Rapid declines in total lymphocyte count and hemoglobin in HIV infection begin at CD4 lymphocyte counts that justify antiretroviral therapy

Stephen J. Gange, Bryan Lau, John Phair, Sharon A. Riddler, Roger Detels and Joseph B Margolick

We investigated whether the onset of rapid declines in total lymphocyte counts and hemoglobin levels might be useful for staging HIV disease and initiating antiretroviral therapy. Using data from the Multicenter AIDS Cohort Study, we found that accelerated declines in both markers generally precede AIDS by 1.2 years and occur when the CD4 lymphocyte counts fall below 350 cells/mm³. These markers may thus be suitable for monitoring disease and timing therapy initiation in resource-limited settings.

There is an emerging consensus that the HIV pandemic in the developing world requires treatment with antiretroviral drugs [1]. However, because of the complexity, expense, and diversity of potentially effective treatments, there is not yet consensus on what treatments should be given and at what stage of HIV infection they should be initiated. In the developed world, guidelines for starting treatment are based on the CD4 lymphocyte counts and plasma HIV-RNA concentration [2], but these markers are considered impractical for widespread use in the developing world due to their expense and other factors.

We modified methods previously applied to the retrospective identification of inflections in total T lymphocyte counts and viral load measurements [15] to determine the occurrence and timing of the onset of rapid declines in TLC and hemoglobin. For each marker, we estimated the magnitude of decline that best distinguished men who did or did not develop AIDS over follow-up. To enhance the stability of our estimates, declines were calculated as the difference between the mean of the two most recent marker values from the mean of all previous marker values. The rates of decline that best (i.e. with maximal sensitivity and specificity) distinguished men with and without the onset of AIDS were 33% per year for TLC and 11.6% per year for hemoglobin. These optimal values provided sensitivity and specificity values of approximately 70% for TLC and 74% for hemoglobin. Similar results were obtained when restricting the analysis to seroconverters (data not shown).

We then identified the time that a decline of these magnitudes began over the course of follow-up (termed 'inflection point'). The median (IQR) time from the inflection point to AIDS was 1.27 years (0.48, 2.64) for TLC and 1.21 years (0.49, 2.49) for hemoglobin. Fig. 1 shows longitudinal markers from two representative
individuals who developed AIDS, displaying the first time that rapid changes (inflections) were identified.

To examine the distribution of these times relative to the first times that the CD4 lymphocyte counts fell below 350 cells/mm³ (i.e. the time at which the initiation of antiretroviral therapy is recommended [2]), we restricted our sample to the 432 men who had a baseline CD4 lymphocyte count greater than 500 cells/mm³, which fell below 350 cells/mm³ at some later time. Our analysis demonstrated that in the vast majority of cases the onset of rapid declines in TLC and hemoglobin occurred within several months of the time when the CD4 lymphocyte counts fell below 350 cells/mm³. The hemoglobin decline preceded the CD4 lymphocyte decline to less than 350 cells/mm³ by a median (IQR) time of 0.35 years (−1.37, +0.64), and the TLC decline followed the time that the CD4 lymphocyte count fell to less than 350 cells/mm³ by 0.11 years (−0.88, +0.62). At the first time when the CD4 lymphocyte counts fell below 350 cells/mm³, the median (IQR) level of TLC was 2088 cells/mm³ (1728, 2499) and hemoglobin was 15.1 g/dl (14.5, 15.8).

These results suggest that the onset of a rapid decline in TLC or hemoglobin could be a clinically valid indicator of a CD4 lymphocyte count that would justify initiating antiretroviral therapy. It is quite likely that the changes in these markers, which are inexpensive and widely available, could have significant value for the prospective monitoring of disease progression and the timing of the initiation of antiretroviral therapy in settings where it is not feasible to follow CD4 lymphocyte counts. The variability in the timing of inflections might be further reduced by considering marker combinations in conjunction with clinical conditions. These observations should be tested in appropriate populations, taking into account factors such as nutrition and endemic diseases that may affect these markers, as well as the frequency of monitoring that would be needed to obtain appropriate precision in the timing of the initiation of therapy.

Acknowledgements

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The effect of TNF polymorphisms on the time-to-onset of lipodystrophy was investigated in 191 Caucasian participants in the western Australian HIV cohort. Carriage of the TNF-α-238G/A polymorphism was found in 13.1% of the cohort, and was associated with an earlier onset of lipodystrophy. The presence of TNF-α-238G/A was associated with lipodystrophy progression in Cox proportional hazards regression analyses, without influencing the relative risk and significance of other predictive host and treatment-related variables.

