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ZIDOVUDINE THERAPY, CD4+ AND CD8+ COUNTS ARE ASSOCIATED WITH A LONGER SURVIVAL FOLLOWING AIDS ONSET

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ABSTRACT

In 60 patients followed from the onset of acquired immunodeficiency syndrome (AIDS) to death, survival was determined by Cox Proportional Hazards Analysis in relationship to seven variables: time-dependent CD4+ and CD8+ peripheral lymphocyte counts, zidovudine treatment, cytomegalovirus (CMV) retinitis, time from AIDS onset, calendar year of AIDS onset (cohort effect), and age. Two significant prognostic variables were identified: zidovudine therapy and either CD4+ or CD8+ counts (the latter could not be distinguished due to concomitant high correlation). Treatment with zidovudine reduced the death rate by 75% compared to no treatment. When included in a proportional hazards regression with all covariates except for the other T lymphocyte count, every increase in CD4+ count of 10 cells was equivalent to a decline in the mortality rate by 13% (p=0.046), and every increase in CD8+ count of 10 cells lowered the mortality by 1.4% (p=0.0031). Patients treated with zidovudine and without CMV retinitis showed the slowest decline of both CD4+ and CD8+ counts. Both CD4+ and CD8+ levels are useful predictors of survival in patients with AIDS.

In HIV-1 infected patients, the fall in peripheral CD4+ lymphocyte counts predicts progression to AIDS and an adverse outcome (1-3). A higher CD4+ count at baseline (4) and improved CD4+ counts with zidovudine therapy (5) are prognostic of longer survival. Nonetheless, CD4+ count below 200 x 10^6 is also compatible with lack of progression to AIDS and prolonged survival (6,7).

In order to predict more accurately the outcome of HIV-1 positive patients treated with antiretroviral therapy, other prognostic indicators besides peripheral counts of CD4+ lymphocytes need to be identified. For example, antiviral activity of CD8+ lymphocytes is a dominant defense mechanism against both HIV-1 retrovirus (8-10) and cytomegalovirus (CMV) (11). We evaluated, therefore, seven variables as potential markers for prolonged survival using Cox Proportional Hazards Analysis. Treatment with zidovudine was associated with maintenance of peripheral CD4+ and CD8+ lymphocyte counts and significantly enhanced survival.

SUBJECTS AND METHODS

Clinical Study

Retrospective analysis of data was performed on 60 patients who satisfied the following criteria: 1) First admission with AIDS to the Eisenhower Medical Center

(EMC), Rancho Mirage, California, between 1986 and 1990 (MF), 2) Survival of at least 60 days from the onset of AIDS, 3) Clinical follow-up since the onset of AIDS regarding opportunistic infections including direct ophthalmoscopic examination regarding CMV retinitis (12), and 4) T lymphocyte counts at the time of AIDS diagnosis and at least 4 subsequent counts. The range of patient survival times after AIDS onset was 81-2405 days. All patients, except one woman, were homosexual men. The median age was 41.0 years compared to the median age, 43.0 years, of 128 patients with AIDS admitted to the hospital of EMC between 1987 and 1990. The mean number of T lymphocyte subset assays obtained in each patient (1- to 3-monthly intervals) was 6.9. The indication for zidovudine therapy (200 mg five times daily) was a peripheral CD4+ lymphocyte count less than 0.2 x 10^9/L, which was almost always accompanied by the onset of signs and symptoms of AIDS. Zidovudine therapy was prescribed for the remaining life-span but the drug was frequently omitted in late stages of AIDS as patient compliance became poorer due, for example, to dementia or intolerance of oral drug medication. Zidovudine therapy was, however, not uniformly available between 1986 and 1987, and both treated and untreated patients with AIDS were encountered in the study population. Fourteen patients did not and 46 patients did receive zidovudine. No other anti-retroviral drug was available during the study period. The treatment modalities of opportunistic infections did not change substantially during the study. Thus (a *Pneumocystis carinii* prophylaxis included, since 1986, oral trimethoprim-sulfamethoxazole or dapsone prescribed according to patient's tolerance; aerosolized pentamidine became available in 1987 but had only limited usage; b) *Pneumocystis carinii* therapy included in all years either intravenous trimethoprim-sulfamethoxazole or intravenous pentamidine or both in succession as tolerated and efficacious, c) CMV retinitis and CMV neurological complications were treated with intravenous ganciclovir since 1996 (12); foscarnet became available in 1991, d) candida esophagitis and cryptococcal meningitis were treated with amphotericin B; fluconazole became available in 1991, e) clofazimine, ethambutol, amikacin, and rifampin were used to treat *Mycobacterium avium-intracellulare* infections; prophylaxis with clarithromycin was not available until 1991. HIV-infected symptomatic patients were anergic to Candida, tetanus toxoid and mumps antigens already at the stage of AIDS-related complex as described previously (13) and, therefore, the effect of anergy on patient survival could not be evaluated.

