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Neurovirological correlation with HIV-associated neurocognitive disorders and encephalitis in a HAART-era cohort

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Abstract

Objective—Replicating HIV-1 in the brain is present in HIV encephalitis (HIVE) and microglial nodule encephalitis (MGNE) and is putatively linked with HIV-associated neurocognitive disorders (HAND). A clinico-neurovirological correlation was conducted to elucidate the relationship between brain viral load and clinical phenotype.

Subjects and assays—HIV gag/pol RNA and DNA copies were quantified with RT-PCR or PCR in 148 HAART-era brain specimens. Comparison to HAND, HIVE and MGNE and correlation with neuropsychological (NP) test scores were done using one-way ANOVA with Tukey-Kramer and Spearman's tests respectively.

Results—Brain HIV RNA was higher in subjects with HAND plus HIVE vs without HAND (delta = 2.48 $\log_{10}$ units, n = 27 vs 36, $p < 0.001$). In HAND without HIVE or MGNE, brain HIV RNA was not significantly different vs without HAND ($p = 0.314$). Worse NP scores correlated significantly with higher HIV RNA and interferon responses in brain specimens ($p<0.001$), but not with HIV RNA levels in premortem blood plasma (n = 114) or cerebrospinal fluid (n = 104). In

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Conflict of interest

For the remaining authors no potential conflicts were declared.

subjects with MGNE, brain HIV RNA was slightly higher versus without MGNE ($p<0.01$), and much lower versus with HIVE ($p<0.001$).

Conclusion—Brain HIV RNA and to a lesser extent HIV DNA are correlated with worse NP performance in the 6 months before death. Linkage occurs primarily in patients with HIVE and MGNE; while on HAART these patients could obtain added NP improvement by further reducing brain HIV. Patients not in those groups are less certain to obtain added NP benefit.

Keywords

Dementia; encephalitis; HIVE; neurocognitive disorders; HAND; Interferon

Introduction

HIV-associated neurocognitive disorders (HAND) in the era of highly active antiretroviral therapy (HAART) occur in an estimated 50% of unselected cohorts (1,2). One concept regarding the pathophysiology of HAND posits that replication of HIV-1 in mononuclear phagocytes of the central nervous system (CNS) is the critical driving force. Neuropathologically that concept was suggested largely using studies conducted prior to the HAART era in subjects with HIV encephalitis (HIVE), and before the development of the most recently applied nosological schema used to diagnose HAND (3). It is well-documented that HIVE is associated with high rates of replicating HIV-1 in the brain (4,5), which suggests that the pathophysiology of HAND might be driven substantially by a high rate of virus replication in the CNS. However, it still remains unclear whether or not mildly increased virus replication can influence HAND, or whether a “no effect” concentration of HIV in the brain can be achieved using HAART. The association of brain viral replication with cognitive impairment has not been broadly documented in humans, in part due to limited brain specimen resources.

Mechanistic scenarios for HAND that have been suggested emphasize the production of neurotoxic HIV-1 proteins in infected macrophages, microglial cells and astrocytes which leads to the production of inflammatory mediators in many types of brain cells (6,7). In turn, virally driven inflammatory cascades in the CNS are believed to produce lasting neurodegenerative-type changes in “bystander” neurons, and dysregulation of glial cells or neurovascular elements which then lead to the neurocognitive dysfunction observed in HAND (8). Evidence obtained prior to the era of HAART suggested that the intensity of the inflammatory reaction in the brain based on cell staining was more strongly correlated with HAND than the load of HIV-1 in the brain (9).

Findings that contradict the prevailing concepts established before HAART have been noted, including decedents with neuropsychological (NP) impairment but not HIVE, and vice versa (10–12). A survey of brain specimens from the National NeuroAIDS Tissue Consortium (NNTC) suggested that the divergence between HAND and the neuropathological diagnosis of HIVE has increased in the HAART era to the point that most patients with HAND do not have HIV-related brain pathologies (13). Mainstream thinking regarding the pathophysiology of HAND has not successfully incorporated the conflicting data concerning the neurovirological correlation (14). It is important to clarify the relationship between CNS virus replication and HAND in order to justify the rationale to further reduce or eradicate virus replication in the CNS compartment. To that end a comparison between brain HIV-1 load and HAND was conducted using clinically-and neuropathologically-characterized subjects.

