Cumulative HIV Viremia during Highly Active Antiretroviral Therapy Is a Strong Predictor of AIDS-Related Lymphoma

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(See the editorial commentary by Sabin, on pages 8–10.)

Background.　AIDS-related lymphoma contributes to significant morbidity and mortality among human immunodeficiency virus (HIV)–infected patients receiving highly active antiretroviral therapy (HAART). We assessed the predictive role of cumulative HIV viremia and other risk factors in the development of AIDS-related non-Hodgkin lymphoma.

Methods.　Data from the Clinical Surveillance of HIV Disease (ClinSurv) study, an ongoing, observational, open cohort study of HIV-infected patients from different urban areas in Germany, were analyzed using a Cox proportional hazards model.

Results.　In the Cox model, which comprised 6022 patients and 27,812 patient-years of follow-up while patients were receiving HAART from 1999 through 2006, cumulative HIV viremia was found to be independently associated with the risk of lymphoma (hazard ratio, [HR], 1.67 [95% confidence interval {CI}, 1.27–2.20]) ($P < .001$). This association differed markedly between lymphoma subtypes. Although the association was more pronounced for Burkitt-type lymphoma (HR, 3.45 [95% CI, 1.52–7.85]) ($P = .003$), there was no association between cumulative HIV viremia and the incidence of primary central nervous system lymphoma (HR, 1.00 [95% CI, 0.39–2.57]) ($P = .997$). Other risk factors associated with an increased risk in a multivariable analysis included the latest CD4 T cell count as well as age per 10-year increment.

Conclusions.　Cumulative HIV viremia is an independent and strong predictor of AIDS-related lymphoma among patients receiving HAART. The influence of cumulative HIV viremia may differ between lymphoma subtypes.

The incidence of opportunistic diseases has significantly decreased among patients infected with human immunodeficiency virus (HIV) since the advent of highly active antiretroviral therapy (HAART); however, the decrease in the incidence of AIDS-related lymphomas has been less marked [1–3]. Malignant lymphomas have become the most common AIDS-related cancers and are associated with poor overall survival. They contribute to significant morbidity and mortality, including up to 30% of AIDS-related deaths [4–7]. Depending on the lymphoma subtype, the risk of developing lymphoma is up to 165 times higher for HIV-infected individuals than for non–HIV-infected individuals, and HAART does not eliminate the risk of lymphoma, despite observations of an ~10-fold reduction in incidence rates when HAART is given over a long period [8–11].

AIDS-related lymphomas represent a heterogeneous entity of diseases, and distinct risk factors may prevail. The influence of immunity on the risk of lymphoma seems to be variable for lymphoma subtypes. In one

large HIV/AIDS Cancer Match Study, Burkitt-type lymphoma was not associated with the CD4 cell count, whereas primary central nervous system (CNS) lymphoma clearly was [12]. Other studies have suggested that HIV replication may influence lymphomagenesis due to consecutive immune activation and B cell stimulation [13, 14]. Viral suppression below the limit of detection of current assays has been shown to be protective, but little is known about the risk of lymphoma among individuals who have viremic episodes while receiving HAART [15]. Intermittent viremia is not uncommon among individuals receiving HAART, and, in one cohort study [16], it was detectable during almost 18% of follow-up while individuals were receiving HAART. In the present study, we analyzed risk factors for the development of AIDS-related lymphoma and lymphoma subtypes in a large cohort of HIV-infected patients receiving HAART, with a particular focus on HIV plasma viremia.

PATIENTS AND METHODS

Cohort. The Clinical Surveillance of HIV Disease (ClinSurv) study is an ongoing, prospective, observational cohort study currently comprising 18,451 HIV-infected patients monitored at 18 clinical centers in different urban areas in Germany. All HIV-infected patients who were consecutively observed beginning on 1 January 1999 were enrolled in the study. Patients are routinely seen in a clinic every 3 months, according to national guidelines. Biannual data collection is performed using local computerized databases, with information on all CD4 T cell counts and viral load measurements gathered, as well as data on all clinical events, antiretroviral therapy (ART) initiation, or changes occurring since the time of the first visit at the reporting center. All time-related variables are collected and referenced according to the day of occurrence. Data are validated and monitored manually as well as electronically at the coordinating center (Robert Koch Institute); source data are verified; and double reporting is excluded. The ClinSurv study has been approved by the Federal Commissioner for Data Protection and Freedom of Information.

