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HIV Viremia and Incidence of Non-Hodgkin Lymphoma in Patients Successfully Treated With Antiretroviral Therapy

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Title: HIV viremia and incidence of non-Hodgkin lymphoma in patients successfully treated with antiretroviral therapy

Running Title: HIV viremia and NHL incidence on ART

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Summary: Non-Hodgkin lymphoma incidence is high in HIV-infected patients successfully treated with ART. HIV replication, even at low levels, may be an important modifiable risk factor for non-Hodgkin lymphoma.

Abstract

Background: The incidence of non-Hodgkin lymphoma (NHL) in HIV-infected patients remains high despite treatment with antiretroviral therapy (ART).

Methods: We evaluated NHL incidence in HIV-infected patients followed in the CFAR Network of Integrated Clinical Systems (CNICS) who started combination ART and achieved suppression of HIV. We estimated the hazard ratio for NHL by time-varying HIV viremia categories accounting for time-varying CD4 cell count using marginal structural models.

Results: We observed 37 incident NHL diagnoses during 21,607 person-years of follow-up in 6,036 patients (incidence rate of 171 per 100,000 person-years (PY) (95% CI: 124, 236)). NHL incidence was high even among patients with nadir CD4 cell count > 200 cells/µl (140 per 100,000 PY (95% CI: 80, 247)). Compared to ≤ 50 copies/mL, hazard ratios (HR) for NHL were higher among those with HIV viremia of 51-500 copies/mL (HR current viremia =1.66; 95% CI: 0.70, 3.94, HR 3-month lag viremia = 2.10; 95% CI: 0.84, 5.22, and HR 6-month lag viremia = 1.46; 95% CI: 0.60, 3.60) and > 500 copies/mL (HR current = 2.39·; 95% CI: 0.92, 6.21, HR 3-month lag = 3.56; 95% CI: 1.21, 10.49, and HR 6-month lag = 2.50; 95% CI:0.91, 6.84). Current HIV RNA as a continuous variable was also associated with NHL (HR = 1.41 per $\log_{10}$ copies/mL; 95% CI: 1.07, 1.85).

Conclusions: Our findings demonstrate a high incidence of NHL among HIV-infected patients on ART and suggest a role of HIV viremia in the pathogenesis of NHL. Earlier initiation of potent ART and maximal continuous suppression of HIV viremia may further reduce NHL risk.

Introduction

Non-Hodgkin lymphoma (NHL) is an AIDS-defining condition[1], for which incidence has declined in the modern era of antiretroviral therapy (ART)[2-7]. However, rates continue to be 4 to 23 times higher in HIV-infected populations compared to HIV uninfected, depending on the proportion receiving ART [4-6,8]. NHL also continues to impact survival of HIV-infected patients with little change in the proportion of NHL-associated deaths from 2000 to 2005 (11% and 10% of deaths, respectively)[9,10].

Immune deficiency, oncogenic viruses (Epstein-Barr and Kaposi sarcoma herpes viruses (EBV and KSHV)), HIV viremia, immune activation, and aging are intersecting factors contributing to the development of NHL in the setting of HIV[8,11-18]. The contribution of each of these factors to NHL risk during ART is unclear. We hypothesized that any detectable HIV viremia is a driver of immune dysfunction, B cell activation, and higher subsequent risk for NHL. We studied the incidence of NHL in a large multi-site cohort of HIV-infected patients on ART and the association with HIV viremia using rigorous methods to control for level of immune deficiency and other known confounders.

Methods

Study population

The Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort includes over 27,000 HIV-infected patients 18 years of age or older in care from 1995 to the present at eight clinical sites across the United States: Case Western Reserve University, Fenway Community Health Center of Harvard University, Johns Hopkins University; University of Alabama at Birmingham, University of California, San Diego, University of California, San Francisco, University of North Carolina, and University of Washington[19]. The frequency of follow-up averages every three months; however, patients can be seen more or less often depending on clinical care. CNICS is a dynamic cohort with approximately 1,800 new

patients enrolling and 13% of existing patients leaving care each year. Institutional review boards at each clinical site approved study protocols.

