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The role of alcohol on platelets, thymus and cognitive performance among HIV-infected subjects: Are they related?

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Abstract

Our objective was to evaluate whether thrombocytopenia and small thymus volume, which may be associated with hazardous alcohol consumption, are predictors of cognitive performance after highly-active antiretroviral treatment (HAART). To achieve this goal 165 people living with HIV starting HAART underwent thymus magnetic resonance imaging, cognitive (HIV Dementia Score [HDS] and the California Verbal Learning Test [CVLT]), immune and laboratory assessments at baseline and after 6 months of HAART. At baseline, hazardous alcohol consumption was significantly correlated with both thymus size ($r = -0.44$, $p = 0.003$) and thrombocytopenia ($r = 0.28$, $p = 0.001$). Of interest, thrombocytopenic patients were characterized by a smaller thymus size. Individuals with and without cognitive impairment differed in alcohol consumption, platelet counts and thymus size, suggesting that they may be risk factors for neurological abnormalities. In fact, after HAART hazardous alcohol use associations with thrombocytopenia were related to cognitive decline (learning = -0.2 ± 0.8 , recall = -0.3 ± 0.1 and HDS = -0.5). This contrasted with improvements on every cognitive measure (learning = 1.6 ± 0.3 , $p = 0.0001$, recall = 2.2 ± 0.4 , $p = 0.0001$ and HDS = 1.0 , $p = 0.05$) in those with neither alcohol use nor thrombocytopenia. In adjusted analyses for sociodemographics, adherence and immune measurements, reduced thymus size was associated with a 90% and thrombocytopenia with a 70% increase in the risk of scoring in the demented range after HAART (RR = 1.9, $p < 0.05$ and RR = 1.7, $p = 0.03$) and with low CLVT scores (thymus volume RR = 2.0, $p = 0.04$, chronic alcohol use $p = 0.05$ and thrombocytopenia $p = 0.06$). Thymus volume and platelet counts were negatively affected by alcohol and were predictors of cognitive performance and improvements after HAART. These results could have important clinical and therapeutic implications.

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Keywords

Thymus; CNS; thrombocytopenia; alcohol; HIV/AIDS

Introduction

More than one million Americans are living with HIV/AIDS, and a considerable number of these persons are suffering from alcohol abuse [1–5]. The overlap of HIV infection and alcohol abuse in the same individual is particularly noteworthy because of the deleterious effects of alcohol consumption on immune response and the central nervous system (CNS) [3–16]. Our most recent study indicates that alcohol negatively impacts thymus size and function, resulting in both immune and neurocognitive impairments [16]. Studies have also demonstrated an increased vulnerability to cognitive declines, when platelet drop occurs [17, 18]. Of interest, this is a condition that relates to alcohol abuse, and we have shown associated with HIV disease progression [19–23]. Nonetheless, published data regarding platelets and HIV-associated neurological disorders are limited, and the mechanism mediating this association is uncertain, highlighting the need for further research [17, 18].

The role of platelets has been expanded from hemostasis to inflammation and host defense [24–27]. Platelets not only phagocyte bacteria and virus, such as HIV, they express immune receptors on their membranes and are the source of a multitude of neurotransmitters [26, 27]. Serotonin, platelet activating factor, brain derived neurotrophic factor, thymus and activation-regulated chemokine, monoamine oxidase and arachidonic acid products are only some of the factors stored in platelets that may negatively impact the development of CNS damage during HIV infection [28–32].

In light of these findings, we hypothesize that both alcohol induced-thrombocytopenia and thymus injury are potential mechanisms mediating cognitive impairment in HIV-infected subjects, and that they are all related (see Figure 1).

Methods

Sampling

Participants aged 18–55 years, who had been diagnosed with HIV and had been receiving a new antiretroviral regimen for less than 10 weeks, were eligible to be enrolled in the Miami Alcohol Research Care for HIV (MARCH) study. Patients were excluded if they were non-ambulatory, or if they had other neuropsychiatric or immune conditions (i.e., current or past CNS opportunistic infection, head injury, tumors, major psychiatric disease or other immune or nutritional illness) that might cause cognitive or immune impairments. Briefly, MARCH was a longitudinal observational study to evaluate the impact of alcohol use on health status of HIV-infected individuals receiving highly-active anti-retroviral treatment (HAART). The definition of HAART for analyses herein was guided by published guidelines as follows: (1) Two or more nucleoside reverse transcriptase inhibitors (NRTIs) with at least one PI or one non-nucleoside reverse transcriptase inhibitor (NNRTI); (2) one NRTI with at least one PI and at least one NNRTI; (3) ritonavir and saquinavir in combination with one NRTI and no

NNRTIs and (4) an abacavir-containing regimen of three or more NRTIs in the absence of both PIs and NNRTIs. Participants were recruited from the University of Miami Miller School of Medicine and Jackson Memorial clinics, where these individuals were followed at regular intervals. The HIV cohort was matched by stratifying gender, age and race with an HIV seronegative (as per laboratory testing during the previous 6 months) community control, consisting of friends and relatives of enrolled participants for comparing study outcomes.

