Role of Zidovudine Antiretroviral Therapy in the Pathogenesis of Acquired Immunodeficiency Syndrome-Related Lymphoma

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The role of zidovudine and other antiretroviral agents in the pathogenesis of acquired immunodeficiency syndrome (AIDS)-related lymphomas has been somewhat controversial. In an attempt to elucidate the precise role of antiretroviral agents in the subsequent development of AIDS-related lymphoma, we performed a population-based, case-control study of human immunodeficiency virus (HIV)-seropositive patients with intermediate- or high-grade lymphoma in Los Angeles County, California, in which information regarding use of antiretroviral medications was ascertained. Diagnostic biopsy material was reviewed to confirm intermediate-grade disease, and patients with intermediate- or high-grade lymphoma in Los Angeles County, in which information regarding use of zidovudine and other antiretroviral agents. A total of 112 HIV-infected homosexual/bisexual men with lymphoma were matched to 112 homosexual/bisexual men with asymptomatic HIV infection; 49 of the lymphoma cases were also matched to 49 additional controls with AIDS, as defined by conditions other than lymphoma. Positive histories of zidovudine use were reported by 44 (39%) lymphoma cases, 24 (21%) asymptomatic HIV controls, and 21 (42%) AIDS controls. The average duration of zidovudine use up to 12 months before lymphoma diagnosis was 19.0 ± 13.0 months (mean ± SD) for the lymphoma cases, 12.6 ± 10.5 months for the asymptomatic controls, and 11.0 ± 7.1 months for the AIDS controls. When comparing the 49 HIV-positive lymphoma cases with their 49 matched AIDS controls, all of whom were diagnosed with AIDS during the same time period, the matched relative odds of lymphoma associated with prior use of zidovudine was 0.43 (95% confidence interval [CI] = 0.17 to 1.12). In comparing all 112 lymphoma cases with 49 AIDS controls, the unmatched relative odds of lymphoma associated with zidovudine use was 0.93 (95% confidence interval = 0.47 to 1.83). One lymphoma case and no AIDS control cases had a history of didanosine use; no lymphoma case or AIDS control cases had taken zalcitabine. We conclude that zidovudine is not associated with an increased risk of development of lymphoma among HIV-infected homosexual or bisexual men.

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The precise etiology of the AIDS-related lymphomas is not fully understood. At the molecular level, it is evident that several distinct aberrations may be found, including presence of monoclonal Epstein-Barr viral genomes in large-cell or immunoblastic lymphomas,7,6 alterations of the c-myc oncogene and p53 suppressor gene in the small noncleaved types,8,9 and alterations of the bcl-6 oncogene in some cases of diffuse large-cell lymphoma.10 Although HIV, per se, is not directly involved in the development of these lymphomas, it may be indirectly involved by inducing expression of multiple cytokines, such as interleukin-6 (IL-6), IL-10, and others, that cause on-going B-cell proliferation, activation, and differentiation.12,13

The epidemiological factors associated with development of AIDS-related lymphoma are largely unknown. All groups at risk for HIV appear to be at approximately equal risk for developing lymphoma,17 in contrast to Kaposi's sarcoma, which occurs primarily in homosexual or bisexual men.18,19 Clinically, the factors associated with development of lymphoma include increasing immune deficiency, as evidenced by CD4 lymphocytes less than 200/μL,6,20 and, in one study, elevated serum IL-6 levels.21 Several studies have suggested that use of zidovudine (AZT) or other antiretroviral medications may be etiologically related to the development of AIDS-related lymphoma, whereas other studies have concluded that AZT was not etiologic.22,23 Herein, we report the results of a population-based, case-control study of patients with intermediate- or high-grade lymphoma, diagnosed in Los Angeles County, in which information regarding use of AZT and other antiretroviral medications was ascertained in an attempt to elucidate the precise role of AZT in the subsequent development of AIDS-related lymphoma. This study was part of a larger case-control study of lymphoma that included both HIV-positive and HIV-negative patients.

