Published in final edited form as:

Future Virol. 2012 February ; 7(2): 205–213. doi:10.2217/FVL.12.3.

The effect of alcohol use on IL-6 responses across different racial/ethnic groups

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Abstract

Aims—Chronic inflammation has become increasingly recognized as a health threat for people living with HIV, given its associations with multiple diseases. Accordingly, the scientific community has prioritized the need to identify mechanisms triggering inflammation.

Participants & methods—A clinic-based case–control study was designed to elucidate the plausible effects of alcohol use on IL-6. Peripheral blood mononuclear cells for measuring IL-6 culture supernatant and plasma for HIV assessments were collected from 59 hazardous alcohol users and 66 nonhazardous alcohol users, who were matched according to their age, gender and US CDC HIV severity status.

Results—Stimulated peripheral blood mononuclear cells produced significantly higher amounts of IL-6 in hazardous alcohol users compared with nonhazardous alcohol users. However, racial status and receiving HAART significantly moderated this effect. Notably, in both HAART and non-HAART scenarios, IL-6 levels were associated with CD4 counts and viral burden. A distinctive IL-6 production pattern across racial/ethnic groups was also evident and showed that, when prescribed HAART, Hispanic hazardous alcohol users have a particularly high risk of morbidity compared with their Caucasian and African–American counterparts. After adjusting for confounders (e.g., sociodemographics and HIV disease status), regression analyses confirmed that chronic inflammation, as indicated by IL-6 levels (log), is associated with alcohol use, race/ethnicity and thrombocytopenia, and tended to be related to concurrent smoking.

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Financial & competing interests disclosure

This manuscript was supported by NIH/NIAAA Grant no. 3R01AA018095-01A1S1. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Conclusion—Our data confirm that, despite HAART, people living with HIV still have a persistent inflammatory response that, in our study, was associated with chronic hazardous alcohol use. The data also highlight racial/ethnic disparities in IL-6 that justify further investigations.

Keywords

AIDS; alcohol; HIV; IL-6; race/ethnicity

HAART has transformed the HIV epidemic by not only dramatically reducing mortality and morbidity, but also modifying the value of virological and immunological disease markers. Accordingly, several virological and immunological markers have been reassessed in the post-HAART era for their significance in predicting both the risk of progression to AIDS and death [1–3]. Among such markers, IL-6 has become a highly valuable predictor of morbidity and mortality [4–8]. The high predictive value of IL-6 has also been evident in epidemiological studies in the general population, where high IL-6 levels have been associated with increased risk of developing multiple infections, cardiovascular disease, diabetes and neuropsychological disorders [9–14]. Notably, these disorders have become relevant for people living with HIV (PLWH) on the basis that HIV is now a chronic disease with modern treatment.

Therefore, it has become very important to expand our current understanding of factors that may be modulating IL-6 levels. Accordingly, several groups have evaluated the potential influence of HAART on IL-6 levels. Unfortunately, they have yielded contradictory results, partly due to using different model systems or study populations [15–17]. In addition to HAART, alcohol and race/ethnicity appear to be important in modifying cytokine production, but have been neglected in HIV studies. In particular, the potential effects of alcohol abuse on cytokine production have not been sufficiently studied, despite considerable alcohol abuse among PLWH. In general, few data are available that have analyzed the relationship between alcohol and cytokines in those free of substantial liver disease. Among them, some studies have indicated that alcohol use has anti-inflammatory properties, including a reduction in IL-6, while animal studies suggest a linear relationship between alcohol drinking and IL-6 [18]. Given the deleterious effects of IL-6 elevations, and the high prevalence of alcohol use among PLWH, it is especially important to clarify its possible effects [18].

Since HIV, similar to other diseases causing premature mortality, exhibits differential impact patterns for different racial/ethnic groups, it is also highly relevant to explore racial differences in the distribution of IL-6 levels. The associations between common gene variations in *IL-6* and race/ethnicity have been examined in a few studies; however, findings have been inconsistent [19–22]. Moreover, none of these studies considered PLWH, or sought to examine divergences in ethnic distribution, which have become characteristic of the HIV epidemic, since they mainly focused on individuals of Japanese, European or Israeli descent [18–23].