Methods

Subjects

HAART-era subjects with HIV/AIDS who underwent autopsy and neuropathological evaluation were examined. Decedents were chosen from the NNTC, which is a multisite consortium of clinical sites that has acquired clinically-characterized tissue specimens (15). Two cohorts were assembled (Table 1). One was focused on subjects with a neuropsychological (NP) diagnosis that was matched to a neuropathological diagnosis (n = 148). A second larger cohort included all of the available neuropathological information regardless of whether matching NP data was available (n = 195). Inclusion in the NP focused cohort was based upon the following criteria: 1) HIV-associated neurocognitive disorder (HAND) that was designated HIV-associated dementia (HAD), or 2) HAND of any severity that was associated with a neuropathological diagnosis of HIV encephalitis (HIVE) or microglial nodule encephalitis (MGNE), or 3) normal NP test performance and no HAND. All qualified subjects were assigned an NP diagnosis. Four subjects who did not have HAND initially had HIVE at autopsy; those four subjects were excluded from group assignment but were not excluded from the correlation analyses. In the second cohort inclusion was based on the following criteria: 1) neuropathologically diagnosed HIVE, with or without available NP testing, and with or without a diagnosis of HAND (n = 38), or 2) MGNE with or without available NP testing, and with or without HAND (n = 13), or 3) other neuropathological diagnoses present in the subjects who already were selected for the NP-based cohort (see Table 1). There were no exclusion criteria.

Neuropsychological testing

Subjects in the NP-focused cohort underwent testing with the NNTC neurocognitive test panel (15,16). Testing was given at six month intervals. Evaluations were scored according to age-, gender-, ethnicity-, and education-adjusted norms and a composite normalized T score for NP performance was assigned to patients who completed at least 10 out of 14 tests. Subjects who completed 9 or fewer tests were given a nosological diagnosis and domain T scores as appropriate but were not assigned a composite NP T score. A diagnosis of HAND was assigned guided by American Academy of Neurology criteria (17) as modified by the Frascati Criteria (3).

Neuropathology

Neuropathological diagnoses were rendered by NNTC site neuropathologists. Criteria used for the nosological diagnosis of HIVE were according to Budka et al. (18). The diagnosis of MGNE was made when the case material contained microglial nodule encephalitis as the predominant finding, with the absence of multinucleated cells and other morphological criteria associated with HIVE.

Brain dissection and extraction of RNA and DNA

Dorsolateral prefrontal cortex (DLPFC) was dissected from Brodmann areas 9 or 10. The Qiagen RNeasy Lipid Tissue Mini Kit (Cat. No. 74804, Valencia, CA, USA) was used to prepare RNA. Briefly, about 100 mg of brain tissue was dissected on dry ice and homogenized in a mini-bead beater. After extraction with chloroform the RNA was centrifuged in RNeasy mini spin columns, washed and eluted. HIV cDNA was prepared using Bio-Rad iScript cDNA Synthesis Kit (Cat. No. 170-8891, Hercules, CA, USA). One μg of brain RNA, 4 μl of 5x iScript reaction mix, 1 μl of iScript reverse transcriptase and 1 μl of 20 μmol/L HIV anti-sense primer 84R was adjusted to 20 μl with nuclease-free water. After incubation DNA was extracted (Stratagene, Cat. #300600, Agilent Technologies, Inc., Santa Clara, CA). Total DNA was extracted from brain tissue in the same way.

Assay of HIV-1 RNA, DNA and inflammatory mRNAs

Brain HIV RNA and DNA were quantified using cDNA and total genomic DNA using HIV gag/pol primer and probe sequences from Palmer et al. (19) by PCR. The reaction contained 4 μl of cDNA or 1 μg of total DNA, 12.5 μl of Sigma JumpStart Taq ReadyMix (Cat. No. D7440, Sigma, St. Louis, MO, USA), 3.5 μl of 25 mmol/L MgCl2, 0.8 μl of 10 μmol/L HIV primer mix and 0.5 μl of 10 μmol/L HIV probe adjusted to 25 μl. Conditions were 2 min. at 95°C, 40 cycles of 15 sec. at 95°C and 60 sec. at 60°C. Real time PCR was run using an Eppendorf RealPlex (Hamburg, Germany). Copies per μg of total RNA were calculated with a standard curve using a previously quantified HIV-positive RNA primary standard (20). The same procedure was used for HIV DNA concentration. The mRNAs for myxovirus resistance 1 (*MX1*), ISG15 ubiquitin-like modifier (*ISG15*), interferon regulatory factor 1 (*IFR1*) and type 2 dopamine receptor long isoform (*DRD2L*) were quantified in cDNA made from mRNA extracts using commercial reagents for RT-PCR. 1μl of 20x *DRD2L* primers and probe mix (Cat. Hs01024460_m1, Applied Biosystems, Foster City, CA, USA) was combined with 1μl of cDNA, 10 μl of 2x JumpStart Taq ReadyMix, 2.5μl of 25 mmol/l $MgCl_2$ adjusted to 20 μl with water. *GAPDH* mRNA was used as the normalizing transcript in reactions analogous to the above using 1μl of 10 μmol/L *GAPDH* primer mix and 0.5μl of 10 μmol/L *GAPDH* probe. For *IRF1* mRNA, *IRF1* mix (Hs00971959_m1), *GAPDH* mix (Hs99999905_m1) and TaqMan Universal PCR Master Mix (Part No. 4304437) were used (Applied Biosystems, Foster City, CA, USA) with conditions as above. For *MX1* mRNA, MX1 mix (Hs00182073_m1) was used. For *ISG15* mRNA, ISG15 mix (Hs00192713_m1) was used. Real time PCR was run in duplicate and relative expression was calculated using the $\Delta\Delta C_t$ method.