Laboratory Study

Lymphocyte subsets reacting to anti-Leu3a/CD4 and anti-Leu2a/CD8 lymphocytes/antibodies (Beckton Dickinson, San Jose, CA) were assayed by flow cytometry on an Epic 5 apparatus (Coulter Corporation, Hialeah, FL) at 1 to 3 month intervals by Immuno, Biogene, Inc., Los Angeles. EDTA-anticoagulated blood samples were processed within 24 hours of collection. The CD4+ count was computed as the number of lymphocytes multiplied by the percentage of CD4+. Comparison of split blood samples showed maximum variation in CD4+ counts of less than 2%.

Statistical Analysis

Cox proportional hazard model

Survival analysis was performed by the Cox multivariate proportional hazard model with 7 time-dependent covariates (14). To calculate the covariates, each patient's follow-up was divided into intervals between clinical visits and between the last visit and the time of death. The time-dependent covariates were: CD4+ and CD8+ average counts in each interval, therapy with zidovudine (by

TABLE 1
Cox Regression with the Covariables CD4+, Zidovudine, CMV Retinitis, Time From AIDS Onset, Onset of AIDS by Year, and Age

Variable	Paramater estimate	Risk ratio	p value
CD4+ (per 10 cells)	-0.013	0.88	0.046
Zidovudine	-1.370	0.25	0.0053
CMV retinitis	-0.43		0.35
Time from AIDS onset	0.16		0.46
AIDS onset by year	0.040		0.78
Age	0.00057		0.98

TABLE 2
Cox Regression with the Covariables CD8+, Zidovudine, CMV Retinitis, Time From AIDS Onset, Onset of AIDS by Year, and Age

Variable	Regression coefficient	Risk ratio	p value
CD8+ (per 10 cells)	-0.0014	0.986	0.021
zidovudine	-1.44	0.24	0.0031
CMV retinitis	-0.47	0.62	0.30
Time from AIDS onset	0.21	1.23	0.32
Calendar year of AIDS onset	0.10	1.11	0.46
Age	-0.0042	0.996	0.83

intention to treat yes/no), CMV retinitis (yes/no), calendar year of AIDS onset, time from AIDS onset, and age. The Cox regression was carried out using the program PROC PHREG in the statistical package SAS. Fitting of the data to the proportional hazards model gives the change in hazard function (instantaneous death rate) with a given change in the value of the covariate. The results were expressed as the risk ratio (the hazard functions with/without a particular variable) which is equivalent to the instantaneous death rate associated with the variable and its associated p value. The proportional hazards assumptions (the ratio of the hazard between two subjects with given covariates being constant with time) were verified for zidovudine, CD4+ and CD8+ counts using the cox.zph function in S+, which determines the regression coefficients as a function of time. Relatively constant regression coefficients were found indicating agreement with the proportional hazards assumptions.