Alcohol consumption assessments included widely-used standardized and validated brief screening questionnaires: Physician's guide of the National Institute on Alcohol Abuse and Alcoholism (NIAAA), American Association, CAGE, AUDIT, and ADS [33, 34]. CAGE is an acronym formed from the italicized letters in the questionnaire (cut-annoyed-guilty-eye) and it is a standardized worldwide use instrument to identify problems with alcohol. The World Health Organization's Alcohol Use Disorders Identification Test (AUDIT) has three questions on alcohol consumption, three questions on drinking behaviour and dependence, and four questions on the consequences or problems related to drinking. The ADS (Alcohol Dependence Scale) is a widely used research and clinical tool that provides a quantitative measure of the severity of alcohol dependence [33, 34]. The 25 items cover alcohol withdrawal symptoms, impaired control over drinking, awareness of a compulsion to drink, increased tolerance to alcohol and salience of drink-seeking behaviour.

Based on criteria established by the NIAAA and American Association guidelines, men who reported >14 and women >7 drinks/week were enrolled in Group 1 (hazardous drinkers), while those who reported fewer drinks were included in Group 2 (non-hazardous drinkers) [33, 34]. Those who provided written informed consent and medical release were enrolled. The Institutional Review Board at the University of Miami approved the study. In the present analysis, baseline data were used to examine the longitudinal relation between cognitive performance and alcohol consumption, thrombocytopenia and thymus size.

Main outcome: Cognition

The National Institute of Mental Health and the National Institute of Neurological Diseases and Stroke have published nomenclature and research case definitions to guide the diagnosis of neurological manifestations of HIV-1 infection [35]. However, the extensive cognitive battery requirements and time consumption limit their use in clinical practice [35]. Thus, two cognitive tests were selected among those included in the recommended battery for initial evaluations of cognitive status initiating HAART.

The HIV Dementia Scale (HDS) was selected for two primary reasons: (1) it was originally designed to serve as a valid screening tool to identify HIV dementia and to monitor therapeutic effects on the central nervous system [36, 37]; and (2) it has been shown as superior to other widely used rapid screening tests (Mini-Mental State Examination, Grooved Pegboard) [36–38]. The HDS has a sensitivity of 80%, a specificity of 91% and a positive predictive value of 78% for identifying HIV dementia [36–38]. The HDS is comprised of four tasks that evaluate the domains of memory (recall of four items at 5 minutes), attention (antisaccadic errors), psychomotor speed (timed written alphabet) and construction (cube copy time).

Since a preferential impairment of memory occurs earlier in the disease process and seems to improve with HAART use, we selected the California Verbal Learning Test (CVLT) as one of the primary outcomes. The CVLT is a multidimensional measure of verbal learning and memory, which includes two word lists, each of which contains 16 shopping items [39]. Items are distributed according to a Monday list and a Tuesday distracter list with scores representing the number of objects that an individual can recall. Participants are asked to recall the Monday list spontaneously, following semantic cues and after a 20-minute delay. Finally, participants are given a “yes/no” recognition test in which they are presented with the 16 target words embedded within a list of 28 non-target words. Scores are based on the number of words provided by the participant on the various trials and on the number of times they correctly identify a word to be a member or not of the first list [39].

At each visit, research data were collected using standardized questionnaires and included: socio-demographic information, medical history, and drug and alcohol use habits. HIV positives were questioned regarding the date of HIV diagnosis, mode of infection and AIDS-defining CDC criteria to establish HIV disease status. Medical prescriptions were documented and a standardized antiretroviral adherence questionnaire administered. After visit procedures were completed, a medical chart and pharmacy records were abstracted and patient information was validated.

Laboratory assessments

Blood samples were collected from all participants and processed within 6 hours. Cell blood counts were obtained using the cell-Dyn 4000, a multi-parameter automated hematology analyzer system, recommended for specimens with low or high platelet concentrations. Thrombocytopenia (absolute thrombocytopenia) was defined as platelet counts below $150 \times 10^3 \text{ cells/mm}^3$ [40, 41].

Isolated peripheral blood mononuclear cells were prepared for four-color direct immunofluorescence procedures (Becton Dickinson, San Jose, CA). Flow cytometry quantified the percentage and absolute numbers of T lymphocyte sub populations CD3+/CD4+ and CD3+/CD8. In addition, HIV viral burden was quantified using the Amplicor HIV monitor test (Roche Diagnostic System, Roche Molecular Systems, Pleasanton, CA).