Demographic, clinical and pathological data

Demographic and medical data were obtained from the NNTC data archive (15) as listed in Table 1. The concentration of HIV gag/pol RNA in blood plasma and cerebrospinal fluid (CSF) was quantified by NNTC sites using the Roche Amplicor HIV-1 Monitor test v1.1 through v1.5 (Basel, Switzerland). With few exceptions the blood and CSF samples were obtained on the day that NP testing was done. Lifetime histories of substance abuse and dependence and of major depression were obtained using the Psychiatric Research Interview for Substance and Mental Disorders (PRISM) or the Composite International Diagnostic Interview (CIDI) (21). HAART status was defined as being active if the subject was given at least 2 nucleoside/nucleotide reverse transcriptase inhibitors (NRTI's) or 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) and 1 protease inhibitor (PI) within one year of death.

Statistics

HIV RNA and DNA levels were logarithm transformed using ($\log_{10} x + 200$) where x is copies of HIV RNA per gram, and 200 represents the observed threshold of HIV RNA detection of the assay. Effects between groups were evaluated using one-way analysis of variance with Tukey-Kramer tests. The normalized composite impairment T scores and seven normalized component domain T scores were correlated with brain HIV RNA and DNA using Spearman's test. The false discovery rate due to multiple comparisons for seven domain T scores was controlled by the method of Benjamini and Hochberg (22). Correlations pertaining to plasma, cerebrospinal fluid (CSF), and inflammatory markers were done using Spearman's test. Fisher r-to-Z transformations were done to determine whether a correlation coefficient from one group was significantly different from another. The significance threshold was $p < 0.05$.

Results

Brain HIV versus the nosological diagnosis of HAND

Brain HIV RNA concentration between four neuropsychologically and neuropathologically classified groups was significantly different ($p < 0.001$) (Figure 1A). The subjects with HAND plus HIVE had substantially higher brain HIV RNA relative to the subjects without HAND (delta = 2.48 $\log_{10}$ units, n = 36 versus 27, $p < 0.001$). Subjects with HAND and MGNE also had a slightly higher brain HIV RNA than those without HAND that was not significant (delta = 0.92 $\log_{10}$ units, n = 36 versus 12, $p = 0.123$). Subjects with HAND but without HIVE or MGNE had no substantial difference in their brain HIV RNA relative the subjects without HAND (delta = 0.385 $\log_{10}$ units, n = 36 versus 69, $p = 0.314$). Results using brain HIV DNA concentration in these comparisons mirrored the HIV RNA results but the group differences were less pronounced (Figs. 1A versus 1B).

Brain HIV versus neuropsychological test scores

To determine if the severity of CNS impairment was related to brain HIV RNA, a correlation analysis was performed using the composite normalized NP T score. A higher level of brain HIV RNA was significantly correlated with having a worse composite NP T-score (rho = −0.290, $p = 0.001$) (Table 2). Correlation coefficients for all of the NP T scores were closer to unity when the subjects with HIVE were considered separately relative to those without HIVE. Fisher r-to-Z transformations showed that the differences with HIVE versus without it were statistically marginal when corrected for multiple comparisons, with the sharpest contrast occurring in the abstract executive ($p = 0.045$) and attention working memory ($p = 0.023$) testing domains.

Brain HIV DNA was less strongly correlated with neurocognitive test scores (see Table S1 in Supplemental Digital Content). One out of seven NP testing domains (learning) was correlated significantly. All of the correlation coefficients were closer to unity in patients with HIVE versus without it and the r-to-Z transformations were statistically significant in several test domains after correction for multiple comparisons, with p values ranging from 0.001 to 0.030.

