Cytomegalovirus (CMV) Viremia and the CD4+ Lymphocyte Count as Predictors of CMV Disease in Patients Infected with Human Immunodeficiency Virus

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We screened 192 patients infected with human immunodeficiency virus (HIV) to examine the relation between CD4+ lymphocyte counts and cytomegalovirus (CMV) viremia and the occurrence of CMV disease and subsequent duration of survival. When we stratified the viremic patients by CD4+ lymphocyte counts, the proportions were as follows: <50/mm3, 20 (25%) of 80 patients; 50-100/mm3, 2 (5.5%) of 36; 101-150/mm3, none of 14; and >150/mm3, 1 (1.5%) of 62. After a mean follow-up period of 8.5 months, 21 (11%) of 192 patients developed CMV disease. The probability of developing CMV disease at 6 months was 13% when the CD4+ lymphocyte count was <50/mm3, 3% when the CD4+ lymphocyte count was 50-100/mm3, and 0 when the CD4+ lymphocyte count was >100/mm3; this probability was 46% for viremic patients and 1% for nonviremic patients. In a multivariate analysis, CMV viremia was independently prognostic of CMV disease (relative risk, 22.03; 95% confidence interval, 6.49-78.97; P < .001), whereas a CD4+ lymphocyte count of <50/mm3 was not (P = .26). These results support the value of CMV viremia for predicting which HIV-infected patients are at risk of developing CMV disease and should therefore receive primary prophylaxis.

Cytomegalovirus (CMV) disease is an important cause of morbidity and mortality among patients with advanced HIV infection. The incidence of CMV disease has increased with the widespread use of antiretroviral therapy and the development of prophylaxis for other opportunistic infections. CMV infection occurs throughout the course of HIV infection in 25%-30% of patients [1]. This high incidence of CMV disease and the short survival after diagnosis [2, 3] emphasize the need for primary prophylaxis. An effective strategy for prophylaxis requires the identification of markers that would be useful for selecting patients at higher risk of developing CMV disease.

We screened 192 patients infected with human immunodeficiency virus (HIV) to examine the relation between CD4+ lymphocyte counts and cytomegalovirus (CMV) viremia and the occurrence of CMV disease and subsequent duration of survival. When we stratified the viremic patients by CD4+ lymphocyte counts, the proportions were as follows: <50/mm3, 20 (25%) of 80 patients; 50-100/mm3, 2 (5.5%) of 36; 101-150/mm3, none of 14; and >150/mm3, 1 (1.5%) of 62. After a mean follow-up period of 8.5 months, 21 (11%) of 192 patients developed CMV disease. The probability of developing CMV disease at 6 months was 13% when the CD4+ lymphocyte count was <50/mm3, 3% when the CD4+ lymphocyte count was 50-100/mm3, and 0 when the CD4+ lymphocyte count was >100/mm3; this probability was 46% for viremic patients and 1% for nonviremic patients. In a multivariate analysis, CMV viremia was independently prognostic of CMV disease (relative risk, 22.03; 95% confidence interval, 6.49-78.97; P < .001), whereas a CD4+ lymphocyte count of <50/mm3 was not (P = .26). These results support the value of CMV viremia for predicting which HIV-infected patients are at risk of developing CMV disease and should therefore receive primary prophylaxis.

CMV disease occurs mainly as a reactivation of latent virus in patients who are seropositive for CMV. Since 85%-95% of HIV-infected patients have antibodies to CMV [4, 5], other markers are required to detect reactivation. It has been shown that the level of immunodeficiency, as measured by the CD4+ lymphocyte count, is of prognostic significance with respect to the occurrence of CMV disease in HIV-infected patients [6, 7]. It has been established that detection of CMV viremia at the time that AIDS is diagnosed is predictive of organ involvement with CMV [8]. Therefore, we designed a prospective study to assess the respective influence of two factors—a low CD4+ lymphocyte count and CMV viremia—on the risk of developing CMV disease. The aim of this study was to determine the usefulness of CMV viremia as a marker for selecting patients who might be candidates for primary CMV prophylaxis.

Methods

Patients. From 1 July 1992 to 31 December 1992, HIV-infected inpatients and outpatients (irrespective of the stage of the infection, based on the guidelines of the Centers for Disease Control and Prevention) seen in the Department of Infectious and Tropical Diseases at Bichat-Claude Bernard Hospital in Paris underwent concomitant (±1 month) screenings that consisted of CMV blood cultures and CD4+ lymphocyte counts. Patients with symptomatic CMV disease before or at the time of the screening period were excluded from the study. Patients were followed up until August 1993 or until they died. Patients who were followed up for <1 month were also excluded from the study.

