Parameters Affecting the Development of Non-Hodgkin's Lymphoma in Patients With Severe Human Immunodeficiency Virus Infection Receiving Antiretroviral Therapy

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Purpose: To investigate the occurrence of non-Hodgkin's lymphoma (NHL) in human immunodeficiency virus (HIV)-infected patients receiving long-term antiretroviral therapy and factors associated with the development of these lymphomas.

Patients and Methods: The charts of 55 patients with advanced HIV infection receiving zidovudine (formerly known as azidothymidine [AZT]-based therapy and 61 patients receiving dideoxyinosine (ddI) were examined for the occurrence of NHL. Stored samples from the AZT-based treatment cohort were examined retrospectively for parameters predictive of the subsequent development of lymphoma.

Results: Eight of 55 patients receiving AZT-based therapy developed NHL, yielding an estimated probability of 12% (95% confidence interval [CI], 4.7% to 27.1%) after 24 months, and 29.2% (95% CI, 15.2% to 48.7%) after 36 months. Four of 61 patients receiving ddI developed NHL, yielding a 6.2% (95% CI, 2.1% to 17%) estimated probability after 24 months, and 9.5% (95% CI, 3.6% to 22.8%) after 36 months. The difference between these cohorts was not significant [two-tailed P (P2) = .13]. Patients with less than 50 CD4 cells/µl developed NHL at a significantly higher rate (P = .0085). This was particularly true for patients who presented with primary CNS lymphoma (PCNSL). For patients receiving AZT-based therapy, pretreatment serum interleukin-6 (IL-6) levels were somewhat higher in those who subsequently developed NHL than in those who did not (P = .048).

Conclusion: HIV-infected patients with profound immunodeficiency, especially those with less than 50 CD4 cells/µl, are at substantial risk of developing NHL and particularly PCNSL. Additional studies are needed to define the role of other factors such as IL-6 in the pathogenesis of these opportunistic tumors.

(29 patients on the phase I study of AZT, 551 eight patients on a pilot study of AZT with simultaneously administered acyclovir, 552 and 18 patients on a pilot study of AZT alternating with zalcitabine (ddC). 553 As noted, we have previously reported on the development of eight cases of NHL in this cohort; we now reexamine this cohort 1 year, 9 months after the previous analysis. Also included in the present analysis were 61 patients enrolled on the phase I studies of ddI and 2',3'-dideoxyadenosine (ddA) between February 1988 and November 1989. 554 Three of the 61 patients included in the ddI cohort received only ddA, an agent that is rapidly converted to ddI by the ubiquitous enzyme adenosine deaminase and can be considered a pro-drug of ddI. 555 An additional five patients in that cohort first received ddA and were then switched to ddI. This report includes all available data on these cohorts up until November 1, 1991.

When they entered onto the antiretroviral therapy protocols, all patients on these cohorts had either AIDS or severe AIDS-related complex (ARC). The ARC patients all had either oral candidiasis, oral hairy leukoplakia, or weight loss of greater than 10% of total body weight. Nearly all patients had less than 350 CD4 cells/µL at the time of entry. Patients with active or previously diagnosed NHL, a Karnofsky performance status of less than 70, active opportunistic infections, or an expected survival of less than 3 months were excluded. The patients receiving AZT-based therapy had not previously received antiretroviral therapy, and those in the ddI cohort generally had received either no prior therapy or less than 4 months of AZT. The median entry CD4 count was 71 cells/µL (range, 0 to 953) for the patients in the AZT-based treatment cohort, and 61 cells/µL (range 4 to 330) for the ddI cohort. In general, surviving patients in the ddI cohort have been monitored for a shorter period of time (maximum, 3 years 8 months) than those in the AZT-based treatment cohort (maximum, 4 years 10 months).