Brain HIV versus neuropathological diagnosis

The neuropathologically-focused cohort contained 65 cases of HIVE, 25 cases of MGNE, and 105 cases with neither diagnosis (Fig. 2A). There were highly significant differences in the levels of brain HIV RNA between the groups ($p < 0.001$). Brain HIV RNA was substantially higher with HIVE versus without it (delta = 2.85 $\log_{10}$ units, n = 65 versus 105, $p < 0.001$). Brain HIV RNA also was higher in the subjects with MGNE versus no MGNE (delta = 1.10 $\log_{10}$ units, n = 26 versus 105, $p < 0.023$). The HIVE and MGNE groups were significantly different from each other ($p < 0.001$). Less pronounced differences were observed in these groups for brain HIV DNA (Figs. 2A versus 2B).

Brain HIV versus HIV in blood plasma, HIV in cerebrospinal fluid (CSF) and plasma CD4+ lymphocytes

Plasma HIV RNA, obtained close to the time of NP testing, was correlated weakly with NP T scores with marginal statistical significance (rho = −0.180, n = 114, $p = 0.055$). Plasma HIV RNA was, however, significantly correlated with brain HIV RNA (rho = 0.371, n = 160, $p < 0.001$). CSF HIV RNA obtained close to the time of NP testing was not correlated with NP T scores significantly (rho = −0.109, n = 84, $p = 0.327$), but was correlated significantly with brain HIV RNA (rho = 0.435, n = 104, $p < 0.001$). Low plasma CD4+ lymphocyte counts close to the time of testing were correlated with worse (lower) NT T scores (0.234, $p = 0.0089$).

Brain HIV versus neuroinflammatory mRNA markers

Brain HIV replication and HIVE are associated strongly with host inflammatory responses including type 1 interferon response genes (IFRG) (9,23,24). *ISG15* and *MX1* mRNAs in the brain specimens both were significantly correlated with composite NP T scores (Figs. 3A–3D). *IRF1* mRNA, which is a predominantly type 2 IFRG (25), was more weakly correlated with NP T scores (Fig. 3E). A neuronally expressed mRNA (*DRD2L*) also is given (Fig. 3F) (20). Correlations between inflammatory markers and brain HIV RNA were highly significant (Figure S1 in Supplemental Digital Content).

Discussion

This is the largest neurovirological survey to date of HIV-infected human brain specimens from subjects who underwent formal neurocognitive testing. The survey confirms that the central nervous system (CNS) is a unique compartment with regard to controlling the rate of virus replication, and supports the hypothesis that virus replication in the CNS contributes to the pathophysiology of HAND in certain subjects. The broad clinical implication is that targeting and reducing HIV replication in the CNS compartment is a logical approach to treat HAND in certain types of neuropathologically characterized patents on HAART. Further decreasing brain HIV loads to levels lower than those currently achieved on HAART could produce beneficial albeit highly variable degrees of improvement in neurocognitive performance. Most of the variation in potential NP benefit segregated according to the neuropathological diagnoses of HIVE and MGNE. Thus, targeting CNS HIV replication therapeutically is likely to produce some NP benefit in patients who 1) have HIVE and MGNE neuropathologically, or 2) have elevated brain type I interferon responses which could drive neurocognitive dysfunction (24), and perhaps 3) have a brain HIV RNA that has “broken out” to above a critical (threshold) value that has not yet been established. In contrast, brain HIV RNA was correlated with NP impairment either minimally or not at all in the numerically largest group of subjects with HAND (i.e. patients lacking HIVE or MGNE) (Figure 1 and Table 1). In those patients the potential NP benefit of further reducing HIV RNA in the brain is not as clear-cut, because they did not have more brain HIV RNA than the group without HAND. Autopsy surveys show that HAART-era subjects in unselected cohorts with HAND are not likely to have a very high prevalence of HIVE or MGNE and/or brain HIV loads in the higher ranges (26,27). In turn reducing residual HIV RNA incrementally, by intensifying virus suppression in the CNS compartment, might not produce substantial NP improvement in subjects already on HAART (2).

Most of the subjects with HAND had neither HIVE nor MGNE, and other neuropathological changes do not segregate with HAND (13). The reasons for that remain unknown (14). It is possible that the early stages of HAND produce neurophysiological changes that cannot be observed histopathologically (10,28). Alternatively, newly recognized histomorphological changes in brain cells remain to be discovered. At present there remains a “gap” between HAND and its putative neurovirological substrate. The disparity is analogous to the therapeutic “gap” that has been recognized between HAND and HAART (2).