Data analysis. This analysis included all patients receiving HAART for whom no diagnosis of lymphoma was made either before or 30 days after HAART initiation, because patients who were given a diagnosis during this period were considered to have prevalent lymphomas. HAART was defined as combination therapy involving at least 3 antiretroviral drugs (either therapy containing a protease inhibitor or nonnucleoside reverse-transcriptase inhibitor or therapy containing 3 nucleoside reverse-transcriptase inhibitors).

For the survival analysis, the length of follow-up was calculated as (1) the time from initiation of HAART (i.e., baseline) to either diagnosis of AIDS-related lymphoma, last follow-up, or 3 December 2006; or (2) a maximum of 3500 days of follow-up (e.g., completion of 10 years), after which time too few

patients were left in the cohort. Patients were included in the analysis only if at least one measurement of the CD4 T cell count and 2 measurements of the plasma viral load were available within this period. Because of the different methods of viral load measurement used and because of previous use of earlier versions of HIV RNA assays that had higher thresholds of detectability, a cutoff level of 500 HIV RNA copies/mL was defined as the limit of detection.

Definition of AIDS-related lymphoma. Incident AIDS-related lymphoma was defined as lymphoma occurring at a minimum of 30 days after initiation of HAART. This period was chosen based on the rapid tumor growth that characterizes these tumors clinically. A sensitivity analysis including only those lymphomas that developed 90 days after of initiation of HAART yielded similar results. Lymphoma classification was performed by local pathologists on the basis of biopsy or autopsy findings, and every participating center vouched for the correct classification [10]. Because central reviewing of specimens was not possible, we restricted lymphoma classification to 4 groups: Burkitt-type lymphoma (Burkitt and Burkitt-like lymphoma), non-Burkitt high-grade B cell lymphoma (including diffuse large B cell lymphoma and primary effusion lymphoma), documented lymphoma for which no histologic diagnosis was available, and primary CNS lymphoma. Low-grade lymphomas were excluded.

As an internal control, the influence of viremia and other risk factors was also analyzed for cases of incident *Candida* esophagitis in the absence of AIDS-related lymphoma, because we assumed no influence of viremia independent of CD4 cell counts for this event. All patients without a diagnosis of lymphoma were included in this analysis. A univariate and multivariable Cox model that incorporated the same variables that were used in the analysis of lymphomas was fitted. Similar to what was done in the analysis of lymphomas, the length of follow-up was calculated as the time from initiation of HAART to either the time that *Candida* esophagitis was newly diagnosed (based on clinical reports), last follow-up, or 31 December 2006. Variables that were significantly associated with an increased risk of *Candida* esophagitis in the univariate analysis were included in the multivariable model.

Analysis of viremia. To analyze the influence of HIV viremia, we first categorized patients with and without a lymphoma diagnosis according to whether viral load measurements >500 copies/mL were noted in <25%, 25%–50%, 51%–75%, or >75% of all available viral load test results. All patients who had at least one CD4 T cell count and one viral load measurement obtained during follow-up were included in the analysis. We noticed a significantly increased risk of lymphoma development in the highest (>75%) category in a Cox proportional hazards model (including 79 patients with lymphoma and 6348 patients without a lymphoma diagnosis) (figure 1, which appears only in the

Figure 1. Influence of persistent viremia

electronic version of the *Journal*). To account for the possibility that successive measurements made during a short period might have biased the results for persistent viremia as described above, we analyzed the area under the curve of the log viral load to quantify cumulative viremia more accurately.

Variable selection and statistical modeling. Factors associated with the diagnosis of AIDS-related lymphoma were assessed in Cox models. Proportional hazards assumptions were confirmed by Schoenfeld's tests. The variables tested included possible confounders or effect modifiers, such as age at baseline, sex, HIV transmission group, origin, AIDS-defining illness before initiation of HAART, previous exposure to antiretroviral monotherapy or dual therapy, and calendar period when HAART was initiated (before 1 January 2001 vs. after 1 January 2001, when modern protease inhibitor therapy became widely available in Germany). To account for the fact that patients could have interrupted therapy during follow-up, the total percentages of follow-up time spent receiving or not receiving HAART were calculated for each individual patient and were introduced into the model as a fixed covariate (table 1). Immunologic variables included the nadir CD4 T cell count before initiation of HAART, the CD4 T cell count at baseline, and the time-updated latest CD4 T cell count.