The primary endpoint was the time to the first occurrence of CMV disease. Survival was considered a secondary endpoint. To assess the value of CMV viremia for predicting the
occurrence of CMV disease, a blood culture that was positive within the month preceding the occurrence of CMV disease was considered a diagnostic marker. Therefore, the patients with positive CMV blood cultures within the month preceding the onset of CMV disease were not considered in the prognostic analysis.

CMV disease was classified as follows: retinitis was defined by characteristic funduscopic findings (exudates, perivascular hemorrhages, and periphlebitis) that had no other apparent etiology. Gastrointestinal involvement was defined by the presence of suggestive gross lesions (colitis or esophagitis) and confirmed on the basis of histological findings (characteristic intracytoplasmic and intranuclear inclusions) and a positive CMV culture [9]. Encephalitis was suspected when suggestive radiological abnormalities were present and a favorable clinical response to specific therapy for CMV disease was observed. CMV meningoencephalopathy was diagnosed on the basis of suggestive neurological signs and the detection of CMV DNA by PCR assay of CSF. CMV pneumonia was suspected when interstitial pneumopathy was present, CMV was isolated from bronchoalveolar lavage fluid, and specific therapy for CMV disease resulted in resolution of these signs.

None of the patients received prophylaxis for CMV disease during the study period, but patients with CMV disease were treated according to the usual recommendations [10].

**Laboratory procedures.** CD4+ lymphocyte counts were measured with use of a routine flow cytometry method. Rapid detection of CMV viremia was performed by using a previously described culture-flask technique [11]. The positive control flask was inoculated with a cell-free suspension of the reference CMV strain AD169. Positive cultures were detected by the staining of at least one cell with use of a monoclonal E13 antibody to immediate early antigen.

**Statistical analysis.** We estimated the time to development of CMV disease by using the Kaplan-Meier product-limit method [12], and we compared these estimates for subgroups of patients according to the results of baseline CMV blood cultures and CD4+ lymphocyte counts by using the logrank test [13]. After a univariate analysis was completed, only significant markers with a P value of .1 were introduced into the multivariate analysis [14]. The CD4+ lymphocyte counts were distributed in two groups (≥50/mm³ or <50/mm³) for the multivariate analysis. For the survival analysis, the occurrence of CMV disease was the only variable considered time dependent in the Cox model.

**Results**

**Characteristics of patients.** During the 6-month study period, 550 patients were seen in our department, and 203 patients were screened with concomitant CMV blood cultures and CD4+ lymphocyte counts. Nine of these 203 patients were excluded because they had had CMV disease before July 1992, and two were excluded because they had been followed up for <1 month. Thus, 192 patients were included in the study. The ratio of males to females was 3.7:1, the mean age (±SD) was 36 ± 10 years (range, 8–68 years), and the mean baseline CD4+ lymphocyte count (±SD) was 148 ± 199/mm³ (range, 2–1,042/mm³). Eighty patients (42%) had CD4+ lymphocyte counts of <50/mm³, 36 (19%) had CD4+ lymphocyte counts of 50–100/mm³, 14 (7%) had CD4+ lymphocyte counts of 101–150/mm³, and 62 (32%) had CD4+ lymphocyte counts of >150/mm³. The mean follow-up time (±SD) was 8.5 ± 3.2 months (range, 1–13 months). Seventeen patients were lost to follow-up before the endpoint date after a mean delay of 4.2 months (range, 1.4–6.6 months).

**CMV blood cultures.** Twenty-three (12%) of the 192 patients had CMV viremia at baseline. Twenty-two (96%) of these 23 patients had CD4+ lymphocyte counts of <100/mm³ (20 had CD4+ lymphocyte counts of <50/mm³). The proportions of patients with CMV viremia, stratified by CD4+ lymphocyte count, are shown in table 1.