Thymus

The magnetic resonance imaging (MRI) was obtained only in a sub-sample of patients that after the first month were adherent to their prescribed medication. The MRI was performed using a thoracic surface coil and electrocardiographic gating and consisted of the following sequences: (1) Sagittal and coronal pilots; (2) T1-weighted axial, slice thickness 6 mm, interslice gap 1 mm, four averages; and (3) T2-weighted axial fat-suppressed, slice thickness 6 mm, interslice gap 1 mm, four averages. Thymic volume can be estimated from the sum of the areas of manually drawn regions-of-interest from each image on which glandular thymic tissue appears. However, the adult thymus is generally fatty involuted, and calculations of true glandular volume may be inaccurate if fatty elements are not excluded. Therefore, we instead performed a quantitative thymic volume calculation by measuring total mediastinal area, mean mediastinal signal intensity (SI (mediastinum)), fat signal intensity (SI(fat)) and

muscle signal intensity (SI(muscle)) on each T1 weighted slice. We assumed that pure glandular (thymic) tissue would have signal intensity similar to that of muscle, whereas a completely fatty involuted thymus would have a signal intensity of fat. For each slice from the left brachiocephalic vein superiorly to the main pulmonary artery inferiorly, we then calculated thymic area using the following formula:

$$\text{Quantitative thymic area} = \text{total mediastinal area} * \frac{SI(fat) - SI(mediastinum)}{SI(fat) - SI(muscle)}$$

The sum of thymic areas was then multiplied by the slice thickness plus interslice gap (7 mm) to obtain total thymic volume.

Statistical analyses

The data were analysed using SAS version 8 and SPSS version 11 and *p* values <0.05 were considered to be statistically significant. Following descriptive statistical analyses, mean variables were compared using Student's *t* test and one-way analysis of variance (ANOVA) procedures. Correlations among the main variables of interest were examined with Pearson's coefficients.

Univariate analyses were used to calculate odds ratios (OR) and 95% confidence intervals (CI). Binary logistic regression analyses were used to evaluate the effects of alcohol (hazardous vs. non-hazardous, thrombocytopenia (thrombocytopenia vs. normal platelet counts), and thymus (<9 vs. >9), and other potential risk factors on cognition. Although no significant differences were observed between groups for education level or CDC status, both variables were controlled for in the final analyses.

In addition, other potential predictors (i.e., gender, race/ethnicity, CDC status, drug use and body mass index) were selected on the basis of the HIV medical literature and were added to the model. Non-significant variables (*p* = 0.05) were removed, beginning with the least significant variable, until the final full model was achieved.

Results

Study population characteristics

This longitudinal study assessed a multiethnic sample of 165 HIV-infected men and women, including 89 hazardous drinkers and 76 non-hazardous drinkers. The sample reported an average of 22 ± 2.5 drinks/week, along with a well-documented history of continuous alcohol consumption averaging 8 ± 7 drinks per day for more than a month prior to sampling. Length of time for alcohol use ranged from 1–32 years (12 ± 8). Approximately one-third of the participants acknowledged current drug abuse, particularly crack/cocaine and marijuana.

Table I summarizes the distributions of several demographic and clinical characteristics of participants by alcohol groups. No significant differences in any of the sociodemographic variables were evident between hazardous and non-hazardous drinkers. Univariate analyses indicated, however, that at baseline hazardous alcohol users were almost three times more

likely to exhibit thrombocytopenia than the non-hazardous group (OR = 2.58, 95% CI: 0.99 = 7.71, $p = 0.05$).

Platelets and alcohol

Most (80%) of the individuals in the study had normal platelets, hemoglobin, and hematocrit measurements, and no significant differences in mean hemoglobin, hematocrit, or proportion of anemia were observed between patients with thrombocytopenia (13.2 ± 2.0) and those with normal platelet counts (13.0 ± 1.9 g/dl).

As displayed in Table II, thrombocytopenic patients were more likely to be older (OR 11.0, 95% CI: 3–18.9, $p = 0.0001$), hazardous alcohol users (OR = 2.4, 95% CI: 1–7.2, $p = 0.05$) and have non-significantly lower CD4 counts. Additional analyses indicated that thrombocytopenic patients significantly differed in their patterns of alcohol consumption, when compared to patients with normal platelet counts. Subjects with thrombocytopenia consumed alcohol more days per week (4.3 ± 2.1 vs. 3.2 ± 2.6 , $p = 0.045$) and for more years (17 ± 11 vs. 10 ± 9.5 , $p = 0.01$). Those with thrombocytopenia tended to have lower mean CD4 cell counts, compared to those with normal platelet counts (see Table II). Moreover, analyses indicated even lower CD4 cell counts in patients who were heavy drinkers and exhibited thrombocytopenia (210 ± 180 cells/ μ l), compared to those non-drinkers with normal platelets (347 ± 181.5 cells/ μ l). Finally, in accord with our previous report [18–20] no significant changes in platelet counts were observed before and after HAART.

Platelets and thymus

The thymus was recognized in 100% of the patients and volumes were highly variable (0.5 – 27 cc³). However, thymus size could not be explained by any sociodemographic characteristics (i.e., age or gender). As previously reported, thymus size differed between hazardous and non-hazardous alcohol users [15].

Of interest, a significant clinical correlation was observed between platelet counts and thymus size ($r = 0.344$, $p = 0.29$), both at baseline and after antiretroviral treatment ($r = 0.342$, $p = 0.22$). Moreover, a smaller thymus size was characteristic of HIV-infected thrombocytopenic patients (4.5 ± 2.9 vs. 8.3 ± 3.9 , $p = 0.03$). To ascertain the role of platelets in the thymus homeostasis, the trajectories of thymus change, as a result of HAART, were compared between thrombocytopenic and non-thrombocytopenic individuals. The mean thymus volume change demonstrated a significant downward slope in thrombocytopenic patients, which contrasted with the increase in thymus volume in those with normal platelet counts (1.3 ± 0.7 vs. -1.1 ± 0.6 , $p = 0.005$).

