Plasma HIV Viral Load in Patients with Hemophilia and Late-Stage HIV Disease: A Measure of Current Immune Suppression

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Background: For patients infected with HIV, plasma HIV viral load in early disease predicts long-term prognosis. However, the implications of viral load measurements late in HIV disease are uncertain.

Objective: To evaluate the relation between plasma HIV viral load and subsequent risk for disease progression in patients with late-stage HIV disease.

Design: Retrospective cohort study.

Setting: 16 treatment centers for patients with hemophilia.

Patients: 389 patients with hemophilia and late-stage HIV disease (CD4 count < 200 cells/mm^3).

Measurements: Plasma HIV viral load was measured at baseline. Patients were followed for AIDS-related illnesses (primary outcome) and, specifically, *Pneumocystis carinii* pneumonia (secondary outcome).

Results: HIV viral load strongly predicted AIDS-related illness. For patients with viral loads less than 4.00 $\log_{10}$ copies/mL, the 1-year actuarial risk was 0% and the 5-year risk was 25%. For patients with viral loads of at least 6.00 $\log_{10}$ copies/mL, the 1-year actuarial risk was 42% and the 5-year risk was 78%. A linear relation existed between viral load and risk for AIDS-related illness (hazard ratio, 2.37 per $\log_{10}$ copies/mL; $P < 0.001$). In addition, viral load most strongly predicted risk for illness immediately after viral load testing; this predictive relation attenuated over time ($P = 0.002$). These findings changed little after adjustment for CD4 cell counts that were updated during follow-up. In the first year after viral load was measured, it predicted occurrence of *P. carinii* pneumonia (hazard ratio, 4.69 per $\log_{10}$ copies/mL; $P < 0.001$).

Conclusions: In patients with hemophilia and late-stage HIV disease, viral load predicts disease progression independently of CD4 cell counts. Because viral load most strongly predicts progression immediately after load is measured, it seems to reflect the current level of immunosuppression.

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For HIV-infected patients, measurement of the circulating virus level has become standard clinical practice (1, 2). High viral loads in HIV-infected patients reflect substantial ongoing viral replication (3) and predict rapid clinical progression (1, 2).

Much of the evidence supporting clinical use of viral load measurements derives from large-scale cohort studies of HIV-infected persons, particularly those with early chronic HIV infection (4–6). In the Multicenter Hemophilia Cohort Study (MHCS) (4), HIV viral load measured within 3 years of seroconversion predicted patients' long-term prognosis. The risk for AIDS-related illness was 16-fold higher among patients with viral loads of at least 10 000 copies/mL than among patients with viral loads less than 1000 copies/mL. In the Multicenter AIDS Cohort Study, early HIV viral load predicted risk for AIDS during 10 years of follow-up (5). High levels of viral replication may decrease production and increase destruction of CD4 lymphocytes (7), and HIV viral loads measured during the early stages of infection may predict the subsequent decrease of CD4 cell count (5). However, it is unknown whether the entire relation between viral load and disease outcome is mediated through these effects on the number of circulating CD4 lymphocytes.

Complementing these cohort studies have been treatment trials showing remarkable benefits for patients receiving highly active antiretroviral therapies. Part of the effect of antiretroviral regimens can be explained by treatment-induced changes in viral load (8, 9). Trial data suggest that risk for clinical progression is elevated in patients who initially respond to therapy but have a subsequent increase in HIV replication (10).

Our analysis of data from the MHCS addresses two poorly understood aspects of the relation between HIV viral load and disease risk. First, we examined viral load and disease risk in patients with late-stage HIV disease who had CD4 counts less than 200 cells/mm^3. Previous studies of patients with low CD4 cell counts have been relatively small (5, 11–13); consequently, the relation between viral load and disease risk in these patients has been imprecisely estimated. A better understanding of this relation could help physicians assess patient prognosis more accurately.

Second, we also studied the time course over which a single measurement of HIV viral load provides prognostic information. We reasoned that demonstration of a relation between viral load and long-term prognosis would indicate that viral loads are related to factors that induce clinical deterioration over several years. This finding would support the hypothesis that high viral loads predict a future decrease in CD4 cell counts. Alternately, demonstration of a relation between viral load and short-term prognosis would provide evidence that HIV viral loads reflect current risk and implicitly measure current immune suppression. Our study therefore provides epidemiologic data relevant to understanding the mechanisms by which high levels of HIV induce disease.

Table 1. Baseline Characteristics*

Characteristic	All Patients (n = 389)
Underlying coagulopathy, *n (%)*	
Hemophilia A	349 (90)
Hemophilia B	32 (8)
Other or unknown	8 (2)
Men, *n (%)*	385 (99)
Median age (interquartile range), *y*	30 (22–38)
Calendar year at baseline, *n (%)*	
1987–1988	48 (12)
1989–1990	125 (32)
1991–1992	113 (29)
1993–1994	81 (21)
1995–1997	22 (6)
Hepatitis infection	
Hepatitis C virus infection, *n (%)*†	380 (98)
Hepatitis B virus chronic carrier, *n (%)*	34 (9)
Median CD4 count (interquartile range), *cells/mm^3*	129 (72–170)
Median HIV viral load (interquartile range), *log_{10} copies/mL*	5.1 (4.5–5.6)
Antiretroviral medication use before baseline visit, *n (%)*	237 (61)
Antiretroviral medication use at baseline, *n (%)*	
None	194 (50)
One drug	162 (42)
Two drugs	30 (8)
Three drugs	3 (1)
Any use of an HIV protease inhibitor	1 (0.3)
Median time from HIV seroconversion to baseline visit (interquartile range), *y*	9 (7–10)