We examined all patients enrolled in CNICS for at least 90 days between 1998 and 2009 who started combination ART (at least three ARV medications) with suppression of HIV viremia and at least three months of follow-up. Suppression of HIV viremia was defined as achieving a single HIV RNA measurement less than 500 copies/mL within the first year of ART initiation. We excluded patients enrolled in CNICS before 1998 to assure HIV viral load testing with ultrasensitive HIV RNA PCR assays (detection limit < 50 copies/mL). Patients who developed NHL prior to or within 14 days of achieving initial suppression of HIV viremia were excluded.

Sources of data

The CNICS data repository captures comprehensive clinical data for HIV-infected patients in care at each CNICS site that include standardized diagnosis, medication, laboratory, and demographic information collected through electronic health records and other institutional data systems[19]. Data quality assessment is conducted at the sites prior to data transmission and at the time of submission to the CNICS Data Management Core. After integration into the repository, data undergo extensive quality assurance procedures and data quality issues are reported to CNICS sites by the Data Management Core to investigate and correct. Data from each site are updated, fully reviewed, and integrated into the repository quarterly. The following variables were included in the analysis: demographics (i.e., year of birth, sex, race/ethnicity), men who have sex with men (MSM) as a risk factor for HIV transmission, Hepatitis B and C co-infection, ART, CD4 cell counts, and HIV viremia determined by quantitative plasma HIV RNA PCR assays. Hepatitis C virus (HCV) infection was defined by positive HCV antibody or HCV RNA testing within six months of HIV RNA suppression. Hepatitis B virus (HBV) infection was defined by positive HBV surface antigen or HBV DNA testing withing six months of HIV RNA suppression. Pre-ART Peak HIV viremia was defined as the highest HIV RNA measurement from the start of CNICS follow-up until ART initiation. Nadir CD4 cell count was defined as the

lowest CD4 cell count from the start of CNICS follow-up until ART initiation. If CD4 cell count was missing at baseline, the CD4 cell count measurement within one month of the date of suppression was used. Single-value imputation with the median was used for missing values of nadir CD4 cell count (<1% missing) and peak HIV viremia (2% missing).

NHL ascertainment

At each CNICS site, incident diagnoses of NHL were reviewed using a standardized protocol to confirm the diagnosis and collect detailed information regarding NHL histopathology subtype[20]. Biopsy results confirmed 87% of systemic NHL and 68% of primary central nervous system (CNS) NHL diagnoses; the remaining NHL cases were diagnosed based on clinical, radiographic, and/or historical information. The NHL ascertainment and verification process was performed at all CNICS sites through December 31, 2009; follow-up was administratively censored after this date.

Statistical analysis

We followed patients from date of suppression of HIV viremia (<500 copies/mL) until incident NHL, death, administrative censoring (defined above), or last follow-up. Loss to follow-up was defined as one year without a clinic visit before the administrative censoring date. Incidence rates were calculated as number of NHL diagnoses per 100,000 person-years (PY) of follow-up time. Considering strong known associations with profound immune deficiency and EBV co-infection, we performed a sensitivity analysis where we censored the 7 cases of primary CNS lymphoma at their date of occurrence.

We used marginal structural models and, for comparison, standard adjusted Cox regression models to examine current and lagged time-varying HIV viremia as the primary predictor of NHL. HIV viremia was modeled as a continuous variable and categorized as ≤ 50 (reference), 51-500, and > 500 copies/mL. In addition to current HIV viremia, we explored 3-, and 6-month lagged values. Current HIV viremia was defined as the HIV RNA measurement at the current visit and lagged HIV viremia as HIV RNA measurements 3 and 6 months prior to

visit. We adjusted for the main covariate of time-varying CD4 cell count through inverse probability (IP) weights, including current and two visit lagged measurements. CD4 cell count was treated as a continuous variable and modeled using a restricted quadratic spline. Other covariates included in adjusted models were age (< 39 vs. ≥ 39 years), sex, race, year of HIV suppression, peak HIV viremia, and HCV co-infection.