To assess the influence of HIV viremia and to find the best possible predictor for lymphoma, we tested the HIV load at baseline, the maximum HIV load, and the time-updated latest HIV load. The latest CD4 cell count and viral load were grouped into clinically meaningful categories (table 2).

In addition, cumulative viremia was modeled as the time-updated area under the curve of the log viral load. Every viral load measured had an attributed period of validity extending to the previous measurement or a maximum of 180 days. A viral load below the limit of detection (500 HIV RNA copies/mL) did not contribute to the calculation of cumulative viremia. The area under the curve was calculated by totaling the product of the log viral load and its corresponding period of validity (figure 2). Using cumulative viremia in the Cox model, we observed risk saturation at ~7000 days × log HIV RNA copies/mL. To explain and further investigate this risk saturation, we constructed a cumulative viremia variable with a finite memory that included only those viral load values that had accumulated in the 3 years before the current follow-up in an alternative model.

Baseline characteristics of patients with lymphoma and of patients with different lymphoma subtypes diagnosed during follow-up were compared with the characteristics of patients without lymphoma, by use of the χ^2 test and the nonparametric rank-sum test. All variables were tested in a univariate analysis and were then included in a multivariable model with stepwise reduction of variables for which $P > .1$. All analyses were performed using Stata software (version 10; Stata). $P < .05$ was considered to denote statistical significance, and all tests of significance were 2-sided.

RESULTS

From 1 January 1999 through 31 December 2006, a total of 6704 HIV-infected patients in the ClinSurv cohort study received HAART. Of a total of 284 cases of AIDS-related lymphoma, 185 were considered to be prevalent at the time of initiation of HAART and were excluded from the study. The remaining 99 cases were diagnosed at least 30 days after initiation of HAART and were considered to be incident cases. A total of 6022 patients, including 66 patients with incident lymphoma, had at least 1 documented CD4 T cell count and 2 viral load measurements during follow-up and were included in the analysis of cumulative viremia (table 2).

Based on a total of 27,812 person-years in this analysis, the incidence of any lymphoma developing was 2.4 cases/1000 patient-years (95% confidence interval [CI], 1.8–3.0 cases/1000 patient-years). Characteristics at baseline, according to lymphoma subtypes, are listed in table 1. In an additional analysis 138 HIV-infected patients with incident *Candida* esophagitis (i.e., cases occurring >30 days after initiation of HAART) and without a diagnosis of lymphoma were identified and analyzed.

Demographic data and HAART. Characteristics at baseline were heterogeneous for patients with lymphoma and those without lymphoma (table 1). There was no difference with respect to the total percentage of follow-up time when HAART was received. The mean observation time was 754 days (interquartile range [IQR], 327–1847 days) in the lymphoma group, compared with 1520 days (IQR, 730.5–2645 days) in the nonlymphoma group ($P < .01$). The frequency of viral load testing per year was similar among patients with lymphoma and lymphoma subtypes, with the exception of patients with Burkitt-type lymphoma, for whom the frequency of testing was higher (5.57 vs. 3.97 measurements/year; $P = .005$) (table 1).

Immunologic and virologic data. The CD4 T cell count nadir before initiation of HAART was significantly lower in the lymphoma group. The CD4 T cell count at baseline was 90 cells/μL (IQR, 38–220 cells/μL) in the lymphoma group versus 204 cells/μL (IQR, 80–340 cells/μL) in the nonlymphoma group ($P < .01$). The log viral load at initiation of HAART (i.e., at baseline) was higher among patients with lymphoma than among patients without lymphoma (5.14 vs. 4.81 HIV RNA copies/mL; $P = .01$).