The mean survival times for patients with and without zidovudine treatment, corrected for the appropriate covariates, were calculated by the Cox regression.

TABLE 3
Cox Regression with the Covariables Zidovudine, CD8+ and CD4+ Counts

Status of CD4+	Status of CD8+	Regression coefficient*	Risk ratio*	p value*
included	excluded	-1.25	0.286	0.0124
excluded	included	-1.29	0.273	0.0093
included	included	-1.14	0.321	0.0236

*for zidovudine

Rate of decline of CD4+ and CD8+ counts and zidovudine therapy

CD4+ and CD8+ data can be approximated by log-normal distributions and were, therefore, analyzed by geometric means. Thirty-one patients were evaluated concurrently regarding T cell status, CMV retinitis and zidovudine use. Mean T cell counts were plotted for each trimester after AIDS diagnosis in patient subgroups divided according to zidovudine use and CMV retinitis.For analysis, patients were assigned to each group according to their status at that point in time. In this analysis were included nine patients (mean age 41.9 years, mean AIDS onset April 1986) who received no antiretroviral therapy, and 22 patients (mean age 41.3 years, mean AIDS onset December 1988) who received zidovudine therapy after the onset of AIDS.

RESULTS

Multivariate Proportional Hazard Analysis of Survival

Cox proportional hazards regression analysis takes into account current CD4+ count, current CD8+ count, zidovudine use, presence of CMV retinitis, time from AIDS onset, calendar year of AIDS onset, and age. Treatment with zidovudine strongly improved survival *(Tables 1,2)*. The risk ratio for persons treated with zidovudine was 0.25 or 0.24 depending on the regression model used with p values of <0.01. Because the likelihood of improved survival using zidovudine increased with the calendar year, the calendar year of AIDS onset (cohort effect) was controlled for by inclusion in the Cox multivariate regression. Even with calendar year of AIDS onset included in the Cox multivariate regression, zidovudine treatment remained highly significant with a risk ratio far below 1.0. Age was also considered as a possible covariate; however, after controlling for other factors, the effect of age was not significant. None of the other variables except T cell counts approached statistical significance *(Tables 1,2)*.

Both CD4+ and CD8+ counts significantly increased survival in the regression with 5 other covariates, but not with each other. CD4+ count reduced the hazard rate by 0.88 and CD8+ by 0.986 per 10 cells, respectively. The CD8+ count was a slightly better predictor of survival than CD4+ count. The p value for the CD8+ was 0.021 and the corresponding likelihood ratio for the model with CD8+ was 292.8 (the lower absolute value corresponds to a better fit), whereas the p value for CD4+ was 0.046 with the likelihood ratio 302.1. It is not possible to determine from this analysis whether the controlling variable is CD8+, CD4+, or both.

