institute of Allergy and Infectious Diseases National Institutes of Health Quality Assurance Program. Plasma HIV RNA levels were measured by using a nucleic acid sequence-based amplification technique (NASBA; Organon Teknika, Durham, North Carolina) with a lower threshold for detection of 4,000 copies/ml (22), equivalent to a viral load of approximately 11,500 copies/ml by reverse transcription polymerase chain reaction (24).

At each visit, participants were asked which antiretroviral medications they had used since their last visit. Interventions were not provided by the study itself, however. Therapy regimens were classified as monotherapy, combination therapy, or potent ART. Monotherapy was defined as the use of a single nucleoside reverse transcriptase inhibitor (zidovudine, stavudine, zalcitabine, didanosine, or lamivudine). Combination therapy was defined as use of two or more nucleoside reverse transcriptase inhibitors. Potent ART was defined, according to 1997 US National Institutes of Health guidelines (5), as use of two or more nucleoside reverse transcriptase inhibitors, with either a protease inhibitor (indinivir, saquinavir, ritonavir, or nelfinavir) or a nonnucleoside reverse transcriptase inhibitor (nevirapine or delavirdine). Women taking a protease inhibitor plus zidovudine and stavudine were not considered to be receiving potent ART, because that combination is antagonistic (5), and thus were classified as receiving combination therapy. Those receiving two or more protease inhibitors were classified as receiving potent ART. Those women receiving only one protease inhibitor or one nonnucleoside reverse transcriptase inhibitor were classified as receiving monotherapy. For this analysis, we considered both monotherapy and combination therapy as nonpotent ART. During each calendar period, therefore, each participant’s therapy use was classified into one of three categories: no ART, nonpotent ART, or potent ART.

The study population pertinent to the question of interest was composed of the 1,777 (86.3 percent) HIV-seropositive participants with at least one study visit following October 1, 1995. This date was chosen as the approximate date on which potent ART became available.

Statistical methods

To characterize the study population eligible to initiate potent ART and contrast it with the complement of the cohort not seen after October 1, 1995, we computed baseline statistics of selected demographic and clinical variables for both groups. Because potent ART was introduced during a defined calendar period, the relevant time scale used for all subsequent analyses was 6-month calendar periods rather than sequential visits.

The mean CD4\(^+\) cell counts of women who reported initiating potent ART (initiators) and of those who could have initiated potent ART but did not (noninitiators) were compared during each calendar period by using the two-sample \(t\) test. Similarly, the chi-square test was used to compare the proportion of women with HIV RNA levels of \(\leq 4,000\) copies/ml. These comparisons between initiators and non-initiators were based on measurements obtained 6 months prior to the assessment of therapy status.

We described the distribution and central tendency of CD4+ cell counts by calculating mean values. Doing so was appropriate because CD4+ cell count values were distributed normally and the assay is sensitive to the full range of possible values. However, because of the relatively high threshold for HIV RNA detection and the fact that a sizable proportion of women had values below the cutoff, mean HIV RNA levels would have been misleading. We therefore derived two complementary statistics to comprehensively describe the distribution of viral load measurements. On the one hand, HIV RNA levels were treated categorically, based on the proportion of participants whose HIV RNA levels were ≤4,000 copies/ml. In addition, HIV RNA levels were described quantitatively, via the mean HIV RNA (in the log base 10 scale) among those with levels of >4,000 copies/ml.