* The baseline visit for this study was the first visit at which HIV-infected patients had a CD4 count less than 200 cells/mm^3 and had stored plasma available for viral load testing. Three hundred seventeen patients (81%) had baseline visits on the first date that their CD4 count was documented as less than 200 cells/mm^3. For the 72 patients whose baseline visit was delayed until stored plasma was available, the median delay was 1.5 years (interquartile range, 0.6–3.1 years).
† Hepatitis C virus infection status for 2 patients (0.5%) was indeterminate on recombinant immunoblot assay testing.

Methods

Patients

The MHCS is a prospective cohort study of patients with hemophilia who were enrolled at 16 treatment sites in the United States and Europe beginning in 1982 (14). Since enrollment, patients have been seen for follow-up every 6 to 12 months. During follow-up visits, clinical outcomes and current treatment are noted and biological specimens are obtained for laboratory testing.

For our retrospective study, we included HIV-infected patients from the MHCS. At each time point, patients who were free of AIDS-related illness were eligible for study entry. Patients were included beginning with the time of the first visit at which they had a CD4 count less than 200 cells/mm^3 and had stored plasma available for viral load measurement (baseline visit). Included patients were then followed for development of an AIDS-related illness (primary outcome) or, in particular, *Pneumocystis carinii* pneumonia (secondary outcome); these outcomes were diagnosed on the basis of Centers for Disease Control and Prevention criteria (15). Patients were censored at death or loss to follow-up. Institutional review boards at the National Cancer Institute, each treatment site, and the coordinating center approved our study.

Laboratory Methods

Plasma was separated from whole blood and frozen on the same day (2 to 8 hours after sampling) or after overnight shipment to a central laboratory. Specimens were stored at −70 °C until testing.

We measured the quantity of HIV RNA circulating in plasma (viral load) using the Amplicor HIV Monitor assay (Roche Molecular Systems, Branchburg, New Jersey). With this assay, a 142 base-pair sequence in the HIV *gag* gene was reverse-transcribed and amplified by polymerase chain reaction (16). Before amplification, heparin, a potential inhibitor, was removed by using silica extraction (17). Dilution of serial samples allowed quantification of HIV viral load over a range of 200 to more than 1 000 000 viral copies/mL. A value of 100 copies/mL was assigned to readings less than 200 copies/mL. We report $\log_{10}$-transformed values of viral load measurements.

Patients were considered HIV-infected if repeated serum or plasma samples were reactive by a commercially licensed HIV enzyme immunoassay and were positive by HIV Western blot or radioimmunoassay (14). CD4 cell counts were determined at study sites by using methods described elsewhere (18, 19).

Statistical Analysis

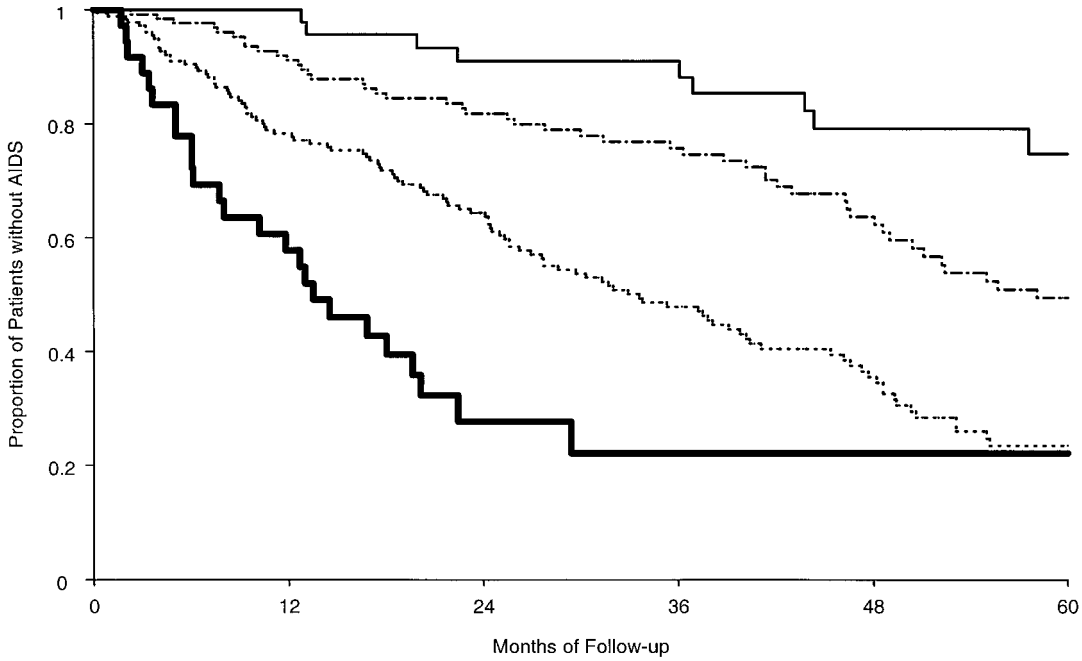
The Kaplan–Meier method was used to estimate the actuarial risk for primary and secondary outcomes for strata defined by baseline CD4 cell counts and viral loads (20). We quantified the effect of clinical characteristics and laboratory measures on risk for AIDS-related illness or *P. carinii* pneumonia using proportional hazards models with baseline and time-dependent covariates (20). We fitted regression splines to check for nonlinearity of the

effects of viral load and CD4 cell count on the hazard for AIDS-related illness (21). Separate models were examined by using baseline CD4 cell counts and CD4 cell counts obtained serially during follow-up (updated every 6 months).

