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Serological Assessment of 18 Pathogens and Risk for AIDS-associated Non-Hodgkin Lymphoma

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Abstract

Background: HIV infection is associated with increased susceptibility to common pathogens which may trigger chronic antigenic stimulation and hyperactivation of B-cells, events known to precede the development of AIDS-associated non-Hodgkin lymphoma (AIDS-NHL).

Methods: To explore whether cumulative exposure to infectious agents contributes to AIDS-NHL risk, we tested sera from 199 AIDS-NHL patients (pre-NHL, average lead-time 3.9 years) and 199 matched HIV-infected controls from the Multicenter AIDS Cohort Study (MACS), for anti-IgG responses to 18 pathogens using multiplex serology. Odds ratios and 95% confidence intervals were estimated using conditional logistic regression models.

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Conflicts of Interest and Source of Funding

Authors have no conflicts of interest to declare.

Results: We found no association between cumulative exposure to infectious agents and AIDS-NHL risk (OR 1.01, 95% CI 0.91–1.12). However, seropositivity for trichodysplasia spinulosa polyomavirus (TSPyV), defined as presence of antibodies to TSPyV capsid protein VP1, was significantly associated with a 1.6-fold increase in AIDS-NHL risk (OR 1.62, 95% CI 1.02–2.57). High Epstein-Barr virus (EBV) anti-VCA p18 antibody levels closer to the time of AIDS-NHL diagnosis (<4 years) were associated with a 2.6-fold increase in AIDS-NHL risk (OR 2.59, 95% CI 1.17–5.74). Additionally, high EBV anti-EBNA-1 and anti-ZEBRA antibody levels were associated with 2.1-fold (OR 0.47, 95% CI 0.26–0.85) and 1.6-fold (OR 0.57, 95% CI 0.35–0.93) decreased risk for AIDS-NHL, respectively.

Conclusions: Our results do not support the hypothesis that cumulative exposure to infectious agents contributes to AIDS-NHL development. However, the observed associations with respect to TSPyV seropositivity and EBV antigen antibody levels offer additional insights into the pathogenesis of AIDS-NHL.

Keywords

AIDS-NHL; HIV; infections; multiplex serology; antibodies

INTRODUCTION

Non-Hodgkin Lymphoma (NHL) is one of the most common AIDS-associated malignancies, and a common cause of death among HIV-infected individuals [1–3]. In fact, NHL incidence is 60- to 200-fold greater among HIV-infected people compared to the general population [2,4–6]. The introduction of highly active antiretroviral therapy (HAART) resulted in, among other benefits, up to 70% decrease of AIDS-NHL incidence compared to the pre-HAART era [7,8]. Nevertheless, NHL risk remains significantly higher in HIV-infected compared to immunocompetent individuals [8–10], and AIDS-NHL is still responsible for 23–30% of AIDS-related deaths in countries with widespread access to HAART [2,7,11–14]. Therefore, the identification and better understanding of risk factors contributing to AIDS-NHL immunopathogenesis remain of great importance.

AIDS-NHLs are a heterogeneous group of tumors that arise from B-cells in >90% of cases [15–17]. The pathogenic events leading to AIDS-NHL are complex and could involve chronic immune stimulation by multiple opportunistic infections [15,16,18–22]. Indeed, while progressive HIV infection itself is a known contributor to chronic B-cell hyperactivation and inflammation [16,23–25], it also provides a setting of increased susceptibility to potential deleterious effects of common pathogens that are mostly harmless in immunocompetent individuals [26]. For example, bacteremias are up to 20 times more prevalent among HIV-infected individuals compared to the general population [27], and opportunistic infections are frequently common cause of death in HIV-infected individuals [28,29].

The most common pathogens linked to AIDS-NHL development are two gamma-herpesviruses; Epstein-Barr Virus (EBV) and Kaposi Sarcoma-associated Herpesvirus (KSHV). Almost all primary central nervous system lymphomas (PCNSLs) are EBV-related, primary effusion lymphomas (PEL) are KSHV-related, and both EBV and KSHV are essential to the development of a subset of immunoblastic diffuse large B-cell lymphomas

(DLBCL) [15,30–34]. In addition, recent large cohort study reported that chronic co-infection with hepatitis B (HBV) and hepatitis C (HCV) viruses also contributes to the AIDS-NHL risk [35].

The association between infectious agents and NHL is not restricted to the setting of HIV, as some chronic infections have also been linked to the development of NHL in immunocompetent people. Chronic HBV infection increases risk for multiple NHL subtypes [36–38]; HCV infection can lead to development of marginal zone B-cell lymphoma (MZL) and DLBCL [39–41]; and chronic infection with *Helicobacter pylori* has been linked to the development of mucosa-associated lymphoid tissue (MALT) lymphoma [42–46].