Cognitive performance by platelet and thymus status

At baseline, 60% of HIV positives had total scores below 9 of 16 possible points, indicating dementia. No significant correlations were found between the HDS scores and age, education, viral load, CD4 count, or drug use.

Table II shows the cognitive characteristics for small thymus vs. large thymus and hazardous vs. non-hazardous drinkers. The non-hazardous drinkers showed significantly better global (HDS), memory learning, and short-term and long-term recalls. As shown in Table III, total and delayed recognition scores were also significantly worse in HIV-infected subjects with a small thymus.

Analyses indicated that patients with thrombocytopenia had lower mean total HDS scores (8.95 ± 3.2), compared to those with platelets above $150,000 \text{ cells/mm}^3$ (9.87 ± 3.7), although this trend was not significant ($p = 0.09$). Univariate analyses indicated that patients with thrombocytopenia were three times as likely to score below 9, relative to those with platelets $> 150,000$ (OR 2.7, 95% CI: 1.0–7.12, $p = 0.003$). When CVLT scores were analysed, thrombocytopenic patients were four times more likely to recall less than eight words in the Monday list (95% CI: 1.9–7.1, $p = 0.0001$).

HDS and CVLT raw scores in each platelet group were also compared in different thymus subgroups after adjusting for the amount of alcohol consumption as depicted in figure 2. In patients with normal platelets, mean HDS scores were significantly lower in the small thymus subgroup than in the large thymus subgroup (9.1 ± 3.6 vs. 13.5 ± 0.5 , $p = 0.04$). They also recalled fewer words from the Monday list (7.5 ± 3.3 words vs. 10.4 ± 2.8 words, $p = 0.06$) and after long delays (8.0 ± 3.2 words vs. 10.9 ± 2.4 words, $p = 0.07$). In the thrombocytopenia group, patients with a small thymus had the lowest HDS scores (7.3 ± 4 words vs. 12.4 ± 2.5 words). Patients with dual comorbidity (small thymus and thrombocytopenia) were five times less likely to memorize more than eight of 16 words in the Monday list (95% CI: 1.2–16.0, $p = 0.02$).

Longitudinal analyses

When a longitudinal analysis was performed on the five recall measures, the summary learning ($F = 2.8$, $p < 0.06$), the short delayed free recall ($F = 3.1$, $p < 0.07$), and the long delayed free recall measures ($F = 4.0$, $p < 0.065$) tended to differ by platelet status. When persistent thrombocytopenia was correlated with the CVLT total learning and HDS scores, the correlations were as follows: $r = -0.21$, $p = 0.03$ for total CVLT; $r = -0.31$, $p = -0.002$ for long delayed recognition; and $r = 0.27$, $p = 0.069$ for HDS scores at the final visit.

To examine the possible effects of thrombocytopenia and alcohol use on HAART response, paired sample *t* tests were used to examine changes over time. After HAART, patients with normal platelet counts significantly improved on both short-term (1.6 ± 0.3 vs. -0.2 ± 0.8 words, $p = 0.0001$) and long delayed free recall (2.2 ± 0.4 vs. -0.3 ± 0.1 , $p = 0.0001$), compared to those with thrombocytopenia who demonstrated a decline in platelets. Total HDS scores improved one point in those with normal platelets; however, a half-point decline was observed in thrombocytopenic patients.

Regression analyses

In the final analyses, age, CDC stage of HIV disease, alcohol consumption, education, and thymus size were included as dichotomous variables in the final regression model, with HDS as the dependent variable. As illustrated in Table IV, hazardous alcohol consumption (RR = 1.2, 95% CI: 1.0–8, $p = 0.05$), small thymus (RR = 1.9, 95% CI: 1.3–4.0, $p = 0.01$), and

thrombocytopenia at the 6 month visit ($RR = 1.7$, $p = 0.04$) were the only variables associated with HDS after adjusting for potential confounders. To delineate the temporal relationship between thrombocytopenia and cognitive status, a variable was created according to the trajectory of platelet change (platelets stayed persistently low, platelets declined at the 6 month visit, or platelets improved from baseline to the 6-month visit) and compared with those with persistently normal platelet counts. Of interest, if the model included persistent thrombocytopenia the risk of scoring in the dementia range was even higher ($RR = 2.5$, $p = 0.03$) and was not significant in those in whom platelet counts normalized ($RR = 1.2$, $p = 0.08$).

Of persistent thrombocytopenia ($RR = 2.1$, $CI: 0.97-48$, $p = 0.05$), more than 20 years drinking ($RR = 1.9$, $CI: 0.95-49$, $p = 0.05$), and thymus size ($RR = 1.96$, $CI: 2.0-9$, $p = 0.04$) were all significant predictors of low total scores on the CVLT after 6 months of HAART.