A biomarker capable of separating specific types of subjects with HAND could be used in clinical surveys to screen patients according to neuropathological risk categories, which would allow for individualizing treatment according to a patient’s likelihood of obtaining added NP benefit from targeting the reduction of CNS HIV loads. There is at present no available laboratory method to fill that niche. Obtaining a brain biopsy lacks feasibility in clinical practice. Measuring CSF HIV RNA is often suggested to be a viable marker for HAND because it was found to be a potentially useful surrogate for HIV in the CNS in a survey before HAART (29). In this survey CSF HIV RNA did not recapitulate the significant neurovirological correlation observed using brain tissue, which agrees with

HAART-era surveys showing that CSF HIV RNA is not correlated with HAND (30). Notably, CSF HIV RNA in this survey was less informative than plasma HIV RNA with regard to neurocognitive dysfunction and the neurovirological correlation. That finding is consistent with studies which show that HAND is correlated with other measurements in the blood compartment including anemia, endotoxin, and soluble CD14 (31–33). Measurement of CSF HIV RNA still is held to be worthwhile in longitudinal research paradigms because it provides access to a sub-compartment of the CNS, can be sampled multiple times and showed linkage with neurocognitive impairment without HAART (29).

The clinical and neurovirological implications of HIVE have undergone elucidation using animal models of lentivirus encephalitis and in vitro HIV-1 infection paradigms (8,11,12,34). The significant neurovirological correlation with HIVE and HAND quantified herein was anticipated from histomorphological observation (10,11). The clinical implications of other neuropathological outcomes including MGNE, diffuse microglial cell activation (DMA), and minimal changes are not as clear (10,14,35–37). MGNE and DMA in HIV/AIDS have been interpreted variously as: 1) responses to opportunistic infections with organisms such as Cytomegalovirus or Toxoplasma ghodii (38), 2) an inflammatory pattern that should be classified together within the broad spectrum of HIVE-related effects (13), 3) a response to the interaction between HIV-associated neurodegeneration and substance use (39) or 4) CNS inflammation induced by lipopolysaccharide or other changes in blood plasma (31,40). The data presented show that brain HIV RNA was higher in subjects with MGNE versus without it (Fig. 2). That suggests that MGNE is a harbinger of unrestrained virus replication and worsening of HAND. Intensified therapy to control CNS viral replication in patients with MGNE would be a logical prophylactic measure to stem progression to more severe HAND and HIVE. Consistent with that suggestion we note that subjects with MGNE were not as likely to be on HAART (Table 1), which could have increased their vulnerability to MGNE.

We explored whether there was a threshold value of brain HIV RNA that can discriminate between subjects likely to obtain NP benefit from targeting CNS HIV, from those less certain to obtain NP benefit. The two populations have overlapping distributions (Fig. 2). The best discrimination occurred at a value of about 15,000 copies per gram of brain neocortical tissue. Above that value 73% of the subjects who are likely to obtain NP benefit are included, while 74% of the subjects with less certain NP benefit are excluded. Based upon the slope of the least squares regression line between brain and CSF HIV RNAs, the suggested “cutoff” value in CSF would be 1,200 copies per ml. Great caution should be exercised regarding the clinical utility of using a cutoff value, because the interrelationship may not be linear and the NP data are quite variable.

Caveats of a retrospective clinicopathological autopsy study should be considered. 1) Clinical evaluations were obtained an average of 7 months before the brain specimens, and the plasma and CSF HIV loads could change with time (Table 1). Pre- versus postmortem CSF and plasma HIV RNA comparisons were available for just 28 and 12 subjects, respectively. CSF HIV RNA was indeed significantly higher postmortem versus premortem (3.58 versus 2.51, $p < 0.01$); the plasma HIV RNA values were not significantly different. 2) Some subjects were not taking HAART when the brain specimen was obtained (Table 1). We evaluated the correlations between brain HIV RNA and NP T scores in the subset of 84 subjects on HAART and obtained the same result as with the entire NP cohort (rho = −0.332, $p = 0.0055$). The same trend was present in the 41 subjects not on HAART (rho = −0.223, $p = 0.1617$). 3) Co-morbid conditions other than HIV infection such as stroke and cardiac disease could contribute to HAND. Pathology in organs other than the CNS including the heart was not significantly more prevalent in HAND (Table 1). Of interest was