Therefore, in order to improve our understanding of the diverse patterns of disease progression, we comprehensively assessed the relationships between IL-6 and alcohol-drinking behaviors by race in PLWH, while controlling for well-known confounding factors. Identification of factors indicative of rapid disease progression would augment preventive strategies and timely interventions.

Participants & methods

Sampling

Our participants were 69 men and 56 women who were consecutively enrolled in the study if they fulfilled inclusion criteria (e.g., at least 18 years old, confirmed HIV-positive and regularly treated at the Jackson Medical Center, FL, USA). We chose participants from an open-access public health system with standard treatment protocols in order to minimize social, medical and treatment inequalities that could confound our outcomes. Men and women were enrolled regardless of clinical (i.e., symptomatic, asymptomatic or AIDS) and treatment status (HAART yes/no). However, the distribution of participants across US CDC categories was well-balanced, with almost half (48%) having AIDS and 60% receiving HAART.

Nonambulatory patients were excluded, as well as those with major comorbidities, such as opportunistic CNS infection, head injury with or without loss of consciousness, tumors, major psychiatric disease, developmental disorders, severe malnutrition or confirmed cardiovascular- or immune-based disease (i.e., malignancies, autoimmune diseases or arthritis) at baseline. To reduce the confounding effects of liver disease and/or illegal drug use, injection and dependent drug users were excluded, as well as any participants who had cirrhosis, active viral hepatitis or liver enzyme levels two standard deviations above normal values. Those individuals who agreed to comply with all the study procedures, to be followed for 6 months and to provide written informed consent were enrolled. Upon entry, standardized research questionnaires were administered and were complemented with a brief physical exam and laboratory testing.

Alcohol exposure

To measure alcohol consumption and alcohol dependence, two widely used assessments were used: the WHO's Alcohol Use Disorders Identification Test and the Alcohol Dependence Scale [24,101]. As established by the National Institute on Alcohol Abuse and Alcoholism and American Psychiatric Association guidelines, men who reported more than 14 drinks per week and women who reported more than seven drinks per week were classified as hazardous drinkers, while those who consumed less alcohol were included in the nonhazardous group. Participants drinking more than five standard drinks in 1 day were considered heavy drinkers [102].

IL-6

We assessed IL-6 using two approaches – plasma and *in vitro* production methods – so that they could be compared. Blood samples were immediately transferred to the laboratory to be processed. Half of the sample was centrifuged to obtain plasma and the other was used for the *in vitro* method. A total of 100 µl of blood was added to each well of a 24-well plate, along with 900 µl medium with antibiotics. Given our interest in determining whether lymphocyte replenishment obtained with HAART affects IL-6 production or not, peripheral blood mono-nuclear cells (PBMCs; 106/ml) were stimulated with plate-bound anti-CD3 monoclonal antibody (10 µg/ml) plus 1 µg/ml soluble anti-CD28 monoclonal antibody for 24 h. We used 10 µl/well, for a final concentration of 5 µg/ml in medium RPMI 1640 with L-glutamine (Gibco BRL, MD, USA), penicillin and streptomycin. A control well (growth medium without stimulant) was included for each blood sample.

Supernatant fluid was harvested after 48 h and levels of IL-6 were determined using a commercial ELISA (Beckman/Coulter Corporation, FL, USA). IL-6 levels were calculated using standard curves and IL-6 levels obtained from the supernatants were normalized to 100,000 lymphocytes. These assays were appropriately quality controlled with different

known sources of recombinant and natural cytokines (including National Institute for Biological Standards and Control/WHO reference standards) and throughout different patient categories.

Virological & immunological assessments

In order to best determine the viroimmune status and HAART effectiveness, T-cell counts and viral burdens were obtained from all the participants. CD4 and CD8 T-cell percentages were determined by flow cytometry, and based on the complete blood cell counts, we determined their absolute counts. Viral loads were measured using a COBAS® Amplicor HIV-1 monitor kit using reverse transcriptase PCR (COBAS Amplicor Analyzer [Roche Molecular Diagnostics, CA, USA]).

The definition of HAART used for analyses was guided by the published recommendations of the Panel on Antiretroviral Guidelines for Adults and Adolescents [103]. Adherence was determined based on pharmacy records and the AIDS Clinical Trials Group antiretroviral adherence questionnaire. This questionnaire specifically measures consistency of behavior and captures patterns of missed doses.

