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Effects of heavy drinking on T-cell phenotypes consistent with immunosenescence in untreated HIV infection

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Abstract

Background—The role of alcohol consumption in HIV-related adaptive immune dysfunction is debated. We hypothesized that heavy drinking would be associated with greater evidence of immunosenescence (i.e. aging related decline of adaptive immune function) among antiretroviral therapy (ART)-naïve HIV-infected individuals.

Methods—Using data from the Russia ARCH cohort study, we conducted a cross-sectional analysis of ART-naïve HIV-infected individuals recruited between 2012–2014.

Independent variable: Heavy drinking defined as > 4 standard drinks in a day (or > 14 standard drinks/week) for men and > 3/day (or > 7/week) for women respectively. Dependent variables: Percentage of CD8+ and CD4+ T-cells with a phenotype consistent with immunosenescence (i.e. expressing CD28-CD57+, or memory [CD45RO+CD45RA+] phenotype and not the naïve [CD45RO-CD45RA+] phenotype). Statistical analysis: Multiple linear regression adjusted for confounders.

Results—Of 214 eligible participants, 61% were heavy drinkers. Mean age was 33 years and the cohort was predominantly male (72%). Hepatitis C prevalence was high (87%) and mean $\log_{10}$ HIV-1 RNA copies/mL was 4.6. We found no significant differences by drinking status in the percentage of immunosenescent, memory or naive CD8+ or CD4+ T-cells.

Conclusions—In this cross-sectional analysis, heavy drinking in the setting of untreated HIV infection did not appear to be associated with alterations in T-cells with phenotypes consistent with immunosenescence. To substantiate these findings, longitudinal studies should assess whether

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changes in alcohol consumption are associated with changes in these and other immunosenescent T-cell phenotypes.

Keywords

Heavy drinking; HIV; immunosenescence; T-cell; Russia

Introduction

Heavy alcohol use occurs more commonly among human immunodeficiency virus (HIV) infected than among uninfected people (Galvan et al., 2002) and is associated with a range of negative health outcomes, including worse medication adherence and increased risk of comorbid conditions. The likely mechanisms driving negative health outcomes among HIV-infected risky drinkers are multifactorial and incompletely understood (Samet et al., 2007, Samet et al., 2003, Braithwaite and Bryant, 2010, Malbergier et al., 2015, Hahn and Samet, 2010).

Immunosenescence, the aging-related decline in adaptive immune function, is associated with negative health outcomes, including cancers, cardiovascular disease and mortality. Since alcohol use and HIV have been independently associated with abnormalities in adaptive immunity (Happel and Nelson, 2005, Szabo and Mandrekar, 2009, Szabo and Zakhari, 2011, Kronfol et al., 1993), a synergistic effect of heavy drinking, a modifiable behavior, and HIV infection may exacerbate immunosenescence leading to poor outcomes.

The aim of this study was to assess the effect heavy drinking on biomarkers consistent with immunosenescence. Immunosenescence is characterized by accumulation of CD28-CD57+ T-cells, decrease in naïve (antigen inexperienced) lymphocytes, and increase in memory (antigen experienced) lymphocytes that are oligoclonally expanded, resistant to apoptosis and functionally incompetent (Weng, 2006, Dock and Effros, 2011). This results in increasing difficulty in recognizing new pathogens and responding appropriately to these pathogens. Such inappropriate responses, including inflammation, which endure over time given senescent cells' resistance to apoptosis, may ultimately be harmful to the host.

We hypothesized that more HIV-infected heavy drinkers (NIAAA, 2005) would have T-cell distributions consistent with immunosenescence compared to HIV-infected non-heavy drinkers and abstainers. We also explored whether this association was modified by HIV-1 RNA.

The Russia ARCH (Alcohol Research Cohort on HIV/AIDS) cohort is an ideal one to study this question. It represents a wide range of well-characterized alcohol use among relatively young (median: 33 years; 25^{th}–75^{th} percentile: 30–36 years), antiretroviral therapy (ART) naïve participants. We conducted this study in the setting of untreated HIV infection to minimize the potential confounding influence of ART and ART adherence on immune parameters. Without first understanding the association of HIV/heavy drinking on immunosenescence in the absence of ART, it would be challenging to interpret these associations in the presence of ART. Further, this cohort has minimal comorbid disease of

aging like cancer, cardiovascular disease and obesity that could confound the association of alcohol and biomarkers consistent with immunosenescence.