The patients in these studies were initially studied at the Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD, and many were monitored there until the time of death. Clinical and laboratory evaluations were performed every 1 to 4 weeks, and patients were admitted for evaluations and treatments when medically indicated. Certain patients were monitored by their referring physician after initially participating in NCI protocols. In these cases, we attempted to ascertain the time of death and information on any malignancies. For those patients who developed NHL, immunologic phenotype of the lymphoma cells was determined by immunoassaying with monoclonal antibodies to antigens expressed on B lymphocytes, T lymphocytes, and mononuclear phagocytes as described previously. 556, 557 Periodic evaluation of lymphocyte subsets reacting to Leu-3 (CD4, T4), helper-inducer T cells) or to Leu-2 (CD8, T8), suppressor-cytotoxic T cells) by flow cytometry were performed in all patients. In addition, serum and Ficoll-Hypaque-separated lymphocytes were obtained and frozen on each patient at entry and whenever lymphocyte subsets were measured. Coded lymphocytes and serum samples were periodically obtained and stored in liquid nitrogen. The 1987 revised CDC case-surveillance definition for AIDS was used to determine the onset of an AIDS-defining illness for each patient. 558

Serum IL-6 Activity

Serum IL-6 levels at entry were determined for 54 of the 55 patients receiving AZT-based antiretroviral therapy using the B9 cell assay with a reference standard IL-6 preparation, as previously described. 559 One IL-6 unit is defined as the quantity that induces one-half maximal stimulation of B9 cells under the conditions used, and corresponds to approximately 10 pg of a recombinant IL-6 preparation (Sandoz Pharma, Basel, Switzerland). Coded samples were stored at - 70° C. They were then heat-inactivated at 56°C for 30 minutes, and IL-6 levels were determined blindly in one large assay. Samples had previously been thawed and frozen only once before this time. Additional serum IL-6 levels at entry into the trial and periodically thereafter were assayed blindly in one batch for the eight patients receiving AZT-based therapy who developed NHL.

Soluble Interleukin-2 Receptors

Serum soluble interleukin-2 (IL-2) receptor levels at entry were determined for 54 of the 55 patients receiving AZT-based therapy using an enzyme-linked immunoadsorbent assay method as previously described. 560 The samples had been stored at -70°C and thawed and frozen twice before assay.

HIV P24 Antigen

Serum HIV p24 antigen (Ag) levels were determined for 54 of the 55 patients receiving AZT-based antiretroviral therapy at entry using a previously described antigen-capture assay. 561 Samples were stored in liquid nitrogen and then assayed blindly in one large batch.

Statistical Analysis

The method of Kaplan and Meier 562 was used to estimate the probability of a NHL developing in patients who remained alive. For both the AZT-based and ddI cohorts, patients were censored at the time of death due to any cause or when they were last known to be alive. Three patients in the AZT-based cohort, none of whom developed lymphoma, received ddI at some point during their treatment. In this analysis, they are considered as being in the AZT-based cohort for the entire length of follow-up. Confidence intervals (CIs) for the Kaplan-Meier analysis were determined using the method reported by Rothman. 563 The Mantel-Haenszel test was used to compare the estimated probability of developing lymphoma for the AZT-based treatment cohort and the ddI cohort over the entire period of follow-up.

The time at which a patient's CD4 count fell below various cutoff levels (200, 150, 100, and 50 cells/µL) was determined for each patient. In doing so, a "running" three-determination mean CD4 cell count was used instead of the actual CD4 count to reduce the effect of transient fluctuations in this parameter, as previously described. 564 CD4 counts increased in most patients after the start of antiretroviral therapy; the time points for falling below various cutoffs were determined after this initial increase. For statistical purposes, patients who were known to have died without developing lymphoma, but who were lost to follow-up before their date of death, were considered to have a CD4 count equal to their last known value until their date of death. This is a conservative estimate that will yield a CD4 count at least as high as the actual count at any given time. There were three such patients in the AZT-based treatment cohort and none in the ddI cohort. One patient in the AZT-based cohort and two in the ddI cohort were lost to follow-up. For statistical purposes, these patients were considered to have died immediately after their last clinical visit.

CIs for the rate of lymphoma developing per patient-year within a range of CD4 cells were likelihood-based, under the assumed Poisson distribution of the number of lymphomas. The overall risk of developing a lymphoma for patients with greater than or less than 50 CD4 cells/µL was evaluated using the test for difference in Poisson rates. 565 The statistical significance of CD4 cells as a risk marker for lymphoma by year of antiretroviral therapy was determined using...
the exact binomial distribution of the difference between the observed and the expected number of lymphomas, as previously described. The incidence of NHL in patients with greater than 50 CD4 cells/µL in the AZT-based treatment cohort compared with the ddl cohort was examined by Fisher's exact test.