Discussion

For the first time, our data in people living with HIV indicate that platelets and thymus are related and closely interact with the CNS, which until recently was unimaginable. Moreover, despite HAART, patients with thrombocytopenia and particularly those with dual comorbidity had a progressive neurocognitive decline. Findings also signify that apart from HIV, alcohol abuse is probably one of the leading causes of thrombocytopenia and thymus damage, and perhaps a mechanism by which alcohol affects the CNS.

While it may be suggested that this is a result of a clustered environment that includes thrombocytopenia, alcohol consumption, and cognitive dysfunction our findings imply more than that. The magnitude of the association between thrombocytopenia and cognitive dysfunction, its independence from traditional risk factors (i.e., CD4, viral load or sociodemographics), and evidence of temporal relationships suggest that these findings are less likely to be due to chance. These results are consistent with previous studies demonstrating a close relationship between platelet counts and the development of cognitive impairment in HIV-infected subjects [16–18]. Further strengthening our findings was the observation that normalization of platelet counts reduced the risks of cognitive abnormalities, while having persistent thrombocytopenia blunted HAART-related immune and cognitive improvements.

Furthermore, a number of possible biological mechanisms exist by which patients with thrombocytopenia may be at increased risk for developing cognitive impairments. For example, our findings indicated that thymus atrophy is more evident in thrombocytopenic patients and that its deleterious effect on the thymus impacts learning and memory. Notably, platelets store a multifunctional protein, known as platelet-derived growth factor (PDGF), which exerts particularly important actions in the CNS. Jenkinson and colleagues defined a population of non-epithelial PDGF α -positive mesenchymal cells in the thymus and showed that these stromal cells regulate normal thymus growth [42]. Thus, under thrombocytopenia conditions, one can expect abnormal PDGF levels causing the thymus to be hypoplastic. Of interest, our data demonstrated that a hypoplastic thymus is associated with cognitive alterations. Even though association does not imply causation, a causal relationship has been

established in animal models demonstrating that thymectomy results in structural and functional alterations in the brain that are reversible if the thymus is re-implanted [13, 43–47]. Similarly in our study after HAART, increases in thymus volume parallel improvements in cognitive performance. On the other hand, persistent thrombocytopenia was accompanied by a simultaneous decline in thymus volume and cognitive performance. These findings necessitate additional studies to corroborate that the thymus alteration may be a mechanism underlying the association between thrombocytopenia and cognitive impairment.

Another potential pathway mediating our findings is interleukin-6 (IL-6), which is elevated in alcohol users, and we have shown it is up-regulated in HIV infected individuals with thrombocytopenia [19, 48]. Unfortunately, in people living with HIV, the effect of IL-6 in platelet repopulation may result in three deleterious effects. First, it may result in CCR4 inducement, which could enhance HIV viral entrance and replication [19–20]. Second, it could decrease levels of thymopoiesis, resulting in thymus atrophy [49]. Finally, it could lead to progressive neurodegenerative disease and alteration of the NMDA receptors, which play a central role in synaptic transmission and calcium influx [50, 52].

The observed relationship among platelets, cognition, and alcohol can also be explained through the role of serotonin (5-HT) in cognition [53, 54]. The synthesis of serotonin is influenced by tryptophan, which is stored in platelets and is decreased in alcoholics [54]. The concomitant presence of thrombocytopenia and alcohol could compromise the supply of tryptophan and reduce cognitive function.

Nevertheless, our results have three limitations: a limited cognitive battery, participants were restricted to those followed at University of Miami/Jackson Memorial facilities, and we cannot establish causality. Nonetheless, these results may have important research, clinical, and therapeutic implications. They improve our understanding of the neuro-immune systems. By identifying those HIV-infected individuals at risk for cognitive declines, such as those with thrombocytopenia, a smaller thymus, and alcohol abuse, the study may permit early clinical follow-ups and interventions. Findings may also guide the development of future neuroprotective therapies.

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References

1. McQuillan, G.; Kruszon-Moran, D. HIV infection in the United States household population aged 18–49 years: Results from 1999–2006. Available from: www.cdc.gov/nchs/data/databriefs/db04.pdf
2. Bean P. HIV and alcohol use: Consequences of comorbidity. *Amer Clin Lab*. 2001; 20:13–16.
3. Isaki L, Kresina TF. Directions for biomedical research in alcohol and HIV: Where are we now and where can we go? *AIDS Res & Hum Retrov*. 2000; 16:1197–1207.
4. Kendall JB. Expanding research on the role of alcohol consumption and related risks in the prevention and treatment of HIV/AIDS. *Subst Use Misuse*. 2006; 41:1465–1507. [PubMed: 17002990]
5. Míguez MJ, Shor-Posner G, Morales G, Rodriguez A, Burbano X. HIV treatment in drug abusers: Impact of alcohol use. *Addict Biol*. 2003; 8:33–37. [PubMed: 12745413]