In multivariate analyses of risk for AIDS-related illness, we controlled for time from HIV seroconversion to baseline (using previously derived seroconversion dates [22]), age at baseline (by quintiles), and current use of antiretroviral therapy (by number of medications and use of protease inhibitors). When examining risk for *P. carinii* pneumonia, we controlled for prophylaxis with trimethoprim–sulfamethoxazole, dapsone, or aerosolized pentamidine.

To determine whether short-term risk or long-term risk was most closely related to baseline viral load and CD4 cell count, we evaluated whether hazard ratios associated with these variables changed over time. At any given time, the hazard ratio quantifies the relation between the variable of interest and instantaneous (ongoing) risk, even if the variable was measured only once, at baseline. Evaluated over time, hazard ratios that approach the null value of 1 suggest that the variable has a decreasing effect on ongoing risk. We examined changes in hazard ratios over time by separating the first 5 years of follow-up into 10 consecutive 6-month intervals, each containing all patients who were event-free and still under follow-up at the start of the interval. For baseline viral load and baseline CD4 cell count, we fitted 10 separate proportional hazards models, 1 for each 6-month interval. We then constructed plots of the hazard ratios for each interval ("interval-specific" hazard ratios). Finally, we examined time trends in interval-specific hazard ratios by evaluating a model, using all 5 years of follow-up, that incorporated a linear interaction between the variable of interest and follow-up time.

High viral load may increase risk for disease by decreasing CD4 cell count (5). We examined this hypothesis by calculating $R = (\beta_0 - \beta_1)/\beta_0$, where β_0 is the log hazard ratio for viral load unadjusted for updated CD4 cell counts and β_1 is the log hazard ratio adjusted for updated CD4 cell counts (23). When R is between 0 and 1, it measures the proportion of the viral load effect explained ("mediated") by CD4 cell counts; values near 1 suggest that CD4 cell counts are on the causal pathway between



Baseline Viral Load	Number of Patients under Follow-up					
< 4.00 $\log_{10}$ copies/mL	47	47	39	32	22	16
4.00–4.99 $\log_{10}$ copies/mL	128	111	90	68	46	31
5.00–5.99 $\log_{10}$ copies/mL	178	133	97	62	36	18
≥ 6.00 $\log_{10}$ copies/mL	36	20	6	4	3	3

Figure 1. Kaplan–Meier curves showing time to AIDS-related illness for strata defined by baseline HIV viral load. The thin solid line represents a viral load less than 4.00 $\log_{10}$ copies/mL, the dotted-and-dashed line represents a viral load of 4.00 to 4.99 $\log_{10}$ copies/mL, the dotted line represents a viral load of 5.00 to 5.99 $\log_{10}$ copies/mL, and the thick solid line represents a viral load of at least 6.00 $\log_{10}$ copies/mL. The numbers of patients under follow-up in each stratum at each 12-month interval are given below the graph.

viral load and disease, and values near 0 suggest separate causal pathways. We calculated a CI for R by using the Fieller theorem (24) and the bootstrap to estimate the covariance between β_1 and β_0.

For other analyses, levels of continuous variables were compared between patient groups by using the Wilcoxon rank-sum test; the relations among continuous variables were examined by using the Pearson correlation coefficient (25). S-Plus (version 4.5, MathSoft, Seattle, Washington) and MATLAB software (version 5, The MathWorks, Natick, Massachusetts) were used for calculations. A P value less than 0.05 was considered statistically significant.

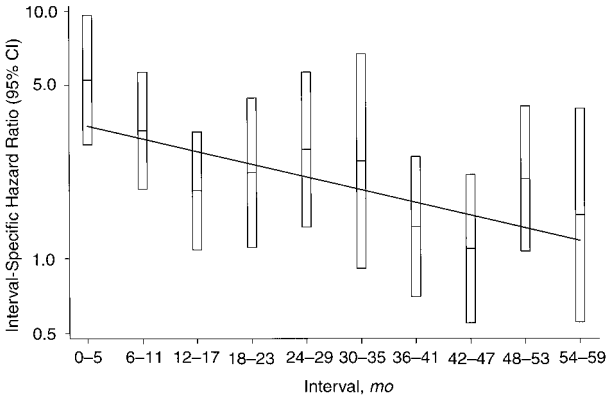


Figure 2. Hazard ratios for AIDS-related illness (unadjusted), corresponding to a 1-$\log_{10}$ increase in baseline viral load, presented separately for each 6-month interval of follow-up. Hazard ratios are shown on a log scale as point estimates and surrounding 95% CIs. Baseline viral load was most strongly associated with risk for disease in early follow-up intervals; hazard ratios, which indicate ongoing disease risk, decrease over time. The diagonal line indicates the linear interaction of $\log_{10}$ viral load with time ($P = 0.002$).