Progressive subcutaneous fat wasting is a component of the antiretroviral therapy-associated lipodystrophy syndrome that is highly prevalent among recipients of highly active antiretroviral therapy (HAART) in white Caucasian populations. The dominant risk factor for the development of fat wasting appears to be the concurrent use of nucleoside analogue reverse transcriptase inhibitors (NRTI) and HIV protease inhibitors (PI), with additional evidence of a differential risk associated with select NRTI drugs [1]. There is increasing awareness, however, that host factors also significantly modify the susceptibility to the development of fat wasting, as well as its severity [2]. Most notably, older age and white racial origin are factors associated with an increased likelihood of developing subcutaneous fat wasting. In the present study, we sought to investigate a possible role for functional promoter polymorphisms in TNF-α and -β loci, as the expression of this cytokine may be involved in adipose tissue pathology associated with lipodystrophy [3], and the carriage frequency of these polymorphisms is divergent among racial populations [4]. In addition, we addressed the hypothesis that these host factors may modify the independent contribution of antiretroviral therapy-specific factors to the progression of lipodystrophy.

In a previous study involving participants in the western Australian HIV Cohort, host and treatment-related factors were found independently to increase the rate of progression to clinically apparent subcutaneous fat wasting in participants on a HAART regimen (n = 230) in a time-to-onset analysis (Cox proportional hazards regression) [5]. An increased relative risk (RR) was associated with older age (RR 1.052/year, P < 0.001), white race (RR 3.9, P = 0.023), duration of dual NRTI therapy before the commencement of triple combination antiretroviral therapy (RR 1.021/month, P = 0.005), and increased cumulative time on a regimen containing stavudine compared with regimens incorporating zidovudine therapy (RR 1.085/month, P < 0.001). The use of the non-nucleoside reverse transcriptase inhibitor nevirapine was associated with a reduced rate of progression, compared with regimens incorporating PI therapy (RR 0.943/month, P = 0.022).

This cohort was utilized for the present study, in which
stored DNA samples of 220 participants were typed for polymorphisms in the promoter regions of TNF-\(\alpha\) (\(-238G/A\) and \(-308G/A\)) and TNF-\(\beta\) (\(-250G/C\)). Results were incorporated into Cox proportional hazards regression analyses (SAS statistical package, SAS Institute, Cary, NC, USA), to assess the effect of variant alleles at these loci in the development of subcutaneous fat wasting, with adjustment for known predictive variables.

Heterozygosity at the \(-238G/A\) TNF-\(\alpha\) polymorphism was found in 25 out of 220 individuals (11.4%), all of whom were of white racial origin. To characterize whether this TNF polymorphism exerted an effect on lipodystrophy progression, analyses were performed in a dataset limited to 191 white Caucasian participants, thereby excluding potential confounding attributable to the role of the TNF-\(\alpha\)-238G/A polymorphism as a surrogate marker of white racial origin. Carriage frequency in this restricted dataset was 25 out of 191 (13.1%). The risk of progression to lipodystrophy was significantly increased in \(-238G/A\) heterozygotes compared with carriers of the wild-type allele \((P = 0.014, \log\) rank, Fig. 1). Incorporation of this variable in Cox proportional hazards analyses demonstrated a significant independent effect of TNF-\(\alpha\)-238G/A heterozygosity \((RR 1.73, P = 0.041)\), which did not impact on the effects of other predictive variables (Table 1).

Promoter polymorphisms at TNF-\(\alpha\)-308G/A and TNF-\(\beta\)-250G/C were found not to contribute significantly to the Cox proportional hazards regressions \((P = 0.22\) and \(P = 0.12,\) respectively). In addition, analyses combining TNF-\(\alpha\)-238G/A heterozygosity and treatment-related variables as interaction terms did not suggest synergistic effects of TNF polymorphism and antiretroviral therapy on the progression of fat wasting.

In this study, carriage of the TNF-\(\alpha\)-238G/A promoter polymorphism independently increased the risk of lipodystrophy progression in a cohort of white Caucasian HAART recipients. Similar results have been presented by Maher and colleagues [6] in a UK case control study involving 96 participants, in which the \(-238G/A\) variant allele was more frequent among HAART recipients with lipodystrophy \((9/61, 14.7\%)\) than in those with no evidence of lipodystrophy \((0/35, 0\%, P = 0.01)\).