While there is ample evidence that individual pathogens confer increased susceptibility to NHL with or without HIV infection, we sought to examine the effects of cumulative exposure to infectious agents in relation to AIDS-NHL risk. We hypothesized that such exposure could contribute to the chronic antigenic stimulation and hyperactivation of B-cells preceding AIDS-NHL development. To test this hypothesis, we measured the presence of antibodies to 38 different antigens of 18 distinct pathogens (14 viruses, 3 bacteria, and a protozoon). The selection of these pathogens was based on: a) previously reported associations with NHL [32,33,35,46–49], and/or b) higher frequency of pathogen or pathogen-associated disease in HIV-infected compared to immunocompetent individuals [50–60], respectively.

MATERIALS AND METHODS

Study population.

The Multicenter AIDS Cohort Study (MACS) is an ongoing prospective cohort study established in 1984 to study the natural and treated history of HIV and AIDS in men who have sex with men (MSM) recruited from four U.S. metropolitan areas (Baltimore/Washington, DC; Chicago; Los Angeles; and Pittsburgh) [61,62]. Study visits are held biannually and include face to face interviews, physical examination, specimen collection and laboratory testing. HIV seropositivity and $CD4^+$ T cell counts are measured at nearly all study visits, and sera are collected and stored in central repositories [63]. All protocols and questionnaires utilized in the MACS have been approved by the Institutional Review Board of each center.

Study Design.

For this present study, we designed a nested case-control study within the MACS. Cases included all participants with a diagnosis of pathologically confirmed AIDS-NHL following enrollment into the MACS and the availability of archival pre-NHL diagnostic serum. Based on these criteria, 200 AIDS-NHL cases were identified. For each case, one HIV-infected participant who did not develop AIDS-NHL up to November 2014 was selected. For cases, serum specimens were selected closest to 4 years prior to AIDS-NHL or any date preceding 4 years. For about half of the cases who did not have archival specimens at least 4 years prior to diagnosis, any pre-diagnosis specimens was utilized. For controls, specimen time-points were matched to each case by visit number. Additionally, controls were matched to

cases on: i) recruitment phase into the cohort (1984–1985, 1987–1991, or 2001+), ii) prior highly active antiretroviral drug use (HAART, ever versus never), and iii) CD4+ T cell counts at the time of AIDS-NHL diagnosis or matched time-point for controls (± 200/μl). In addition, cases who became HIV-infected after recruitment into the cohort were matched to controls by their seroconversion date, and cases treated with HAART were matched to controls on time since their first therapy. The definition of HAART was guided by the DHHS/Kaiser Panel [64] guidelines and defined as three or more antiretroviral (ART) drugs consisting of one or more protease inhibitors (PIs), or one non-nucleoside reverse transcriptase inhibitor (NNRTI), or the nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs), or an integrase inhibitor (II), or an entry inhibitor (including fusion inhibitors; EI). One case/control set was excluded from analysis due to insufficient specimen volume leaving a total of 199 cases and 199 controls for the final analysis.

Serological Methods.

Frozen serum samples were shipped on dry ice to the German Cancer Research Center (Heidelberg, Germany) for serological testing for IgG antibodies to 38 previously well-defined and specific antigens of 18 pathogens (Supplementary Table S1). Analysis included: i) human herpesviruses: Herpes Simplex Virus 1 and 2 (HSV-1, −2), Epstein Barr Virus (EBV/HHV4), Human Cytomegalovirus (HCMV/HHV5), Human Herpesviruses 6 and 7 (HHV-6, −7), Kaposi's sarcoma-associated herpesvirus (KSHV/HHV8); ii) human hepatitis viruses: Hepatitis B Virus and Hepatitis C Virus (HBV and HCV); iii) human polyomaviruses (HPyV): BKPyV, JCPyV, Merkel cell polyomavirus (MCPyV), and Trichodysplasia spinulosa-associated polyomavirus (TSPyV); iv) Human Papillomavirus type 16 (HPV16); v) bacteria: Helicobacter pylori, Chlamydia trachomatis, and Mycoplasma genitalium; and iv) parasite Toxoplasma gondii. Antigen preparation and serological techniques have been previously described [65–69]. Briefly, serum samples (1:1000 dilutions) were incubated with antigen-loaded fluorescently labeled beads and analyzed on a Luminex 200 analyzer. As output, bead-bound fluorescence-stained human antibodies to each of the antigens of interest were quantified as median fluorescence intensity values (MFI) in a single reaction for each sample [69,70]. Following quantification, standard cut-offs for seropositivity were applied for each antigen by visual inspection of frequency distribution curves (percentile plots), as previously described [71–74]. Quality controls used on every tested plate included previously tested serum samples with known reactivity profiles. Coefficients of variation (CVs) for infection antibodies ranged from 6–29%, with a median of 18%. Eighty percent of markers tested had a CV less than 20%.