Race/ethnicity

Data on race and ethnicity were categorized in accord with the 1997 Office of Management and Budget Directive. We used the following mutually exclusive categories and participant self-reports to categorize participants as: Caucasian (white non-Hispanic), African-American and Hispanic (whites). Participants from other racial ethnic groups were not analyzed because their numbers were insufficient to produce meaningful results.

Other risk factors

To investigate risk factors associated with increased levels of IL-6, potentially confounding variables such as anthropometrics, age, gender, nutritional status (albumin and BMI), liver enzymes, smoking or drug use were assessed. Weight was measured to the nearest 0.1 kg on a balance beam scale, with the participant dressed in indoor clothing without shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BMI was calculated as weight in kilograms divided by height in meters squared. Tobacco and drug use habits were assessed using standardized questionnaires. In addition, a detailed current and past medical history was obtained. Variables were first analyzed as continuous, and then dichotomized to facilitate analyses.

Statistical analyses

Data were analyzed using SAS 8.1 (SAS Institute Inc., NC, USA) and SPSS 15 (SPSS Inc., IL, USA). Associations between the main variables of interest were examined with Pearson's correlation coefficient analyses. Both HIV viral load and IL-6 levels in the supernatants of PBMCs were asymmetrically distributed and were analyzed after log transformation. The plasma levels were approximately normally distributed and were not transformed. IL-6 differences between subgroups were compared using analysis of variance.

Multivariate regression analysis was used to describe the associations between IL-6 elevations, race and alcohol behaviors, while controlling for all variables that were statistically significant in the univariate analyses. Multiple logistic regression analyses were used to analyze the effects of race/ethnicity, alcohol and other potential risk factors on IL-6. The initial predictive model included age, socioeconomic status, BMI, CD4 cell counts, gender, viral loads, illicit drugs and HAART; nonsignificant variables ($p = 0.05$) were removed, beginning with the least significant variables. These factors were selected because

they were either significant ($p \leq 0.05$) in the univariate analysis or they were recognized risk factors. Regression output was reported as adjusted odds ratios, accompanied by 95% CIs.

Results

Sociodemographics by alcohol groups

With an overall participation rate of 98%, 125 HIV-infected men and women were enrolled in the study. The descriptive characteristics of the 125 participants according to alcohol consumption are shown in Table 1, indicating that the two groups were very similar.

IL-6 by sociodemographic characteristics & treatment

Induction of IL-6 by PBMCs after stimulation varied from 8–24,293 levels in pg to 1.8–5.2 IL-6 logs. There was no significant association between IL-6 levels and gender, age, calory intake, dietary fat or BMI. However, there was a significant association between IL-6 levels and smoking, in which IL-6 production was increased in smokers compared with nonsmokers (3.6 ± 0.8 vs 3.3 ± 1 log). Smokers were three-times more likely to have >3.9 IL-6 logs than nonsmokers (95% CI: 1.05–9.00; $p = 0.03$).

Since a growing body of literature indicates that HAART reduces the inflammatory response, the relationship between HAART and IL-6 was assessed. Consistent with the SMART study, analyses indicated that IL-6 values were significantly lower in HAART-treated individuals (2.8 ± 1.5 vs 3.6 ± 0.68 log; $p = 0.001$).

Since HIV and other diseases associated with premature mortality are known to exhibit differential impact patterns across racial/ethnic groups, we also explored the race-specific distribution of study variables. As summarized in Table 2, the only difference across the racial/ethnic groups was the prevalence of smoking. We then explored racial/ethnic difference in IL-6 production and, indeed, analyses indicated that there were differences between the three subgroups (Figure 1). The PBMC production of IL-6 in Caucasian participants was significantly different from that in African-American participants, even when controlling for treatment and sociodemographic variables. Regardless of HAART status, African-American participants had lower levels of IL-6 (without HAART 1.98 ± 1.53 pg). Among non-HAART-treated individuals, Caucasians exhibited enhanced release of IL-6 in the culture supernatants compared with Hispanics (4.6 ± 1.1 vs 2.8 ± 1.3 pg/105 Lym; $p = 0.07$). However, upon antiretroviral treatment, Hispanics exhibited the highest IL-6 levels (3.8 ± 0.68 log), followed by Caucasians (3.6 ± 0.6 log) and then by African-Americans (2.8 ± 0.7 log; $p = 0.03$).