6. Chander G, Josephs J, Fleishman JA, Korthuis PT, Gaist P, Hellinger J, Gebo K. HIV Research Network. Alcohol use among HIV-infected persons in care: Results of a multi-site survey. *HIV Med.* 2008; 9:196–202. [PubMed: 18366443]
7. Altura BM, Altura BT. Association of alcohol in brain injury, headaches, and stroke with brain-tissue and serum levels of ionized magnesium: A review of recent findings and mechanisms of action. *Alcohol.* 1999; 19:119–130. [PubMed: 10548155]
8. Meadows GG, Wallendal M, Kosugi A, Wunderlich J, Singer DS. Ethanol induces marked changes in lymphocyte populations and natural killer cell activity in mice. *Alcohol Clin Exp Res.* 1992; 16:474–479. [PubMed: 1626648]
9. Saad AJ, Jerrells TR. Flow cytometric and immunohisto-chemical evaluation of ethanol-induced changes in splenic and thymic lymphoid cell populations. *Alcohol Clin Exp Res.* 1991; 15:796–803. [PubMed: 1755511]
10. Watzl B, Lopez M, Shahbazian M, Chen G, Colombo LL, Huang D, Way D, Watson RR. Diet and ethanol modulate immune responses in young C57BL/6 mice. *Alcohol Clin Exp Res.* 1993; 17:623–630. [PubMed: 8333593]
11. Lopez MC, Watzl B, Colombo LL, Watson RR. Alterations in mouse Peyer's patch lymphocyte phenotype after ethanol consumption. *Alcohol.* 1997; 14:107–110. [PubMed: 9085709]
12. Bagasra O, Howedy A, Dorio R, Kajdacsy-Balla A. Functional analysis of T-cell subsets in chronic experimental alcoholism. *Immunol.* 1987; 61:63–69.
13. Szabo G. Consequences of alcohol consumption on host defense. *Alcohol Alcohol.* 1999; 34:830–841. [PubMed: 10659718]
14. Míguez MJ, Shor-Posner G, Morales G, Rodriguez A, Burbano X. HIV treatment in drug abusers: Impact of alcohol use. *Addict Biol.* 2003; 8:33–37. [PubMed: 12745413]
15. Miguez-Burbano MJ, Lewis JE, Moreno J, Fishman J. cognitive performance and the thymus among HIV infected subjects receiving HAART. *Journal of Biologics: Targets and Therapy.* 2008; 2:321–327. [PubMed: 19707364]
16. Wachtman LM, Skolasky RL, Tarwater PM, Esposito D, Schifitto G, Marder K, McDermott MP, Cohen BA, Nath A, Sacktor N, et al. Platelet decline: An avenue for investigation into the pathogenesis of Human Immunodeficiency Virus associated dementia. *Arch Neurol.* 2007; 64:1264–1272. [PubMed: 17846264]
17. Wachtman LM, Tarwater PM, Queen SE, Adams RJ, Mankowski JL. Platelet decline: An early predictive hemato-logic marker of simian immunodeficiency virus central nervous system disease. *J Neurovirol.* 2006; 12:25–33. [PubMed: 16595371]
18. Burbano X, Míguez MJ, Lecusay R, Rodriguez A, Ruiz P, Morales G, Castillo G, Baum M, Shor-Posner G. Thrombocytopenia in HIV-infected drug users in the HAART era. *Platelets.* 2001; 12:456–461. [PubMed: 11798394]
19. Míguez MJ, Rodríguez A, Hadrigan S, Asthana D, Burbano X, Fletcher MA. Interleukin-6 and platelet protagonists in T lymphocyte and virological response. *Platelets.* 2005; 16:281–286. [PubMed: 16011978]
20. Miguez-Burbano MJ, Jackson Jr J, Hadrigan S. Thrombocytopenia in HIV disease: Clinical relevance, physiopathology and management. *Curr Med Chem Cardiovasc Hematol Agents.* 2005; 10:365–376. [PubMed: 16250867]
21. Latvala J, Parkkila S, Niemelä O. Excess alcohol consumption is common in patients with cytopenia: Studies in blood and bone marrow cells. *Alcohol Clin Exp Res.* 2004; 28:619–624. [PubMed: 15100613]
22. Rauchenzauner M, Kountchev J, Ulmer H, Pechlaner Ch, Wiedermann ChJ, Bellmann R, Joannidis M. Disturbances of electrolytes and blood chemistry in acute alcohol intoxication. *Wien Klin Wochenschr.* 2005; 2(117):83–91. [PubMed: 15773422]
23. Von Hundelshausen P, Weber Ch. Platelets as immune cells bridging inflammation and cardiovascular disease. *Circ Res.* 2007; 100:27–40. [PubMed: 17204662]
24. Gear AR, Camerini D. Platelet chemokines and chemokine receptors: Linking hemostasis, inflammation, and host defense. *Microcirculation.* 2003; 10:335–350. [PubMed: 12851650]