A two-group analysis of the statistical significance for various entry parameters of the 55 AZT-based treatment patients was performed using the Wilcoxon rank-sum test for the null hypothesis that a parameter was unrelated to the development of NHL during the first 3 years of follow-up. A paired analysis of the IL-6 levels at entry and within 4 months of lymphoma was performed for the eight patients who developed NHL, using the exact Wilcoxon signed-rank test.

RESULTS

Development of Lymphomas

We previously reported that as of February 1990, eight of 55 patients in the AZT-based treatment cohort developed NHL. On reanalysis of this cohort 1 year, 9 months later, we find that none of the 15 patients who were alive at the time of the initial analysis have since developed lymphoma, and eight have subsequently died of other AIDS-related illnesses. In all, eight of the 55 patients (14.5%) have developed lymphoma, 39 have died without developing lymphoma, seven are still alive, and one has been lost to follow-up. These patients have been monitored for up to 4.8 years. Based on the updated data, the probability of a patient in that cohort developing NHL after 24 months of AZT-based antiretroviral therapy is 12% (95% CI, 4.7% to 27.1%), increasing to 29.2% (95% CI, 15.2% to 48.7%) after 36 months (Fig 1).

Of the 61 patients in the ddl cohort (now monitored for up to 3.7 years), NHL has so far developed in four patients (6.6%) (Table 1; patients no. 9 through 12). These four lymphomas were diagnosed a median of 17.4 months (range, 1.1 to 26.6) after the patients' entry onto the study. Histologically, they were all high-grade, B-cell tumors; three were small noncleaved-cell non-Burkitt's lymphoma (SNCCCL) and one was a Burkitt's lymphoma (BL) (Table 1). One tumor presented as a primary CNS lymphoma (PCNSL), and the other three presented with visceral disease (including one patient with primary hepatic lymphoma). This last patient developed fevers and hepatic dysfunction within weeks of starting on ddA (a pro-drug of ddl), and it is possible that he had preclinical lymphoma when he entered the protocol. Three patients died 1.3, 3.7, and 4.5 months after the diagnosis of lymphoma (patients no. 10, 9, and 11, respectively). The fourth patient (patient no. 12) is still alive 8.5 months following diagnosis. In all, 28 patients in the ddl cohort died without developing NHL, 27 patients are still alive, and two patients were lost to follow-up. The estimated probability of developing a NHL within 2 years of starting therapy was 6.2% (95% CI, 2.1% to 17%), increasing to 9.5% (95% CI of 3.6% to 22.8%) after 3 years (Fig 1). While this figure is somewhat lower than that in the AZT-based treatment population, the difference between the two cohorts is not statistically significant (two-tailed \( P = 0.13 \)). The risk of lymphoma in this cohort may change as the patients are monitored for a longer period of time.

If we combine the AZT-based and ddl cohorts to form one large 116-patient cohort, a total of 12 patients developed NHL (10.3%). Overall, the estimated probability of a patient in this combined cohort developing NHL within 2 years of starting antiretroviral therapy was 8.4% (95% CI, 4.1% to 16.6%), increasing to 19.4% (95% CI, 10.9% to 32%) after 3 years (Fig 2).

CD4 Counts and NHL

When we previously reported on the development of NHL in the cohort of patients on AZT-based therapy, we noted that the patients tended to have CD4 counts of less than 50 cells/µL at the time lymphoma developed. We have now analyzed the relationship between CD4 counts and the hazard of lymphoma in this cohort over an extended observation period. In all, the hazard of developing lymphoma in patients with less than 50 CD4 cells/µL in this cohort was 0.164 lymphomas per patient-year (95% CI, 0.075 to 0.306 lymphomas/patient-year), as compared with a rate of 0 lymphomas per patient-year (95% CI, 0 to 0.033 lymphomas/patient-year) in patients with greater than 50 CD4 cells/µL (\( P = 0.008 \)). The estimated risk of a patient in this cohort developing NHL after having less than 50 CD4 cells/µL for 24 months was 26.9% (95% CI, 12.7% to 48.2%). Forty-one of the 55 patients in this cohort had a CD4 count greater than 50 cells/µL at some time during the follow-up period (28 of these patients had > 50 CD4 cells/µL at entry). Six of these 41 patients subsequently developed NHL. All six of these patients had less than 50 CD4 cells/µL at the time of NHL diagnosis.
Table 1. NHLs in HIV-Infected Adult Patients Receiving Long-Term Antiretroviral Therapy at the NCI