Lymphoma characteristics. Of the 66 patients with lymphoma who fulfilled the study inclusion criteria, 11 (16.7%)

Table 1. Patient characteristics

Characteristic	Patients without a lymphoma diagnosis (n = 5956)	Patients with a lymphoma diagnosis				
		All (n = 66)	Burkitt-type NHL (n = 11)	Non-Burkitt high-grade B cell NHL (n = 32)	Primary CNS lymphoma (n = 8)	AIDS-related lymphoma[a] (n = 15)
Days of observation, median (IQR), no.	1520 (731–2645)	754 (327–1847)[b]	464 (227–1528)[b]	930 (559–1934)[c]	329.5 (183–1444)[c]	742 (362–1898)[c]
Viral load measurements per year, mean ± SD, no.	3.91 ± 2.17	4.45 ± 2.65	5.57 ± 2.05[b]	4.33 ± 2.56	5.07 ± 3.59	3.54 ± 2.55
Sex, male	79.8	92.4[c]	90.9	87.5	100	100
Risk of transmission						
Among MSM	52.6	65.2[c]	72.7	68.8	62.5	53.3
Among IDUs	8.1	4.6	9.1	3.1	0	6.7
AIDS-defining illness[d]	28.7	53.0[b]	54.6	56.3[b]	75.0[b]	33.3
Age at baseline, mean ± SD, years	39.4 ± 1.4	43.7 ± 1.2[b]	42.8 ± 3.0	43.5 ± 1.8[c]	47.6 ± 4.4[c]	42.8 ± 2.3
Geographic origin						
Germany	75.6	83.3	72.7	84.4	87.5	86.7
Sub-Saharan Africa	10.3	6.0	9.1	0	12.5	13.3
HAART initiation before 1 Jan 2001	45.2	69.7[b]	45.5	75.0[b]	87.5[c]	33.3
ART naive at HAART initiation	53.6	42.4[b]	54.6	37.5	75.0	26.7[c]
Follow-up time receiving HAART, mean ± SD, %	86.5 ± 0.3	88.4 ± 2.8	96.3 ± 2.8	93.2 ± 2.7	75.3 ± 13.7	79.4 ± 7.7
Log viral load at HAART initiation, median (IQR), copies/mL	4.81 (4.03–5.33)	5.14 (4.79–5.48)[c]	5.03 (4.92–5.24)	5.36 (4.9–5.7)[c]	5.08 (4.34–5.14)	5.00 (4.00–5.48)
CD4 T cell count, median (IQR), cells/μL						
Nadir before HAART	186 (70–300)	60 (30–137)[b]	44 (29–71)[c]	60 (30–137)[b]	96 (15–106)	93.5 (60–185)
At baseline	204 (80–340)	90 (38–220)[b]	93.5 (42–284)	77 (27–208)[b]	68.5 (18–106)	107 (83–415)

NOTE. Data are the % of patients, unless otherwise indicated. Proportions and means of patients with AIDS-related lymphoma subtypes are compared with those of patients without a lymphoma diagnosis, by use of the Wilcoxon rank sum test and the χ^2 test. ART, antiretroviral therapy; CNS, central nervous system; HAART, highly active antiretroviral therapy; IDUs, injection drug users; IQR, interquartile range; MSM, men who have sex with men; NHL, non-Hodgkin lymphoma; SD, standard deviation.

[a] Not otherwise specified.
[b] $P \leq .01$.
[c] $P < .05$.
[d] Before HAART initiation.

Table 2. Cox proportional hazards model for all incident AIDS-related lymphomas (*n* = 66) and cases of incident *Candida* esophagitis (*n* = 138).