25. Youssefian T, Drouin A, Masse JM, Guichard J, Cramer EM. Host defense role of platelets: Engulfment of HIV and Staphylococcus aureus occurs in a specific subcellular compartment and is enhanced by platelet activation. *Blood*. 2002; 99:4021–4029. [PubMed: 12010803]
26. Zucker-Franklin D, Seremetis S, Zheng ZY. Internalization of human immunodeficiency virus type I and other retroviruses by megakaryocytes and platelets. *Blood*. 1990; 75:1920–1923. [PubMed: 2337668]
27. Youssefian T, Drouin A, Mass JM, Guichard J, Cramer EM. Host defense role of platelets: Engulfment of HIV and Staphylococcus aureus occurs in a specific subcellular compartment and is enhanced by platelet activation. *Blood*. 2002; 99:4021–4029. [PubMed: 12010803]
28. Asojo OA, Oluwatoyin A, Boulègue C, Hoover DM, Wuyuan L, Jacek L. Structures of thymus and activation-regulated chemokine (TARC). *Acta Crystallogr D Biol Crystallogr*. 2003; 59:1165–1173. [PubMed: 12832759]
29. Perry SW, Hamilton JA, Tjoelker LW, Dbaibo G, Dzenko KA, Epstein LG, Hannun Y, Whittaker JS, Dewhurst S, Gelbard HA. Platelet-activating factor receptor activation. An initiator step in HIV-1 neuropathogenesis. *J Biol Chem*. 1998; 273:17660–17664. [PubMed: 9651362]
30. Abi-Younes S, Si-Tahar M, Luster AD. The CC chemokines MDC and TARC induce platelet activation via CCR4. *Thromb Res*. 2001; 101:279–289. [PubMed: 11248289]
31. Bachis A, Major EO, Mocchetti I. Brain-derived neurotrophic factor inhibits Human Immunodeficiency Virus-1/gp120-mediated cerebellar granule cell death by preventing gp120 internalization. *J Neurosci*. 2003; 23:5715–5722. [PubMed: 12843275]
32. Gurgis, G. Platelets in Neuropsychiatric disorders.. In: Michelson, AD., editor. *Platelets*. Vol. 43. Academic Press; Boston: 2006. p. 790-806.
33. National Institute on Alcohol Abuse and Alcoholism. *The Physicians' Guide to Helping Patients with Alcohol Problems*. Government Printing Office; Washington, DC: 1995. p. 3769
34. Carrington RM, Fiellin DA, O'Connor P. Hazardous and harmful alcohol consumption in primary care. *Arch Intern Med*. 1999; 159:1681–1689. [PubMed: 10448769]
35. Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M, Gisslen M, Grant I, Heaton RK, Joseph J. Updated research nosology for HIV-associated neurocognitive disorders. *Neurology*. 2007; 69:1789–1799. [PubMed: 17914061]
36. Power C, Selnes OA, Grim JA, McArthur JC. The HIV Dementia Scale: A rapid screening test. *J AIDS*. 1995; 8:273–278.
37. Dougherty RH, Skolasky RL, McArthur JC. Progression of HIV-associated dementia treated with HAART. *AIDS Read*. 2002; 12:69–74. [PubMed: 11905143]
38. Smith CA, van Gorp WG, Ryan ER, Ferrando SJ, Rabkin J. Screening subtle HIV-related cognitive dysfunction: The clinical utility of the HIV Dementia Scale. *J Acquir Immune Defic Syndr*. 2003; 33:116–118. [PubMed: 12792365]
39. Delis, DC.; Kramer, JH.; Kaplan, E.; Ober, BA. *California verbal learning test*. The Psychological Corporation; New York: 1987.
40. Medical Dictionary. Thrombocytopenia. Available from: URL:<http://medical-dictionary.thefreedictionary.com/thrombocytopenia>
41. Peltz S. Severe thrombocytopenia secondary to alcohol use. *Postgrad Med*. 1991; 89:75–76. [PubMed: 2020651]
42. Jenkinson WE, Rossi SW, Parnell SM, Jenkinson EJ, Anderson G. PDGFR-expressing mesenchyme regulates thymus growth and the availability of intrathymic niches. *Blood*. 2007; 109:954–960. [PubMed: 17008543]
43. Torre D, Pugliese A. Platelets and HIV-1 infection: Old and new aspects. *Curr HIV Res*. 2008; 9:411–418. [PubMed: 18855651]
44. Nishiyama N. Thymectomy-induced deterioration of learning and memory. *Cell Mol Biol*. 2001; 47:161–165. [PubMed: 11292251]
45. Gaufo GO, Diamond MC. Thymus graft reverses morphological deficits in dorsolateral frontal cortex of congenitally athymic nude mice. *Brain Res*. 1997; 756:191–199. [PubMed: 9187332]
46. Saito H, Nishiyama N, Zhang Y, Abe Y. Learning disorders in thymectomized mice: A new screening model for cognitive enhancer. *Behav Brain Res*. 1997; 83:63–69. [PubMed: 9062662]