that brain infarcts tended to be more common with HAND versus without it ($p < 0.11$), which suggests that brain ischemia could have influenced the prevalence of HAND.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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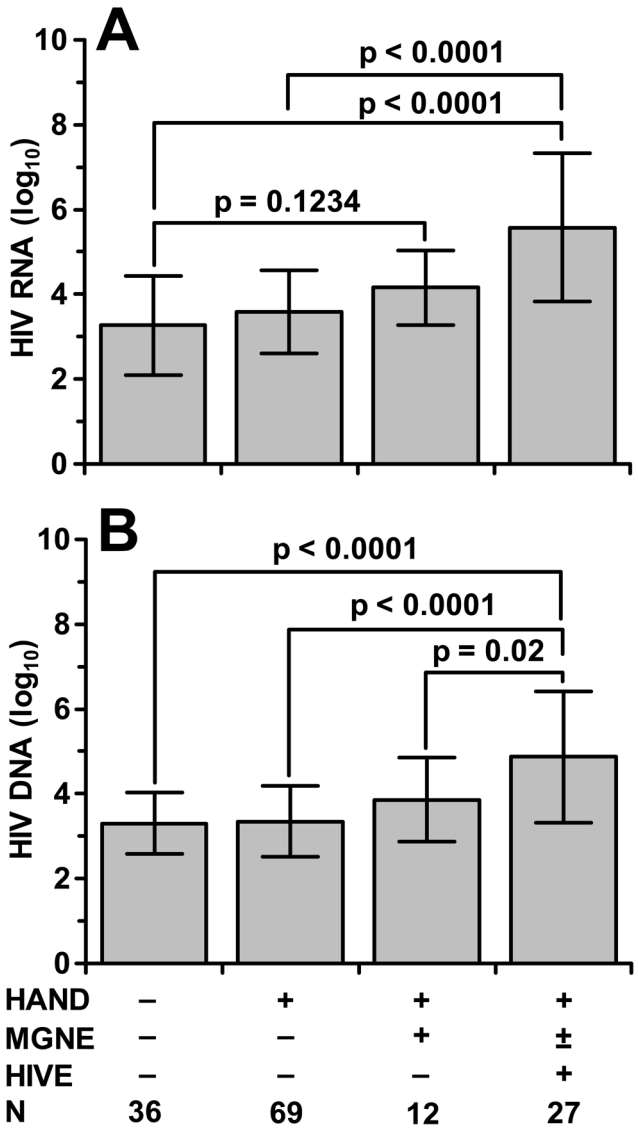

Figure 1.

Brain HIV RNA (A) and DNA (B) expressed as copies per gram of brain tissue in the neuropsychologically-focused cohort. One-way analysis of variance showed a significant group effect ($p < 0.0001$). P-values for the post-hoc Tukey-Kramer tests are shown. Brain HIV RNA and DNA both were significantly higher in subjects with HAND plus HIVE. Brain HIV RNA was marginally higher in HAND with MGNE. Brain HIV RNA and DNA were not significantly higher in HAND without HIVE or MGNE. The number of subjects without HAND shown here differs from Table 1 because four subjects with HIVE did not have HAND and could not be assigned to a group (see Methods). HAND, HIV associated neurocognitive disorder; MGNE, microglial nodule encephalitis; HIVE, HIV encephalitis; N, number of subjects in each group.

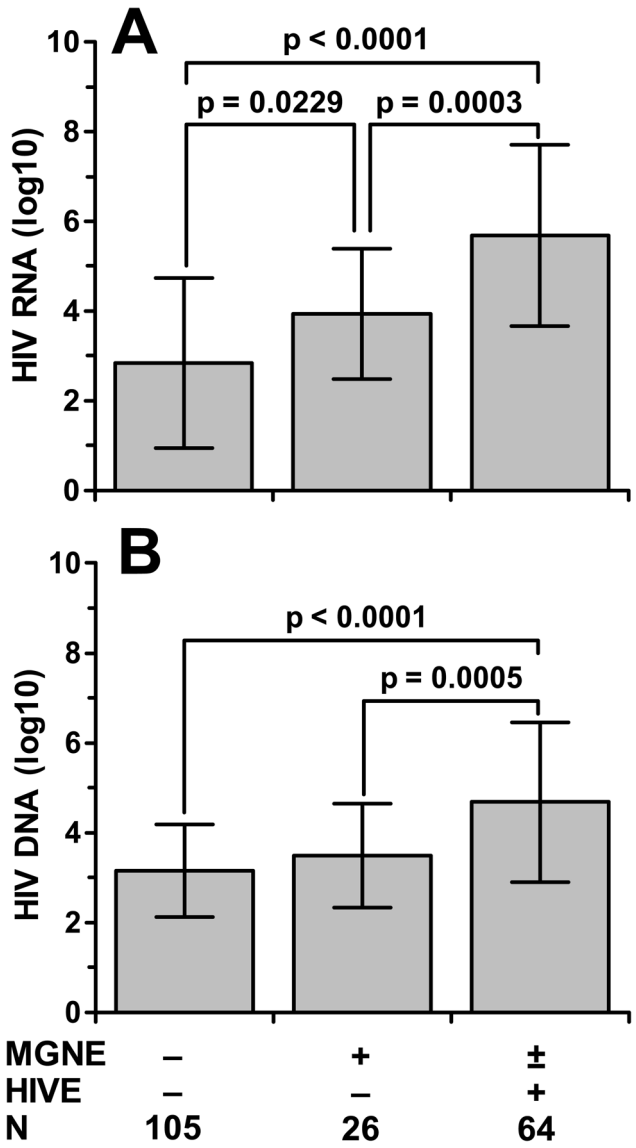

Figure 2.