We subsequently considered the joint contributions of CD4+ cell count and HIV RNA level on initiation of potent ART. Specifically, we calculated the probability of initiating potent ART during each calendar period and used logistic regression to determine the relation between initiation of therapy and a 100-cell decrease in CD4+ cell count and a 1-log10 increase in HIV RNA level. Specifically, if \( p \) denotes the probability of initiating potent ART, the logistic regression model that we used was

\[
\log \left( \frac{p}{1-p} \right) = \beta_0 + \beta_1((\text{CD4}^+ \text{ cell count} - 500)/100) + \beta_2 \log_{10}(\text{HIV RNA}/5,000),
\]

where \((1 + \exp(-\beta_1))^{-1}\) represents the probability of starting potent ART for a CD4+ cell count of 500 cells/\( \mu \)l and HIV RNA level of 5,000 copies/ml, \( \exp(-\beta_2) \) represents the odds ratio for starting potent ART associated with a 100-cell decrease in CD4+ cell count, and \( \exp(\beta_2) \) represents the odds ratio for starting potent ART corresponding with a 1-log10 increase in HIV RNA level (e.g., 10,000–100,000 copies/ml). For this analysis, persons whose HIV RNA levels were 4,000 copies/ml were recoded as having levels of 3,000 copies/ml to account for the skewed distribution of HIV RNA levels among those broadly classified as undetectable (i.e., ≤4,000 copies/ml) by using nucleic acid sequence-based amplification.

During any calendar period, those women who used potent ART may have been reporting potent ART for the first time (i.e., potent ART initiators) or, alternatively, may have been continuing use of potent ART from the preceding period. To more fully evaluate antiretroviral use, it was of interest to document the number of participants who transitioned to other therapies subsequent to using potent ART during a given period.

Finally, we illustrated the pitfalls of an analysis that ignores selection by indication by comparing levels of markers of disease progression at a given point in time among nontherapy users, nonpotent ART users, and potent ART users. Logistic regression models were used for the binary outcome (i.e., HIV RNA level ≤4,000 copies/ml), and multiple linear regression was used for the continuous outcomes (CD4+ cell count and log10 HIV RNA level of those women with >4,000 copies/ml).

**RESULTS**

The study population for this analysis consisted of the 1,777 HIV-infected participants with at least one visit after October 1, 1995. There were 282 participants not seen after this date and therefore not in study follow-up during the period when potent ART was introduced. In both groups, the median date and age at enrollment was March 1995 and 37 years, respectively, and there were no differences in HIV exposure category (table 1). Significant differences were found between the two groups with regard to level of infertility at study enrollment. Specifically, a higher proportion of the study population (35.1 percent) than the complement of the cohort (18.8 percent) had HIV RNA levels of ≤4,000 copies/ml (\( p < 0.01 \)). Furthermore, among those with HIV RNA levels of >4,000 copies/ml, median HIV RNA levels were lower in the study population, 53,289 copies/ml, than in the complement of the cohort, 196,479 copies/ml (\( p < 0.01 \)). Additional evidence of less-advanced HIV disease in those women seen after October 1, 1995, consisted of higher CD4+ cell counts (346 vs. 130 cells/\( \mu \)l, \( p < 0.01 \)) and a lower prevalence of those who reported AIDS (\( p < 0.01 \)). Furthermore, a lower proportion of those seen after October 1, 1995, were diagnosed with AIDS (48.0 vs. 57.5 percent, \( p < 0.01 \)) or died (9.8 vs. 49.3 percent, \( p < 0.001 \)) during study follow-up. Therapy use at study enrollment was comparable between the two groups; most of those women receiving treatment were using a single antiretroviral medication (monotherapy). Table 1 also shows the number of participants who contributed information on use of treatment during each calendar period. Among the study population, the proportion of persons who contributed data on therapy use increased during the first three periods because of ongoing enrollment; the subsequent decline was due to death and loss to follow-up.