IL-6 & HIV disease

Considering that increased IL-6 concentrations might be related to more severe disease, we also assessed the association between IL-6 and HIV disease markers. The Pearson coefficients for correlations of log HIV viral load with IL-6 were 0.23 ($p = 0.05$) for *in vitro* production and 0.21 for plasma ($p = 0.08$). IL-6 production in stimulated PBMCs and CD4 cells were significantly intercorrelated (Pearson correlation coefficient: 0.30; $p = 0.02$), but not with plasma levels (0.23; $p = 0.1$).

To further assess the impact of disease status in IL-6 production, participants were dichotomized at the CD4 count cut-off point of 200 cells/mm³. We observed increased IL-6 production in stimulated PBMCs in those with >200 CD4 cell counts, compared with individuals with <200 cells/mm³ (3.7 ± 0.57 vs 2.8 ± 1.35 log; $p = 0.01$). Thus, IL-6 is regulated differently with respect to disease state.

IL-6 in relation to alcohol

Mean levels of IL-6 in the supernatants of hazardous alcohol users were significantly higher compared with nonhazardous users (3.6 ± 0.6 vs 3.1 ± 1.3 log; $p = 0.04$). While an overall relationship of alcohol intake was observed with concentrations of circulating IL-6, this did not reach statistical significance (151 ± 33 vs 66.1 ± 6.5 ; $p = 0.07$). However, the heaviest liquor intakes were associated with higher circulating IL-6 concentrations (p for interaction = 0.01). Therefore, additional analyses were performed using only IL-6 production by PBMCs, which indicated distinctive scenarios in HAART- and non-HAART-treated PLWH. Figure 2 shows the crude association of alcohol intake with IL-6 in antiretroviral-treated and -nontreated individuals. We found an inverse relationship in nontreated individuals, with minimum levels in participants who were heavy drinkers, followed by hazardous drinkers. Notably, former drinkers had similar values to subjects who were not hazardous users, indicating that the alcohol effect is reversible. The profile of non-HAART hazardous drinkers was also of interest, revealing the lowest IL-6 values (1.6 ± 1.3 vs 3.8 ± 0.7 pg/104 Lym; $p = 0.001$), the lowest CD4 cell counts (69.7 ± 52.6 vs 145.5 ± 126 pg/104 Lym; $p = 0.004$) and the highest viral loads ($934,144 \pm 770,388$ vs $345,713 \pm 255,657$ HIV copies; $p = 0.006$).

Among those receiving HAART, stimulated PBMCs from heavy and hazardous drinkers produced significantly higher amounts of IL-6 compared with nonhazardous individuals (4.4 ± 0.75 or 3.75 ± 0.8 vs 3.24 ± 0.9 pg/104 Lym; $p = 0.05$). This residual inflammation was associated with higher viral loads ($21,112 \pm 8950$ vs 8191.5 ± 1947 pg/104 Lym; $p = 0.05$), with similar CD4 cell counts (396.5 ± 320 vs 346.5 ± 249 ; $p = 0.2$) and significantly lower CD8 percentages (47 ± 18 vs $56 \pm 13\%$; $p = 0.05$). This suggests that even when treated with HAART, individuals with excessive alcohol intakes (heavy and hazardous drinkers) maintain a proinflammatory status that is significantly related to higher viral loads.

Regression-adjusted analyses

Regression analyses were used to determine the effects of age, HIV disease, HAART, alcohol use and ethnicity status on IL-6. The results are summarized in Table 3 and indicate that hazardous alcohol use, being white, having thrombocytopenia and smoking remained independently associated with IL-6 levels after adjusting for gender, HAART, CDC stage of HIV disease and BMI.