**Occurrence of CMV disease.** Until August 1993, CMV disease had been diagnosed in 21 (11%) of 192 patients, for an overall incidence of 17% per patient-year. Manifestations included retinitis (14 patients), colitis (two), esophagitis (one), encephalitis (two), meningoencephalopathy (one), and interstitial pneumonia (one). At the time that CMV disease was diagnosed, the mean CD4+ lymphocyte count (±SD) was 34 ± 54/mm³ (range, 2–232/mm³). At baseline, 16 (76%) of these patients had CD4+ lymphocyte counts of <50/mm³, 4 (19%) had CD4+ lymphocyte counts of 50–100/mm³, none had CD4+ lymphocyte counts of 101–150/mm³, and one (5%) had a CD4+ lymphocyte count of >150/mm³. Among the 21 patients who developed CMV disease, 14 (67%) had CMV viremia at baseline. The mean delay (±SD) between the time of CMV viremia and the diagnosis of CMV disease was 3.5 months ± 3 months (range, 15 days–9.5 months). Of the 171 patients who did not develop CMV disease, nine (5.3%) had CMV viremia at baseline.

Five of the 21 patients with CMV disease had blood cultures performed within the month preceding the onset of CMV disease and were not included in the prognostic analysis, as previously stated. Thus, 187 patients, 16 of whom had CMV disease, were considered for this analysis. The probabilities of developing CMV disease, based on the baseline CD4+ lymphocyte count and the results of CMV blood culture, are presented...
in figures 1 and 2, respectively. The probability of developing CMV disease at 6 months reached 13% for patients with baseline CD4+ lymphocyte counts of <50/mm³, whereas the probability was 3% for patients with CD4+ lymphocyte counts of 50–100/mm³; there was no probability of developing CMV disease at 6 months for patients with CD4+ lymphocyte counts of >100/mm³. The probability of developing CMV disease at 6 months was 46% for patients with CMV viremia vs. 1% for patients without CMV viremia.

CD4+ lymphocyte count and CMV viremia as predictors of CMV disease and their relationship. In the univariate analysis, age (P = .28) and sex (P = .31) were not predictors of CMV disease. Both the CD4+ lymphocyte count (P < .001) and CMV viremia (P < .001) were found to be significantly associated with the development of CMV disease. When the CD4+ lymphocyte count was considered as a qualitative variable (with a cutoff at 50/mm³) in the multivariate analysis (RR, 2.32; 95% CI, 0.53–10.19; P = .26), only CMV viremia was statistically significant (RR, 22.03; 95% CI, 6.49–78.97; P < .001).

Survival of patients according to CMV viremia and CD4+ lymphocyte count. Forty-six patients died after a mean follow-up (±SD) of 5.6 ± 3.2 months (range, 1.2–12.6 months). Seventeen (81%) of the 21 patients who developed CMV disease died, whereas 29 (17%) of the 171 patients who did not develop CMV disease died after a mean follow-up (±SD) of 8.5 ± 3.2 months. The median survival (±SD) after the diagnosis of CMV disease was 7.4 ± 3.3 months (range, 43 days–13 months). In the univariate analysis, the CD4+ lymphocyte count, CMV viremia, and CMV disease were significant predictors of survival (P < .001 for each of the three variables). In the multivariate analysis, the factors associated with higher mortality were both a CD4+ lymphocyte count of <50/mm³ (RR, 6.37; 95% CI, 2.74–14.82; P < .001) and the occurrence of CMV disease (RR, 3.63; 95% CI, 1.68–7.84; P = .001); however, the presence of CMV viremia was no longer significant (RR, 1.71; 95% CI, 0.80–3.67; P = .17).

Discussion

Our study of 192 HIV-infected inpatients and outpatients who were screened concomitantly for CD4+ lymphocyte counts and the presence of CMV viremia showed that the overall risk of developing CMV disease for this population was 17% at 1 year. However, this risk varied according to the detection of CMV in blood and the CD4+ lymphocyte count. Although the presence of viremia was clearly related to the CD4+ lymphocyte count, only CMV viremia appeared to be an independent predictor of the occurrence of CMV disease.

There are several limitations to this prospective observational study. It was difficult, even prospectively, to reach an exhaustive method for concomitant assessment of the CD4+ lymphocyte counts and CMV blood culture results for all inpatients and outpatients followed up in our department. However, 130 patients (66%) with CD4+ lymphocyte counts of <150/mm³ were included in this assessment. Thus, only one-third of this subset of patients at risk for CMV disease were not screened. Furthermore, we did not assess some variables that could have influenced the occurrence of CMV disease such as the stage of HIV infection, risk factors for HIV infection, CMV serology results, and antiretroviral therapy at the time of enrollment in the study.