Materials and Methods

Participants

We analyzed baseline data from the Russia ARCH cohort, a study that aims to evaluate the longitudinal association between alcohol consumption and biomarkers of innate and adaptive immune alterations. Participants were recruited between November 2012 and October 2014 from clinical care sites and non-governmental organizations serving HIV-infected persons in St. Petersburg, Russia. After signing informed consent, participants were administered an interview assessment and provided a blood sample.

Eligibility criteria included the following: documented HIV infection and ART-naïve at enrollment; 18–70 years old; provision of contact information of two relatives or friends who could be contacted to assist with follow-up; stable address; possession of a telephone (home or cell); fluent in Russian; and ability to provide informed consent. Institutional Review Boards of Boston University Medical Campus and Pavlov State Medical University approved this study.

Assessments

Assessments were administered by a trained research associate in a face-to-face interview. Particularly sensitive sections of the assessment (e.g. HIV stigma, sexual behaviors) were self-administered by the participant.

This study analyzed data obtained from the following instruments: 30 Day Timeline Follow Back for alcohol use (Sobell et al., 1986), the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998), Fagerström Test for Nicotine Dependence (Heatherton et al., 1991), survey of co-morbidities adapted from the Veterans Aging Cohort Study patient questionnaire (Justice et al., 2006) and HIV Risk Behavior Survey (RBS) (Darke et al., 1991).

Flow Cytometry

Heparin anticoagulated whole blood was processed within 4 hours of collection at the St. Petersburg Pasteur Institute Central Clinical Diagnostic Laboratory. All reagents were from BD Biosciences. Whole blood was labeled with FITC anti-CD8, PE anti-CD45RO, PeCy5 anti-CD45RA and APC-H7 anti-CD4, or FITC anti-CD8, PE anti-CD57, PeCy5 anti-CD28 and APC-H7 anti CD4 or appropriate isotypes. After incubation, red blood cells were lysed and washed and samples were fixed in paraformaldehyde. Flow cytometry was performed on a BD FACS Canto and analyzed with BD FACS DIVA software.

Independent variables, Outcomes and Covariates

The main independent variable was past 30-day heavy drinking based on Timeline Followback for alcohol use (Sobell et al., 1986) and categorized per National Institute on Alcohol Abuse and Alcoholism (NIAAA) criteria for risky alcohol use: > 4 standard drinks

in a day (or > 14 standard drinks/week) for men and > 3/day (or > 7/week) for women respectively (NIAAA, 2005). Secondary analyses assessed tertiles of alcohol use quantity (average daily alcohol volume) and frequency (number of drinking days per month) separately.

The outcome variables were CD8+ and CD4+ T-cell phenotypes consistent with immunosenescence. Immunosenescence is the ageing related decline of adaptive immune function. It is characterized by accumulation of CD28-CD57+ T-cells, decrease in naïve (antigen inexperienced) lymphocytes, and increase in memory (antigen experienced) lymphocytes that are oligoclonally expanded, resistant to apoptosis and functionally incompetent (Weng, 2006, Dock and Effros, 2011).

Thus, the two primary outcomes were the percentage of CD8+ and CD4+ T-cells expressing the CD28-CD57+ phenotype. T-cells that lose CD28 (CD28-) or gain CD57 (CD57+) expression have experienced chronic antigenic stimulation, undergone multiple rounds of antigen-specific replication, have decreased ability to replicate further, are cytotoxic or immunosuppressive, and have subsets that are poorly regulated (Strioga et al., 2011). The CD28-CD57+ phenotype is associated with alcoholism (Cook et al., 1995), intracellular infections like HIV (Vivar et al., 2008, Brenchley et al., 2003), and diseases of aging like cancer (Strioga et al., 2011). Secondary outcomes were the percentage of CD8+ and CD4+ T-cells expressing the CD45RO+CD45RA- or CD45RO-CD45RA+ phenotype. Memory T-cells typically express CD45RO but not CD45RA and respond more rapidly than naïve T-cells (CD45RO-CD45RA+) to previously encountered antigens. Memory T-cells are preferentially infected and compromised by HIV, and expressed with higher frequency in people with alcoholism versus those without (Cook et al., 1994, Cook et al., 1995).