The possible explanations for the effect of the TNF-\(\alpha\)-238G/A promoter polymorphism on lipodystrophy progression are speculative at present. Altered TNF-\(\alpha\) expression may modulate the ‘downstream’ effects of antiretroviral therapy in adipose tissue, where this cytokine is involved in determining insulin sensitivity and the differentiation of adipocytes [7], as well as transducing apoptotic stimuli [8]. Consistent with this possibility, significantly elevated adipose TNF-\(\alpha\) protein expression has been found in individuals affected with lipodystrophy [3], whereas synergistic antidiapogenic effects of PI therapy and exogenous TNF-\(\alpha\) have also been noted in vitro [9]. Morphological studies of affected adipose tissue [10,11] in lipodystrophic HIV-infected individuals have also revealed the presence of macrophage-derived lipogranulomata that appear to be

### Table 1. Cox proportional hazards regression analyses of factors influencing progression to lipodystrophy in a cohort of 191 white Caucasian individuals.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>No TNF variable in model</th>
<th>With TNF variable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk ratio</td>
<td>P value</td>
</tr>
<tr>
<td>Age</td>
<td>1.055/year</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Time on dual NRTI</td>
<td>1.024/month</td>
<td>0.002</td>
</tr>
<tr>
<td>Time on stavudine</td>
<td>1.085/month</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Time on nevirapine</td>
<td>0.933/month</td>
<td>0.017</td>
</tr>
<tr>
<td>TNF-238 heterozygote</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

NRTI, Nucleoside reverse transcriptase inhibitors.
recruited to apoptotic cells, consistent with a role for inflammatory cytokines in tissue pathology. These results suggest that the presence of the -238G/A promoter polymorphism may influence lipodystrophy progression through a stimulatory effect on TNF-α transcription and expression. However, there is a lack of consensus regarding the effect of this polymorphism on TNF regulation, with evidence for both increased and decreased transcriptional activity in restricted cell lines [4]. Therefore, it is plausible that the -238 variant allele, which has strong linkage disequilibrium with specific allelic combinations within the major histocompatibility complex [4], may be acting as a surrogate marker in these analyses for an unknown genetic factor.

We conclude that the TNF-α-238G/A promoter polymorphism defines a host factor that may influence the progression of lipodystrophy, although the mechanism awaits characterization. The influence of treatment-related variables on lipodystrophy progression was not altered after adjustment for the presence of the TNF-α-238G/A polymorphism, indicating that the effects of therapy are not contingent on the presence of this host factor.

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References


Serum cortisol in HIV-infected patients with and without highly active antiretroviral therapy

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Cortisol serum levels were evaluated in clinically stable HIV-infected patients. Patients with metabolic disturbances had higher cortisol concentrations than those without (P = 0.007). Cortisol levels were associated with non-nucleoside reverse transcriptase inhibitors (NNRTI) (P < 0.0001) and protease inhibitors (P = 0.006), but not with nucleoside reverse transcriptase inhibitors only (P = 0.96). Untreated patients who started therapy experienced an increase in serum cortisol (P = 0.01). Only NNRTI (P = 0.01) and metabolic disturbances (P = 0.02) were significantly associated with cortisol levels in multivariate analysis.

Although many studies have analysed the adrenal function in HIV-infected patients [1–7], to our knowledge only two small studies have been performed in patients receiving highly active antiretroviral therapy (HAART) with contradictory results [8,9].

We prospectively studied a large number of stable patients, who underwent sequential measurements of serum cortisol to ascertain the prevalence of adrenal dysfunction, to analyse the factors that could be associated with cortisol levels, and in particular, to examine the possible influence of antiretroviral therapy.

The study group was composed of 197 adult patients (188 men, mean age 36.8 years), who underwent a total of 351 determinations of cortisol, as well as of other clinical and laboratory parameters, each separated by a mean interval of 28.1 weeks. The patients were seen at our outpatient clinic and none of them had identifiable severe, acute diseases at the time of sampling. All laboratory determinations were performed in the same blood samples collected between 08:00 and 08.30 a.m. because of the circadian rhythms. Cortisol was determined by immunochemoluminescence (auto-
matic analyser ADVIA: CENTAUR; Bayer Diagnos-
tics, Tarrytown, NY, USA). The range of normality was 166–773 nmol/l.

The distribution of cortisol values was Gaussian. Therefore, the Pearson’s correlation coefficient, Student’s t-test, one-way analysis of variance, and paired t-test were used as appropriate. The independent influence of diverse variables on cortisol concentrations was evaluated by a stepwise multiple linear regression analysis.