	All AIDS-related lymphomas				*Candida* esophagitis, multivariable model	
	Univariate model		Multivariable model			
Risk factor	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age at baseline[a]	1.43 (1.16–1.76)	<.001	1.42 (1.15–1.75)	.001	...	...
Sex, male	2.78 (1.12–7.14)	.03	...	...	...	...
Transmission risk among MSM[b]	1.44 (0.87–2.39)	.16	...	...	...	...
Germany as origin[c]	1.37 (0.72–2.62)	.34	...	...	...	...
AIDS-defining illness before HAART initiation	2.78 (1.71–4.51)	<.001	...	...	...	...
ART naive at HAART initiation[d]	0.94 (0.57–1.55)	.81	...	...	...	...
HAART initiation on or after 1 Jan 2001[e]	0.91 (0.51–1.62)	.75	...	...	...	...
Follow-up time receiving HAART,[f] %	1.06 (0.95–1.19)	.23	...	...	0.86 (0.81–0.91)	<.001
CD4 T cell count, cells/μL						
Nadir of <200 before HAART initiation[g]	5.91 (2.51–13.91)	<.001	...	...	...	...
Latest			...	...	...	...
<200[h]	19.19 (6.76–54.48)	<.001	8.16 (3.90–17.10)	<.001	7.64 (4.74–12.29)	<.001
201–350[h]	10.35 (3.57–30.06)	<.001	5.21 (2.46–11.03)	<.001	2.38 (1.39–4.05)	.01
351–500[h]	2.60 (0.73–9.21)	.14	...	...	...	...
Latest viral load, copies/mL						
500–10,000[i]	0.84 (0.30–2.34)	.73	...	...	2.05 (1.15–3.65)	.01
10,001–100,000[i]	3.14 (1.67–5.90)	<.001	...	...	1.96 (1.11–3.48)	.02
>100,000[i]	5.60 (2.86–10.97)	<.001	...	...	6.27 (3.69–10.64)	<.001
Cumulative HIV viremia[j]	2.17 (1.66–2.85)	<.001	1.67 (1.27–2.20)	<.001	1.11 (0.91–1.36)	.30

NOTE. ART, antiretroviral therapy; CI, confidence interval; HR, hazard ratio; MSM, men who have sex with men.

[a] Per 10-year increment.
[b] Compared with other transmission groups.
[c] Compared with other countries of origin.
[d] Compared with prior exposure to antiretroviral monotherapy or dual therapy and unknown pretreatment status.
[e] Compared with HAART initiation before 1 January 2001.
[f] Per 10%.
[g] Compared with a CD4 T cell count nadir of ≥200 CD4 T cells/μL.
[h] Reference group, >500 CD4 T cells/μL.
[i] Reference group, ≤500 copies/mL.
[j] Per 2000 days × log HIV RNA copies/mL (reference group, 0 days × log HIV RNA copies/mL). For the alternative model of cumulative viremia in the past 3 years, the HR was 1.81 (95% CI, 1.32–2.49) (*P*<.001) in the multivariable analysis.

had Burkitt-type lymphoma, 32 (48.5%) had non-Burkitt high-grade B cell lymphoma, 8 (12.1%) had primary CNS lymphoma, and 15 (22.7%) had AIDS-related lymphoma, not otherwise specified. Four of the patients with AIDS-related lymphoma (6% of the 66 patients with lymphoma included in the study) had clinically suspected lymphoma. When these 4 patients with clinically suspected lymphoma were excluded, a sensitivity analysis yielded similar results (data not shown). Compared with the 66 patients with lymphoma who were included in the analysis, the 33 patients with lymphoma who were excluded from the study because they lacked CD4 cell counts or viral load data were significantly younger (mean age, 39.2 vs. 43.7 years; *P* = .03), less often received a diagnosis of AIDS before initiation of HAART (mean frequency, 27.3% vs. 53.0%; *P* = .02), and had a shorter time to lymphoma diagnosis (mean, 261 vs. 754 days; *P*<.001). There was no difference in terms of transmission risk, sex, country of origin, year

of initiation of HAART, ART-naive status, or percentage of follow-up time during which HAART was received.

Risk factors in the univariate and multivariable analyses. In a univariate analysis, baseline risk factors that were associated with an increased risk for the development of any lymphoma were age per 10-year increment, male sex, AIDS-defining illness before initiation of HAART, and a CD4 T cell count nadir of <200 cells/μL. CD4 T cell counts in the lowest 2 categories (<200 cells/μL and 201–350 cells/μL) and a latest plasma viral load of ≥10,001–100,000 HIV RNA copies/mL were also associated with the risk of lymphoma. The hazard ratio (HR) for the incidence of lymphoma increased per 2000 days × log HIV RNA copies/mL (table 2).