Additional exploratory outcomes included CD28- cells, CD57+ cells, proportion of CD28- cells that expressed CD57, elevated levels of CD28-CD57+T-cells (defined as greater than 75^{th} percentile), and the ratio of naïve to memory CD8+ (or CD4+) T-cells. We also examined absolute counts of CD28-CD57+ T-cells. Absolute CD4 T-cell counts were only available for a subset of participants who had a complete blood count (CBC) measurement performed.

Covariates included in regression models were self-reported and included demographics (age, gender); comorbid infections (history of hepatitis C, hepatitis B, herpes zoster, and tuberculosis); cancer, and any past 30-day injection drug use. In exploratory analyses, $\log_{10}$ HIV-1 RNA was assessed as a potential effect modifier of the relationship between heavy drinking and markers of immunosenescence.

Statistical analysis

Descriptive statistics were used to characterize subjects by heavy drinking status. Variables were compared by heavy drinking status using t-tests, Wilcoxon rank sum, chi-square or Fisher's exact tests as appropriate. Spearman correlations between independent variables and covariates were calculated and no pair of variables included in the regression models had correlation higher than 0.40.

Multiple linear regression models were used to evaluate associations between heavy drinking status and percentage of each T-cell subset. Some T-cell subsets were log-transformed due to skewness in their distributions. In such cases, regression coefficients were back-transformed for ease of interpretation and the measures of effect reported are ratio of means for heavy versus non-heavy drinkers. Initial regression models were fit, first adjusting for age only and then a final model was fit controlling for all covariates. To assess possible effect modification by HIV-1 RNA, an interaction term for alcohol use and HIV-1 RNA was included in regression models. If the interaction term was significant, subsequent models were fit and stratified by high vs. low viremia (dichotomized at median) to describe associations within each subgroup. Confirmatory analyses were performed using median regression models, which are more robust to departures from normality and outliers than linear regression models (Hao LN, 2007, R, 2005). Secondary analyses were conducted using multiple logistic regression models to test whether heavy drinking status was associated with having elevated (highest quartile) CD28-CD57+ T-cells.

Prior to assessing whether average daily alcohol volume and number of drinking days per month were associated with altered immune function, we constructed separate generalized additive models (GAMs) (Hastie and Tibshirani, 1986, Hastie and Tibshirani, 1990) with smoothing splines for each alcohol variable to assess whether there appeared to be a linear relationship between these continuous alcohol measures and the primary outcomes. The assumption of linearity of the relationship between the continuous alcohol measures and primary outcomes did not appear to hold. Thus these alcohol measures were categorized into tertiles for subsequent regression analyses.

Inverse probability weighting (IPW) was applied to account for unavailable CBC data (34% of the sample) required for calculation of absolute CD4+ T-cell counts. Using IPW, subjects with complete data are weighted by the inverse of the probability of their being a complete case (Seaman and White, 2013, Vansteelandt, 2010).

P-values less than 0.05 were considered statistically significant except in testing for interaction terms where an α level of 0.10 was used to identify potential effect modification. Due to the exploratory nature of these analyses, no adjustment was made for multiple comparisons. SAS 9.3 statistical software was used.

Results

Participant characteristics

Complete flow cytometry data were available for 253 ART naïve HIV-infected participants. Thirty-nine people who had undetectable HIV-1 RNA were excluded from analyses. Of the remaining 214 individuals, 61% were heavy drinkers. Participants' characteristics were similar by heavy drinking status except hepatitis C and HIV-1 RNA (Table 1). Participants were young (mean age 33 years) and predominantly male (72%). Hepatitis C prevalence was high (87%), past 30-day injection drug use was common (37%), and mean $\log_{10}$ HIV-1 RNA copies/mL was 5. Almost 85% of the cohort identified as current smokers. Heavy drinkers drank alcohol an average of 17 days (out of 30) and consumed just over 60 grams of alcohol (~4 drinks) daily compared to 3 days and 4 grams for non-heavy drinkers, respectively.