MATERIALS AND METHODS

Study design and eligibility requirements. Patients living in the County of Los Angeles, between the ages of 20 and 74 years, who were newly diagnosed with lymphoma were identified by the Cancer Surveillance Program, the population-based cancer registry for Los Angeles County, using a rapid case reporting system. To be eligible,
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a case must have been diagnosed with high- or intermediate-grade lymphoma\textsuperscript{27} between April 1989 and November 1992, must speak English or Spanish, must be a resident of Los Angeles County at the time of lymphoma diagnosis, must be willing to undergo a personal interview, and must sign an informed consent, approved by the Human Research Committee of the University of Southern California School of Medicine.

One HIV-positive asymptomatic control subject was individually selected and matched to each eligible HIV-positive lymphoma case, based on year of birth within 3 years of the case, race and ethnicity, gender, language of interview, and specific mode of transmission of initial HIV infection. These asymptomatic HIV-infected controls were identified at a County- or University-affiliated clinic in the community. Furthermore, using the same matching criteria, 50 of the HIV-infected lymphoma cases were also matched to a second control, who was a patient with AIDS, based on a diagnosis other than lymphoma, and who was identified through the records at the Los Angeles County-University of Southern California Medical Center.

Interview. Personal interviews were conducted with each case and control in a matched pair (HIV-positive lymphoma case plus HIV-positive asymptomatic control) or triplet (case, HIV-positive asymptomatic control, and AIDS without lymphoma control) by two trained nurse-epidemiologists. The structured questionnaire obtained information on the respondents’ lifetime history of medication usage; medical history; hospitalizations and special treatments such as radiotherapy, blood transfusions, and anesthetic exposures; smoking and alcohol intake history; use of illicit drugs; family medical history; and occupational and household exposure to a series of substances. Specific medications were selected for inclusion in the questionnaire based on several a priori criteria, including prior evidence of an association with lymphoma, established effects on the immune system, or use to treat diseases that themselves may be associated with greater risk of lymphoma. Exposure information was collected up to the date that was 12 months before the date of the patient’s being diagnosed with lymphoma, or, for matched controls, up to the date that was 12 months before their matched lymphoma patient’s diagnosis.

The specific questions regarding medication use were as follows, “Prior to (reference date), did you ever take or use any of the following medications, for at least one month continually, or for at least a one month’s treatment regimen? If yes, what were the first and last years you took the drug? How many months in all did you take it? Why did you take this drug?” The following drugs were specifically mentioned: cortisone ointments (ie, Aristocort, Celestone, decadron, diconason, Kenalog, metiderm, pramosone, topical, topicort, valisone); estrogens; androgens/body builders; atabrine; weight loss drugs and shots; diabase, tolaine, or other oral drugs for diabetes; insulin shots; valium, librium, or drugs for “nerves”; aspirin daily for at least one month; morin or indocin; butazolidine; flagyl; AZT; other antiretroviral drugs; other drugs (specify).

Although internal checks and balances within each interview were performed, to assure consistency of data reported, we did not routinely perform chart reviews to verify the accuracy of information reported by the patients or controls.

Confirmatory testing of HIV status. Blood samples were obtained from all patients and controls. HIV status was determined by enzyme-linked immunosorbent assay, with confirmatory Western blot, performed by standard methods. A total of 120 eligible cases were HIV-seropositive. Of these, 113 were homosexual or bisexual men.

Assessment of lymphoma pathology. Diagnostic pathology materials were requested from all study participants to allow uniform review and to restrict eligibility to patients with high- or intermediate-grade lymphoma.\textsuperscript{27} All materials received were reviewed and classified by two expert hematopathologists (B.N.N. and D.S.). Pathology materials were available and reviewed in 112 (93.3\%) of the 120 HIV-infected lymphoma cases. All were confirmed to have high- or intermediate-grade lymphomas. In the absence of available materials, the original pathology reports and results of immunophenotypic analyses were carefully reviewed. Based on this evaluation, the remaining 8 HIV-infected lymphoma patients were also determined to be eligible for study.

Statistical analyses. The individual matching of cases and controls was retained in the statistical analyses, except when all cases were compared with AIDS controls. Odds ratios (ORs) were estimated using logistic regression methods.\textsuperscript{28} The 95\% confidence intervals (CIs) for the ORs were estimated using the logarithm of the OR and its standard error. Where appropriate, a single degree of freedom test was used to assess the significance of linear trend with increasing exposure.