Discussion

This study demonstrates, for the first time, that in PLWH, hazardous alcohol use is a major modifiable risk factor for abnormal IL-6 production. In a similar search for factors explaining the phenomenon of chronic inflammation in PLWH, a recent publication comparing three national cohorts (CARDIA, MESA and SMART) demonstrated that HAART and viral load were closely related to IL-6 [25]. They also observed an unexplained ongoing inflammation in PLWH, even when receiving HAART [25]. Our findings expand upon those previous attempts by providing evidence that, in HAART recipients without clinical evidence of liver disease, hazardous alcohol use is associated with both subclinical inflammation and increased viral replication. Additional analyses also demonstrate that race/ethnicity significantly modifies this relationship. Our findings suggest several outstanding clinical and public health implications. First, based on the SMART study findings, it may be posited that avoiding hazardous alcohol use will significantly reduce IL-6 and premature mortality in PLWH [25]. Accordingly, our study further emphasizes the benefit of reducing alcohol use among PLWH. Second, these findings also suggest the value of further study of racial/ethnic differences in similar populations. It also highlights the importance of carefully

selecting how cytokines will be assessed and indicates the advantage of measuring cytokines in the supernatant of PBMCs, rather than in serum or plasma.

Equally importantly, the analyses showed a distinctive relationship between alcohol and IL-6 according to treatment status. Although alcohol intake downregulates IL-6 production in the natural course of HIV disease, among treated individuals, the relationship was bell-shaped. The inverse relationship between alcohol and IL-6 observed in nontreated individuals was accompanied by a deleterious viroimmune profile. Based on Szabo's study [25], this profile could be attributed to alcohol inducing an aberrant superantigen presentation that leads to limited IL-6 production and T-cell proliferation, thus reducing viral containment. It may also be related to alcohol's enhanced effects on IL-6 uptake and metabolism [25,26]. Although a high proportion of PLWH undergoing HAART achieve viral suppression and extended life expectancy, a subset of this population maintain a proinflammatory state, enhancing the risk for AIDS and non-AIDS-associated diseases (i.e., cognitive impairment, kidney damage and cardiovascular diseases) [27–29]. The national studies also revealed that IL-6 elevations after HAART were associated with an increased risk of all-cause mortality [12,17]. Unfortunately, the mechanisms mediating this persistent immune activation are not fully understood. However, our study contributes to this knowledge gap by demonstrating that, in HAART-treated individuals, hazardous alcohol use maintains a chronic proinflammatory state (higher IL-6 values). IL-6 elevations associated with alcohol use may be multifactorial and reflect a compensatory response to thrombocytopenia and/or a common hematologic disorder manifested in PLWH and hazardous alcohol users that is related to increased morbidity and mortality. This may be a sign of early liver damage or cholestasis, associated with the interactive effects of alcohol and HAART, and requiring further research [29–31]. It could also be speculated that ethanol can activate the IL-6 transcription factor NF- κ B and thus the virus replication machinery [32]. Further supporting this hypothesis is the enhanced ongoing viral replication observed among those with IL-6 elevations. Consistent with prior studies of the general population, our data indicate racial/genetic modulation of alcohol health effects, underscoring the importance of race analyses, particularly among PLWH. While confirming lower IL-6 levels among African-Americans, our study extends earlier findings by demonstrating, for the first time, that among anti-retroviral-treated individuals, Hispanics who abused alcohol had higher IL-6 responses, suggesting chronic inflammation. These results complement our previous findings that lipid disturbances were also more frequently observed among Hispanic hazardous drinkers. Taken together, they strongly emphasize this population's special need for careful evaluation of HAART-associated complications, such as cardiovascular diseases and metabolic syndrome.

Several factors may limit our findings. A longitudinal study, instead of a cross-sectional study, may more precisely estimate key parameters. Although the correlative nature of this study precludes causal inferences, the robust associations between alcohol and IL-6 regulation, its biological plausibility and its persistent relationship after controlling for confounders support the notion that they are closely related. Our findings are limited by the specific site of the study, and the relatively small sample size. Furthermore, the sample was restricted to Hispanic whites, Caucasians and African-Americans, which may further limit the generalizability of these findings. Therefore, a study with a larger, more ethnically diverse sample and with a longer follow-up duration would strengthen our results.

In conclusion, these results may be highly relevant for clinicians, since PLWH with inefficient viral control and with chronic inflammation constitute a major clinical hurdle given the threat of disease progression, medical complications and increased mortality. Moreover, our data suggest the benefit of alcohol screening and close monitoring of Hispanics and non-Hispanic Caucasian hazardous drinkers receiving HAART.