Among those with available data, low CD4 to CD8 T-cell ratio was more prevalent among heavy versus non-heavy drinkers.

Unadjusted regression analyses

In preliminary unadjusted analyses, compared to non-heavy drinkers and abstainers, the distribution of biomarkers associated with immunosenescence did not differ significantly by heavy drinking status (Table 2a, b). Mean ±SD absolute counts of CD8+CD28-CD57+ T-cells were similar among non-heavy drinkers (226 ±181) and heavy drinkers (285 ±180 p=0.13). Naïve and memory CD8+ or CD4+ T-cells and their ratios did not differ by heavy drinking status. The percentage of CD8+ T-cells with the CD28- phenotype appeared higher (63 ±13% vs. 59 ±11%, p=0.03, Table 2) among heavy drinkers.

Adjusted regression analyses

In adjusted linear regression models, there were no significant differences by heavy drinking status in the percentage of CD28-CD57+, memory or naïve T-cell phenotypes (Table 2), or in the ratio of naïve to memory CD8+ or CD4+ T-cells (Table 2a, 2b). Median regression results for the primary outcomes were consistent with results obtained from linear regression models. Similar results for the primary outcomes were obtained when we analyzed absolute T-cell counts. Results for absolute counts were consistent when inverse probability weighting was applied to account for missingness in CD4+ T-cell counts (data not shown).

We constructed logistic regression models to investigate the association of heavy drinking and elevated CD28-CD57+T-cells categorized as highest versus lower three quartiles. There was no difference by heavy drinking status in the proportion of participants with CD8+CD28-CD57+ or CD4+CD28-CD57+ T-cell percentages in the highest quartile (adjusted odds ratio (95% CI): 1.17 (0.59, 2.34) and 1.01 (0.49, 2.08) respectively (Table 2a, 2b).

There was also no significant association between quantity or frequency of alcohol consumption (analyzed separately as tertiles) and these immunosenescence biomarkers with two exceptions: CD8+ CD28-CD57+ cells were significantly higher among those who drank 16–36g of alcohol (intermediate tertile) per month compared to those who drank less than 16g of alcohol (lowest tertile; adjusted mean difference 5.53 (0.08, 10.98) Table 3a). Those who drank intermediate or high (tertile) quantities of alcohol had significantly higher CD4+ CD28-CD57+ cells (Table 3b).

We assessed interactions between heavy drinking and HIV-1 RNA on T-cell outcomes in all fully adjusted models. No significant interactions were reported for any outcome except CD4+ CD28- T-cell percentage ($p<0.10$). In subsequent models stratified by log HIV-1 RNA (dichotomized at median of 4.65 $\log_{10}$ copies/mL), the association between heavy drinking status and CD4+CD28- T-cells was 0.78 (0.42, 1.44) among highly viremic compared to 1.32 (0.71, 2.44) among less viremic participants.

Discussion

Among young HIV-infected Russians not receiving ART, heavy drinking was not significantly associated with alterations in CD28-CD57+, naïve or memory T-cell phenotypes associated with immunosenescence.

Limited data exist on alcohol's effects on T-cell subsets in humans in the context of untreated HIV infection. The present study extends the existing literature by examining alcohol's effect on specific T-cell subsets among HIV-infected people using validated measures of alcohol consumption.

A potential reason why our hypothesis was not supported by these findings is that the effect of unsuppressed HIV replication (and HCV) on these T-cell subsets may be so great that it drowns out any potential effect associated with heavy drinking. While we report increased CD8+CD28- T-cell expression among heavy drinkers, it is conceivable that the higher HIV-1 RNA in heavy drinkers diminished CD57 expression on CD8+CD28- cells (Lee et al., 2014) masking an alcohol-related increase in overall CD28-CD57+ expression. Our effect modification analysis also supports this explanation since it suggests that effects of heavy drinking on CD4+CD28- T-cell proportions may be of a smaller magnitude among highly viremic compared to less viremic people.