In a multivariable analysis, age per 10-year increment (HR, 1.42 [95% CI, 1.15–1.75]; *P* = .001) and CD4 T cell counts of <200 cells/μL (HR, 8.16 [95% CI, 3.90–17.10]; *P*<.001) and 201–350 cells/μL (HR, 5.21 [95% CI, 2.46–11.03]; *P*<.001)
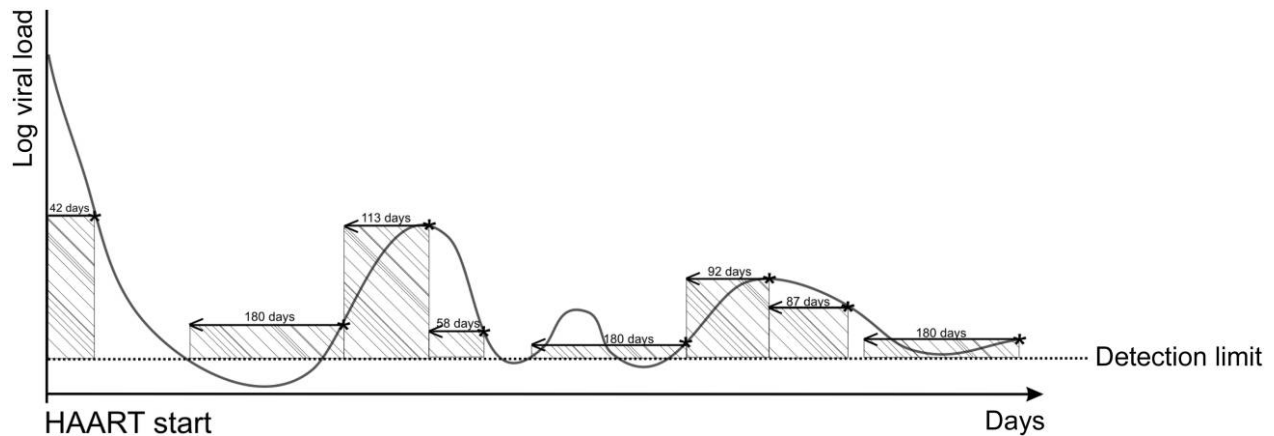
Figure 2. Construction of cumulative viremia, with calculation of the time-updated area under the curve of the log-viral load. The log viral load (*) was multiplied by the corresponding period of validity (←) extending to the previous viral load measurement (*) or a maximum of 180 days. The gray-shaded area under the curve denotes cumulative viremia. The area below the detection limit (<500 human immunodeficiency virus RNA copies/mL) was not calculated.

remained independently associated with the risk of development of any lymphoma. In addition, cumulative viremia was highly associated with the risk of lymphoma (HR per 2000 days × log HIV RNA copies/mL, 1.67 [95% CI, 1.27–2.20]; $P<.001$). The influence of cumulative viremia, as stratified by a CD4 T cell count of <200 cells/μL, is depicted in figure 3.

Analysis of lymphoma subtypes and Candida *esophagitis.* Additional univariate and multivariable analyses were performed to analyze the risk factors for lymphoma subtypes and *Candida* esophagitis. Time receiving HAART, the latest CD4 T cell count assigned to the 2 lowest categories (<200 cells/μL and 201–350 cells/μL), and cumulative HIV viremia were all significantly associated with the risk for non-Burkitt high-grade lymphoma. In contrast, cumulative HIV viremia was the only risk factor highly associated with a diagnosis of Burkitt-type lymphoma. The HR for cumulative HIV viremia was higher for Burkitt-type lymphoma (HR per 2000 days × log HIV RNA copies/mL, 3.45 [95% CI, 1.52–7.85]; $P = .003$) than for non-Burkitt high-grade lymphoma (HR per 2000 days × log HIV RNA copies/mL, 2.02 [95% CI 1.37–2.98]; $P<.001$).

There was no association between cumulative HIV viremia and diagnosis of primary CNS lymphoma. Age per 10-year increment (HR, 1.83 [95% CI, 1.03–3.24]; $P = .040$) and a CD4 cell count <200 cells/μL (HR, 6.53 [95% CI, 1.44–29.51]; $P = .015$) were the only risk factors significantly associated with a diagnosis of CNS lymphoma (table 3).

In a multivariable analysis, there was no association between cumulative HIV viremia and diagnosis of *Candida* esophagitis. Duration of HAART and a CD4 T cell count of <200 cells/μL or 201–350 cells/μL, as well as the latest viral load in 3 categories above the threshold of detection (500–10,000 copies/mL, 10,001–100,000 copies/mL, and >100,000 copies/mL) were all associated with the risk of *Candida* esophagitis (table 2).