Statistical analyses.

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using conditional logistic regression models. The case-control matching by design was incorporated into the models by adding a grouping variable for matched set. In addition to the matching factors, all models were adjusted for covariates selected *a priori* for their previously described associations with AIDS-NHL and included race/ethnicity (categorical: Hispanic white, non-Hispanic white, Hispanic black, non-Hispanic black, Asian/Pacific Islander) and age (continuous) at the date of serum collection for serological testing in this study.

To address our primary hypothesis, we examined the association between cumulative exposure to infectious agents and AIDS-NHL risk. This exposure was modeled as a continuous variable and defined as the number of pathogens found to be seropositive based on a predefined number of antigens testing positive, as well as a categorical variable (seropositive for 10–18 pathogens versus ≤9 pathogens). These categories were determined by the median number of seropositive pathogens in the control group (10 pathogens) and not by a *prior* biological rationale. Secondarily, we also examined the association between AIDS-NHL risk and seropositivity to each of the 18 pathogens individually (seropositive versus seronegative). We also examined quartiles of antibody levels to each antigen of the two herpesviruses that have been etiologically linked to AIDS-NHL (KSHV and EBV), among those participants who were seropositive for that antigen, using logistic regression models adjusting for the matching factors as covariates in the model. Quartiles of antibody levels to TSPyV VP1 antigen were also examined due to recent data suggesting influence on lymphoma pathogenesis [75]. Quartiles for antibody levels were determined by the distribution within the control group, and are presented as $<25^{th}$, 25^{th}-75^{th}, and $>75^{th}$ percentile for comparability with a prior study [76]. In addition, we examined patterns of AIDS-NHL risk associated with EBV antibody levels according to the time interval (or lead-time) between serum sample collection and AIDS-NHL diagnosis (<4 years or ≥4 years). The categories for lead-time were selected according to the natural distribution of the data and to ensure an approximately equal number of participants in each category. Due to the exploratory and hypothesis generating nature of these secondary aims, we did not correct for multiple-hypothesis testing.

Correlation matrix for all infections.

We have run a correlation matrix for all infections measured in our study (Supplementary Table S2). Bonferroni correction was applied for multiple comparisons. Significant positive correlation was found between seropositivity to HBV and HSV2, and HBV and KSHV, respectively; as well as between seropositivity to HSV2 and KSHV and HSV2 and Chlamydia trachomatis.

RESULTS

Study population.

Cases and controls were similar in their distributions by recruitment year, antiretroviral drug therapy, and CD4+ T-cell count, as expected based on the matching criteria (Table 1). The majority of cases and controls were enrolled into the MACS in the initial recruitment wave (1984–1986, 84.6% for each group), were HAART naïve (94.5% for each group) and had >400 CD4+ T-cells/mm^3 (46.2% of cases and 41.3% of controls, respectively). The majority of cases and controls were non-Hispanic white (70.9% and 79.9% respectively). Cases tended to be slightly older than controls; 35.2% of cases were 40 years or older, compared to 30.7% of controls.

Among the cases, the mean time from blood draw to NHL diagnosis was 3.9 years; ranging from 1 month to 12 years (standard deviation 1.6 years). The majority of cases were systemic lymphomas (69.8%), among which DLBCL was the most common subtype

(49.6%). For 82.4% of cases, AIDS-NHL was the first primary cancer. Kaposi sarcoma preceded AIDS-NHL in 32 out of the 35 cases where AIDS-NHL was a second primary cancer (Table 1).

Cumulative exposure to infectious agents.

Supplementary Table S1 lists the names of 18 pathogens and 38 antigens tested in this study. Cumulative exposure to infectious agents (defined as the number of pathogens found to be seropositive) was not associated with AIDS-NHL risk when examined as a continuous variable (OR 1.01, 95% CI 0.91–1.12) (Table 2). Seropositivity for a higher number of pathogens (10–18 versus ≤9), was not significantly associated with an increased AIDS-NHL risk (OR 1.35, 95% CI 0.78 – 2.32) (Table 2).

Individual pathogen seropositivity.