The mean serum cortisol for all determinations was 577 nmol/l (95% confidence interval, 555–599 nmol/l). Increased serum cortisol was found in 45 determinations (12.8%), and decreased concentrations in 10 determinations (2.8%). None of these patients had symptoms of hyper- or hypocortisolism. There was no significant correlation between cortisol and age ($P = 0.9$), duration of HAART ($P = 0.06$), or CD4 cell counts ($P = 0.9$).

Patients who had a past diagnosis of AIDS had significantly higher cortisol concentrations than patients without such a diagnosis (mean 599 versus 549 nmol/l, $P = 0.02$), and patients with an undetectable viral load had higher cortisol levels than patients with a detectable viral load (mean 612 versus 519 nmol/l, $P < 0.0001$).

Patients with metabolic disturbances had higher cortisol concentrations than patients without (mean 607 versus 546 nmol/l, $P = 0.007$). Among patients receiving HAART, those who had lipodystrophy as the only metabolic disturbance had lower cortisol levels than those with other metabolic disturbances (diabetes or hyperlipidemia) that did not include lipodystrophy (mean 579 versus 679 nmol/l, $P = 0.01$), and similar concentrations to patients who did not develop metabolic disturbances (560 nmol/l, $P = 0.6$).

Patients on HAART had higher cortisol values than untreated patients (mean 596 versus 486 nmol/l, $P < 0.0001$). Fig. 1 shows the mean values of cortisol according to different treatment groups. Patients on efavirenz had significantly higher cortisol concentrations than those on nevirapine (mean 668 versus 596 nmol/l, $P = 0.026$), and than those treated with protease inhibitors (PI) (mean 571 nmol/l, $P = 0.003$). Patients treated with nucleoside reverse transcriptase inhibitors (NRTI) plus PI who switched to NRTI plus non-nucleoside reverse transcriptase inhibitors (NNRTI) experienced an increase in their cortisol levels with respect to patients who continued with NRTI plus PI (mean 668 versus 541 nmol/l, $P = 0.049$). Finally, cortisol values clearly increased after the onset of HAART (from a mean of 455 to

![Fig. 1. Mean cortisol values in the five treatment groups.](image)

Fig. 1. Mean cortisol values in the five treatment groups. NNRTI, Non-nucleoside reverse transcriptase inhibitors; NO, no antiretroviral drug; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitors. Vertical bars represent the standard error.
651 nmol/l, \( P = 0.01 \) in 15 patients who had determinations before and after the initiation of therapy.

There was a trend towards higher cortisol concentrations over time \( (P = 0.056) \), which reached statistical significance between the first and the third determination \( (P = 0.02) \). A multiple regression analysis of all parameters evaluated revealed that only treatment with NNRTI \( (P = 0.01) \) and the presence of metabolic disturbances \( (P = 0.02) \) were significantly associated with cortisol levels.

This series constitutes, to our knowledge, the largest study approaching the adrenal function in stable, HAART-treated patients. Our results suggest that HAART increases cortisol levels, and this was confirmed by the subset of patients who started treatment during the study period. A direct effect of antiretroviral drugs on the cortisol metabolic pathways seems the most likely explanation for these findings, as all PI inhibit to different degrees the CYP3A4 isozyme \([10]\). In addition, PI have been found to inhibit the metabolism of other sterols, such as testosterone \([11]\).

Similarly to others \([12]\), we did not find significant differences in cortisol levels between patients treated with NRTI only and those untreated. However, the NNRTI-treated patients had higher cortisol values than the group taking PI. This difference was more marked in efavirenz-treated patients, a drug that both induces and inhibits CYP3A4. Efavirenz increases the area under the curve of ethynyl estradiol, a synthetic estrogen \([13]\), and the serum levels of 17β-estradiol \([14]\), but, to our knowledge, no effects on cortisol or other corticosteroids have been reported. Why patients treated with nevirapine, a pure CYP3A4 inducer, had similar levels to those treated with PI is unclear, but this observation suggests that the mechanism is not mediated through CYP3A4 inhibition.

Two previous studies failed to demonstrate any significant difference in cortisolemia between lipodystrophic patients and either non-lipodystrophic patients \([8]\) or healthy HIV-negative individuals \([9]\). Our study also demonstrates significantly higher cortisol levels in patients with laboratory abnormalities as the sole metabolic side-effect, than in those with lipodystrophy not accompanied by biochemical abnormalities.