Results

Patient Characteristics

The study sample consisted of 389 patients with hemophilia (**Table 1**). Patients had advanced HIV infection at baseline (median duration of HIV infection, 9 years; median CD4 count, 129 cells/mm^3). The median baseline viral load was 5.1 $\log_{10}$ copies/mL. A relatively small but highly significant inverse correlation existed between baseline $\log_{10}$ viral load and baseline CD4 cell count ($r = -0.23$; $P < 0.001$). The median number of CD4 cell count measurements after baseline was 3 (interquartile range, 2 to 6); among patients who had more than one measurement of CD4 cell count, the median time between measurements was 10 months.

Most patients entered our study in the late 1980s or early 1990s. At baseline, half of the patients used antiretroviral medications (**Table 1**). During follow-up, 303 patients (78%) used at least one antiretroviral medication, 126 (32%) used at least two antiretroviral medications in combination, and 32 (8%) used at least three antiretroviral medications in combination.

For study patients, the median time from baseline to AIDS-related illness, death, or loss to follow-up was 31 months. During 1158 person-years of follow-up, 211 patients (54%) developed an AIDS-related illness (incidence, 0.18 cases per person-year).

Predictors of AIDS-Related Illness

Kaplan–Meier curves indicating time to AIDS-related illness are shown in **Figure 1**; patient strata are defined by baseline viral load (<4.00 $\log_{10}$ copies/mL, 4.00 to 4.99 $\log_{10}$ copies/mL, 5.00 to 5.99 $\log_{10}$ copies/mL, and ≥6.00 $\log_{10}$ copies/mL). Higher viral loads were associated with shorter time to disease progression: At 1 year of follow-up, actuarial risks for AIDS-related illness in each stratum were 0%, 9%, 12%, and 42%, respectively. At 5 years, corresponding risks were 25%, 51%, 76%, and 78%, respectively. In a proportional hazards survival model, the unadjusted hazard ratio for a 1-$\log_{10}$ increase in viral load was 2.37 (95% CI, 1.91 to 2.94).

Viral load immediately affected disease progression. Illness-free survival for patients with high baseline viral loads diverged rapidly from that for patients with lower viral loads (**Figure 1**). Over time, this divergence was less apparent, implying

Table 2. Proportional Hazards Models for Time to AIDS-Related Illness

Characteristic	Unadjusted Hazard Ratio (95% CI)	*P* Value	Adjusted Hazard Ratio (95% CI)*	*P* Value	Multivariate Model Hazard Ratio (95% CI)†	*P* Value
Baseline viral load with time interaction						
Main effect (per $\log_{10}$ copies/mL)	3.64 (2.56–5.18)	<0.001	3.58 (2.51–5.10)	<0.001	2.82 (1.99–4.00)	<0.001
$\log_{10}$ viral load × time interaction (per year)	0.79 (0.68–0.91)	0.002	0.79 (0.69–0.92)	0.002	0.77 (0.67–0.89)	<0.001
Baseline CD4 count (per decrease of 50 cells/mm^3)	1.60 (1.41–1.82)	<0.001	1.61 (1.42–1.83)	<0.001	–	–
CD4 count updated every 6 months (per decrease of 50 cells/mm^3)	1.68 (1.51–1.87)	<0.001	1.70 (1.52–1.90)	<0.001	1.59 (1.42–1.78)	<0.001

* Adjusted for baseline age (by quintiles), time between HIV seroconversion and baseline, and current use of antiretroviral therapy (<3 or ≥3 antiretroviral medications). Controlling for use of antiretroviral medications with the variables "1 or more medications compared with none" or "protease inhibitor compared with none" produced almost identical results (data not shown).

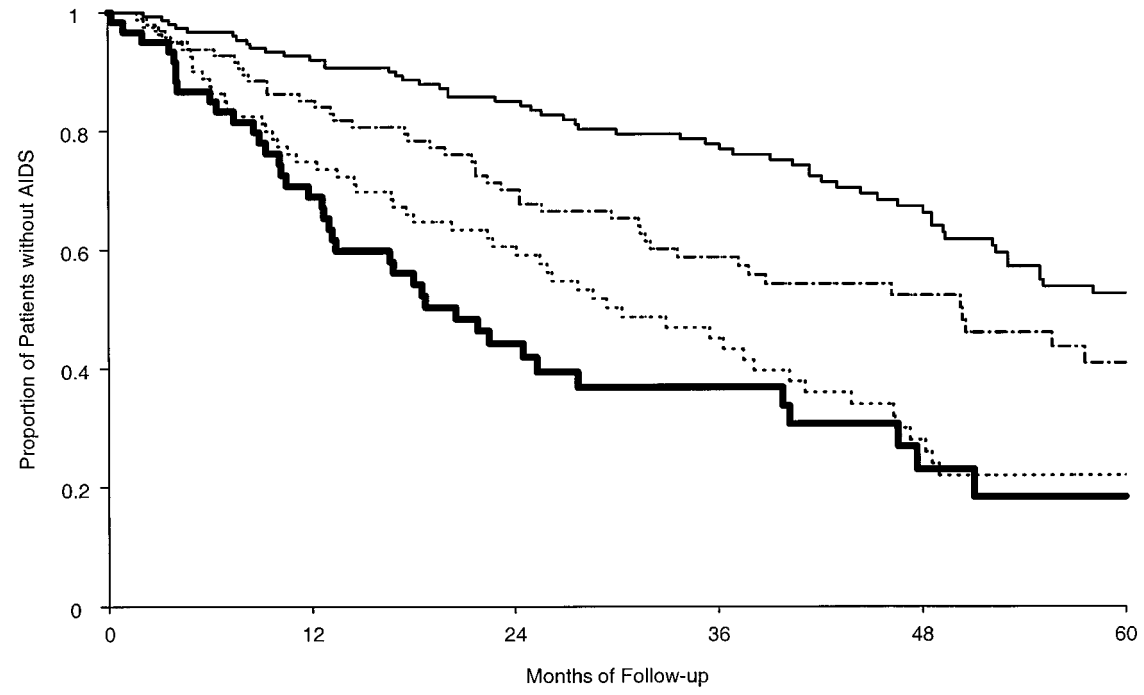
† The multivariate model included baseline $\log_{10}$ viral load and its interaction with time, updated CD4 cell count, baseline age (by quintiles), time between HIV seroconversion and baseline, and current use of antiretroviral therapy (<3 or ≥3 medications). Comparison of the hazard ratio for baseline $\log_{10}$ viral load in the multivariate model with the corresponding hazard ratio in the model without updated CD4 cell counts shows that updated CD4 counts explain 19% of the effect of baseline viral load, with $R = [\log(3.58) - \log(2.82)]/\log(3.58) = 0.19$.