Tables 2 and 3 describe characteristics of participants who initiated potent ART within a given calendar period and compares them with participants who did not begin potent ART. Table 2 presents findings on CD4+ cell count, and table 3 shows findings on plasma HIV RNA level. During any calendar period, potent ART initiators, as compared with noninitiators, had significantly lower CD4+ cell counts and significantly higher HIV RNA levels 6 months preceding initiation of therapy. The relative difference in mean CD4+ cell counts between initiators and noninitiators decreased over time. Furthermore, participants who initiated potent ART during the later calendar periods had higher CD4+ cell counts than those who initiated therapy earlier. However, CD4+ cell count continued to predict potent ART initiation during the 6 months preceding March 1998: the mean CD4+ cell count of the 145 initiators was 381 cells/\( \mu \)l, significantly lower (\( p = 0.031 \)) than the mean CD4+ cell count of the 603 women who could have started potent ART but did not (434 cells/\( \mu \)l). Table 3 also shows that the percentage of women with HIV RNA levels of ≤4,000 copies/ml was significantly smaller among initiators during each calendar period and that, over time, an increasing proportion of potent ART initiators had HIV RNA levels of ≤4,000 copies/ml. In contrast to the association of CD4+ cell count with potent ART initiation, however, a trend toward
Table 4 presents the results from logistic regression models that summarize the effects of CD4+ cell count and HIV RNA level on initiation of potent ART during different calendar periods. In univariate analyses, the odds of potent ART initiation were highly associated with both a decrease in CD4+ cell count and an increase in HIV RNA levels during each calendar period. Estimates from the multivariate model indicated that decreases in CD4+ cell count were independently associated with potent ART initiation, regardless of HIV RNA level. However, once CD4+ cell count was adjusted for, increases in HIV RNA level were no longer associated with potent ART initiation except during the last calendar period, when HIV RNA test results were most readily available to clinicians and the turnaround time for receiving the results was the shortest. To illustrate the temporal trends between calendar periods, we estimated the probability of potent ART initiation for a given CD4+ cell count of 500 cells/µl and an HIV RNA level of 5,000 copies/ml. Table 4 shows that this probability increased steadily from 0.5 percent during the October 1995–March 1996 period to 16.9 percent during the October 1997–March 1998 period.

To further characterize potent ART initiation, it was central to our analysis to consider adherence to and continual...
Selection by Indication of Potent ART Initiation

use of therapy over time. Figure 1 illustrates transitions between calendar periods from potent ART to other therapy use. During each calendar period, potent ART users were unlikely to switch from potent regimens, and approximately 85 percent of those using potent ART continued to do so between calendar periods. Very few women who used potent ART completely discontinued therapy from one calendar period to the next but rather switched to nonpotent ART. Of note, however, during the last three periods, there was a significant trend ($p < 0.03$) toward more women switching from potent ART to either nonpotent ART or no therapy.

Figures 2–4 describe temporal changes in markers of HIV disease progression according to concurrent reports of therapy use, and they illustrate the impact of such therapy at the population level. Prior to the fourth calendar period (April 1996–September 1996), no significant changes over time occurred in the proportion of participants who had HIV RNA levels of $\leq 4,000$ copies/ml among those using either nonpotent ART or no ART (figure 2). However, when potent ART became widely available in early 1996, an increase in the proportion of women with HIV RNA levels of $\leq 4,000$ copies/ml in these two groups became evident.

**TABLE 2.** Mean CD4$^+$ cell count (cells/µl) 6 months preceding initiation of potent antiretroviral therapy (ART) among those eligible to start, Women’s Interagency HIV Study, 1995–1998

<table>
<thead>
<tr>
<th>Calendar period</th>
<th>Started potent ART</th>
<th>Did not start potent ART</th>
<th>Difference in mean CD4$^+$ cell counts</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 1, 1995–March 31, 1996</td>
<td>230 (186)</td>
<td>379 (307)</td>
<td>–149</td>
<td>0.061</td>
</tr>
<tr>
<td>April 1, 1996–September 30, 1996</td>
<td>250 (215)</td>
<td>393 (278)</td>
<td>–143</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>October 1, 1996–March 31, 1997</td>
<td>312 (202)</td>
<td>434 (286)</td>
<td>–122</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>April 1, 1997–September 30, 1997</td>
<td>375 (245)</td>
<td>427 (269)</td>
<td>–52</td>
<td>0.012</td>
</tr>
<tr>
<td>October 1, 1997–March 31, 1998</td>
<td>381 (247)</td>
<td>434 (267)</td>
<td>–53</td>
<td>0.031</td>
</tr>
</tbody>
</table>

$^*$ HIV, human immunodeficiency virus; SD, standard deviation.
† A limited number of participants were missing CD4$^+$ cell count data.