We found progressively increasing cortisol levels over time. This increase cannot be attributed to a worsening in the condition of the patients and the subsequent stress, because they were clinically stable and, in fact, their immunological and virological parameters improved over time (data not shown). Probably, the reason for this increase was related to the higher frequency of antiretroviral treatment in subsequent determinations, which was related to increasing metabolic disturbances. In fact, both antiretroviral therapy and metabolic disturbances were associated with higher cortisol values in both the univariate and multivariate analyses.

We conclude that hypercortisolemia is commonly observed in stable HIV infection in the absence of clinical manifestations, whereas the prevalence of hypocortisolemia is substantially lower. Metabolic disturbances and antiretroviral treatment with drugs other than NRTI, particularly NNRTI, are associated with higher cortisol concentrations. Although alterations in the metabolic pathways of cortisol by these drugs could account for our findings, the intimate mechanism responsible for these interactions remains to be elucidated.

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In summary, the clinical consequences of treatment interruptions may include increased risk of advanced disease and CD4 cell loss, which can be significant. The results highlight the importance of monitoring patients closely during treatment interruptions and the need for patient education and support to encourage adherence to treatment regimens. Further research is needed to fully understand the impact of treatment interruptions on patient outcomes and to develop strategies to mitigate these risks.

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**Immunological changes during treatment interruptions: risk factors and clinical sequelae**

Mary B. Poulton, Caroline A. Sabin and Martin Fisher

This study highlights risk factors for CD4 cell losses and clinical consequences of antiretroviral treatment interruptions. The largest decreases in CD4 cell counts occur in patients with lower nadir CD4 cell counts or more advanced disease. Falls are greater in those with higher rates of CD4 cell loss before highly active antiretroviral therapy, and are associated with a significant increase in clinical/AIDS events during treatment interruption. Caution is therefore advised when considering treatment interruption in those with advanced disease.

Although the possible beneficial effects of treatment interruptions remain unproved, many patients stop therapy for a variety of reasons [1,2]. However, the information available to guide patients and clinicians with regard to the risks of CD4 cell loss or disease progression when taking a treatment interruption is limited [3–6]. In particular, few studies have assessed the clinical sequelae of treatment interruptions, and to date there are limited data suggesting clinical progression when therapy is stopped [2,7].

To study the possible risk factors for CD4 cell loss during treatment interruption and to assess the clinical sequelae, we identified patients attending the HIV clinic in Brighton who had taken a treatment interruption of at least 2 months. All patients had to have received highly active antiretroviral therapy (HAART) with three or more drugs for 3 or more months before taking a treatment interruption. In addition, patients had to have at least two CD4 cell counts off therapy during treatment interruption. Data were collected on demographic, treatment, CD4 cell count and viral load history, and clinical events, both on and off treatment.

Linear regression methods were used to assess the predictors of CD4 cell change. Univariate regression models considered the effects of the following variables on outcome: CD4 cell count; percentage and viral load at the start of treatment interruption; nadir CD4 cell count before treatment interruption; rate of CD4 cell loss before HAART (available for 28 patients); age; number of drugs and drug classes exposed to at the start of treatment interruption; time since first starting antiretroviral treatment; clinical stage at baseline; and whether the patient took a treatment interruption because of adverse events. Those factors significant in univariate models were included in a multivariable regression analysis. All analyses were performed using PROC REG in the statistical software package, SAS. To assess the risk of clinical progression during the treatment interruption, the event rate was calculated as the number of events divided by the person-years of follow-up, after starting HAART but before treatment interruption, and during treatment interruption itself. Differences in these rates were tested for significance using Poisson regression.

Forty patients met the criteria for inclusion. The median age of patients was 38 years (range 24–62). Thirty-nine were men (predominantly homosexual) and 33 were born in the UK (82.5%). The median nadir CD4 cell count was 182 cells/mm³ (range 6–523), with a median previous yearly rate of CD4 cell change of −59 cells/mm³ (range −294, 100). At the start of treatment interruption patients had been on treatment for a median of 2.6 years (range 0.5–10.5) and on HAART for a median of 1.5 years (range 0.5–4.0). Patients had been exposed to a median of six different antiretroviral agents (3–11), with 17 (42.5%) having been exposed to all three drug classes. At the time of treatment interruption, the majority of patients were receiving three drugs (36; 90%). Eighteen patients (45%) had a viral load of less than 500 copies/ml and 13 (32.5%) had a history of an AIDS-defining illness at baseline. The median length of treatment interruption was 214 days (61–1036).