Seropositivity to trichodysplasia spinulosa polyomavirus (TSPyV) was significantly associated with AIDS-NHL (OR 1.62; 95% CI 1.02–2.57, Table 3). No other associations were observed regarding seropositivity of remaining 17 pathogens tested. Interestingly, when HCV and HBV were examined together, there was a suggestion of an increased risk of AIDS-NHL associated with seropositivity for both viruses compared to seronegativity for both (OR=1.51, 95% CI=0.63–3.61).

TSPyV, EBV- and KSHV-specific antigens.

Among 199 cases, 151 (76%) were defined as TSPyV seropositive compared to 134 (67%) of controls (p=0.037). Though seropositivity to TSPyV was significantly associated with AIDS-NHL risk (Table 3), we did not observe any significant associations between TSPyV antibody levels and AIDS-NHL risk (Table 4).

Seroprevalence of the four specific EBV antigens measured (VCA p18, EA-D, ZEBRA, and EBNA-1), was similar between cases and controls and ranged from 81–100% among cases and 86–100% among controls (data not shown). Among the EBV VCA p18 seropositives, high antibody levels (levels >75th percentile) were associated with a 2.6-fold increase in AIDS-NHL risk when measured within four years prior AIDS-NHL diagnosis (OR 2.59; 95% CI 1.17 – 5.74, Table 4). In contrast, EBV anti-ZEBRA and EBV anti-EBNA-1 antibody levels had significant inverse associations with AIDS-NHL risk, with 1.6 to 2.1-fold decreased risks associated with the 25^{th}-75^{th}, and $>75^{th}$ percentile categories, respectively, compared with those with levels in the $<25^{th}$ percentile category (OR 0.47; 95% CI 0.26 – 0.85 and OR 0.57; 95% CI 0.35 – 0.93, Table 4).

Presence of antibodies to either LANA or K8.1 antigen was required to define the subject as KSHV seropositive. There was a non-significant dose-response between anti-LANA antibody levels and increased AIDS-NHL risk; high KSHV anti-LANA antibody levels ($>75^{th}$ percentile) was associated with a non-significant 1.9-fold increased risk for AIDS-NHL overall (OR 1.9; 95% CI 0.87 – 4.20, Table 4). Higher anti-K8.1 antibody levels also appeared to be modestly, but non-significantly associated with increased AIDS-NHL risk.

DISCUSSION

To explore the impact of common infections to the development of AIDS-NHL, we utilized multiplex serology approach and measured antibodies to 18 different pathogens commonly found at higher frequencies in HIV-infected compared to the non-HIV-infected individuals. Using sera collected prior to AIDS-NHL diagnosis, we found that cumulative exposure to pathogens we measured for was not associated with AIDS-NHL risk. However, novel observations include findings on seropositivity to TSPyV, and high antibody levels of EBV anti-VCA p18 antibodies, to be significantly associated with increased AIDS-NHL risk, whereas high levels of EBV anti-EBNA-1 and anti-ZEBRA antibodies were significantly associated with decreased AIDS-NHL risk.

Association of TSPyV with AIDS-NHL lymphoma is novel. TSPyV is a polyomavirus discovered in skin lesions of immunosuppressed patients which causes a rare skin disease trichodysplasia spinulosa [77,78]. In contrast to other polyomaviruses, TSPyV does not seem to be a part of the skin microbiome in healthy people [55], and Wieland and colleagues reported that TSPyV DNA was more frequently found on the skin of HIV-infected compared to non-HIV-infected men (3.8% vs. 0.8%) [55]. Indeed, when we stratified AIDS-NHL in our study into systemic and CNS lymphomas, we observed that the increased AIDS-NHL risk was restricted to systemic lymphomas (OR 2.03, 95% CI 1.17–3.53) and not to CNS lymphomas (OR 0.77, 95% CI 0.29–2.04). However, B-cell AIDS-NHL located in the skin are rare [79–81], and in our study only 3% (5/151) of TSPyV seropositive cases, and 2% (1/48) of TSPyV seronegative cases had skin-associated AIDS-NHL. Using the same multiplex serology assay for polyomaviruses, Teras and colleagues found no significant association between TSPyV seropositivity and NHL in immunocompetent people [82].