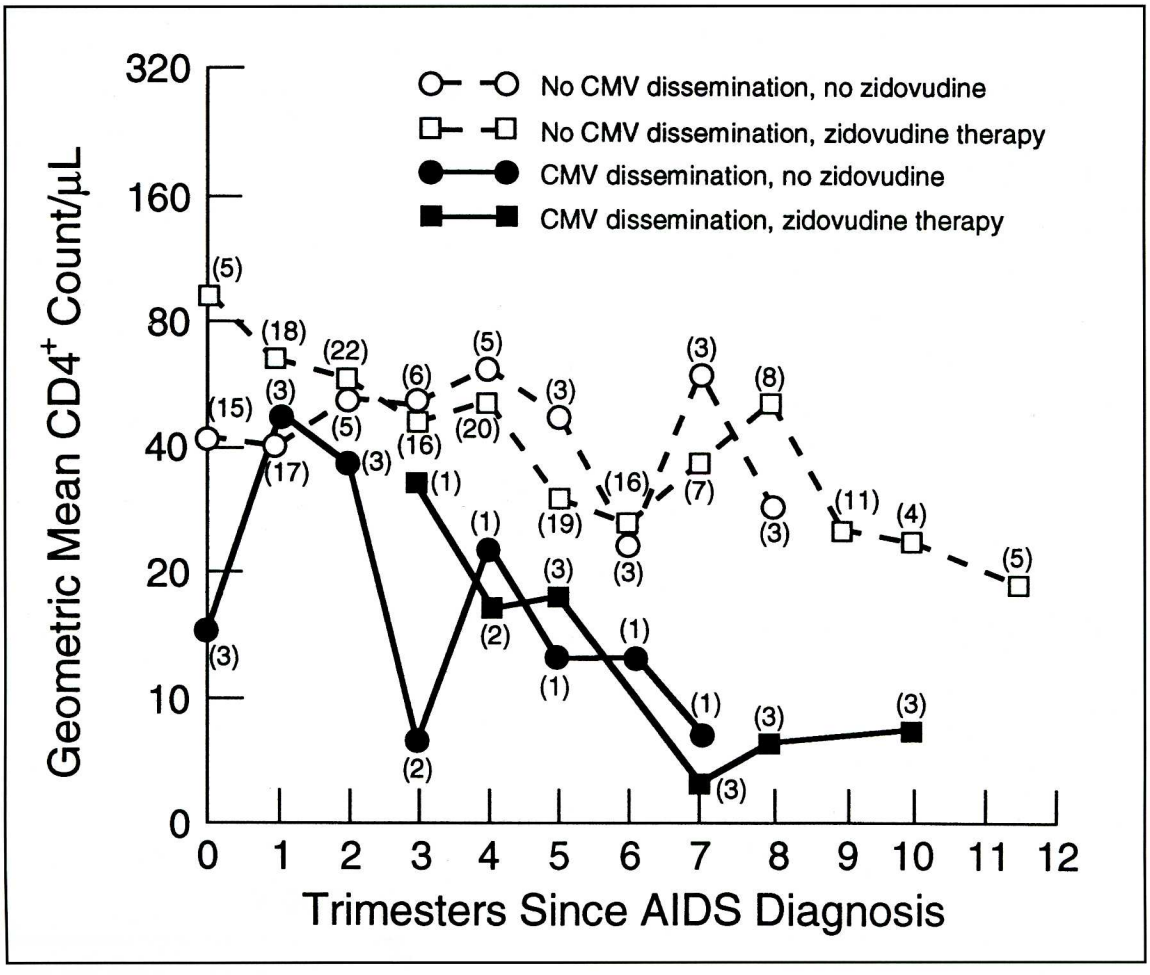

Fig. 1. The course of peripheral CD4+ lymphocyte counts after AIDS onset in patient groups subdivided with respect to current zidovudine use and current CMV dissemination (the number at each symbol indicate the number of patients in each group at that time): Geometric mean counts charted at three-monthly intervals (standard error of the means was approximately 50%).

They both had a correlation coefficient of 0.40. The significance, regression coefficient, and risk ratio for the other variables were similar, regardless of whether the regression included CD4+ alone, CD8+ alone, or both together *(see Table 3)* using the covariate zidovudine.

For patients without zidovudine treatment, the mean patient survival (calculated by the Cox regression) was 0.68 years (median survival—0.80 years), whereas for those treated the mean survival was 1.92 years (median survival—1.50 years) after onset of AIDS. The calendar year of treatment alone had no impact on survival. When both zidovudine treatment and calendar year of treatment onset were included, the effect of zidovudine treatment was highly significant (p value = 0.0093), whereas the p value for the calendar year alone was 0.67. If zidovudine treatment was excluded, the calendar year still showed no effect on survival (p value = 0.23). These findings suggest that the beneficial effect of zidovudine was real and not merely a consequence of a presumed correlation of