that the effect of baseline viral load on ongoing disease risk became attenuated. Equivalently, **Figure 2** shows that hazard ratios for baseline viral load were greatest in early intervals of follow-up. Over time, a linear decrease was seen in the interval-specific hazard ratios for baseline viral load ($P = 0.002$) (**Figure 2**). In a survival model with an interaction term between $\log_{10}$ viral load and time since baseline, baseline viral load remained important, but the magnitude of its effect decreased about 20% per year (hazard ratio, 0.79 per year) (**Table 2**).

The time to AIDS-related illness also differed across strata defined by baseline CD4 counts (150 to 199 cells/mm^3, 100 to 149 cells/mm^3, 50 to 99 cells/mm^3, and <50 cells/mm^3) (**Figure 3**). Time to illness was shorter for persons with lower baseline CD4 cell counts. At 1 year of follow-up, actuarial risks for AIDS-related illness in each stratum were 8%, 15%, 25%, and 31%, respectively; at 5 years, the corresponding risks were 47%, 59%, 78%, and 82%.

A linear relation was seen between baseline CD4 cell count and risk for AIDS-related illness (hazard ratio, 1.60 per each decrease of 50 cells/mm^3 in baseline CD4 count) (**Table 2**). Similar to the relation between viral load and risk for AIDS, the effect of baseline CD4 cell count waned with increasing follow-up time, although this trend was not statistically significant (linear interaction of baseline CD4 cell count with time, $P = 0.08$) (**Figure 4**). Using updated CD4 cell counts to model risk did not affect the strength of this relation (**Table 2**).

After adjustment for age, time from HIV seroconversion, and use of antiretroviral therapy, the hazard ratios for baseline $\log_{10}$ viral load and its linear interaction with time did not change appreciably, nor did those for baseline or updated CD4 cell counts (**Table 2**). In a multivariate model, baseline $\log_{10}$ viral load, its interaction with time, and updated CD4 cell count independently predicted AIDS-related illness (**Table 2**). When updated CD4 cell counts were included in the multivariate model, the hazard ratio for $\log_{10}$ viral load changed from 3.58 to 2.82 (**Table 2**), implying that CD4 cell counts obtained during follow-up explained 19% (CI, 12% to 29%) of the effect of baseline viral load.



Baseline CD4 Count		Number of Patients under Follow-up				
150–199 cells/mm^3	151	136	112	88	61	42
100–149 cells/mm^3	97	78	59	41	26	15
50–99 cells/mm^3	81	59	42	25	14	8
0–49 cells/mm^3	60	38	19	12	6	3

Figure 3. Kaplan–Meier curves showing time to development of AIDS-related illness for strata defined by baseline CD4 cell count. The thin solid line represents a CD4 count of 150 to 199 cells/mm^3, the dotted-and-dashed line represents a CD4 count of 100 to 149 cells/mm^3, the dotted line represents a CD4 count of 50 to 99 cells/mm^3, and the thick solid line represents a CD4 count less than 50 cells/mm^3. The numbers of patients under follow-up in each stratum at each 12-month interval are given below the graph.