47. Zhang Y, Saito H, Nishiyama N. Thymectomy-induced deterioration of learning and memory in mice. *Brain Res.* 1994; 658:127–134. [PubMed: 7834333]
48. Jerrard-Dunne P, Sitzler M, Risley P, Steckel DA, Buehler A, von Kegler S, Markus HS. Interleukin-6 promoter polymorphism modulates the effects of heavy alcohol consumption on early carotid artery atherosclerosis: The Carotid Atherosclerosis Progression Study (CAPS). *Stroke.* 2003; 34:402–407. [PubMed: 12574550]
49. Gregory D, Sempowski GD, Hale LP, Sundry JS, Massey JM, Koup RA, Douek DC, Patel DD, Haynes BF, Haynes BF. Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy. *J Immunol.* 2000; 164:2180–2187. [PubMed: 10657672]
50. Nath A, Conant K, Chen P, Scott C, Major EO. Transient exposure to HIV-1 Tat protein results in cytokine production in macrophages and astrocytes. A hit and run phenomenon. *J Biol Chem.* 1999; 274:17098–17102. [PubMed: 10358063]
51. Merrill JE, Chen IS. HIV-1, macrophages, glial cells, and cytokines in AIDS nervous system disease. *FASEB J.* 1991; 5:2391–2397. [PubMed: 2065887]
52. Qiu Z, Sweeney DD, Netzeband JG, Gruol DL. Chronic interleukin-6 alters NMDA receptor-mediated membrane responses and enhances neurotoxicity in developing CNS neurons. *J Neurosci.* 1998; 18:10445–10456. [PubMed: 9852582]
53. Blakely RD, Berson HE, Freneau RT, Caron MG, Peek MM, Prince HK, Bradley CC. Cloning and expression of a functional serotonin transporter from rat brain. *Nature.* 1991; 354:66–70. [PubMed: 1944572]
54. Abdulla A, Badawy B. Tryptophan metabolism in alcoholism. *Nutr Res Rev.* 2002; 15:123–152. [PubMed: 19087401]

Figure 1. Proposed Model. In HIV infected individuals alcohol a wide spectrum of damage over the platelets and the thymus (indicated by red arrows). Then, thrombocytopenia and thymus injury, alone or in concert, induced CNS damage manifested in cognitive impairments (illustrated with purple and orange arrows).

Figure 2.
Cognitive improvement with HAART by platelets and thymus status.

Table I

Socio-demographic information of HIV infected hazardous and non-hazardous users at baseline.

Variables	HIV infected hazardous drinkers <i>N</i> = 89	HIV infected non-hazardous drinkers <i>N</i> = 75
Age in years	40 ± 7.2	41 ± 6.7
Gender		
• Male	62%	70%
• Female	38%	30%
Race/Ethnicity		
• African American	64%	52%
• Hispanic	29%	34%
• Caucasian	7%	12%
• Others	0%	2%
Annual income		
• <\$10,000	89%	88%
• \$11,000–\$20,000	9%	10%
• >\$20,000	2%	2%
Level of education		
• <11 years of education	53%	56%
• >12 years of education	47%	44%
Marital/partner status		
• Single, separated or widowed	89%	84%
• Married or with a partner	11%	16%
Number of years diagnosed with HIV	8.7 ± 5.3	9.7 ± 6.5
CD4+ lymphocyte percentages	14.9 ± 9.6	13.1 ± 8.1
Body Mass Index kg/m ²	26.7 ± 6.7	27 ± 5.6
Thrombocytopenia	17%	8%

Values are means ± SD or percentages.

No significant differences in sociodemographic characteristics were found between groups.

Table II

Socio-demographic and clinical characteristics of HIV infected by platelet counts.

Variables	Thrombocytopenia <150,000 cells/mm ³	Normal platelets
Gender		
• Male	61%	69%
• Female	39%	31%
Age groups		
• <49 years old	94%	64%
• >50 years	6%	36%
Alcohol groups		
• Hazardous alcohol	74%	56%
• Non-hazardous	26%	44%
• >20 years drinking	41%	14%
CD4 by groups		
• <200 CD4 cells/μl	48%	39%
• >200 CD4 cells/μl	52%	61%
Anemia	19%	13%
Hematocrit (%)	33.4 ± 8.7	34.2 ± 6.5

Values are percentages.

Table III

Cognitive baseline characteristics.

Characteristics	Non-hazardous drinkers	Hazardous drinkers	<i>p value</i>	Small thymus	Normal thymus	<i>p value</i>
HIV dementia scores (HDS)	10.4 ± 4	8.4 ± 4	0.05	7.0 ± 3	12 ± 2	0.005
CVLT total scores	11.3 ± 3	9.6 ± 3	0.05	7.5 ± 3	10.4 ± 3	0.06
CVLT long delay recall	10 ± 4	13 ± 3	0.03	8 ± 3	11 2	0.07

Data are mean (SD) or numbers (%), unless otherwise indicated.

HDS = HIV Dementia Scale; CVLT = California Verbal Learning Test.

Table IV

Multivariate analyses for global and verbal learning assessment.

Model	Multivariater	95% CI	<i>p value</i>
HIV Dementia Scale			
Hazardous alcohol use	1.2	1.0–8.0	0.05
Small thymus	1.9	1.3–4.0	0.01
Persistent TCP	1.7	1.0–3.5	0.04
California Verbal Learning Test			
Persistent TCP	2.1	1.0–48	0.05
>20 years drinking	1.9	1.0–49	0.05
Small thymus	1.96	2.0–9.0	0.04

Participants with platelets counts <150,000 cells/mm³ both at baseline and at the 6 month visits was defined as persistent TCP.

Thymus volumes <9cc³ (mean of the study population) were defined as small.