Brain HIV RNA and DNA expressed as copies per gram of brain tissue in the larger neuropathologically-focused cohort. One-way analysis of variance showed a significant group effect ($p < 0.0001$). P-values for the post-hoc Tukey-Kramer tests are shown. Brain HIV RNA was higher in the subjects with MGNE and HIVE versus neither (panel A). The group with MGNE had significantly lower HIV RNA than the group with HIVE (panel A). Results using HIV DNA (panel B) are similar with smaller effect sizes. MGNE, microglial nodule encephalitis; HIVE, HIV encephalitis; N, number of subjects in each group.

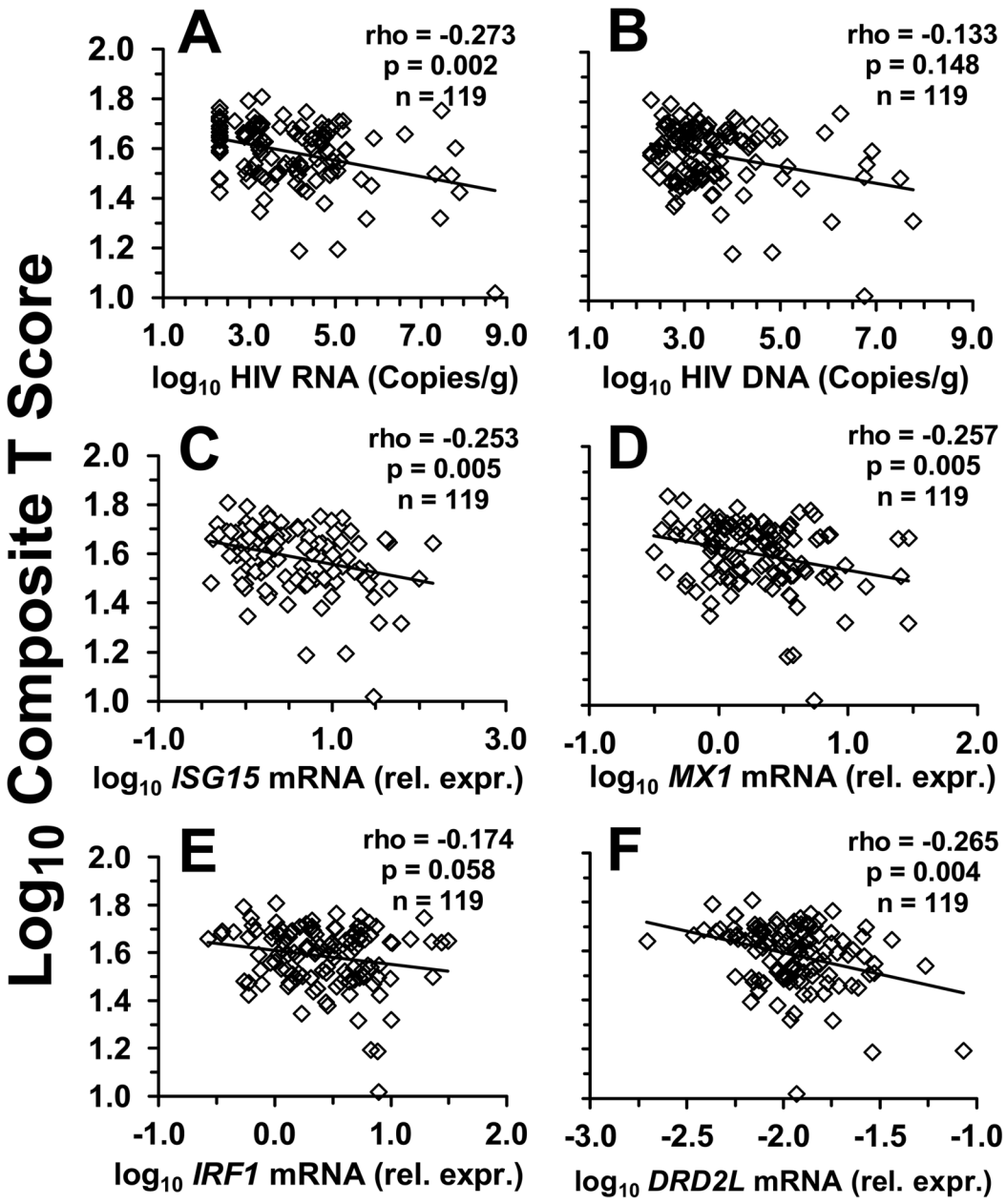

Figure 3.