These data should be interpreted in the context of other substances used in this cohort in which over a third report past 30-day injection drug use. Opioids and cannabis, for example, have immunosuppressive properties (Sacerdote, 2008, Rieder et al., 2010), which may also be masking potential effects of heavy drinking on these T-cells.

We speculate that alcohol withdrawal could provide another possible explanation of our results. Alcohol withdrawal is a potential unmeasured confounder in this cohort with 57% of participants reporting an alcohol use disorder. Alcohol withdrawal alters CD4, CD8, natural killer and monocyte cell counts (Kutscher et al., 2002, Mili et al., 1992). If alcohol withdrawal prevalence differed by heavy drinking status, and withdrawal differentially reduced expression of T-cells consistent with immunosenescence, this could have confounded the association between heavy drinking and these T-cells. Future studies should measure alcohol withdrawal and explicitly test this hypothesis.

Prior studies have shown expansion of CD28-, CD57+, and CD45RO and contraction of CD45RA T-cell populations with alcohol consumption in HIV uninfected populations (Cook, 1998, Arosa et al., 2000, Cook et al., 1995). In HIV-infected populations, the available human data describe effects on absolute CD4 or CD8+ T-cell count (Samet et al., 2007, Baum et al., 2010, Ghebremichael et al., 2009, Miguez-Burbano et al., 2009, Conen et al., 2013) but not specific T-cell subsets in the setting of HIV infection. These limitations affect our ability to generate specific hypotheses from prior work about immunobiological mechanisms linking alcohol to HIV disease progression.

Data from non-human primates supplement human data and suggest important differences in T-cell populations sampled from peripheral blood versus within organs. There is a lower frequency of central memory (CD62L+CD45RA-) and effector memory (CD62L-CD45RA-)

CD4+ T-cells (in blood, liver, spleen and lymph nodes) and effector memory CD8+ T-cells (in liver) in alcohol-receiving macaques (Marcondes et al., 2008). These animal data suggest that our results in peripheral blood may not reflect T-cell subset biology within organs like the intestines and the liver where alcohol and HIV-related disruption may be occuring via microbial translocation (Brenchley et al., 2006) and hepatocyte injury (Szabo and Zakhari, 2011).

While there were no significant associations between heavy drinking defined using quantity and frequency of alcohol consumption, we did find some associations when quantity and frequency were considered separately. Intermediate volumes and intermediate or high frequency of alcohol consumption were associated with higher levels of T-cells phenotypes consistent with immunosenescence. However, there was no evidence of a dose response for either quantity or frequency. While it is unclear what to make of this finding, future work should assess whether a threshold effect for alcohol consumption on immune function may exist, which may not be evident at the NIAAA thresholds of > 4 drinks in a day (or > 14 standard drinks/week) for men and > 3/day (or > 7/week) for women.

Limitations of our study warrant discussion. T-cell biomarkers were selected to define T-cell subsets consistent with immunosenescence based on best available data at the time the study was designed. Novel T-cell biomarkers that distinguish immunosenescent and naïve versus effector or central memory versus effector memory subtypes (e.g. CD27, CCR7, CD62L) were unavailable for this study. There was no HIV uninfected comparator group to distinguish alcohol versus HIV-related effects on T-cell subsets, however the objective of this study is to evaluate the effect of alcohol among those with HIV infection. Alcohol use was not confirmed using serum biomarkers; however, we relied on a validated and a widely used instrument to measure alcohol exposure. HCV prevalence was high overall (87%) and may have reduced our ability to detect differences in T-cell subsets by heavy drinking status. Comorbidity data was obtained via self-report and are thus subject to reporting bias. As with all observational studies, there is a possibility of unmeasured confounding.

In summary, these cross-sectional data suggest that heavy drinking does not significantly alter immunosenescent T-cell subsets among ART-naive HIV-infected drinkers. Future studies should confirm these findings in treated HIV-infected drinkers, assess alternative markers of adaptive immunity, assess organ specific T-cell changes, and further consider whether changes in alcohol use over time or alcohol withdrawal alters adaptive immunity in the context of HIV infection.