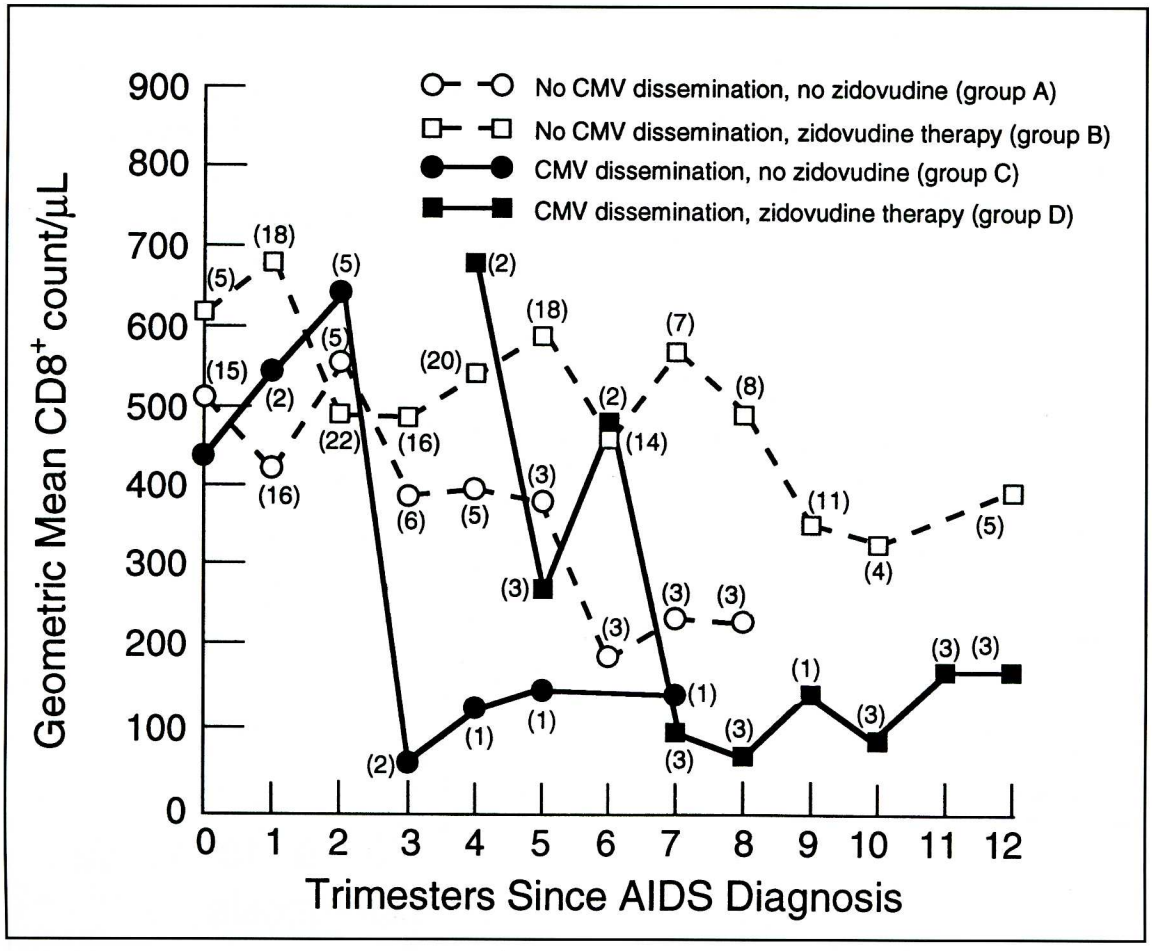

Fig. 2. The course of peripheral CD8+ lymphocyte counts after AIDS onset in patient groups subdivided with respect to current zidovudine use and current CMV dissemination (the numbers at each symbol indicate the number of patients in each group at that time): Geometric mean counts charted at three-monthly intervals (standard error of the means was approximately 50%).

probability of zidovudine treatment and general improvement in AIDS treatment with an increasing calendar year.