HIV Viral Loads in Severely Immunocompromised Patients

Sixty patients (15%) had baseline CD4 counts less than 50 cells/mm^3 (median baseline CD4 count for this group, 24 cells/mm^3). Baseline viral loads were higher for this group than for other patients (median, 5.3 $\log_{10}$ copies/mL compared with 5.1 $\log_{10}$ copies/mL; $P = 0.01$). For patients with a baseline CD4 count of 0 to 24 cells/mm^3 and a viral load less than 5.3 $\log_{10}$ copies/mL (the median value), the 1-year actuarial risk was 29%; for patients with a baseline CD4 count of 0 to 24 cells/mm^3 and a viral load of at least 5.3 $\log_{10}$ copies/mL, the 1-year actuarial risk was 60% (**Figure 5**). Similarly, for patients with a baseline CD4 count of 25 to 49 cells/mm^3 and a viral load less than 5.3 $\log_{10}$ copies/mL, the 1-year risk was 8%; for patients with a baseline CD4 count of 25 to 49 cells/mm^3 and a viral load of 5.3 $\log_{10}$ copies/mL or greater, the 1-year risk was 25%. Viewed as a continuous variable, viral load predicted AIDS-related illness among these 60 patients with advanced immune compromise (hazard ratio, 1.88 per $\log_{10}$ copies/mL [CI, 1.07 to 3.29]; $P = 0.03$). The effect of viral load did not change after adjustment for baseline CD4 cell count, age, time since seroconversion, and antiretroviral therapy (hazard ratio, 2.02 [CI, 1.08 to 3.76]; $P = 0.03$).

Viral Load as a Predictor of *Pneumocystis carinii* Pneumonia

Because our data indicate that HIV viral load predicts disease risk most strongly in the first year after measurement, we examined risk for *P. carinii* pneumonia during the first year of follow-up. We chose *P. carinii* pneumonia as a secondary outcome because it was the most common AIDS-related illness in our study and because decisions about prophylaxis can be guided by data on disease risk. In the first year, 25 patients (6%) developed *P. carinii* pneumonia; this diagnosis accounted for 39% of 64 AIDS-related illnesses in the first year of follow-up. For viral loads less than 4.00 $\log_{10}$ copies/mL, 4.00 to 4.99 $\log_{10}$ copies/mL, 5.00 to 5.99 $\log_{10}$ copies/mL, and at least 6.00 $\log_{10}$ copies/mL, actuarial risks for *P. carinii* pneumonia were 0%, 2%, 10%, and 14%, respectively. After we controlled for the use of *P. carinii* pneumonia prophylaxis, baseline viral load (hazard ratio, 4.69 per $\log_{10}$ copies/mL [CI, 2.31 to 9.49]; $P < 0.001$) and baseline CD4 cell count (hazard ratio, 1.52 per each decrease of 50 cells/mm^3 [CI, 1.05 to 2.21]; $P = 0.03$) independently predicted development of *P. carinii* pneumonia.

Discussion

An HIV viral load measurement taken late in HIV disease predicts subsequent disease risk. In our study of 389 patients with hemophilia and CD4 counts less than 200 cells/mm^3, one measure of HIV viral load identified risk over a median follow-up of 31 months. Baseline viral loads predicted disease progression, especially during early follow-up. Each $\log_{10}$ increase in baseline viral load was associated with a fivefold increase in risk for AIDS-related illness during the first 6 months of follow-up (**Figure 2**). Even in patients with CD4 counts less than 50 cells/mm^3, viral load was strongly associated with disease risk.

Previous analyses of the effect of viral load and CD4 cell count on progression of HIV disease have assumed that relative risks associated with these factors remain constant over time. However, this assumption, which underlies the proportional hazards survival model, may not be realistic because hazard ratios, which quantify ongoing risk associated with a variable of interest, may change over time. For example, if a cumulative effect of the risk factor is necessary before risk increases, the risk associated with this factor will be lowest during early follow-up. Alternatively, the risk factor could affect risk immediately. In that case, if levels of the risk factor change over time and only a single baseline measurement is used to predict risk during the entire follow-up period, the association between the risk factor and disease will be most apparent early in the follow-up period; during later periods, the association between the baseline levels of the risk factor and ongoing risk, measured by the hazard ratio, will be attenuated.

In our study of patients with hemophilia and late-stage HIV disease, a simple proportional hazards analysis did not adequately depict the relation between viral load and risk for disease. High viral loads were most predictive of AIDS-related illness