Conclusion

Similar to prior studies, in this contemporary sample of HIV-infected individuals, marked elevations of IL-6 were evident. Such a persistent inflammatory response is of concern given its consistent association with a number of adverse health outcomes. It is relevant that our data indicated a dynamic relationship between HIV, HAART, alcohol intake and IL-6. This association was not affected by controlling for potential confounders, including a number of social and lifestyle indicators, and was consistent in treated and nontreated individuals. Although several mechanisms are likely to be involved, the relationship between alcohol intake and levels of IL-6 is highly relevant as it offers a modifiable target. It also provides a biologically plausible explanation for the observed health disparities.

References

Papers of special note have been highlighted as:

■ of interest

■ of considerable interest

1. Fauci AS. Multifactorial nature of human immunodeficiency virus disease: implications for therapy. *Science*. 1993; 262:1011–1018. [PubMed: 8235617]
2. Fauci AS. Host factors and the pathogenesis of HIV-induced disease. *Nature*. 1996; 384:529–534. [PubMed: 8955267]
3. Poli G, Fauci AS. Cytokine modulation of HIV expression. *Semin Immunol*. 1993; 5:165–173. [PubMed: 7688596]
4. Poli G.; Fauci, AS. Role of cytokines in the pathogenesis of human immunodeficiency virus infection. In: Aggarwal, BB.; Puri, RK., editors. *Human Cytokines: Their Role in Disease and Therapy*. Blackwell Science; MA, USA: 1995. p. 421–449.
5. Poli G, Kinter AL, Fauci AS. Interleukin 1 induces expression of the human immunodeficiency virus alone and in synergy with interleukin 6 in chronically infected U1 cells: inhibition of inductive effects by the interleukin 1 receptor antagonist. *Proc Natl Acad Sci USA*. 1994; 91:108–112. [PubMed: 7506410]
6. Zaitseva M, Lee S, Lapham C, et al. Interferon gamma and interleukin 6 modulate the susceptibility of macrophages to human immunodeficiency virus type 1 infection. *Blood*. 2000; 96(9):3109–3117. [PubMed: 11049991]
- 7■. Rodger AJ, Fox Z, Lundgren JD, et al. INSIGHT Strategies for Management of Antiretroviral Therapy (SMART) Study Group. Activation and coagulation biomarkers are independent predictors of the development of opportunistic disease in patients with HIV infection. *J Infect Dis*. 2009; 200(6):973–983. One of the first publications recognizing a chronic inflammatory response in HAART-treated individuals and its potential consequences. [PubMed: 19678756]
8. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta*. 2011; 1813(5):878–888. [PubMed: 21296109]
9. Marconi C, de Andrade Ramos BR, Peraçoli JC, Donders GG, da Silva MG. Amniotic fluid interleukin-1 beta and interleukin-6, but not interleukin-8 correlate with microbial invasion of the amniotic cavity in preterm labor. *Am J Reprod Immunol*. 2011; 65(6):549–556. [PubMed: 21214658]
10. Cussigh A, Falletti E, Fabris C, et al. Interleukin 6 promoter polymorphisms influence the outcome of chronic hepatitis C. *Immunogenetics*. 2011; 63(1):33–41. [PubMed: 21072509]
11. Nishida H, Horio T, Suzuki Y, et al. Interleukin-6 as an independent predictor of future cardiovascular events in high-risk Japanese patients: comparison with C-reactive protein. *Cytokine*. 2011; 53(3):342–346. [PubMed: 21190868]
12. SMART Study Group. Risk for opportunistic disease and death after reinitiating continuous antiretroviral therapy. *Ann Int Med*. 2008; 149:299–303.