Adjusted and IP weighted Cox models were fit with and without peak pre-ART HIV viremia and HCV co-infection and the results were comparable; peak pre-ART HIV viremia and HCV co-infection were removed from the final model. A polytomous logistic regression was used to model HIV viremia categories for IP weights. Robust estimates of the variance were used in marginal structural models to account for the estimated IP weights. The distributions of the IP weights are summarized in the Supplemental Table. Marginal structural Cox models were used to generate curves of NHL incidence over time from HIV suppression. All statistical analyses were done with SAS version 9.3 (Cary, NC).

Results

Among 18,382 HIV-infected patients enrolled in CNICS between 1998 and 2009, we studied 6,036 individuals who were treatment naïve at enrollment, initiated ART and achieved suppression of HIV viremia to less than 500 copies/mL (Figure and Table 1). The median age at HIV suppression was 39 years, 51% were white, 80% were male, 56% reported MSM as a risk factor for transmission of HIV infection, 7% were co-infected with HBV, and 13% with HCV. Prior to treatment with ART, nadir CD4 cell count was 180 cells/μl, median peak pre-ART HIV viremia was 5.0 $\log_{10}$ copies/mL, and median peak HIV viremia after ART initation was 2.60 $\log_{10}$ copies/mL. The median time from ART initiation until suppression of HIV viremia to ≤ 500 copies/mL was 1.86 (IQR 1.09, 3.25) months. During follow-up, 2,411 (40%) patients experienced a rebound in HIV RNA level over 500 copies/mL at any time point and the median time from initial suppression to rebound for these patients was 8.25 months (IQR 3.64, 18.09).

The initial ART regimen was anchored with a non-nucleoside reverse transcriptase inhibitor (NNRTI) in 2,964 patients (49%), a ritonavir boosted protease inhibitor (PI) in 1,964 (33%), an unboosted PI in 561 (9%), PI and NNRTI in 121 (2%), and other classes of ART in 426 (7%).

There were 37 incident NHL diagnoses during 21,607 person-years of follow-up for a crude incidence rate of 171 per 100,000 PY (95% CI: 124, 236) with higher incidence in patients with nadir CD4 cell count ≤ 50 cells/μl (223 per 100,000 PY (95% CI: 132, 376)), as compared to those with higher nadir CD4 (Table 2). The median time between HIV suppression and incident NHL was 0.8 years (range 0.1, 7.5). Of the 37 incident NHL cases, 21 (57%) were diffuse large B cell, 7 (19%) were primary CNS, 4 (11%) Burkitt, and 5 (14%) other or unspecified lymphoma subtypes. There were 2,441 patients (40%) lost to follow-up, as defined above, over the 12-year study period. The median observation time was 2.8 years with a maximum of 11.8 years.

The crude and adjusted hazard ratios (HR) for NHL were higher among those with current, 3- and 6-month lagged HIV viremia of 51-500 copies/mL and > 500 copies/mL compared to those with current HIV viremia ≤ 50 copies/mL (Table 3). The adjusted hazard ratio for low-level viremia (51-500 copies/mL) reached statistical significance only in the 3-month lagged Cox model (covariate adjusted HR = 2.66, 95% CI: 1.08, 6.57). Current HIV viremia modeled as a continuous variable showed an increased hazard (per $\log_{10}$ copies/mL, Cox-adjusted HR = 1.41; 95% CI: 1.07, 1.85). The proportional hazards assumption was not violated for either the covariate-adjusted or IP-weighted Cox models (p-value = 0.442 and p-value = 0.745, respectively). Cumulative NHL incidence curves for current, 3- and 6-month lagged HIV viremia are displayed in Figure A-C.