The observed associations between EBV antigens and AIDS-NHL risk may provide insight into pathogenic effects of EBV. EBV is a herpesvirus that causes lifelong infection and undergoes cycles of viral reactivation [83,84]. We found high levels of EBV anti-VCA p18 antibodies to be associated with increased AIDS-NHL risk, but only when measured closer to AIDS-NHL diagnosis date (<4 years). Detection of high EBV anti-VCA p18 IgG has been associated with high EBV loads in HIV carriers [85,86], and is thought to reflect an active EBV infection (loss of control of EBV infection) or EBV viremia [87]. Indeed, the loss of immunoregulatory control of EBV-infected B-cells, resulting from an impaired T-cell function, is one of the two major mechanisms underlying genesis of AIDS-NHL [22,31,88]. Modest positive associations of EBV VCA p18 and increased NHL risk were also found in immunocompetent people [76].

IgG antibodies to another EBV antigen, EBV EBNA-1, also persist throughout the lifetime among EBV-infected individuals. In contrast to anti-VCA p18, anti-EBNA-1 IgG antibodies are not present during the acute phase of EBV infection but develop in a later course of the infection [89]. EBNA-1, the EBV nuclear antigen, contains critical epitopes which can elicit cytotoxic T lymphocyte (CTL) responses to EBV infection, crucial for infection control [90,91]. In contrast to EBV VCA p18 findings, we found that high levels of anti-EBNA-1 IgG were associated with decreased AIDS-NHL risk, with associations being stronger when anti-EBNA-1 antibodies were detected >4 years prior diagnosis. We also observed an inverse

association between higher EBV anti-ZEBRA antibody levels and AIDS-NHL risk. The ZEBRA protein is one of the early encoded EBV proteins which activates a switch from the latent to the lytic viral gene expression [92,93]. We hypothesize that the observed inverse associations represent consumption of anti-EBNA-1 and anti-ZEBRA antibodies required to counteract chronic EBV viral infection preceding AIDS-NHL, possibly through antibody-dependent cell-mediated cytotoxicity [94]. Indeed, decreased anti-EBNA-1 antibody levels were shown to be associated with low CTL responses in children with chronic EBV infection, and in multiple diseases [95–98].

Our data on significant inverse association between high levels of antibodies to EBV ZEBRA and AIDS-NHL risk stand in contrast to increased NHL risk with high EBV ZEBRA antibodies observed in recent Western and Asian cohorts [75,76], respectively. These different findings might be reflective of different biology between NHL in immunosuppressed versus immunocompetent populations. Indeed, the observed positive association with EBV ZEBRA and EA_D in prior studies was specific for chronic lymphocytic leukemia/small lymphocytic (CLL/SLL) and follicular lymphoma (FL) NHL subtypes, which represented less than 1% of cases in our study [76].

Although the associations were not significant, there was a suggestive association of high levels of KSHV anti-LANA and anti-K8.1 antibodies and AIDS-NHL risk. KSHV is a causative agent of Kaposi sarcoma (KS) [34,99,100], and KS and AIDS-NHL represent the two most commonly occurring cancers among HIV-infected people [7]. KSHV is also the main cause of Primary Effusion Lymphoma (PEL) and Castleman's disease (CD), two rare AIDS-NHL subtypes [34,101]. The active role of KSHV has also been proposed in the immunoblastic variant of DLBCL [30,102–104]. We were unfortunately unable to define the DLBCL in our cohort further as immunoblastic, centroblastic or anaplastic [105] and therefore we could not confirm if it were the immunoblastic DLBCL variant that were KSHV seropositive. LANA, a latency-associated nuclear antigen, is one of the few KSHV encoded proteins that are highly expressed in latently infected tumor cells and acts as a regulator of viral transcription [106,107]. Its direct role in oncogenesis can be linked to binding and inactivation of the two major tumor suppressor proteins; p53 and pRb, respectively [108,109]. K8.1 glycoprotein is a structural component of KSHV expressed only during viral replication; therefore, it does seem plausible that the presence of KSHV K8.1 antibodies, or high levels of these, could indicate individuals who are at a greater risk for development of KSHV-associated malignancies [15,33,49,102].

NHL are a heterogeneous group of cancers both in general population, although less so in the setting of HIV. The two most common AIDS-NHL subtypes are DLBCL and BL. Also in our cohort DLBCL represented 69/139 (50%) and BL 23/139 (16%) of the systemic AIDS-NHL cases. Exploratory analysis in our cohort found that when these case groups were compared to one another, that there were not significant differences in antigen exposure. In addition, a fraction of AIDS-NHL in our study were second primary tumors (35/199, 18%). A subgroup analysis restricted to the 164 AIDS-NHL as a first primary cancer only, showed no significant differences in pathogen seropositivity or antibody levels to specific antigens compared to all AIDS-NHL.