13. Patanella AK, Zinno M, Quaranta D, et al. Correlations between peripheral blood mononuclear cell production of BDNF, TNF-alpha, IL-6, IL-10 and cognitive performances in multiple sclerosis patients. *J Neurosci Res.* 2010; 88(5):1106–1112. [PubMed: 19885866]
14. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing Type 2 diabetes mellitus. *JAMA.* 2001; 286:327–334. [PubMed: 11466099]
15. Amirayan-Chevillard N, Tissot-Dupont H, Capo C, et al. Impact of highly active antiretroviral therapy (HAART) on cytokine production and monocyte subsets in HIV-infected patients. *Clin Exp Immunol.* 2000; 120:107–112. [PubMed: 10759771]
16. Stone SF, Price P, Keane NM, Murray RJ, French MA. Levels of IL-6 and soluble IL-6 receptor are increased in HIV patients with a history of immune restoration disease after HAART. *HIV Med.* 2002; 3(1):21–27. [PubMed: 12059947]
17. Neuhaus J, Jacobs DR, Baker JV, et al. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. *J Infect Dis.* 2010; 201:1788–1795. [PubMed: 20446848]
18. Dai J, Lin D, Zhang J, et al. Chronic alcohol ingestion induces osteoclastogenesis and bone loss through IL-6 in mice. *J Clin Invest.* 2000; 106:887–895. [PubMed: 11018077]
19. Liu Y, Berthier-Schaad Y, Fallin MD, et al. IL-6 haplotypes, inflammation, and risk for cardiovascular disease in a multiethnic dialysis cohort. *J Am Soc Nephrol.* 2006; 17:863–870. [PubMed: 16467451]
20. Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. *Arterioscler Thromb Vasc Biol.* 2002; 22:1668–1673. [PubMed: 12377747]
21. Saijo Y, Yoshioka E, Fukui T, Kawaharada M, Sata F, Sato H. Effects of the interaction between interleukin-6 -634C/G polymorphism and smoking on serum C-reactive protein concentrations. *Hypertens Res.* 2007; 30:593–599. [PubMed: 17785926]
22. Friedlander Y, Kark JD, Sinnreich R, Tracy RP, Siscovick DS. Fibrinogen and CRP in Israeli families, genetic and environmental sources of concentrations and longitudinal changes. *Atherosclerosis.* 2006; 189:167–177.
23. Cohen MS, Hellmann N, Levy JA, DeCock K, Lange J. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *J Clin Invest.* 2008; 118(4):1244–1254. [PubMed: 18382737]
24. Skinner HA, Allen BA. Alcohol dependence syndrome: measurement and validation. *J Abnorm Psychol.* 1982; 91:199–209. [PubMed: 7096790]
25. Szabo G, Mandrekar P, Catalano D. Inhibition of superantigen-induced T cell proliferation and monocyte IL-1 beta, TNF-alpha, and IL-6 production by acute ethanol treatment. *J Leukoc Biol.* 1995; 58:342–350. [PubMed: 7665990]
26. Deaciuc IV, Alappat JM, McDonoug KH, D'Souza NB. Effect of chronic alcohol consumption by rates on tumor necrosis factor-alpha and interleukin-6 clearance *in vivo* and by isolated, perfused liver. *Biochem Pharmacol.* 1996; 52:891–899. [PubMed: 8781508]
27. Narkiewicz K, van de Borne PJ, Hausberg M, et al. Cigarette smoking increases sympathetic outflow in humans. *Circulation.* 1998; 98:528–534. [PubMed: 9714109]
28. Stengel B, Couchoud C, Cénée S, Hémon D. Age, blood pressure and smoking effects on chronic renal failure in primary glomerular nephropathies. *Kidney Int.* 2000; 57:2519–2526. [PubMed: 10844621]
29. Wesson DE. The relationship of cigarette smoking to end-stage renal disease. *Semin Nephrol.* 2003; 23:317–322. [PubMed: 12838500]
30. Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl J Med.* 2000; 343:1467–1476. [PubMed: 11078773]
31. Te HS. Cholestasis in HIV-infected patients. *Clin Liver Dis.* 2004; 8:213–228. VIII–IX. [PubMed: 15062202]
32. Mandrekar P, Catalano D, Szabo G. Alcohol-induced regulation of nuclear regulatory factor kappa beta in human monocytes. *Alcohol Clin Exp Res.* 1997; 21:988–994. Classical basic science article regarding the broad mechanisms underlying the effects of alcohol. [PubMed: 9309306]