Finally, the quantitative value of the relative risks of developing CMV disease based on a CD4+ lymphocyte count of
<50/mm\(^3\) and the presence of CMV viremia should be considered with caution. Indeed, the precision of these results is relatively low, as evidenced by the wide confidence intervals, because of the relatively low prevalence of CMV disease. However, this low precision does not modify the general conclusion of the study, i.e., that the presence of CMV viremia has prognostic value, while the CD4\(^+\) lymphocyte count appears to be a weaker prognostic marker for CMV disease.

The value of the CD4\(^+\) lymphocyte count for predicting the occurrence of CMV disease is now well established. The results of studies by Pertel et al. [6] and Galland et al. [7] as well as those of the present study show that the risk of developing CMV disease is significantly higher for patients with CD4\(^+\) lymphocyte counts of <100/mm\(^3\). This risk varies in the three studies from \(~\)5% to 11% at 6 months. The risk increases significantly (13%–15%) at 6 months when the CD4\(^+\) lymphocyte count decreases to <50/mm\(^3\).

The predictive value of CMV viremia in HIV-infected patients has been a point of controversy in the literature [15–17]; however, the value of this marker has been established for bone marrow transplant recipients [18]. Zurlo et al. [15] reported that CMV viremia had poor diagnostic and predictive values in a retrospective study of 322 HIV-infected patients. CMV disease developed in 35% of patients with CMV viremia after a mean delay of 84 days, whereas it developed in 15% of patients with negative blood cultures after a mean delay of 260 days [15]. On the other hand, the predictive value of CMV viremia at the onset of AIDS has been previously reported by Salmon et al. [8], who prospectively followed up 71 HIV-infected patients. The rate of occurrence of CMV disease was 50% after 8 months for patients with CMV viremia and 9% for patients with negative blood cultures.

It might be argued that the value of CMV viremia for predicting the occurrence of CMV disease is due to the increased frequency of CMV viremia among patients with decreased CD4\(^+\) lymphocyte counts [19]. This was the reason we analyzed the influence of these two prognostic factors separately in the univariate analysis and then assessed their relationship in the multivariate analysis. This analysis showed that only CMV viremia is an independent predictor of CMV disease, while the CD4\(^+\) lymphocyte count did not reach a level of statistical significance; on the other hand, this level might have been reached with a more powerful analysis, e.g., one in which sequential assessments were made over time. Nevertheless, these data suggest that both markers—a low CD4\(^+\) lymphocyte count and CMV viremia—could be considered in identifying the patients with the highest risk of developing CMV disease, i.e., those with CMV viremia and CD4\(^+\) lymphocyte counts of <50/mm\(^3\).

The short mean survival (±SD) of patients in this population, especially after the occurrence of CMV disease (i.e., 7 months ± 3 months), confirms that CMV disease is associated with a poor prognosis in the course of HIV infection, as previously described [7, 20]. The results of the present study confirm that both the CD4\(^+\) lymphocyte count and the presence of CMV disease are predictors of survival [7]. As expected, this study shows that CMV viremia influences survival only in the presence of CMV disease and is not an independent predictor of survival.

In conclusion, our data suggest that CMV viremia is both a marker of advanced immunosuppression related to HIV infection and independently predictive of the development of CMV disease. Since it has recently been shown that primary prophylaxis for CMV disease in HIV-infected patients is feasible [21], it is necessary to identify as accurately as possible the patients who are candidates for prophylaxis. Whether prophylaxis should be offered to all patients with antibodies to CMV and CD4\(^+\) lymphocyte counts of <50/mm\(^3\) or whether such prophylaxis should be offered only to patients with CMV viremia remains questionable.

New techniques such as the detection of CMV antigenemia [22] or the detection of CMV DNA from leukocytes or plasma by PCR assay [23, 24] could also allow detection of CMV reactivation. Assessment of the prognostic value of these techniques is under investigation. Early detection of CMV reactivation should allow targeted prophylactic strategies that are directed at the patients with the highest risk of developing CMV disease. This targeted approach could prevent the potential adverse events associated with such therapies in patients with a lower risk of developing CMV disease, increase the quality of the lives of such patients, and decrease the cost of CMV prophylaxis. In the future, these advantages should be taken into account in adapting prophylaxis for all opportunistic infections, when possible, to the risk factors for individual patients.

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References