After censoring the 7 cases of primary CNS NHL, we observed a loss of precision (these 7 cases comprised 19% of the 37 NHL cases), and for those with 3- or 6-month lagged CD4 count >500 cells/mm^3 we also observed an attenuation on the size of association. For those

with a 3-month lagged CD4 count 51-500 cells/mm^3, the loss of precision resulted in a lack of statistical significance (HR = 2.11; 95% CI: 0.79, 5.64).

Discussion

NHL continues to be an important cause of morbidity and mortality among HIV-infected individuals in the era of potent combination ART. We observed a high overall NHL incidence of 171 per 100,000 PY in a large cohort of patients receiving effective ART; far exceeding that reported in HIV uninfected populations of approximately 10 to 20 per 100,000 PY[5,8,21,22]. This incidence is comparable to rates of the two most common cancers in the general U.S. population: prostate and breast cancer with age-adjusted incidence rates of 152 and 124 per 100,000 PY, respectively[22]. A high incidence of NHL was noted even among patients with nadir CD4 cell count > 200 cells/μl (140 per 100,000 PY) suggesting this cancer is associated with HIV infection above and beyond the level of immunodeficiency categorized as "AIDS-defining" by the CDC in 1993[1]. Moving forward, studies should include NHL as an important clinical endpoint along with non-AIDS defining cancers.

In other cohort studies, high-level HIV viremia (over 10,000 copies/mL), cumulative HIV viremia, and lack of ART was predictive of NHL independent of nadir and time-varying immunodeficiency[8,11-15]. In contrast to prior studies, we focused our investigation on whether low-level detectable HIV viremia, as measured with modern ultrasensitive HIV RNA assays was an independent predictor of NHL among patients who demonstrated HIV suppression on ART. We believe this population is most representative of the current era of HIV treatment in which the majority of patients are offered ART resulting in an effective initial response[23]. Prior studies examined heterogeneous populations with regard to ART use and were unable to examine lower levels of HIV viremia as a predictor of clinical events such as NHL. We postulated that viral replication drives immune dysfunction and B cell activation, which increases NHL risk. Our findings support this hypothesis. After adjusting for known confounders of older

age, white race, male sex, HCV co-infection, and time-varying CD4 cell count, risk of NHL was higher when HIV viremia was above the limit of detection (50 copies/mL) in a dose-dependent relationship, albeit results were imprecise due to the relatively small number of NHL cases (n = 37). Replication in other cohorts, perhaps with extended follow up, is required to further refine the association between low-level HIV viremia and NHL during ART.

Other investigations have postulated a biologic mechanism by which HIV increases NHL risk independent of its effects on T cells and resulting immune deficiency. B cells are highly activated in the setting of untreated HIV and biomarkers of B cell activation are associated with AIDS-associated NHL (AIDS-NHL)[18,24]. Activation induced cytidine deaminse (AID), a DNA-mutating enzyme up regulated by B cell activation, is central to the development immunoglobulin heavy-chain gene class switch recombination or somatic hypermuatation in germinal center B cells [25]. Increased AID gene expression is found prior to AIDS-NHL and is induced by many viruses: EBV, HCV, HPV, and HIV[18,26]. HIV envelope can acquire CD40 ligand (CD40L) from host cell membrane and CD40L is a potent B cell stimulator[27]. *In vitro* experiments found CD40L-positive HIV virions induced AID gene expression and CD40L-negative HIV virions did not[27]. This induction was mediated by a direct interaction between CD40L in HIV envelope and CD40 receptor on B cells. Therefore, it is biologically plausible that HIV virions directly promote the development of B cell NHL through stimulation of the CD40 receptor and activation of B cells.

The precision of our results was limited by the relatively few observed NHL cases. Beyond the issue of precision, there are several possible reasons why we did not observe even stronger associations between HIV viremia and NHL risk among patients on ART. First, we were unable to assess compartmentalized HIV replication in lymphoid tissues. Throughout HIV infection and treatment with ART, lymphatic tissues, particularly gut-associated lymphoid tissue (GALT), are a major site of HIV replication, T cell depletion and virus persistence[28-33]. ART interruptions or poor ART penetration into GALT or other lymphatic tissue may result in

preferentially high levels of HIV in these areas and localized inflammation with B cell activation leading to lymphomagenesis. Alternatively, HIV may not be the only virus driving this process. Persistent defects in T cell immunity could result in increased lytic EBV replication and B cell activation or latent EBV mediated genetic changes leading to NHL.