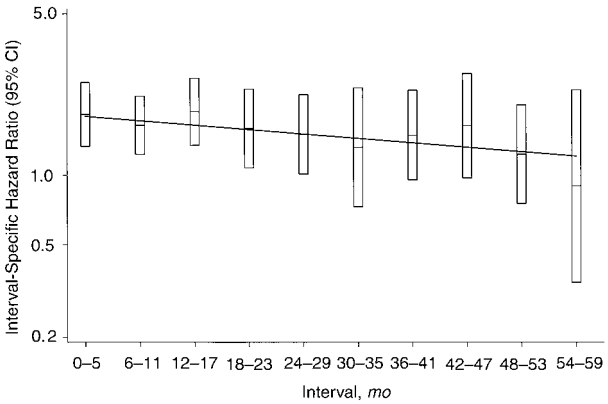


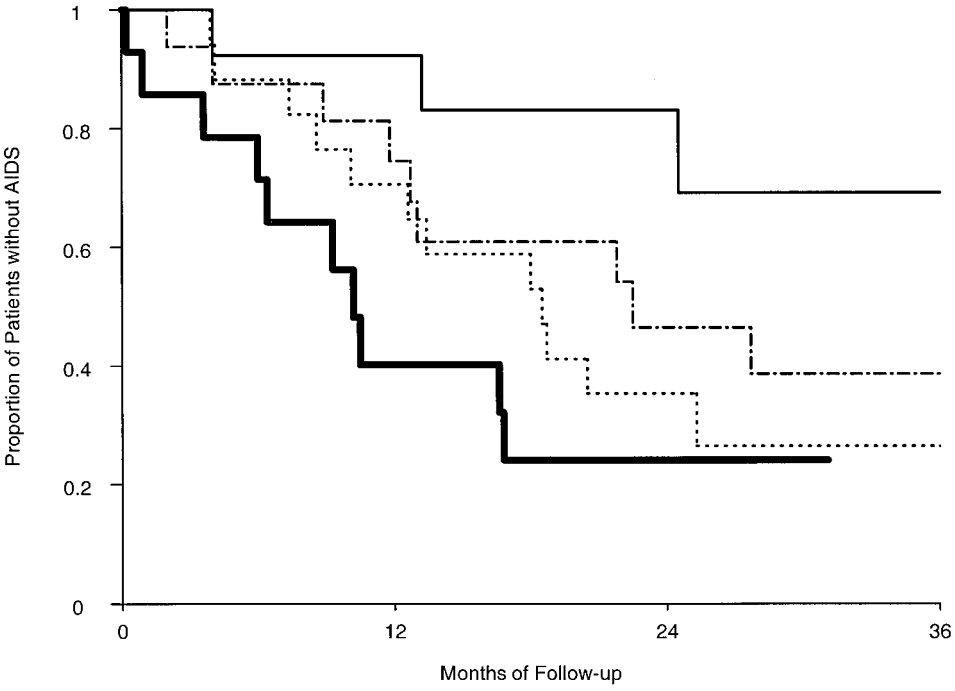
Figure 4. Hazard ratios for AIDS-related illness (unadjusted), corresponding to a decrease of 50 cells/mm^3 in baseline CD4 count, presented separately for each 6-month interval of follow-up. Hazard ratios are shown on a log scale as point estimates and surrounding 95% CIs. Baseline CD4 cell count was most predictive of disease in early follow-up intervals; hazard ratios, which indicate ongoing disease risk, decrease over time. The diagonal line indicates the linear interaction of CD4 cell count with time ($P = 0.08$).

immediately after they were measured (**Figure 2**). This finding strongly suggests that viral load reflects the current level of immune dysfunction. Consistent with this hypothesis, viral loads tend to increase over time among untreated patients (26, 27). Because HIV viral loads may vary in this way, a single baseline viral load measurement offers progressively less information about ongoing disease risk after several years have passed. Similarly, the prognostic information provided by a single CD4 cell count is greatest immediately after it is measured and attenuates with time (**Figure 4**).

Our results do not support the notion that the effect of viral load on disease risk is mediated entirely through decreases in CD4 lymphocyte counts (5). If this effect explained the relation between viral load and disease risk, the hazard ratios for viral load would be expected to increase over time. It would take some time for high viral loads to exert a deleterious effect on CD4 cell counts. Because the hazard ratios for baseline viral load actually decrease over time, this explanation seems untenable. Further evidence that high viral loads predict the development of AIDS independently of decreases in CD4 cell count is provided by our multivariate analysis; serial CD4 cell counts during follow-up explained only 19% of the predictive effect of the baseline measurement of viral load.

Previous studies (5, 11–13), all of which were smaller than our study, have examined viral load measured late in disease and the risk for illness or death. These studies reported relative hazards of 1.5 to 3.7 per $\log_{10}$ copies/mL—findings that are generally consistent with our results—but were too small to definitively examine whether viral loads provide information about short-term risk for disease (11–13). However, for strata defined by viral load, the Kaplan–Meier curves presented in these reports show early separation in risk and are similar to the curves in **Figure 1** (5, 12, 13). These studies therefore support our conclusion that, among patients with late-stage disease, high viral load is a marker for current immune dysfunction.

The reasons that a high viral load is associated



Baseline CD4 Count	Baseline Viral Load		Number of Patients under Follow-up		
25–49 cells/mm^3	< 5.30 $\log_{10}$ copies/mL	13	10	6	4
25–49 cells/mm^3	≥ 5.30 $\log_{10}$ copies/mL	16	11	6	5
0–24 cells/mm^3	< 5.30 $\log_{10}$ copies/mL	17	12	5	3
0–24 cells/mm^3	≥ 5.30 $\log_{10}$ copies/mL	14	5	2	0

Figure 5. Kaplan–Meier curves showing time to development of AIDS-related illness for patients with baseline CD4 counts less than 50 cells/mm^3. The thin solid line represents patients with a baseline CD4 count of 25 to 49 cells/mm^3 and a viral load less than 5.3 $\log_{10}$ copies/mL, the dashed-and-dotted line represents patients with a baseline CD4 count of 25 to 49 cells/mm^3 and a viral load greater than or equal to 5.3 $\log_{10}$ copies/mL, the dotted line represents patients with a CD4 count of 0 to 24 cells/mm^3 and a viral load less than 5.3 $\log_{10}$ copies/mL, and the thick solid line represents patients with a CD4 count of 0 to 24 cells/mm^3 and a viral load greater than or equal to 5.3 $\log_{10}$ copies/mL. The numbers of patients under follow-up in each stratum at each 12-month interval are given below the graph.

with current immune compromise need elucidation. In late-stage disease, HIV infection may directly cause defective immune function. Macrophages and CD4 lymphocytes from HIV-infected persons display qualitative defects toward various pathogens (28, 29), and high HIV viral load may be associated with anergy to recall antigens (30). Patients with active cytomegalovirus disease have diminished cytomegalovirus-specific CD4 cell responses, but patients with a history of cytomegalovirus disease who are treated with highly active antiretroviral therapy (which presumably reduces HIV viral load) have improved responses (31). Immune dysfunction may be mediated by the HIV envelope glycoprotein gp120, which inhibits macrophage phagocytosis of such opportunistic organisms as *Cryptococcus neoformans* and *Mycobacterium avium* (32, 33).