There was a drop in the median CD4 cell count from 348 to 216 cells/mm³ during the treatment interruption, with a median individual patient drop of 88 cells/mm³ (drop of 443 to increase of 133) (P = 0.0001). CD4 cell percentages decreased from 17.5 to 12.5%, with a median patient drop of 5% (drop of 17% to increase of 4%, P = 0.0001). The viral load increased from 2.81 to 5.17 log₁₀ copies/ml, with individual changes ranging from a drop of 1.22 log₁₀ copies/ml to an increase of 4.08 log₁₀ copies/ml (median increase of 2.38 log₁₀ copies/ml, P = 0.0001). The individual plots of CD4 cell changes (Fig. 1) did not suggest any changes in the rate of CD4 cell loss over treatment interruption, but these results should be interpreted cautiously because of the possibility of information censoring as those with more rapid CD4 cell losses restart therapy. In a multivariable analysis, those with higher baseline CD4 cell counts (P = 0.02), but lower CD4 cell nadir (P = 0.02) lost more CD4 cells. In addition, the CD4 cell loss was greater in those with a higher rate of CD4 cell loss before starting HAART.
Patients were followed for a total of 41.7 person-years over treatment interruption. During this time, 31 patients (77.5%) experienced at least one clinical event, with 65 events occurring in total, of which six were AIDS-defining. The median time to the first clinical event (Kaplan–Meier) was 121 days, with 15, 30, 43 and 6 months after the start of the treatment interruption, respectively. This gave incidence rates of 1.56 per year for clinical events and 0.14 per year for AIDS events. Between starting antiretroviral therapy and the start of the treatment interruption, patients had experienced 106 clinical events, of which five were AIDS-defining, over a total follow-up period of 135.29 years. Incidence rates before the treatment interruption were 0.78 per year for clinical events and 0.04 per year for AIDS events. These rates were significantly lower ($P = 0.0001$ for all clinical events, $P = 0.03$ for AIDS events) than those seen during the treatment interruption.

Event rates in the different CD4 cell strata are shown in Table 1. At CD4 cell counts of less than 50 cells/mm$^3$, the event rate is actually higher while on treatment than during the treatment interruption, although this is not statistically significant. This probably reflects a selection bias, whereby of those with very low CD4 cell counts, only those without clinical events may risk stopping treatment. At higher CD4 cell counts, the event rate is higher in those taking a treatment interruption, and this is statistically significant for CD4 cell counts greater than 200 cells/mm$^3$.

In line with findings from other studies [4,5], our results suggest that those at most risk of a large CD4 cell loss are those who currently have high CD4 cell

<table>
<thead>
<tr>
<th>CD4 cell count (cells/mm$^3$)</th>
<th>While on treatment before treatment interruption</th>
<th>During treatment interruption</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>15</td>
<td>6</td>
<td>0.18</td>
</tr>
<tr>
<td>Patient-years</td>
<td>6.19</td>
<td>4.92</td>
<td></td>
</tr>
<tr>
<td>Rate/year (95% CI)</td>
<td>2.42 (1.36–4.00)</td>
<td>1.22 (0.45–2.65)</td>
<td>0.18</td>
</tr>
<tr>
<td>50–199</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>19</td>
<td>12</td>
<td>0.13</td>
</tr>
<tr>
<td>Patient-years</td>
<td>25.78</td>
<td>9.36</td>
<td></td>
</tr>
<tr>
<td>Rate/year (95% CI)</td>
<td>0.74 (0.44–1.15)</td>
<td>1.28 (0.66–2.24)</td>
<td>0.13</td>
</tr>
<tr>
<td>More than 200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events</td>
<td>70</td>
<td>47</td>
<td>0.001</td>
</tr>
<tr>
<td>Patient-years</td>
<td>93.49</td>
<td>27.46</td>
<td></td>
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<tr>
<td>Rate/year (95% CI)</td>
<td>0.75 (0.57–0.92)</td>
<td>1.71 (1.22–2.20)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

CI, Confidence interval.
counts but previously had much lower nadir values before starting treatment. These results may suggest some irreversible loss of immune function in those starting treatment at very low CD4 cell counts, and may thus have important implications for patients who choose to delay therapy to a late stage, or those who present late for treatment.

We have found an increase in the rates of clinical and AIDS events during treatment interruption. As clinical/ AIDS events are far more pertinent to patients than CD4 cell loss, these results are important. Our results suggest that treatment interruptions are independently associated with an increased risk of clinical events regardless of the CD4 cell loss, as the difference in risk is apparent in most CD4 cell strata. A finer stratification of CD4 cell counts is desirable, but with the sample size in this study, we were unable to do this. Larger studies that consider this issue are needed.