In HIV infection, chronic antigenic stimulation (as in cases with multiple infections), and lack of CD4+ T-cell help, can lead to T-cell exhaustion, i.e. disruption of memory T-cell function and defects in memory T-cell responses necessary to combat and eliminate infectious agents [110–112]. Exhausted CD8+ T-cells exhibit a loss of cytotoxic function [113] and decreased mitogen-induced proliferation [114]. But, importantly, virus-specific CD8+ T-cell response can be restored, either through a period of rest from antigenic stimulation or through inhibition of the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) apoptotic pathway. Also, antiretroviral therapy helps restore virus-specific CD8+ T cells [115,116]. Thus HAART in combination with strategies to reduce antigenic stimulation may help to reduce risk of AIDS-NHL. Indeed, association of EBV reactivation and T-cell exhaustion has been demonstrated in several diseases [98,117]. Further studies are required to investigate if reactivation of EBV or KSHV is associated with a T-cell exhaustion profile (upregulation of checkpoint inhibitors such PD-1, LAG-3, Tim-3, and CTLA-4 on T-cells), and AIDS-NHL risk.

Our study has few limitations. One limitation is the possibility that assessment of antibodies to different pathogens in HIV-infected people could be complicated by HIV-associated premature exhaustion of B-cells leading to impaired antibody responses [118–121]. Such impairment of serologic memory confers additional risk for HIV related opportunistic infections and mortality. Although premature exhaustion of immune cells can be reversed by antiretroviral therapy [115,116], a minority of cases and controls in out cohort received HAART. Another potential limitation is that our study consisted largely of white men who have sex with men, potentially limiting the generalizability of study findings. Also, 42/199 (21%) of the AIDS-NHL cases in our cohort were pathologically classified as “NHL not otherwise specified (NOS)”, making it difficult to evaluate NHL subtype-specific associations with seropositivity to certain pathogens or their antigens.

To our knowledge, this is the first comprehensive examination of seropositivity to multiple pathogens, including 14 different viruses, three bacteria, and a protozoon, in an attempt to better define cumulative pathogen exposures as well as individual pathogen/antigen associations with AIDS-NHL risk. Sensitive serological assays for detection of antibodies to infections can be a powerful tool for identification of cancer biomarkers [122]. In addition to the prior reports demonstrating that AIDS-NHL development is preceded by high serum levels of several inflammatory cytokines and chemokines indicative of B-cell hyperactivation [16,20,123], as well as microbial translocation [124], our results contribute data on association of well-known (KSHV and EBV) and potentially novel lymphomagenic agents (TSPyV) with AIDS-NHL risk. Therefore, a possible strategy to reduce underlying immune activation in HIV-infected persons as a strategy to reduce AIDS-NHL risk, may involve a multi-pronged approach including earlier access to HAART, use of anti-inflammatory agents to dampen immune activation, as well as treatment of co-infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Selected characteristics of the 199 AIDS-NHL cases and 199 matched HIV-infected controls from the Multicenter AIDS Cohort Study

	AIDS-NHL cases (N = 199)	HIV-infected controls (N = 199)
	N *(%)*	**N *(%)***
Recruitment Cohort		
1984–1985	168 *(84)*	168 *(84)*
1987–1991	24 *(12)*	24 *(12)*
2001+	7 *(4)*	7 *(4)*
Race		
White, non-Hispanic	161 *(81)*	159 *(80)*
Black, non-Hispanic	17 *(8)*	23 *(11)*
Hispanic	21 *(11)*	16 *(8)*
Asian or Pacific Islander	0 *(0)*	1 *(1)*
Age[1]		
< 30	28 *(14)*	31 *(15)*
30 – 39	83 *(42)*	93 *(47)*
40 – 49	70 *(35)*	61 *(31)*
≥ 50	18 *(9)*	14 *(7)*
CD4+ T-cells/mm3[1]		
< 200	44 *(22)*	40 *(20)*
200 – 399	63 *(32)*	57 *(29)*
≥400	92 *(46)*	102 *(51)*
Prior HAART exposure[1]		
No	188 *(95)*	188 *(95)*
Yes	11 *(5)*	11 *(5)*
Time from serum date until NHL diagnosis, years (mean ± SD)	3.9 ± 1.6	N/A
NHL Site / ICD-O-3 code[2]		
Systemic / all beside 71.0–71.9 and 72.0–72.9	139 *(70)*	
Central Nervous System / 71.0–71.9, 72.0–72.9	60 *(30)*	
NHL Subtype (systemic only) / ICD-O-3 code[3]		
Diffuse large B-cell lymphoma / 9680.3, 9684.3	69 *(50)*	
Burkitt Lymphoma / 9687.3	23 *(16)*	
Lymphoplasmacytic lymphoma / 9671.3	1 *(1)*	
Mature T-cell lymphoma / 9702.3	2 *(1)*	
Primary effusion lymphoma / 9678.3	1 *(1)*	