A similar influence of cumulative HIV viremia was observed for all lymphomas (HR per 2000 days × log HIV RNA copies/mL, 1.81 [95% CI, 1.32–2.49]; $P<.001$) and for Burkitt-type lymphomas (HR per 2000 days × log HIV RNA copies/mL, 3.46 [95% CI, 1.51–7.92]; $P = .003$), when analyzing cumulative HIV viremia only in the 3 years preceding the time of the most recent follow-up, the date of lymphoma diagnosis, or the censoring date, respectively.

DISCUSSION

The present study provides evidence supporting the strong influence of HIV replication on lymphomagenesis. The HR for the development of lymphoma increased with the accu-
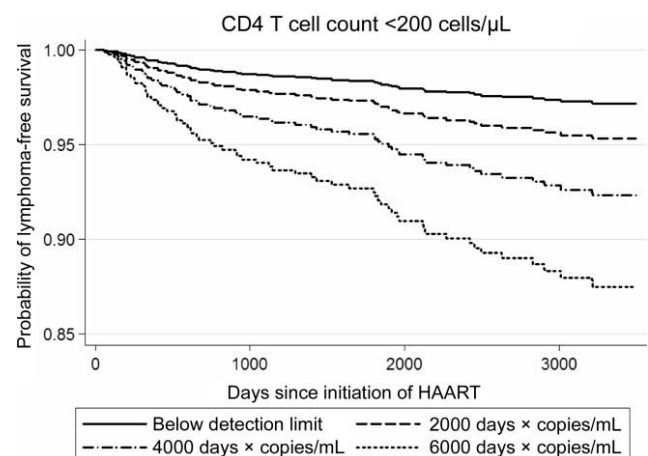

Figure 3. Cox proportional hazards model illustrating the influence of cumulative human immunodeficiency virus viremia on lymphoma-free survival. Stratum: CD4 T cell count <200 cells/μL.

Table 3. Multivariable Cox proportional hazards model for lymphoma subtypes.

Risk factor	Burkitt-type NHL		Non-Burkitt high-grade B cell NHL		Primary CNS lymphoma	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Age at baseline[a]	…	…	…	…	1.83 (1.03–3.24)	.04
Follow-up time receiving HAART,[b] %	…	…	1.48 (1.10–1.98)	.01	…	…
Latest CD4 T cell count,[c] cells/μL						
<200	…	…	9.95 (3.14–31.51)	<.001	6.53 (1.44–29.51)	.02
201–350	…	…	7.78 (2.50–24.19)	<.001	…	…
Cumulative HIV viremia[d]	3.45 (1.52–7.85)	.003	2.02 (1.37–2.98)	<.001	1.00 (0.39–2.57)	1.00

NOTE. CI, confidence interval; CNS, central nervous system; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HR, hazard ratio; NHL, non-Hodgkin lymphoma.

[a] Per 10-year increment.
[b] Per 10%.
[c] Reference group, CD4 T cell count >500 cells/μL.
[d] Per 2000 days × log HIV RNA copies/mL (reference group, 0 days × log HIV RNA copies/mL).

mulation of HIV viremia during follow-up, independent of the latest CD4 T cell count.

Previous cohort study analyses suggested that the risk of lymphoma was lower among patients who had a viral load of <500 HIV RNA copies/mL and that plasma viremia might be an independent risk factor, but these possibilities were not further investigated in a multivariable analysis [15, 17]. In another study, an HIV load above the limit of detection was associated with the development of new opportunistic diseases in patients receiving HAART [18]. However, in our study, cumulative HIV viremia (rather than the latest viral load) best predicted the development of lymphoma, suggesting an important effect of sustained viremia over time and a distinct pathogenesis of AIDS-related lymphoma, compared with other opportunistic diseases.

The present study had several limitations, which were mainly the result of the study design. First, we included only patients who were receiving HAART. We were unable to analyze how much viremia had accumulated before the initiation of HAART. Of note, however, the effect of cumulative viremia remained highly significant when only viremia accumulated in the 3 years before current follow-up was included. This finding indicates that recent viremia might be most relevant for lymphomagenesis and that the risk of lymphoma is reduced when full viral suppression is sustained over a minimum of 3 years.