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Table 1

Description of Russia ARCH cohort

Characteristic	Not Heavy Drinking (N=83)	Heavy Drinking (N=131)	p-value
Mean age (SD), years	34.3 (5.4)	32.9 (6.0)	0.09
Female	18 (22%)	41 (31%)	0.13
IDU (Past 30 days)	27 (33%)	53 (41%)	0.24
Cannabis (Past 30 days)	10 (12%)	24 (18%)	0.22
Hepatitis C	79 (95%)	107 (82%)	<0.01
History of hepatitis B	36 (43%)	49 (37%)	0.38
Herpes Zoster	21 (25%)	28 (21%)	0.51
Tuberculosis	3 (4%)	14 (11%)	0.06
Cancer	0 (0%)	0 (0%)	--
Mean $\log_{10}$ HIV-1 RNA (SD)	4.4 (0.9)	4.7 (0.9)	<0.01
Median drinking days (past 30 days) (p25, p75)	0 (0.0, 3.0)	14.0(10.0, 26.0)	<0.01
Median average daily alcohol (grams) (1 drink = 14g) (p25, p75)	0.0(0.0, 3.7)	41.8(22.6, 74.7)	<0.01
Mean years since HIV diagnosis (SD)	7.7 (4.3)	6.8 (4.9)	0.15
Current regular smoking	68 (82%)	114 (87%)	0.31
* Absolute CD4 count	637 (312)	533 (267)	0.08
* Absolute CD8 count	718 (295)	804 (351)	0.24
* Absolute CD4/Absolute CD8 ratio <1	16 (57.1%)	76 (76.0%)	0.05

* N for absolute CD4 and CD8 values was 128 (28 not heavy drinkers, 100 heavy drinkers)

Table 2a

Association of heavy drinking with CD8+ T-cell subsets (linear and logistic regression)

Outcome		T-cell distributions[b]		Adjusted mean (%)difference in outcomes in heavy vs. non-heavy drinkers (95% CI)
Denominator	**T-cell subset**	**Not Heavy Drinking**	**Heavy Drinking**	
CD8+	[a] CD8+ CD28- CD57+	34 (14)	36 (15)	2.38 (−2.00, 6.75)
	CD8+CD45RO+CD45RA- (memory)	34 (14)	34 (14)	−1.13 (−5.14, 2.88)
	CD8+ CD45RO+CD45RA- (naïve)	52 (14)	53 (14)	1.79 (−2.34, 5.91)
	CD8+ CD28-	59 (11)*	63 (13)*	3.07 (−0.52, 6.65)
	CD8+ CD57+	40 (19)	42 (20)	2.10 (−3.76, 7.97)
	N Top Quartile of CD8+CD57+CD28- (%)	20 (24%)	34 (26%)	1.17 (0.59, 2.34)[c]
	N (CD8 Naive / CD8 Memory) <1 (%)	22 (27%)	32 (24%)	0.58 (0.28, 1.21)[c]
	CD8 Naive / CD8 Memory ratio	2.33 (3.32)	2.09 (2.03)	1.03 (0.83, 1.28)[d]
CD8+CD28-	CD8+ CD57+	56 (19)	56 (20)	0.94 (−4.78, 6.65)

Adjusted for age, gender, hepatitis C, hepatitis B, herpes zoster, tuberculosis, cancer, past 30 day injection drug use

[a] Pre-specified primary outcome. All other outcomes considered secondary or exploratory

[b] Values are mean percentages (standard deviation) unless otherwise specified

[c] Logistic regression results

[d] Represents ratio of means (outcome was analyzed as log transformed variable)

* p=0.03 for comparison of means by heavy drinking status

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Table 2b

Association of heavy drinking with CD4+ T-cell subsets (linear and logistic regression)