**TABLE 3.** Percentage of participants with an HIV$^+$ RNA level of $\leq 4,000$ copies/ml 6 months preceding initiation of potent antiretroviral therapy (ART) among those eligible to start, Women’s Interagency HIV Study, 1995–1998

<table>
<thead>
<tr>
<th>Calendar period</th>
<th>Started potent ART</th>
<th>Did not start potent ART</th>
<th>Odds ratio</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 1, 1995–March 31, 1996</td>
<td>9</td>
<td>36</td>
<td>0.18</td>
<td>0.064</td>
</tr>
<tr>
<td>April 1, 1996–September 30, 1996</td>
<td>26</td>
<td>39</td>
<td>0.56</td>
<td>0.024</td>
</tr>
<tr>
<td>October 1, 1996–March 31, 1997</td>
<td>36</td>
<td>49</td>
<td>0.61</td>
<td>0.002</td>
</tr>
<tr>
<td>April 1, 1997–September 30, 1997</td>
<td>42</td>
<td>50</td>
<td>0.72</td>
<td>0.067</td>
</tr>
<tr>
<td>October 1, 1997–March 31, 1998</td>
<td>41</td>
<td>63</td>
<td>0.41</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$^*$ HIV, human immunodeficiency virus.
† A limited number of participants were missing HIV RNA data.

**TABLE 4.** Probability of initiating potent antiretroviral therapy (ART) and the multivariate (univariate) odds ratios associated with changes in CD4$^+$ cell count (cells/µl) and HIV$^+$ RNA (copies/ml), by calendar period, Women’s Interagency HIV Study, 1995–1998

<table>
<thead>
<tr>
<th>Calendar period</th>
<th>Multivariate (univariate) odds ratio</th>
<th>Probability of initiating potent ART (%)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 1, 1995–March 31, 1996</td>
<td>Each 100-cell decrease in CD4$^+$ cell count</td>
<td>1.08 ($1.30^*$)</td>
</tr>
<tr>
<td>April 1, 1996–September 30, 1996</td>
<td>Each 1 log$_{10}$ increase in HIV RNA</td>
<td>1.70 ($1.62^*$)</td>
</tr>
<tr>
<td>October 1, 1996–March 31, 1997</td>
<td>Each 1 log$_{10}$ increase in HIV RNA</td>
<td>1.44 ($1.43^*$)</td>
</tr>
<tr>
<td>April 1, 1997–September 30, 1997</td>
<td>Each 1 log$_{10}$ increase in HIV RNA</td>
<td>1.17 ($1.21^*$)</td>
</tr>
<tr>
<td>October 1, 1997–March 31, 1998</td>
<td>Each 1 log$_{10}$ increase in HIV RNA</td>
<td>1.17 ($1.23^*$)</td>
</tr>
</tbody>
</table>

$^*$ p < 0.05.
† HIV, human immunodeficiency virus.
‡ Given a CD4$^+$ cell count of 500 cells/µl and an HIV RNA level of 5,000 copies/ml.

Although approximately 40 percent of participants had HIV RNA levels of ≤4,000 copies/ml when potent ART was initiated, this proportion increased to approximately 65 percent among potent ART users (figure 2), documenting the effect of potent ART in reducing HIV RNA levels to ≤4,000 copies/ml. During each calendar period, a significantly ($p < 0.012$) greater proportion of potent ART users than nontherapy users had HIV RNA levels of ≤4,000 copies/ml.
Furthermore, while in the April 1996–September 1996 period the proportion of nonpotent ART users, as compared with potent ART users, with HIV RNA levels of ≤4,000 copies/ml was significantly lower (odds ratio = 0.64, \( p = 0.033 \)), the proportions were very similar during the most recent period (October 1997–March 1998) (odds ratio = 0.88, \( p = 0.403 \)). Thus, although potent ART users as a group included women with the most advanced disease, the proportion whose HIV RNA levels were undetectable remained relatively stable and high compared with the other groups, overcoming the confounding by severity previously documented in this population.