We have demonstrated that immunological changes seen in individuals during treatment interruption do indeed translate into clinical deterioration. The CD4 cell loss is greatest in those individuals with a history of advanced clinical disease or low nadir CD4 cell counts. We would urge caution in such individuals if treatment interruption is considered, and close monitoring during this time is essential to ensure that the risk of disease progression is minimized.

References


Older HIV-positive patients in the era of highly active antiretroviral therapy: changing of a scenario

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To determine the impact of highly active antiretroviral therapy (HAART) on the viro-immunological response of older HIV-positive patients, we prospectively selected 58 older cases and 116 younger controls. The CD4 T-cell count at baseline was lower in cases, whereas the HIV viral load was similar in both groups. Cases were more frequently affected by co-morbid conditions. Under HAART, a significant increase in CD4 T-cell numbers was observed in both groups, but there was no statistical difference regarding the response to HAART.

Most of the known epidemiological features of older HIV-infected patients were determined before the introduction of highly active antiretroviral therapy (HAART) in 1996 [1–5]. Since then a less beneficial effect of HAART on the immunological outcome in older compared with younger patients has been reported [6–8]. In fact, both the intensity [6,7] and the rapidity [8] of the immunological response appeared to be reduced in older patients, with few exceptions [4]. Interestingly, older age did not seem to affect the long-term virological outcome of HAART-treated patients significantly [6,9,10].

The aim of this study was to determine using a prospective case–control study the impact of HAART on the virological and immunological response in a cohort of older HIV-positive patients, when confounding variables such as adherence to therapy, side-effects and non–HIV-related co-morbidities were evaluated.

All patients presenting with a first HIV-positive test from January 1997 to December 2000, admitted to our in- and outpatient care unit, were considered.

Older (≥ 50 years) and younger (20–35 years) patients who were given HAART regularly and with a follow-up of at least 6 months were included as cases and controls, respectively (ratio 1 : 2). The control group
was matched by sex, year of HIV diagnosis and the presence of AIDS-defining conditions. A patient was considered regularly HAART-treated if he or she was under HAART for at least 3 months.

Three outcomes were considered: immunological success; virological success; and viro-immunological success, defined as a CD4 T-lymphocyte count greater than 200 cells/mm$^3$ and an HIV viral load less than 50 copies/ml, both conditions together, respectively, at the end of the follow-up.

In order to evaluate the significance of non-HIV-related co-morbid conditions, we used a modified version of the Charlson co-morbidity index [11].

Fifty-eight older patients (cases) were compared with 116 younger individuals (controls). Cases had a median age of 57.5 ± 5.5 years and controls 30.9 ± 3.6 years. Seventy-six percent of subjects in both groups were men and 48% were in stage C of HIV infection. The mean of CD4 T cells was significantly lower in cases (108 ± 111 versus 187 ± 199 cells/mm$^3$; $P = 0.005$), whereas the mean of the HIV viral load was similar in the two groups (4.97 ± 0.78 versus 4.88 ± 0.86 copies/ml; $P = NS$).

Cases had more co-morbid conditions than controls (44.8 versus 15.5%; $P < 0.0001$). One-third suffered from cardiovascular diseases, a rare condition among controls. Cases also had a higher mean Charlson index compared with controls (0.55 ± 0.98 versus 0.16 ± 0.5; $P < 0.0001$).

No statistically significant difference between cases and controls was observed in the type, number and duration of HAART regimens. The first-line antiretroviral therapy included protease inhibitors in more than 80% of cases and controls. Patients who required a second-line therapy received a cocktail including protease inhibitors in 43 versus 53% ($P = NS$) and non-nucleoside reverse transcriptase inhibitors in 57 versus 47% ($P = NS$) of cases and controls, respectively. A high level of adherence to HAART was observed in both groups (98 versus 93%; $P = NS$). The more frequent adverse reactions were dyslipidemia, digestive intolerance and lipodystrophy ($P = NS$).

Comparing the mean baseline with the end of follow-up values, a statistically significant increase in CD4 T-cell numbers was observed. There was a progressive linear enhancement at any individual time of the follow-up (Table 1).

Immunological success was observed in 69% of the cases and 79% of the controls ($P = NS$). The following variables were significantly associated with immunological success at univariate analysis: Centers for Disease Control and Prevention (CDC) stage A ($P = 0.01$), low Charlson index ($P = 0.01$), CD4 T-cell count at the beginning and at 6, 12, 18, 24, 30 and 36 months of HAART ($P < 0.01$), months to achieve CD4 T-cell count greater than 200 cells/mm$^3$ ($P = 0.001$), enhancement of CD4 T-cell count in the first 6 months ($P = 0.0003$), HIV viraemia at 30 months ($P = 0.003$).