Websites

101. Babor, TF.; Higgins-Biddle, JC.; Saunders, JB.; Monteiro, MG. [Accessed 20 June 2010] AUDIT. http://whqlibdoc.who.int/hq/2001/WHO_MSD_MSB_01.6a.pdf
102. [Accessed 20 June 2010] NIAAA and American Psychiatric Association guidelines. <http://rethinkingdrinking.niaaa.nih.gov/IsYourDrinkingPatternRisky/WhatsAtRiskOrHeavyDrinking.asp>
103. Department of Health and Human Services. [Accessed 1 June 2010] Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Nov 3. 2008 www.fda.gov/ForConsumers/ByAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm121604.htm

Executive summary

- This study highlights the limitations of using plasma or serum to assess cytokines.
- The data show that IL-6 is associated with both viral load and CD4 cell counts, suggesting that IL-6 is still a valid marker of immune disturbances in HAART recipients.
- Consistent with prior studies, the data indicate race differences in IL-6 levels, underscoring the importance of such analyses.
- Hazardous alcohol use among people living with HIV was associated with high IL-6 levels, reinforcing the need for alcohol programs (screening, prevention and interventions) among this population.
- IL-6 responses differed across HIV treatment groups, highlighting the need for performing separate analyses in future studies.

Figure 1. IL-6 according to race/ethnicity and HAART

Values are the means of the IL-6 logarithm. The data represent descriptive statistics and comparisons of baseline values among race/ethnic groups both in HAART- and non-HAART-treated subgroups. The levels are dissimilar between race/ethnic groups.

Figure 2. IL-6 logarithmic values according to HAART and patterns of alcohol consumption
Values are the means of the IL-6 logarithm. The data represent baseline values between heavy, hazardous and nonhazardous drinkers and those who have quit.

Table 1

Baseline sociodemographic information for the HIV-infected participants with and without hazardous alcohol use.

Variable	Hazardous drinkers (n = 59)	Nonhazardous drinkers (n = 66)	p-value
Age (years)	41.5 ± 7.5	42 ± 10	NS
Men	58%	53%	NS
Women	42%	47%	NS
African-American	37%	25%	NS
Hispanic	56%	65%	NS
Caucasian	7%	10%	NS
Income < US\$20,000/year	89%	87%	NS
HAART	21%	19%	NS
Albumin (g/dl)	4.0 ± 0.2	4.0 ± 0.5	NS
Smoking	72%	59%	NS
<i>Liver enzymes</i>			
SGOT (IU/l)	71.4 ± 14	71 ± 13	NS
SGPT (IU/l)	56.2 ± 11	42.7 ± 8	NS

The data represent descriptive statistics and comparisons of baseline key variables between hazardous drinkers and nonhazardous drinkers. Subgroups only tended to be dissimilar in race/ethnicity.

NS: Nonsignificant.

Table 2

Baseline sociodemographic information for the HIV-infected participants by race/ethnicity.

Variables	People living with HIV (n = 125)			p-value
	Hispanic white	Non-Hispanic white	African-American	
Age (years)	43.4 ± 10	47.9 ± 9	42.6 ± 9.4	0.1
Male	73%	70%	60%	0.7
Female	27%	30%	40%	
Income < US\$20,000/year	93%	80%	91%	0.8
CD4 cell count/mm ³	276.6 ± 56	336 ± 105	278.9 ± 33	0.5
Viral load	131,510 ± 41,523	232,083 ± 153,895	145,278 ± 49,849	0.6
HAART yes	69%	80%	75%	0.7
HAART no	31%	20%	25%	
Alcohol	51%	30%	39%	0.2
Smoking	61%	33%	69%	0.03
Albumin (g/dl)	4 ± 0.1	4.2 ± 0.3	3.9 ± 0.2	0.3

Table 3

Multivariate analyses for IL-6 levels.

Model	Multivariate RR	95% CI	p-value
Hazardous liquor use	8.4	1.3–25	0.03
Race/ethnicity (African–American/Caucasian + Hispanic)	9.5	2.2–40	0.002
Thrombocytopenia	4.6	1.4–14.1	0.01
Smoking	1.6	1.0–2.3	0.056

RR represents the final logistic regression model predicting IL-6 values. Significance was set at 0.05. The model statistics computed include adjusted odds ratios, 95% CIs and their corresponding p-values. Hazardous liquor use, thrombocytopenia and smoking were set as dichotomous variables.

RR: Relative risk.