We also considered the implications of confounding by severity on mean HIV RNA levels among participants with HIV RNA levels of >4,000 copies/ml, according to therapy use (figure 3). It is of note that this analysis excluded those women for whom therapy was most effective and whose HIV RNA levels were brought to ≤4,000 copies/ml and focused instead on women with detectable HIV RNA levels. In contrast to the information shown in figure 2, potent ART did not overcome confounding by severity for this outcome of interest. Indeed, during each calendar period, potent ART users did not significantly differ from the other two groups (i.e., computed \( p \) values for all eight comparisons were non-significant; the lowest was 0.160). Thus, although women who used potent ART were most likely to have HIV RNA levels of ≤4,000 copies/ml, those potent ART users with levels of >4,000 copies/ml did not necessarily have the lowest HIV RNA levels. Thus, if potent ART did not bring HIV RNA levels to ≤4,000 copies/ml, mean HIV RNA levels among those using potent ART were simply brought down to those lower levels present among participants who did not transition to potent ART.

Prior to the introduction of potent ART, there were no changes in CD4+ cell count among participants using no therapy or nonpotent ART (figure 4). The subsequent rise following the April 1996–September 1996 calendar period may give the false impression that nonpotent ART is increasingly effective in reversing immunosuppression. The more likely explanation is that a significant number of women transitioned from nonpotent ART to potent ART regimens during the later periods. The number of women using nonpotent ART thus decreased from 697 (April 1996–September 1996) to 414 (April 1997–September 1997), while the number of women reporting use of potent ART increased from 118 to 519. Because women with the lowest CD4+ cell counts transitioned to potent ART, CD4+ cell counts among potent ART users were consistently and significantly (\( p < 0.017 \)) lower.
than they were among the other two groups except during the most recent period, when potent ART users had on average 21 fewer cells/µl than nonpotent ART users did ($p = 0.246$).

**DISCUSSION**

In this large cohort study of women with HIV infection, those receiving potent ART were more likely to have advanced HIV disease than were their counterparts who were not receiving potent ART. From the clinical perspective, this finding is not surprising, because immunologic and virologic parameters are key criteria used to initiate, monitor, and modify ART (5). From the epidemiologic perspective, however, the fact that ART use is not random but rather selected for has important implications for the interpretation of results regarding the effect of therapies. Since CD4$^+$ cell count and HIV RNA level are confounders for potent ART use, we illustrated that conclusions drawn from cross-sectional analyses alone underestimate the population-level effectiveness of potent ART.

An important component of this analysis was to characterize potent ART initiation among a cohort of HIV-infected women in the United States, and several observations can be made in this regard. First, we found that during each calendar period, women who initiated potent ART had lower CD4$^+$ cell counts (table 2) and higher HIV RNA levels (table 3) than those who did not initiate therapy but were in a position to do so. The probability of starting potent ART increased over time, after holding CD4$^+$ cell count at 500 cells/µl and HIV RNA level at 5,000 copies/ml (table 4). On the other hand, mean CD4$^+$ cell counts and the percentage of participants with HIV RNA levels of ≤4,000 copies/ml preceding potent ART initiation increased significantly during the follow-up period. Although there is no consensus at present regarding when to initiate potent ART in asymptomatic persons, we found that during the most recent periods, potent regimens were being made available relatively earlier in the course of HIV disease. Randomized clinical trials designed to investigate benefits of early treatment of asymptomatic persons with zidovudine had equivocal results (26, 27), and findings of similar trials of potent antiretrovirals have yet to be reported (28, 29).