A statistically significant reduction in the HIV viral load was observed in both cases and controls when comparing baseline with the end of the follow-up values. In particular, the means of the HIV-RNA viral load log decreased from 4.97 ± 0.78 to 1.7 ± 0.1 copies/ml in cases ($P < 0.0001$) and from 4.88 ± 0.86 to 1.97 ± 0.67 copies/ml in controls ($P < 0.0001$).

Virological success was observed in 79% of cases and 72% of controls ($P = NS$). The following variables were significantly associated with virological success at univariate analysis: CDC stage A ($P = 0.02$), high adherence to HAART ($P = 0.01$), dyslipidemia as an adverse effect of HAART ($P = 0.01$), HIV viral load at 6 ($P = 0.004$) and 24 months ($P = 0.02$).

Viro-immunological success was observed in 64% of cases and 62% of controls ($P = NS$). The following variables were significantly associated with viro-immunological success at univariate analysis: CDC stage A

<table>
<thead>
<tr>
<th>Months</th>
<th>n</th>
<th>Cases Mean ± SD</th>
<th>n</th>
<th>Controls Mean ± SD</th>
<th>$P$</th>
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<th>n</th>
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<th>$P$</th>
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<td>116</td>
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<td>237 ± 178</td>
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<td>62</td>
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<tr>
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<td>40</td>
<td>461 ± 235</td>
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<tr>
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<td>413 ± 167</td>
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<td>524 ± 225</td>
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<td>303 ± 183</td>
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<tr>
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<td>291 ± 117</td>
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</tr>
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</table>
high adherence to HAART \((P = 0.007)\), dyslipidemia as an adverse effect of HAART \((P = 0.006)\), CD4 T-cell numbers at baseline and at 6, 12, 18, 24, 30 and 36 months of HAART \((P < 0.01)\), enhancement of CD4 T-cell count in the first 6 months \((P = 0.001)\), HIV viral load at 6 \((P = 0.04)\) and 30 months \((P = 0.01)\).

Multivariate analysis showed that, after adjustment for sex and Charlson index, there was no statistically significant difference between cases and controls for immunological, virological and viro-immunological success \((P = \text{NS})\). Similar results were obtained when HIV- and HAART-related variables were added to the model (Table 2).

The benefits of antiretroviral therapy in HIV-positive older patients have not been up to now completely elucidated. In fact, clinical trials often exclude older patients because of multiple medical problems or co-existing non-HIV-related therapeutic regimens [12].

Since HAART became available, few reports have regarded its effects in older HIV-positive patients. A poor immunological response under HAART was observed [6,7], possibly caused by the depressed thymic function of older patients [6–8]. Only one report showed a similar virological and immunological outcome in older HIV-positive patients compared with younger individuals [9].

Our study confirms this preliminary observation and clearly demonstrates that older patients under HAART experienced a successful immunological response comparable to that of younger individuals. In fact, our older patients, starting HAART with a lower level of CD4 T cells, obtained a significant increment in their CD4 T-cell count, with the same rapidity, intensity and persistence in time as observed in younger patients. Previous observations have reported that high adherence to HAART allowed HIV-positive patients to achieve successful response [13,14]. Our findings indicate that this goal could be achieved independently of age. In fact, a substantial output of CD4 T cells can be maintained into late adulthood, thus contributing to HAART-related immune recovery in older HIV-positive patients [15–17].

It is of note that no differences were observed in virological response as indicated by viral load reduction in older patients compared with younger patients. This result is in line with previous reports [6,9], including a large study that indicated that older age decreased the risk of having a viral load greater than 1000 copies/ml in HIV-positive patients under HAART [10].

Univariate analysis showed an association of low Charlson index with immunological success. However, considering that the Charlson index was higher in older patients, none of the models of multivariate analysis indicated an association between co-morbidity and virological or immunological response. Interestingly, cardiovascular disease and diabetes were the more frequent co-morbidities in our older population. In view of the recent reports of premature atherosclerosis in younger HAART-treated patients [18,19], an important increase in coronary events in older HIV-positive patients under HAART could occur in the forthcoming years.

In conclusion, an early diagnosis of HIV infection in older patients is mandatory because the use of HAART allows them to achieve the same viro-immunological response as younger individuals.

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