	AIDS-NHL cases (N = 199)	HIV-infected controls (N = 199)
Follicular lymphoma / 9691.3	1 *(1)*	
NHL, NOS / 9590.3, 9591.3	42 *(30)*	
Cancer diagnosis prior to NHL[4]		
NHL is first primary cancer	164 *(82)*	
NHL is second primary cancer	35 *(18)*	
Tumor EBV status		
Negative	28 *(14)*	
Positive	60 *(30)*	
Unknown	88 *(44)*	

Abbreviations: AIDS, Acquired Immunodeficiency Syndrome; NHL, non-Hodgkin lymphoma; SD, standard deviation; HAART, highly active antiretroviral therapy; EBV, Epstein-Barr virus

[1]The reference date for these variables is the collection date of a blood sample used for testing in this study

[2]ICD-O-3 topographical codes: http://codes.iarc.fr/topography

[3]ICD-O-3 morphological codes: http://codes.iarc.fr/codegroup/2, here for systemic NHLs only

[4]Kaposi sarcoma preceded AIDS-NHL in 32 out of the 35 cases where AIDS-NHL was a second primary cancer

Table 2.

Association between pathogen burden and AIDS-NHL risk

	AIDS-NHL cases	HIV-infected controls	OR	95% CI
	N *(%)*	N *(%)*		
Risk per seropositive antigen	199 *(100)*	199 *(100)*	1.01	0.91–1.12
Categories of seropositive pathogens				
≤ 9	36 *(18)*	44 *(22)*	1	
10 – 18	163 *(82)*	155 *(78)*	1.35	0.78–2.32

Table 3.

Associations between seropositivity for each of the 18 pathogens and subsequent AIDS-NHL risk in pre-diagnostic sera from 199 AIDS-NHL cases and 199 HIV-infected matched controls from the Multicenter AIDS Cohort Study

	AIDS-NHL cases N *(%)*	HIV-infected controls N *(%)*	OR	95% CI
Human papillomaviruses				
HPV16 L1 negative	158 *(79)*	165 *(83)*	1	
HPV16 L1 positive	41 *(21)*	34 *(17)*	1.33	0.75 – 2.37
Human polyomaviruses				
BKPyV VP1 negative	32 *(16)*	32 *(16)*	1	
BKPyV VP1 positive	167 *(84)*	167 *(84)*	1.10	0.61 – 2.01
JCPyV VP1 negative	145 *(73)*	140 *(70)*	1	
JCPyV VP1 positive	54 *(27)*	59 *(30)*	0.85	0.52 – 1.38
TSPyV VP1 negative	48 *(24)*	65 *(33)*	1	
TSPyV VP1 positive	151 *(76)*	134 *(67)*	**1.62**	**1.02 – 2.57**
MCPyV VP1 negative	64 *(32)*	71 *(36)*	1	
MCPyV VP1 positive	135 *(68)*	128 *(64)*	1.20	0.76 – 1.90
Human hepatitis viruses				
HBV negative	48 *(24)*	44 *(22)*	1	
HBV positive	151 *(76)*	155 *(78)*	1.00	0.60 – 1.65
HCV negative	174 *(88)*	181 *(91)*	1	
HCV positive	25 *(12)*	18 *(9)*	1.23	0.25 – 5.98
Human herpesviruses[1]				
HSV1 negative	47 *(24)*	53 *(27)*	1	
HSV1 positive	152 *(76)*	146 *(63)*	1.11	0.69 – 1.79
HSV2 negative	64 *(32)*	60 *(30)*	1	
HSV2 positive	135 *(68)*	139 *(70)*	0.81	0.51 – 1.27
EBV negative	0 *(0)*	1 *(1)*	1.0	
EBV positive	199 *(100)*	198 *(99)*	NE	
HCMV negative	0 *(0)*	1 *(1)*	1.0	
HCMV positive	199 *(100)*	198 *(99)*	NE	
HHV6 negative	96 *(48)*	80 *(40)*	1	
HHV6 positive	103 *(52)*	119 *(60)*	0.71	0.46 – 1.10
HHV7 negative	52 (26)	44 *(22)*	1	
HHV7 positive	147 *(74)*	155 *(78)*	0.76	0.47 – 1.24
KSHV negative	81 *(41)*	90 *(45)*	1	
KSHV positive	118 *(59)*	109 *(55)*	1.18	0.76 – 1.83
Bacterial infections				
H. pylori negative	165 *(83)*	166 *(83)*	1	
H. pylori positive	34 *(17)*	33 *(17)*	1.02	0.55 – 1.88
C. trachomatis negative	26 (13)	31 *(16)*	1	