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Antiretroviral Regimen</th>
<th>CD4 Cells/µL at NHL Diagnosis</th>
<th>Cell Type</th>
<th>Immunologic Phenotype</th>
<th>Stage at Diagnosis</th>
<th>Site of Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AZT</td>
<td>14</td>
<td>LCIBL</td>
<td>B cell</td>
<td>IE</td>
<td>Lung</td>
</tr>
<tr>
<td>2</td>
<td>AZT</td>
<td>8</td>
<td>LCIBL</td>
<td>B cell</td>
<td>IV</td>
<td>Esophage, liver, spleen</td>
</tr>
<tr>
<td>3</td>
<td>AZT</td>
<td>21</td>
<td>LCIBL</td>
<td>Null cell</td>
<td>IV</td>
<td>Ascitic fluid, pleural fluid</td>
</tr>
<tr>
<td>4</td>
<td>AZT and acyclovir</td>
<td>4</td>
<td>SNCCl</td>
<td>B cell</td>
<td>IE</td>
<td>Brain</td>
</tr>
<tr>
<td>5</td>
<td>AZT and d4C</td>
<td>4</td>
<td>SNCCl</td>
<td>B cell</td>
<td>IE</td>
<td>Brain</td>
</tr>
<tr>
<td>6</td>
<td>AZT and acyclovir</td>
<td>19</td>
<td>LCIBL</td>
<td>B cell</td>
<td>IE</td>
<td>Brain</td>
</tr>
<tr>
<td>7</td>
<td>AZT and d4C</td>
<td>4</td>
<td>SNCCl</td>
<td>B cell</td>
<td>IE</td>
<td>Leptomeningias</td>
</tr>
<tr>
<td>8</td>
<td>AZT and acyclovir</td>
<td>4</td>
<td>SNCCl</td>
<td>B cell</td>
<td>IV</td>
<td>Liver</td>
</tr>
<tr>
<td>9</td>
<td>dda*</td>
<td>236</td>
<td>SNCCl</td>
<td>B cell</td>
<td>IV</td>
<td>Lung, liver, rectus muscle</td>
</tr>
<tr>
<td>10</td>
<td>ddi</td>
<td>10</td>
<td>SNCCl</td>
<td>B cell</td>
<td>IE</td>
<td>Brain</td>
</tr>
<tr>
<td>11</td>
<td>ddi</td>
<td>11</td>
<td>SNCCl</td>
<td>B cell</td>
<td>IV</td>
<td>Liver</td>
</tr>
<tr>
<td>12</td>
<td>ddi</td>
<td>220</td>
<td>BL</td>
<td>B cell</td>
<td>IV</td>
<td>Liver, tonsil, pelvis</td>
</tr>
</tbody>
</table>

Abbreviations: LCIBL, large-cell immunoblastic lymphoma; BL, Burkitt's lymphoma; SNCCl, small noncleaved-cell lymphoma.
*dda is rapidly converted to ddi in the body.

(P2 = .0066 for the null hypothesis that having > or < 50 CD4 cells/µL did not influence the incidence of lymphoma in this cohort of 41 patients). Thus, in this study, patients in the AZT-based treatment cohort with greater than 50 CD4 cells/µL at entry or at any time after initiating antiretroviral therapy did not develop NHL until their CD4 counts fell below 50 cells/µL.

We have now performed additional analyses and developed new data regarding the relationship between CD4 count and the development of lymphoma in the combined cohort of patients on AZT-based and ddl therapy (Table 2). The median CD4 cell count for the 12 patients who developed lymphoma at the time of lymphoma diagnosis was 11 cells/µL (range, 4 to 236). Patients in this combined cohort developed two lymphomas during the period they had greater than 50 CD4 cells/µL; representing 125.9 patient-years of observation (0.016 lymphomas/patient-year; 95% CI, 0.003 to 0.049 lymphomas/patient-year). The patients developed 10 lymphomas during the period they had less than 50 CD4 cells/µL, representing 102.4 patient-years of observation (0.098 lymphomas/patient-year; 95% CI, 0.049 to 0.171 lymphomas/patient-year). The null hypothesis that a CD4 count of greater or less than 50 CD4 cells/µL does not affect the probability of lymphoma developing is rejected, with a two-tailed P value of .0085. The effect of CD4 count on the hazard of developing NHL was still significant after adjusting for length of time on antiretroviral therapy (P2 = .015).