Outcome		T-cell distributions[b]		Adjusted Ratio of means for heavy vs. non-heavy drinkers (95% CI)
Denominator	**T-cell subset**	**Not Heavy Drinking**	**Heavy Drinking**	
CD4+	[a] CD4+CD28-CD57+	5.17 (10.69)	4.41 (5.89)	1.12 (0.69, 1.82)
	CD4+ CD45RO-CD45RA+ (memory)	52 (15)	54 (17)	1.70 (−3.05, 6.46)[c]
	CD4+CD45RO+CD45RA- (naïve)	39 (15)	39 (16)	−0.29 (−4.68, 4.09)[c]
	CD4+ CD28-	6.28 (12.24)	5.80 (7.04)	1.07 (0.71, 1.63)
	CD4+ CD57+	7.48 (11.05)	6.64 (6.54)	0.96 (0.69, 1.32)
	N Top Quartile of CD4+CD57+CD28- (%)	20 (24%)	34 (26%)	1.01 (0.49, 2.08)[d]
	N (CD4 Naive / CD4 Memory) <1 (%)	52 (63%)	93 (71%)	1.33 (0.69, 2.58)[d]
	CD4 Naive / CD4 Memory ratio	0.95 (0.79)	0.92 (0.87)	0.92 (0.72, 1.18)
CD4+CD28-	CD4+ CD57+	62 (31)	63 (29)	2.27 (−6.60, 11.13)[c]

Adjusted for age, gender, hepatitis C, hepatitis B, herpes zoster, tuberculosis, cancer, past 30 day injection drug use

[a] Pre-specified primary outcome. All other outcomes considered secondary or exploratory

[b] Values are mean percentages (standard deviation) unless otherwise specified

[c] Represents mean percent difference (outcome was analyzed as log transformed variable)

[d] Logistic regression results

Table 3a

Association between quantity and frequency of drinking with CD8+ T-cell subsets (linear regression)

Denominator	T-cell subset (relative frequencies (%)unless otherwise specified)	Adjusted mean (%) difference by drinking days (Low tertile (0–8 days) is referent group)		Adjusted mean (%) difference by drinking volume (Low tertile (0–15.7g) is referent group)	
		Mid tertile (9–14 days)	High tertile (15–30 days)	Mid tertile (16.0–35.9g)	High tertile (39.0–449.5g)
CD8+	[a] CD8+CD28-CD57+	−0.72 (−5.97, 4.53)	2.39 (−2.57, 7.35)	5.53 (0.08, 10.98)	2.44 (−2.42, 7.31)
	CD8+CD45RO+CD45RA- (memory)	−1.03 (−5.84, 3.79)	−0.74 (−5.29, 3.81)	−0.39 (−5.41, 4.63)	−1.76 (−6.24, 2.73)
	CD8+CD45RO-CD45RA+ (naïve)	1.43 (−3.52, 6.38)	1.53 (−3.15, 6.21)	1.90 (−3.26, 7.06)	2.72 (−1.89, 7.33)

Analyses adjusted for age, gender, hepatitis C, chronic hepatitis B, herpes zoster, tuberculosis, injected drug use in the past 30 days, and cancer

[a] Pre-specified primary outcome. All other outcomes considered secondary

Table 3b

Association between quantity and frequency of drinking with CD4+ T-cell subsets (linear regression)

Denominator	T-cell subset (relative frequencies (%)unless otherwise specified)	Adjusted mean (%) difference by drinking days (Low tertile (0–8 days) is referent group)		Adjusted mean (%) difference by drinking volume (Low tertile (0–15.7g) is referent group)	
		Mid tertile (9–14 days)	High tertile (15–30 days)	Mid tertile (16.0–35.9g)	High tertile (39.0–449.5g)
CD4+	[a] CD4+CD28-CD57+	0.65[b] (0.36, 1.16)	0.74[b] (0.43, 1.28)	0.98[b] (0.48, 2.04)	0.94[b] (0.49, 1.79)
	CD4+CD45RO+CD45RA- (memory)	2.64 (−3.05, 8.34)	2.33 (−3.05, 7.71)	2.92 (−3.03, 8.86)	2.46 (−2.85, 7.77)
	CD4+CD45RO-CD45RA+ (naïve)	−1.06 (−6.32, 4.20)	−0.90 (−5.87, 4.07)	0.00 (−5.50, 5.49)	−0.98 (−5.88, 3.93)

Analyses adjusted for age, gender, hepatitis C, chronic hepatitis B, herpes zoster, tuberculosis, injected drug use in the past 30 days, and cancer

[a] Pre-specified primary outcome. All other outcomes considered secondary

[b] Ratio of means (outcome was analyzed as a log transformed variable)