One HIV-positive lymphoma case did not provide information regarding antiretroviral drug use. Therefore, the analyses presented herein are restricted to the 112 HIV-infected homosexual/bisexual men with lymphoma and their matched controls. In evaluating use of antiretroviral medications among lymphoma cases and controls, differences were strongly dependent on the year of study entry. Thus, subjects who entered study within the first 2 years were more likely to have taken AZT and less likely to have taken other antiretrovirals, such as didanosine (ddI) or zalcitabine (ddC). Furthermore, routine use of AZT changed over the course of this study; after approximately 1990, it became accepted medical practice to use AZT in asymptomatic seropositive individuals with less than 500/\mu{}L CD4 cells.\textsuperscript{29} Before that time, AZT use was primarily restricted to patients with AIDS or symptomatic HIV disease. Thus, comparisons of cases with asymptomatic controls could provide misleading estimates of relative risk. Furthermore, exposure information was collected up to the date that was 1 year before diagnosis of lymphoma, and appropriate evaluation of CD4 counts in our patients was not uniformly performed at that time, nor was it available retrospectively from medical records. Thus, to control for the changing patterns of antiretroviral use and for our inability to control for specific CD4 counts among subjects and controls, we restricted the detailed analyses of AZT usage to the 49 lymphoma cases in homosexual/bisexual men in whom case-specific AIDS controls had been obtained. AZT use in cases with AIDS-lymphoma was thus compared with use of the drug in controls who had full-blown AIDS diagnosed within the same time period but based on a diagnosis other than lymphoma.

RESULTS

A total of 527 patients with high- and intermediate-grade non-Hodgkin’s lymphoma who met all eligibility requirements for the study underwent personal interview. An additional 658 patients with high- or intermediate-grade lymphoma had died before the time that an interview could be performed; 44 further cases were too ill to be interviewed, and 145 refused to participate. Primary physicians denied permission to contact an additional 57 patients who had been identified by the Cancer Surveillance Program.

Serum for assessment of HIV status was obtained on all 527 interviewed patients with lymphoma. HIV seropositivity was confirmed in 120 patients, of whom 113 were homosexual or bisexual males. Distribution by age and by racial and ethnic group for the 113 HIV-seropositive lymphoma cases and their asymptomatic seropositive and AIDS controls is presented in Table 1. The median age for lymphoma cases was 38.4 years (range, 21.5 to 66.8 years), whereas that for asymptomatic controls was 38.2 years (range, 22.3 to 64.5

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years). Median age for the AIDS controls was 36.4 years (range, 24.1 to 59.8 years). Caucasians comprised 68% of the cases and 71.2% of the combined control groups, whereas 26.6% of cases and 25.8% of controls were Latino and 3.5% of cases and 2.5% of controls were African-American.

Results of consensus pathological review are presented in Table 2. High-grade lymphoma was diagnosed in 78 of the cases (65%), whereas intermediate-grade was confirmed in 33 (28%). One case was diagnosed as intermediate-high-grade lymphoma, not otherwise specified, and pathological material was inadequate for consensus review in 8 (7%).

Initial analyses on AZT usage are presented for 112 homosexual men with lymphoma and their 112 asymptomatic and 49 AIDS controls; 1 case did not provide information regarding AZT, and both the case and his 2 controls were removed from the analyses. A history of AZT use was found in 44 (39%) lymphoma cases, 24 (21%) asymptomatic HIV controls, and 21 (42%) AIDS controls. The average duration (mean ± SD) of AZT use up to 12 months before lymphoma diagnosis was 19.0 ± 13.0 months in the lymphoma cases, 12.6 ± 10.5 months in the asymptomatic controls, and 11.0 ± 7.1 months in the AIDS controls.

More detailed analyses of AZT usage were restricted to 49 AIDS-lymphoma cases and their 49 matched controls, all of whom had full blown AIDS based on a diagnosis other than lymphoma. Six case/control pairs took AZT as the only antiretroviral drug; 23 case/control pairs did not take AZT; 6 lymphoma cases took AZT, whereas the matched AIDS control did not; and 14 control subjects took AZT, whereas the matched lymphoma case did not. Among these 12 lymphoma cases and 20 AIDS controls who used AZT, the average months of use were 13.1 ± 10.5 for cases and 11.0 ± 7.1 for controls.