Potential reasons for detectable HIV viremia in this study included ART non-adherence, interruptions in drug supply, virologic failure, or intermittent release from latent reservoirs. Population-based studies report approximately 25% of HIV-infected individuals in the United States lack health insurance and this factor has been shown to increase risk of ART discontinuation and suboptimal virologic suppression [34,35]. Another reason for non-adherence and viremia before NHL could be symptomatic illness from an underlying undiagnosed (subclinical) lymphoma; however, we think this was unlikely in our study as associations were stronger in our 3- and 6-month lagged HIV viremia analyses. We would expect weaker associations of detectable viremia further preceding NHL as symptoms leading to non-adherence should present or intensify in days to week immediately preceding definitive clinical diagnosis.

This study has several limitations. First, associations presented here reflect observational evidence and therefore, could be influenced by unmeasured confounding. Second, 40% of included patients experienced loss to follow-up, likely due to transfer of care, and could have developed NHL outside of CNICS. Lastly, we were unable to provide direct comparison of NHL incidence and risk with a matched population without HIV infection. Despite these limitations, this work has several strengths. This was a multi-site study from a large and diverse population with NHL diagnoses rigorously ascertained to minimize misclassification. Second, CNICS contains comprehensive and standardized information on ART, CD4 cell counts, and HIV RNA levels determined by modern ultrasensitive assays. Finally, distinct from previous studies, our study was the first to use marginal structural models to estimate the effect

of time-varying HIV viremia while adjusting appropriately for confounding by time-varying immune deficiency and other known risk factors.

In conclusion, our findings highlight the importance of prompt, maximal, and sustained HIV suppression with potent combination ART. Current HIV treatment guidelines in the United States recommend initiation of ART and maximum virologic suppression for nearly all infected patients, regardless of CD4 cell count, to reduce transmission, minimize AIDS and non-AIDS clinical events, and maximize life expectancy[36]. Our study supports these recommendations and further contributes to mounting evidence that HIV replication, even at low levels, is associated with increased morbidity and mortality[14,37-41]. In clinical practice, this will require earlier initiation of ART, maintaining insurance coverage and drug supply, switching to or intensification with modern potent ART regimens, and patient education on the importance of strict ART adherence. However, complete normalization of risk of NHL and other diseases may not be possible unless ART strategies or novel therapeutics are developed that reverse immune dysfunction and activation. Until then, providers need to be particularly vigilant for early signs and symptoms of lymphoma and prompt diagnosis in HIV-infected patients aging in the modern era of effective ART.

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Authorship Contributions and Conflict of Interest

C.J.A., A.L.B. and S.R.C. designed the study and acquired the data from the CNICS data management core. A.L.B. and S.R.C. selected the appropriate statistical analyses and executed them with the assistance of C.J.A. The initial draft of the manuscript was written by C.J.A., A.L.B., and S.R.C. Throughout the study, all authors participated in discussions about the design, statistical analyses, and interpretation of findings. All authors were also were involved in the review and editing process of the final manuscript for submission. All authors declare that they have no conflicts of interest.

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Table 1. Characteristics of analyzed patients categorized by incident NHL status after achieving HIV suppression on antiretroviral therapy (ART).