	AIDS-NHL cases N *(%)*	HIV-infected controls N *(%)*	OR	95% CI
C. trachomatis positive	173 *(87)*	168 *(84)*	1.18	0.63 – 2.18
M. genitalium negative	113 *(57)*	105 *(53)*	1	
M. genitalium positive	86 *(43)*	94 *(47)*	0.80	0.52 – 1.22
Parasitic infections				
Toxoplasma gondii negative	182 *(91)*	185 *(93)*	1	
Toxoplasma gondii positive	17 *(9)*	14 *(7)*	1.22	0.55 – 2.72

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Table 4.

Association between antibody levels of TSPyV, EBV, KSHV antigens in seropositive AIDS-NHL cases and HIV-infected matched controls from the Multicenter AIDS Cohort Study overall, and stratified by lead-time

	All AIDS-NHL				< 4 year lead-time				≥ 4 years lead-time			
	AIDS-NHL cases (N)	HIV-infected controls (N)	OR	95% CI	AIDS-NHL cases (N)	HIV-infected controls (N)	OR	95% CI	AIDS-NHL cases (N)	HIV-infected controls (N)	OR	95% CI
TSVPyV VP1												
< 25th	49	34	1		26	16	1		23	18	1	
25th - 75th	75	67	0.84	0.47–1.50	38	37	0.55	0.16–1.82	37	30	1.89	0.54–6.60
≥ 75th	27	33	0.59	0.29–1.20	14	18	0.29	0.05–1.57	13	15	1.40	0.22–8.50
EBV EA-D												
< 25th	48	43	1		19	18	1		29	25	1	
25th - 75th	64	85	0.63	0.37 – 1.07	35	50	0.56	0.25 – 1.26	29	35	0.70	0.33 – 1.45
≥ 75th	51	42	1.02	0.56 – 1.86	28	20	1.22	0.50 – 2.96	23	22	0.90	0.39 – 2.08
EBV VCA p18												
< 25th	39	50	1		19	31	1		20	19	1	
25th - 75th	102	100	1.31	0.78 – 2.12	48	48	1.73	0.84 – 3.53	54	52	0.85	0.39 – 1.81
≥ 75th	58	49	1.49	0.84 – 2.65	36	24	**2.59**	**1.17 – 5.74**	22	25	0.74	0.31 – 1.78
EBV ZEBRA												
< 25th	66	47	1		26	23	1		40	24	1	
25th - 75th	76	92	**0.57**	**0.35 – 0.93**	41	54	0.66	0.33 – 1.34	35	38	0.56	0.28 – 1.12
≥ 75th	42	46	0.63	0.36 – 1.13	25	20	1.10	0.48 – 2.53	17	26	0.39	0.17 – 0.89
EBV EBNA-1												
< 25th	66	48	1		31	26	1		35	22	1	
25th - 75th	94	96	0.71	0.44 – 1.15	51	53	0.78	0.40 – 1.51	43	43	0.62	0.31 – 1.24
≥ 75th	31	48	**0.47**	**0.26 – 0.85**	17	21	0.67	0.29 – 1.56	14	27	**0.32**	**0.13 – 0.75**
KSHV LANA												
< 25th	19	26	1		9	12	1		10	14	1	
25th - 75th	57	52	1.46	0.72 – 2.98	27	30	1.24	0.43 – 3.60	30	22	1.86	0.68 – 5.09
≥ 75th	38	26	1.91	0.87 – 4.20	22	14	2.01	0.64 – 6.30	16	12	1.88	0.60 – 5.92
KSHV K8.1												

	All AIDS-NHL				< 4 year lead-time				≥ 4 years lead-time			
	AIDS-NHL cases (N)	HIV-infected controls (N)	OR	95% CI	AIDS-NHL cases (N)	HIV-infected controls (N)	OR	95% CI	AIDS-NHL cases (N)	HIV-infected controls (N)	OR	95% CI
< 25th	14	13	1		8	6	1		6	7	1	
25th - 75th	42	24	1.78	0.70 – 4.49	18	13	1.03	0.28 – 3.78	24	11	3.15	0.76 – 13.4
≥ 75th	18	12	1.31	0.44 – 3.91	11	5	1.70	0.37 – 7.72	7	7	0.87	0.16 – 4.80