We further examined this relationship for various subsets of lymphomas. Each of the six patients who presented with PCNSL had less than 50 CD4 cells/µL at the time of diagnosis, and the null hypothesis that a CD4 count of greater or less than 50 CD4 cells/µL does not affect the probability of developing a PCNSL was rejected, with a two-tailed P value of .016. By contrast, two of six patients who presented with systemic lymphoma had greater than 50 CD4 cells/µL at the time of diagnosis, and the relationship between CD4 count and the probability of developing systemic lymphoma was not statistically significant in this small subset (P2 = .42). Overall, patients diagnosed with a PCNSL had significantly lower CD4 counts at the time of diagnosis than those with systemic lymphoma (P2 = .019). By contrast, there was no statistically significant relationship between the CD4 count at diagnosis and the histologic subtype of lymphoma (SNCCl v large-cell immunoblastic lymphoma [LCIBL]; P2 = .34).

Entry Parameters and the Subsequent Risk of Lymphoma

Several lines of evidence have suggested that stimulation by IL-6 may play a role in the development of B-cell tumors.30-34 Moreover, most HIV-infected patients have elevated IL-6 levels compared with non–HIV-infected controls.21-35 With this background, we explored the possibility
Table 2. Relationship Between CD4 Count and the Immediate Risk of Lymphoma in Patients Receiving Long-Term Antiretroviral Therapy

<table>
<thead>
<tr>
<th>CD4 Count Range (cells/µL)</th>
<th>Patient-Years of Observation</th>
<th>No. of Lymphomas</th>
<th>Hazard Rate for the Development of Lymphoma (lymphomas/patient-year)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 200</td>
<td>63.3</td>
<td>2</td>
<td>0.032</td>
<td>0.005-0.098</td>
</tr>
<tr>
<td>150 to 200</td>
<td>16.5</td>
<td>0</td>
<td>0</td>
<td>0-0.116</td>
</tr>
<tr>
<td>100 to 150</td>
<td>20.3</td>
<td>0</td>
<td>0</td>
<td>0-0.094</td>
</tr>
<tr>
<td>50 to 100</td>
<td>25.8</td>
<td>0</td>
<td>0</td>
<td>0-0.074</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>102.4</td>
<td>10</td>
<td>0.098</td>
<td>0.049-0.171</td>
</tr>
</tbody>
</table>

that there might be a relationship between the serum IL-6 levels at entry in our patients and the development of NHL over a 3-year period. This analysis was performed only for the AZT-based treatment cohort, as a number of patients in the ddl cohort had not yet been monitored for 3 years. Entry serum samples were available for all but one of the patients in the AZT-based treatment cohort. The mean entry serum IL-6 level for the eight patients who developed NHL was 13.1 U/mL ± 1.3 U/mL (arithmetic mean ± SEM), while the entry serum IL-6 level of 46 assessable patients who did not develop NHL was 10.7 U/mL ± 0.3 U/mL (Table 3). This difference was statistically significant, with a two-tailed P value of .048. When we examined IL-6 levels in patients who were alive 1 year after entering the study, the mean level in those who subsequently developed lymphoma was still higher than the mean IL-6 levels in those who did not, although the difference was not statistically significant (P2 = .27) (data not shown). For those patients who developed lymphoma, there was generally an increase in IL-6 levels from entry until the time that lymphomas developed (Fig 3). Much of this increase occurred after the first year on therapy. However, patients who did not develop lymphomas also had an increase in IL-6 levels (data not shown). Analysis of later time points in these two groups is complicated by the number of patients who either died or for whom later specimens were not available, and the significance of this finding is not clear.

We next examined a variety of other parameters at the time of entry for evidence of a relationship to the development of NHL. The other entry parameters examined were quantitative immunoglobulin G and A levels, Epstein-Barr virus titers, soluble IL-2 receptor levels, CD4 and CD8 cell counts, and HIV p24 Ag levels (Table 3), While there were some differences in the mean values of these parameters between the patients who did and did not develop lymphoma, none of these differences were statistically significant. However, it should be noted that all of the patients in this study had either AIDS or advanced ARC at entry, and it is possible that a statistically significant relationship would be apparent in cohorts of HIV-infected patients monitored from an earlier stage of disease.