Conversely, a patient's immune deficiency could itself increase HIV viral load. However, this seems to be disproved by results of trials (8, 9) that show a dramatic decrease in risk for AIDS when HIV viral load is reduced. The findings of these trials suggest that HIV replication or the resulting high levels of circulating virus cause immunosuppression.

Because HIV viral load reflects current immune competence, it can indicate the need for prophylaxis against opportunistic infections. For our patients, HIV viral load predicted *P. carinii* pneumonia when it was used in conjunction with CD4 cell count. Although current recommendations suggest that all patients with CD4 counts less than 200 cells/mm^3 receive *P. carinii* pneumonia prophylaxis (34), we found that few patients developed *P. carinii* pneumonia when their viral loads were less than 5.00 $\log_{10}$ copies/mL. It is interesting to note that among our patients, a half-$\log_{10}$ increase in viral load carried a risk for *P. carinii* pneumonia equivalent to that conferred by a decrease in CD4 count of 92 cells/mm^3. Our results, which are based on relatively few episodes of *P. carinii* pneumonia, should be considered preliminary. However, if they are confirmed (particularly for patients receiving highly active antiretroviral therapy), new guidelines for prophylaxis that incorporate information on HIV viral load may help target prophylaxis regimens in a safer and more effective manner. Use of viral load as a measure of immune function that is complementary to CD4 cell count may also facilitate evaluation of patients who present with symptoms, such as fever or cough, that have broad differential diagnoses.

One limitation of our study is that our patients were mostly studied before combination antiretroviral therapy became standard clinical practice in the mid-1990s. We therefore could not examine the effects of currently used therapies, which can often reduce viral load to levels that are undetectable by extremely sensitive assays. Future research should address the time course over which treatment benefits accrue to determine whether, in effectively treated patients, reductions in viral load result in decreased immunosuppression or instead affect other aspects of HIV disease pathogenesis. Our results should also be replicated in HIV-infected persons with HIV risk factors other than hemophilia.

Serial viral load measurements provide more information than one measurement (26). For patients receiving combination antiretroviral therapy, pretreatment viral load and CD4 cell count predict risk, but the magnitude and duration of treatment-induced changes in viral load are also important (8, 9, 35, 36). Because we measured viral load only at baseline, we could not study changes in viral load. However, our relatively simple study design highlighted the time course over which a single measure of viral load gives useful information on disease risk.

Viral load measurements in patients with late-stage HIV disease offer information on risk for AIDS-related illness. Viral load most strongly reflects current risk and thus provides information on current immune dysfunction. The information given by viral load measurements complements that given by CD4 cell counts and could be incorporated as an independent measure into patient management decisions about diagnosis, prophylaxis, and therapy.

Appendix: Institutions and Investigators in the Multicenter Hemophilia Cohort Study

Research Triangle Institute, Rockville, Maryland: Barbara L. Kroner, PhD, and Susan E. Wilson. National Cancer Institute, Rockville, Maryland: Eric A. Engels, MD, MPH; Mitchell Gail, MD, PhD; James J. Goedert, MD; Thomas R. O'Brien, MD, MPH; Charles Rabkin, MD, MSc; and Philip S. Rosenberg, PhD. Mount Sinai Medical Center, New York, New York: Louis M. Aledort, MD, and Stephanie Seremetes, MD. Pennsylvania State University School of Medicine, Hershey, Pennsylvania: M. Elaine Eyster, MD. Cornell University Medical Center, New York, New York: Donna Di Michele, MD, and Maragaret W. Hilgartner, MD. Cardeza Foundation Hemophilia Center, Philadelphia, Pennsylvania: Barbara Konkle, MD. Christiana Hospital, Newark, Delaware: Phillip Blatt, MD. University of North Carolina, Chapel Hill, North Carolina: Gilbert C. White II, MD. Children's Hospital of Philadelphia, Philadelphia, Pennsylvania: Alan R. Cohen, MD. Children's Hospital National Medical Center, Washington, D.C.: Anne L. Angiolillo, MD, and Naomi Luban, MD. Georgetown University Medical Center, Washington, D.C.: Craig M. Kessler, MD. Case Western Reserve University, Cleveland, Ohio: Michael M. Lederman, MD. Tulane University Medical School, New Orleans, Louisiana: Cindy Leissinger, MD. University of Colorado, Denver, Colorado: Marilyn Manco-Johnson, MD. University of Texas Health Science Center, Houston, Texas: W. Keith Hoots, MD. Hospital Cantonal Univer-

sitaire, Geneva, Switzerland: Philippe de Moerloose, MD. Athens University Medical School and Laikon General Hospital, Athens, Greece: Angelos Hatzakis, MD; Anastasia Karafoulidou, MD; and Titika Mandalaki, MD. Universität München, Munich, Germany: Wolfgang Schramm, MD. University of Vienna, Vienna, Austria: Sabine Eichinger, MD.

From the National Cancer Institute, Rockville, Maryland.

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