	Overall (n=6,036)	NHL (n=37)	No NHL (n=5,999)
Male sex	4,824 (80%)	34 (92%)	4,790 (80%)
White race	3,104 (51%)	22 (60%)	3,082 (51%)
MSM	3,386 (56%)	21 (57%)	3,365 (56%)
HBV	397 (7%)	3 (8%)	394 (7%)
HCV	801 (13%)	9 (24%)	792 (13%)
Age (years)	39 (33, 46)	42 (39, 46)	39 (33, 46)
ART initiation year	2005 (2002, 2008)	2003 (2001, 2005)	2005 (2002, 2008)
Time from ART initiation to HIV suppression (months)	1.85 (1.09, 3.25)	1.86 (1.03, 3.11)	1.86 (1.09, 3.25)
Peak pre-ART HIV viremia* (log_{10} copies/mL)	5.0 (4.6, 5.5)	5.4 (5.0, 5.7)	5.0 (4.6, 5.5)
Peak HIV viremia after ART initiation** (log_{10} copies/mL)	2.60 (1.94, 4.28)	2.67 (2.26, 4.84)	2.60 (1.94, 4.28)
Nadir CD4 cell count* (cells/µl)	180 (50, 280)	100 (30, 250)	180 (50, 280)
CD4 cell count at HIV suppression (cells/µl)	290 (160, 430)	160 (70, 350)	290 (160, 430)

Data are number (%) or median (IQR). MSM=men who have sex with men. ART=antiretroviral therapy. *103 (2%) missing peak pre-ART HIV viremia and 29 (<1%) missing nadir CD4 cell count. **Highest HIV RNA level at any time during study follow-up.

Table 2. NHL incidence rates by nadir CD4 cell count

	n	NHL events	Person-years (PY)	Rate (95% CI) (per 100,000 PY)
Overall	6,036	37	21,607	171 (124, 236)
Nadir CD4 cell count (cell/µl) ≤ 50	1,566	14	6,280	223 (132, 376)
51-200	1,855	11	6,783	162 (90, 293)
>200	2,615	12	8,543	140 (80, 247)

Table 3. Unadjusted and adjusted hazard ratios for NHL categorized by current, three month lagged, and six month lagged HIV viremia

HIV viremia (copies/mL)	Crude HR (95% CI)	Covariate-adjusted HR (95% CI)*	IP-weighted HR (95% CI)**
Current			
≤ 50 (Reference)	-	-	-
51-500	1.98 (0.90, 4.37)	1.92 (0.87, 4.26)	1.66 (0.70, 3.94)
> 500	4.10 (1.77, 9.51)	3.05 (1.22, 7.63)	2.39 (0.92, 6.21)
Three month lagged			
≤ 50 (Reference)	-	-	-
51-500	2.81 (1.14, 6.91)	2.66 (1.08, 6.57)	2.10 (0.84, 5.22)
> 500	5.70 (2.23, 14.61)	4.21 (1.56, 11.34)	3.56 (1.21, 10.49)
Six month lagged			
≤ 50 (Reference)	-	-	-
51-500	2.28 (0.96, 5.43)	2.13 (0.89, 5.10)	1.46 (0.60, 3.60)
> 500	3.84 (1.55, 9.52)	2.83 (1.10, 7.28)	2.50 (0.91, 6.84)

*Cox model adjusted for age, race, sex, current CD4 cell count, and year of HIV suppression; restricted quadratic splines fit on year of suppression variable and current CD4 cell count.

**Marginal structural model (monthly structure) adjusted using inverse probability weighting (IPW) with combined weight for binary age, race, sex, CD4 cell count history, and year of suppression; restricted quadratic splines fit on year of HIV suppression, monthly visit variables, and CD4 cell count. IPW models included two lagged values from the monthly visits. p-value = 0.11, 0.04, and 0.10 for trend in NHL hazard across the three HIV viremia categories for current, three month lagged, and six month lagged, respectively.

Figure Legends

Figure 1. Patient selection

CNICS=Centers for AIDS Research (CFAR) Network of Integrated Clinical Systems

ART=Antiretroviral therapy

Figure 2. Non-Hodgkin lymphoma incidence curves by HIV viremia category. Inverse probability weighting (IPW) adjusted estimated survival curves categorized by (A) current, (B) three month lagged, and (C) six month lagged HIV viremia (copies/mL).