Table 3. Entry Parameters in 55 Patients Who Did or Did Not Go on to Develop Lymphoma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients Who Developed NHL (n = 8)</th>
<th>Patients Who Did Not Develop NHL (n = 47)</th>
<th>P* (two-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-6 (U/mL)</td>
<td>13.1 ± 1.3</td>
<td>10.7 ± 0.3</td>
<td>.048</td>
</tr>
<tr>
<td>Serum IgG (mg/dl)</td>
<td>1,775 ± 220</td>
<td>1,760 ± 105</td>
<td>.91 (NS)</td>
</tr>
<tr>
<td>Serum IgA (mg/dl)</td>
<td>428 ± 60</td>
<td>421 ± 32</td>
<td>.75 (NS)</td>
</tr>
<tr>
<td>Epstein-Barr virus titer (G.M.)</td>
<td>1.550 X± 1.15</td>
<td>1.761 X± 1.19</td>
<td>.34 (NS)</td>
</tr>
<tr>
<td>Serum IL-2 receptors (U/mL)</td>
<td>1,755 ± 337</td>
<td>1,599 ± 218</td>
<td>.35 (NS)</td>
</tr>
<tr>
<td>CD4+ cells/µL</td>
<td>46 ± 18</td>
<td>134 ± 25</td>
<td>.21 (NS)</td>
</tr>
<tr>
<td>CD8+ cells/µL</td>
<td>527 ± 94</td>
<td>569 ± 58</td>
<td>.90 (NS)</td>
</tr>
<tr>
<td>Serum HIV p24 Ag (pg/ml)</td>
<td>517 ± 391</td>
<td>129 ± 30</td>
<td>.82 (NS)</td>
</tr>
</tbody>
</table>

Abbreviations: IgG, immunoglobulin G; IgA, immunoglobulin A; G.M., geometric mean; Ag, antigen; NS, not significant.

*P values for the null hypothesis that the parameter was unrelated to the development of NHL during 3 years of follow-up.

DISCUSSION

We previously reported on the development of NHL in eight patients in a cohort of 55 patients with AIDS or ARC receiving AZT-based therapy.9 We now report the occurrence of four NHLs in a separate cohort of 61 HIV-infected patients receiving ddl-based therapy. In addition, we document a relationship between a low CD4 count and the occurrence of these lymphomas, particularly in patients presenting with PCNSL. Finally, we provide evidence suggesting that patients with high serum IL-6 levels may have a greater risk of later developing NHL.

These results highlight the importance of NHL as a late complication in patients with HIV infection. Overall, in the combined cohort of 116 patients with AIDS or ARC receiving antiretroviral therapy, the estimated probability of a NHL developing after 3 years was 19%, with an overall yearly incidence of 5.6% per patient-year. (However, it
should be noted that the risk of NHL was not constant, but rather increased over time on antiretroviral therapy.)

An association between tumors, particularly NHL, and primary or secondary immunodeficiency states has been appreciated for some time.37,39 However, as seen here, this association has taken on a new significance with the advent of the AIDS epidemic. The association between HIV infection and aggressive NHL has been appreciated for some time15; indeed, the CDC included certain types of NHL as an AIDS-defining complication in 1985,4 and, in 1991, they documented that 3.4% of adult patients in the United States who developed AIDS had NHL as their initial AIDS-defining illness.7 However, this figure generally does not include NHL developing after another AIDS-defining complication, and we believe it is certainly an underrepresentation of the actual incidence.

On the other hand, the incidence of NHL in our cohort is somewhat higher than that found in reports of certain other cohorts. For example, Moore et al40 identified only 24 NHLs in a review of the clinical records of 1,030 patients with advanced HIV infection receiving AZT therapy, yielding an estimated risk of 3.2% after 2 years. However, the patients in their study had a somewhat better clinical status at entry than those in our cohorts (a median CD4 count of 104 cells/µL in their study compared with 62 cells/µL in our combined cohort). Another possibility is that some lymphomas may have been missed outside of a research setting; indeed, several of the lymphomas in our study could have easily been mistaken for other manifestations of HIV infection in patients with advanced AIDS. For example, two PCNSLs were diagnosed in patients with previously treated cerebral toxoplasmosis upon biopsy of enlarging brain lesions, and one PCNSL was only diagnosed at necropsy. In support of this possibility is the relatively high incidence of NHL reported in necropsy series of patients with HIV infection.41-44 For example, in one autopsy series of 101 patients with AIDS, 20 patients had NHL, and five of these patients had PCNSL diagnosed only at necropsy.41 At the same time, it should be noted that the denominator of patients on our study was relatively small. Prospective studies of larger cohorts followed carefully until death will be needed to get a better sense of the overall incidence of this condition.