When comparing the 49 HIV-positive lymphoma cases with their 49 matched AIDS controls, the relative odds of lymphoma associated with prior use of AZT was 0.43 (95% CI = 0.17 to 1.12). In comparing the 112 lymphoma cases with each of their controls (asymptomatic HIV-seropositive and HIV-seropositive AIDS without lymphoma), the matched relative risk of lymphoma associated with any use of AZT was 1.64 (95% CI = 0.94 to 2.85). This elevated OR reflects the greater likelihood over time of asymptomatic controls being treated with AZT. In using all 112 lymphoma cases but retaining only the AIDS controls and no longer retaining the individual matching in the analyses, the relative odds of lymphoma associated with AZT use was 0.93 (95% CI = 0.47 to 1.83).

Because patients with AIDS-related primary CNS lymphoma may have different biological and immunological characteristics of disease when compared with those with systemic lymphomatous disease, we also ascertained the specific extrastral sites of lymphoma in our subjects. Among the 112 HIV-positive case-control pairs, 12 had CNS lymphoma. Among the 49 HIV-positive cases matched to specific AIDS controls, 5 had CNS lymphoma. Among the 12 total CNS lymphoma cases, 8 received AZT and 4 did not. Of the 5 CNS lymphoma cases who were matched to specific AIDS controls, 3 had taken AZT, whereas 2 had not.

We also evaluated any relationship between ddI use and subsequent development of lymphoma. One case of AIDS-lymphoma took ddI alone, whereas no AIDS-control subject had taken this drug.

The role of ddC as a risk factor for AIDS-lymphoma could not be ascertained, because no AIDS-lymphoma case and no matched AIDS-control had taken this drug, which was licensed for use in HIV infection in 1992.

**DISCUSSION**

The potential role of AZT in the development of AIDS-lymphoma has been controversial. In 1992, an autopsy series of 637 consecutive Italian patients with AIDS showed that 42 (6.6%) had primary CNS lymphoma, whereas an additional 53 (8.3%) had systemic AIDS-related lymphoma. The frequency of primary CNS lymphoma was found to correlate with higher cumulative doses of AZT received, occurring in 6.3% of patients who had not received AZT, in 8% of those who had received a total cumulative dose less than 300 grams, and in 12.5% of those who had received a cumulative dose of greater than 300 grams of AZT (P = .06). No correlation was found between cumulative AZT use and subsequent development of systemic lymphoma. An additional study by Pluda and colleagues also suggested the possibility that, based on the long-term follow-up of 116 patients with symptomatic HIV disease who had been accrued on various trials of antiretroviral agents (primarily treatment).

| Table 1. Demographic Data on Cases and Controls |
|-----------------|-----------------|-----------------|
| Age Group (yr)  | No. of Lymphoma Cases (%) | No. of AIDS-Controls (%) | No. of Asymptomatic HIV Controls (%) |
| 20-29           | 16 (14.2)        | 11 (22.0)        | 21 (18.6)        |
| 30-39           | 53 (46.9)        | 24 (48.0)        | 49 (43.4)        |
| 40-49           | 30 (26.6)        | 10 (20)          | 30 (26.6)        |
| 50+             | 14 (12.4)        | 5 (10)           | 13 (11.5)        |
| Racial/ethnic group |                |                  |                  |
| Caucasian       | 77 (68.1)        | 36 (72.0)        | 80 (70.8)        |
| Latino/Hispanic | 30 (26.6)        | 12 (24.0)        | 30 (26.6)        |
| African-American| 4 (3.5)          | 1 (2.0)          | 3 (2.7)          |
| Asian           | 2 (1.8)          | 1 (2.0)          | 0 (0.0)          |