Relationship of CD8+ and CD4+ status to zidovudine use, CMV retinitis and survival

Previously we observed a temporal relationship between the decline of CD8+ below 0.500 x 10^9 and the onset of late opportunistic infections with CMV and *M. avium-intracellulare* (15). In this study we charted the decline of CD4+ and CD8+ separately for patients with or without zidovudine therapy, as well as with or without CMV dissemination. The decline of CD4+ preceded that of CD8+ (note the different vertical scales in *Figs. 1 and 2*). Patients treated with zidovudine and without CMV retinitis had the slowest decline of CD4+ counts *(Fig. 1)*. These patients also displayed the slowest fall of CD8+ counts, whereas those without zidovudine therapy and with CMV dissemination had the fastest decline of CD8+ counts *(Fig.2)*.

DISCUSSION

Survival analysis using the Cox multivariate regression method with time dependent covariates takes proper account of censored data and covariate values changing with time. The results showed that both zidovudine, and CD4+ and CD8+ time-dependent counts increased patient survival after onset of AIDS. Although the treated and untreated patient groups did not differ with respect to age, duration of AIDS-related complex, or manifestations of AIDS, the study lacked randomization. The treatment modalities, apart from zidovudine, however, did not change substantially during the study and no significant effect of the calendar year (cohort effect) was demonstrated. The median survival of zidovudine-treated patients after AIDS onset noted in this study, 1.50 years, was similar to that observed by the German AIDS study group (16),namely 1.56 years, and by Vella et al in Italy (17), namely 1.76 years. The median survival times of untreated patients were 0.80 years in this study, approximately 0.68 in the "German study," and 0.8 years in the "Italian study."

CD8+ lymphocyte counts have seldom been evaluated in HIV-infected patients for prediction of survival or development of opportunistic infection. One study implicated increased CD8+ counts at an early stage of HIV infection in hemophiliac patients to a more rapid CD4+ decline (18). Two recent studies showed that a higher CD8+ count (after 24 weeks of therapy or at baseline) predicted better prognosis in multivariate or univariate models (4,5). In our study, zidovudine use was associated with maintenance of a better CD8+ count for as long as 2 years from the onset of AIDS, but with only a brief effect on CD4+ counts as observed by others (19-24). The association between maintenance of CD8+ and zidovudine therapy was most apparent in the subgroup of patients without CMV retinitis suggesting the two processes are interrelated — that is, control of CMV dissemination by zidovudine therapy and decreased risk of CMV dissemination in patients with maintenance of CD8+ status. Maintenance of CD8+ by zidovudine may explain the protective effect of zidovudine therapy on survival extending beyond the putative supporting effect on CD4+. Therapeutical approaches designed to increase the number and function of circulating CD8+ lymphocytes may benefit AIDS patients (24-26).

Because HIV does not destroy CD8+, another virus, such as CMV, capable of infecting CD8+ cells and shortening patient survival, should be considered as a cofactor in CD8+ destruction. CMV infected CD8+ lymphocytes in transplant patients (27), involved a high proportion of CD4+ and CD8+ lymphocytes, macrophages and polymorphonuclear leukocytes in patients with AIDS (28), and participated in lysis of HIV-infected lymphoblasts *in vitro* (29). HIV infection of CD8+ cells was found only in a small fraction of CD8+ cells and was not cytopathic (30).

Our findings suggest that the peripheral CD8+ lymphocyte count, in addition to the CD4+ count, should be further pursued as a predictor of survival in patients with AIDS. The favorable but time-limited effect of zidovudine on CD4+ and CD8+ may explain why early treatment with zidovudine fails to improve patient survival compared with delayed therapy (31). We speculate that the survival benefit after early zidovudine therapy derives primarily from the salutary effect on CD4+ cells, whereas later therapy derives from the beneficial effect on CD8+, which often persists even in the late stage of AIDS.

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