It is noteworthy that many of the lymphomas in our study occurred in patients with less than 50 CD4 cells/µL. The patients in these cohorts were among the first to receive active antiretroviral therapy and to have the advantage of recent advances in the prophylaxis and treatment of opportunistic infections. It is reasonable to speculate that a prolongation of survival, particularly in patients with less than 50 CD4 cells/µL, may have contributed to the high cumulative incidence of NHL seen in this study. Before the availability of antiretroviral therapy and effective prophylaxis (and therapy) of opportunistic infections, patients with AIDS commonly died, even those with comparatively high CD4 counts. Now, in the United States death from any cause is comparatively uncommon until the CD4 count falls below 50 cells/µL.27,45,46 Thus, as we have improved our antiretroviral and supportive therapies for HIV infection, CD4 has, in effect, become a better mortality risk indicator. The relationship between improved survival of patients with AIDS and the development of NHL is analogous to that previously observed in certain primary immunodeficiency diseases, such as Wiskott-Aldrich syndrome, in which the cumulative incidence of NHL increased as patients lived longer as a result of improved supportive therapies.47 However, while the incidence of NHL in our cohort is certainly high, it is difficult to identify similarly monitored historical cohorts before the use of antiretroviral therapy for comparison.

When we initially observed a higher incidence of NHL in our AZT-based treatment cohort, we wondered whether the dideoxynucleoside anti-HIV drugs might have had a direct effect on the development of lymphomas. Indeed, animal carcinogenesis studies have shown that rats and mice administered lifelong high-dose AZT develop vaginal malignancies.48 However, the metabolism of AZT in humans differs from that in rodents that humans glucuronidate AZT in the liver to 5'-O-glucuronyl-AZT (GAZT), while rodents do...
produce high levels of IL-6, and that it can increase reactive cells of patients with high-grade lymphomas. B-cell lines and tumors, that tumor-infiltrating finding that it can act as an autocrine growth factor for other settings. In particular, a potential role of IL-6 in the pathogenesis of NHL was supported by the report by Neri et al of monoclonal, episomal Epstein-Barr virus genome in HIV-associated NHL, suggesting that Epstein-Barr virus infection precedes the clonal B-cell expansion. Also, the overall high levels of IL-6 in patients with HIV infection suggested that this cytokine may indeed be contributing to the higher incidence of NHL. Stimulation of certain B-cell subpopulations by IL-6 may increase the probability of other tumorigenic events, such as translocations involving c-myc. The finding here that patients who went on to develop lymphoma had somewhat higher IL-6 levels at entry than those who did not develop lymphoma adds some additional weight to this hypothesis. However, it should be noted that the difference in IL-6 levels between the groups in our study was relatively small, and it is possible that this relationship was a chance occurrence. (Since we initially examined only IL-6 levels at entry, we did not formally correct our statistical analysis for the other parameters subsequently examined.) Other well-defined cohorts should be examined to determine if this relationship is consistent. It will also be important to examine a range of other lymphokines and cytokines for a possible relationship to the development of NHL.

More striking than the association of high IL-6 levels with the subsequent development of NHL was the finding that these tumors (particularly those of the CNS) were most frequent in patients with CD4 counts less than 50 cells/µL. An association between CNS lymphomas and very low CD4 counts has also been noted by Levine et al. As noted previously, there is evidence from our group and others that a CD4 count of less than 50 cells/µL is a good hazard marker for the risk of death in HIV-infected patients. As such, the development of effective antiretroviral therapy may also have an impact on the incidence of NHL. While a prolongation of life in patients with less than 50 CD4 cells/µL may at the same time increase the cumulative incidence of NHL, newer antiretroviral treatments and strategies that can maintain CD4 cells greater than 50 CD4 cells/µL may in fact prevent or delay the occurrence of these tumors. In addition, identification of parameters predictive for the development of NHL in HIV-infected patients may allow closer surveillance for lymphomas and may lead to strategies to prevent their occurrence.

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