| Table 2. Results of Pathological Diagnosis: 120 Cases of AIDS-Lymphoma |
|-----------------|-----------------|
| Specific Diagnosis                      | Frequency |
| Intermediate grade                      |            |
| Follicular, large cell                  | 3           |
| Diffuse, small cleaved                   | 1           |
| Diffuse, mixed small and large          | 5           |
| Diffuse, large cell                     | 24          |
| High grade                              |            |
| Immunoblastic, large cell               | 24          |
| Small non-cleaved                       | 50          |
| Small non-cleaved, Burkitt              | 3           |
| Lymphoblastic, non-convoluted           | 1           |
| High/Intermediate, NOS                  | 1           |
| Material inadequate for diagnosis       | 8           |
consisting of AZT), AZT might be involved in the pathogenesis of AIDS-related lymphoma. At 36 months of follow-up, 19% of the cohort had developed lymphoma, consisting of primary CNS lymphoma in the majority, thus suggesting the possibility that AZT may have contributed to the pathogenesis of these tumors. However, the clinical, pathological, and immunological characteristics of the lymphomas were identical to those that had been described in HIV-infected lymphoma patients who had not received AZT,16,23 and the investigators concluded that AZT usage was probably not an etiologic factor, although this question could not be addressed with certainty. The unexpected occurrence of thymic lymphoma developing among laboratory mice who received a closely related antiretroviral drug, ddC (dideoxyctydine), provided additional support for the concept that antiretroviral compounds might play a role in the subsequent development of AIDS-related lymphoma,19 especially because two of the eight lymphoma patients in Pluda’s cohort had received both AZT and ddC.21 Of further importance, AZT itself can act as a weak mutagen.31

In contrast to these data, additional studies have suggested that AZT probably does not play a role in the pathogenesis of AIDS-related lymphoma. Thus, Moore et al studied 1,030 patients with symptomatic HIV disease, all of whom had received AZT and were followed up for 1,463 person years.25 The median time from onset of AZT therapy to diagnosis of lymphoma was 308 days (range, 14 to 683 days). The probability of lymphoma was 0.8% at 6 months, 1.6% at 12 months, 2.4% at 18 months, and 3.2% at 24 months after institution of AZT therapy. Thus, the relative risk of developing lymphoma was stable at 0.8% for each additional 6 months of AZT therapy. Furthermore, the relative hazard of developing lymphoma was the same in the first 6 months of AZT therapy as that after 18 months, with no relationship to total cumulative dose. Because the time between initiation of AZT and first diagnosis of AIDS was longer in patients who developed lymphoma than in those who did not, the investigators speculated that the lymphomas occurred as a consequence of prolonged survival in a state of profound immunocompromise. It was concluded that AZT, per se, was not etiologic in the development of AIDS-related lymphoma.25

Similar to the data discussed above, the current large, carefully controlled study has not shown a statistical relationship between prior use of AZT or duration of AZT use and subsequent development of AIDS-lymphoma. Because the widespread use of AZT in asymptomatic HIV-infected individuals changed during the time course of this study so that asymptomatic subjects accrued later in the study were more likely to have received the drug, and because retrospective analyses of CD4 cell counts were not available to provide a matching criterion for our patients and controls, the most valid control group for our patients with AIDS-lymphoma appeared to be case-specific matched controls with full-blown AIDS, as defined by conditions other than lymphoma. In comparing these pairs, there was no evidence, either in subjects with primary CNS or with systemic lymphomatous disease, that AZT use was associated with an increased risk of development of AIDS-lymphoma. Although it is possible that the role of AZT as a causative factor could have been masked by the fact that the nonlymphoma AIDS controls had lower CD4 counts and, thus, were potentially on AZT longer, this was not, in fact, the case, because the average months of AZT use was similar among lymphoma cases and nonlymphoma AIDS controls. Furthermore, there is evidence that AIDS-lymphoma occurs when CD4 counts are extremely low10,21,24 and certainly not higher than in the majority of other AIDS-defining conditions. In terms of power calculations, for the comparison of 49 lymphoma cases and 49 individually matched AIDS controls, this study had sufficient power to rule out a threefold increased risk of lymphoma associated with AZT use. When comparing the 112 lymphoma cases with 49 AIDS controls, adequate statistical power was present to rule out a 2.5-fold increased risk of lymphoma associated with use of AZT. The roles of ddI and ddC could not be ascertained, because only 1 AIDS-lymphoma patient had taken ddI (versus no control) and no lymphoma case or matched AIDS control had taken ddC, presumably secondary to the time frame during which the study was conducted. Because the incidence of lymphoma increases with the degree of immunocompromise,25,26 it is probable that AZT and other antiretroviral agents may simply function to prolong survival, providing additional time in which multiple immunological, virologic, and genetic factors may converge, leading eventually to the development of AIDS-related lymphoma.

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