Second, although the CD4 cell count and the viral load are routinely checked every 3 months, in accordance with local guidelines, there may have been inconsistencies in the frequency and number of data collection time points, as well as in the types of assays used for analysis in the participating centers. We constructed the cumulative viremia, assuming the comparability of licensed and validated viral load tests, to account for missing viral load measurements during an individual's follow-up. This was done in an attempt to include the largest number of patients

possible and not to restrict this analysis to patients who had a complete follow-up. However, exclusion of patients with lymphoma who were lacking CD4 cell count or viral load data could have led to an overestimation of the influence of age and a previous AIDS diagnosis on lymphomagenesis.

Third, although end points were carefully verified by reviewing clinical reports and results of histologic examination, central reviewing of lymphoma histopathologic findings and further classification of lymphomas with unknown histology were not possible in this clinical cohort analysis. Given the difficulties in the diagnosis and correct classification of AIDS-related lymphoma, we cannot entirely exclude the possibility that some cases had not been classified correctly. Burkitt-type lymphoma, however, is an entity that is more clearly defined and has more distinct clinical and histopathologic findings than do other types of high-grade non-Hodgkin lymphoma (NHL) [19]. Furthermore, high-grade malignant primary cerebral NHL is characterized by its almost exclusive localization within the CNS. Therefore, despite the possibility of some misclassification, we regarded it as justified to group the tumors for the analysis as outlined above.

Finally, in the subtype analysis, only a small number of cases of Burkitt lymphoma and primary CNS lymphoma were available, resulting in wider confidence intervals and a less reliable multivariable model. This warrants further investigation in even bigger cohorts.

Despite these limitations, which are typical of cohort studies, we regard these results as important. To our knowledge, this is the first study to show that uncontrolled HIV replication during HAART, as assessed by cumulative HIV viremia, is predictive of the development of AIDS-related lymphoma. This effect seems to be strongest for high-grade NHL other than primary cerebral lymphoma. By contrast, no independent in-

fluence of cumulative viremia on incident primary CNS lymphoma or *Candida* esophagitis was observed.

The underlying mechanisms, however, remain unclear. Persistently elevated serum globulin levels, an indicator of chronic B cell stimulation, are associated with an increased risk of lymphoma, probably because of an increased turnover of B cells and accumulating genetic aberrations, giving rise to malignant B cell clones [13]. B cell stimulation may persist for a long period, even when viral replication is profoundly suppressed by HAART [14]. The clear influence of cumulative viremia on the risk of Burkitt-type NHL in our study suggests that this tumor entity might be especially promoted by factors associated with viral replication, such as B cell activation and accumulation of viral proteins in germinal centers, the anatomical site of B cell maturation and differentiation. In non–HIV-infected patients, Epstein-Barr virus may drive development of classical Burkitt lymphoma; however, this virus has been shown to be associated with only 50% of HIV-associated Burkitt-type lymphomas, and its role in lymphomagenesis is not entirely clear [20, 21]. In fact, ongoing replication of HIV might similarly trigger development of Burkitt-type lymphomas in HIV-infected patients.

Low CD4 T cell counts were shown to be associated with several AIDS-defining diseases, including AIDS-associated malignancies [12, 22–25]. Therefore, reduced immunosurveillance apparently contributes to an increased risk for the development of tumors. In this study, the risk of lymphoma was highest when the latest CD4 cell count was <200 cells/μL (HR, 8.16). Of note, 10 (15.2%) of 66 lymphomas occurred at a CD4 cell count of >350 cells/μL, indicating a significant influence of risk factors other than the latest CD4 cell count.

In conclusion, providing longitudinal data from a large multicenter clinic-based cohort, we are able to demonstrate that uncontrolled HIV replication during HAART, as assessed by cumulative HIV viremia, is predictive of the development of lymphoma. The effect seems to be strongest for Burkitt-type lymphoma, whereas primary CNS lymphoma may be more similar to an opportunistic infection such as *Candida* esophagitis. In line with other studies, the latest CD4 cell count and age were also associated with the risk of lymphoma. Therefore, cumulative HIV viremia during HAART represents an additional (yet undescribed) and potentially modifiable risk factor.

These results should encourage physicians to optimize HAART for patients with persisting HIV replication, in an attempt to achieve complete viral suppression and to minimize the risk of this life-threatening complication.

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