Univariate analyses found that both CD4$^+$ cell count and HIV RNA level were associated with potent ART initiation; however, when the two variables were evaluated simultaneously, we found that once CD4$^+$ cell count was accounted for, HIV RNA level no longer was associated with therapy initiation. This finding suggests that during the period of this study (October 1995–March 1998), it was primarily immunologic deficiency that prompted clinicians to prescribe, and participants to initiate, potent ART. The observation that HIV RNA level was independently associated with...
potent ART initiation during the most recent calendar period suggests that with the increasing availability and use of HIV RNA testing, both patients and clinicians may be increasingly likely to make therapy-related decisions based on viral load results. It is also possible that an independent contribution of HIV RNA level to potent ART initiation may have been observed during earlier periods if we had used an assay with a level of detectability substantially lower than 4,000 copies/ml.

The cohort in which this analysis was based included women from six study centers throughout the United States. To address whether possible regional differences in access to care and laboratory methods for the assessment of biomarkers may confound the study’s inferences, we extended the model used for table 4 to include five indicator variables for the six centers. We found that the inferences relative to CD4+ cell count and HIV RNA level were identical after adjustment for center. Furthermore, the magnitude of the associations between these markers and potent ART initiation was practically unchanged: the odds ratios associated with each 100-cell decrease in CD4+ cell count were 1.76, 1.49, 1.21, and 1.20 and the corresponding odds ratios associated with each 1-log10 increase in HIV RNA level were 0.74, 0.94, 1.14, and 1.44 for each of the last four calendar periods.

It should be noted that although, in some cases, clinicians use changes in CD4+ cell counts or HIV RNA levels, rather than cross-sectional values, as an indication for treatment, we restricted our analysis to cross-sectional values because guidelines for initiating potent ART are based on this criterion. It is quite possible that women who began potent ART at relatively high CD4+ cell counts (i.e., >500 cells/µl) and low HIV RNA levels (i.e., <10,000 copies/ml) were those who experienced a steep decline in CD4+ cell counts (e.g., from 750 to 500 cells/µl) or a large increase in HIV RNA levels (e.g., from <50 to 8,000 copies/ml) during the previous 6 months.

The effectiveness of therapies is likely to depend heavily on the continual use of prescribed regimens. We have shown (figure 1) that the majority of women reporting potent ART continued to use this type of therapy between periods. Nonetheless, some women discontinued use of potent antiretroviral regimens, and future studies are needed to characterize and better understand the underlying reasons for abandoning potent ART. It is of concern that the number of women switching from potent ART appears to be increasing: 10 women (10 percent) in the April 1996–September 1996 period to 37 women (12 percent) in the October 1996–March 1997 period to 70 women (16 percent) in the April 1997–September 1997 period. Such an upward trend in the number of women switching from potent ART may have the greatest impact on those markers of disease progression that require ongoing and long-term therapy use to show improvement. This possibility may explain, in part, why trends in mean CD4+ cell count over time do not suggest a strong immune reconstitution (figure 4). Another potential contributor may be less-than-optimal adherence to the complexities of potent ART regimens.

These analyses of the initiation and maintenance of potent ART were drawn from comparisons between calendar periods in which the unit of analysis was the individual person. Given the effect of confounding by severity in this setting, if our inferences had relied on cross-sectional comparisons alone, derived conclusions regarding the association of therapy use with immunologic/virologic parameters would have vastly underestimated the benefits of potent ART in this population. Although women initiating potent ART had higher HIV RNA levels compared with those not initiating potent ART, a greater proportion of potent ART users had RNA levels of ≤4,000 copies/ml during each calendar period. In effect, confounding by severity was “overcome” because potent ART was so effective for this outcome (figure 2). Thus, although the collective group of women starting potent regimens was weighted with participants who had high HIV RNA levels, potent ART was able to suppress virus below detectable levels. On the other hand, among those with HIV RNA levels of >4,000 copies/ml, mean HIV RNA levels of those using potent ART were only at the level of those using nonpotent ART; that is, the well-known effectiveness of potent ART in reducing viral load was masked (figure 3). Finally, confounding by severity had the most serious impact on trends in CD4+ cell count, clearly illustrating that cross-sectional ecologic analyses are uninformative, given the selection biases at play (figure 4). In sum, while potent ART is so effective that it was able to overcome confounding by severity in reducing HIV RNA levels to ≤4,000 copies/ml, it does not overcome this confounding on the scales of mean detectable HIV RNA levels and mean CD4+ cell counts.

Our ability to document and account for selection by indication for potent ART initiation is contingent on the appropriateness and quality of the measures we have chosen to gauge disease progression and clinical severity (17). In this regard, an important strength of the present analysis is that it was conducted within a large cohort study of HIV-infected women, from which repeated biologic measurements have been collected longitudinally. Studies have consistently demonstrated that these two variables are highly predictive of both survival and disease progression (30–32). The relatively high threshold for HIV RNA detectability is an acknowledged limitation of the analysis.

It was reassuring to find that over time, an increasing proportion of study participants reported receiving potent ART and that CD4+ cell counts were high for those women who did not receive therapy. It is of concern, however, that during the two most recent calendar periods, mean CD4+ cell counts declined in untreated women, suggesting that stable levels may not remain for prolonged periods of time (figure 4). This late-occurring decline of CD4+ cell counts in those who initially maintain stable, high levels underscores the risk of late progression by persons who have previously shown clinical stability for long periods of time (33).

A common feature illustrated in figures 2–4 is that before potent ART was introduced in early 1996, measures of disease progression did not change significantly among nonpotent ART users. It was only after the introduction of potent regimens that the percentage of participants with HIV RNA levels of ≤4,000 copies/ml increased, mean HIV RNA levels declined, and CD4+ cell counts increased, concomitant
with the initiation of potent ART by women with the most advanced disease. This finding confirms previous observations that potent ART, rather than monotherapy or combination therapy, is most effective in impacting the progression of HIV disease, as well as survival (14, 34). In this regard, it is of note that the vast majority of women initiating potent ART in this population had prior experience with either monotherapy or combination therapy. It is also possible that population-level markers of disease progression “improved” as only the healthiest cohort members survived. While this finding was true, it should be noted that the analysis did exclude those women not seen after October 1, 1995, a group demonstrated here to have the most advanced HIV disease.

In summary, the fact that persons who are the most ill are those who are “selected” for therapy must be acknowledged when observational data are used to either estimate individual-level efficacy or evaluate the effectiveness of therapy at the population level. For the former approach, our ability to ascertain the impact of potent antiretroviral regimens by using simple comparisons of immunologic and virologic markers of disease progression among those exposed and those unexposed will inevitably be compromised by the operation of confounding by severity. As a result, measurements of the impact of therapy on disease progression will underestimate the efficacy of such therapies. Thus, when data from observational studies are used to confirm estimates of efficacy, disease severity operates as a confounder that must be adjusted for.

Alternatively, when observational data are used to describe effectiveness at the population level, it becomes essential to document, by using rigorously collected data in the context of a prospective observational study, the profile of those who are selected to receive therapy and in which ways such persons have more advanced and severe disease. In this setting, disease severity is not a confounder to be adjusted for; rather, the goal is to document that such selection in fact exists. This distinction is important when interpreting the extent to which disease is reduced in the context of the documented selection by severity of those who receive treatment. Such analyses of therapeutic effectiveness at the population level (S. J. Gange, The Johns Hopkins University School of Public Health, unpublished manuscript) generate important public health information that complements the individual-level inferences of efficacy generated from randomized clinical trials (35–37).

REFERENCES