Composite performance on neuropsychological testing (ordinate) was plotted with neurovirological and neuroimmunological measurements in the brain specimens (abscissa) of 119 HIV-infected subjects. High brain HIV RNA load was significantly correlated with lower composite neuropsychological T scores (A). High brain HIV DNA load had a similar but weaker pattern that was not significant statistically (B). High levels of *ISG15* and *MX1* mRNAs were significantly correlated with low T scores (C,D). The levels of *IRF1* mRNA had a weaker correlation that was marginal statistically (E). High expression of *DRD2L* mRNA, which is a dopamine receptor transcript expressed primarily in neurons, was correlated with worse impairment (F). Relative expression of mRNAs was normalized to *GAPDH* mRNA. Correlations used Spearman's test and were log transformed on both axes to achieve a uniformly-scaled comparison panel.

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Table 1

Cohort data

Characteristic	Neuropsychological cohort			Neuropathological cohort			
	HAND versus without[1]			HIVE or MGNE versus without[1]			
	No HAND	HAND	All subjects	No HIVE or MGNE	No HIVE with MGNE	HIVE[2]	All subjects
Number of subjects	40	108	148	105	26	64	195
Age at death, mean (SD)[3]	51 (10)	45 (9)	47 (10)	48 (10)	43 (10)	43 (7)	46 (9)
Hours postmortem, mean (SD)[4]	21 (28)	11 (11)	14 (18)	14 (16)	15 (20)	16 (21)	15 (18)
Months from last NP assessment to death, mean (SD)[4]	10 (10)	6 (7)	7 (8)	8 (9)	8 (9)	6 (5)	8 (8)
Gender, N (%)							
Female	4 (10)	21 (19)	25 (17)	22 (21)	3 (12)	6 (9)	31 (16)
Male	36 (90)	87 (81)	123 (83)	83 (79)	23 (88)	58 (91)	164 (84)
Race, N (%)							
White	33 (83)	67 (62)	100 (68)	69 (66)	18 (69)	45 (70)	132 (68)
Black	6 (15)	30 (28)	36 (24)	26 (25)	5 (19)	15 (23)	46 (24)
Native American	0 (0)	6 (6)	6 (4)	6 (6)	1 (4)	0 (0)	7 (4)
Other	1 (3)	5 (5)	6 (4)	4 (4)	2 (8)	4 (6)	10 (5)
Hispanic or Latino, N (%)	6 (15)	29 (27)	35 (24)	29 (28)	4 (15)	17 (27)	50 (26)
Years of education, median	13.0	12.0	12.0	12.0	12.0	12.0	12.0
HIV risk factor, N (%)							
Intravenous Drug Use	7 (18)	31 (29)	38 (26)	31 (30)	9 (35)	9 (14)	49 (25)
Male-to-male sex	27 (68)	47 (44)	74 (50)	47 (45)	13 (50)	36 (56)	96 (49)
Heterosexual sex	5 (13)	19 (18)	24 (16)	18 (17)	1 (4)	12 (19)	31 (16)
Other	1 (3)	11 (10)	12 (8)	9 (9)	3 (12)	7 (11)	19 (10)
Years HIV-1 infected, mean (SD)[4]	15 (5)	11 (5)	12 (5)	12 (5)	9 (5)	11 (6)	12 (6)

Characteristic	Neuropsychological cohort			Neuropathological cohort			
	HAND versus without[1]			HIVE or MGNE versus without[1]			
	No HAND	HAND	All subjects	No HIVE or MGNE	No HIVE with MGNE	HIVE[2]	All subjects
Active on HAART[1], N (%)[5]	29 (73)	67 (62)	96 (65)	71 (68)	12 (46)	28 (44)	111 (57)
Log plasma HIV c/ml, mean (SD)[5]	4.0 (1.5)	4.3 (1.4)	4.2 (1.4)	4.0 (1.4)	4.0 (1.4)	5.1 (1.0)	4.3 (1.4)
Log plasma HIV undetectable[7,8], N (%)	8 (22)	16 (17)	24 (18)	19 (20)	6 (30)	1 (2)	26 (16)
Log CSF HIV c/ml, mean (SD)[5]	2.3 (1.0)	2.3 (0.9)	2.3 (0.9)	2.1 (0.7)	2.1 (0.8)	3.4 (1.1)	2.4 (1.0)
Log CSF HIV undetectable[7,8], N (%)	12 (55)	24 (48)	36 (50)	30 (60)	6 (46)	1 (5)	37 (45)
CD4+ lymphocytes/mm^3, mean (SD)[6]	152 (175)	109 (183)	121 (181)	135 (192)	131 (230)	54 (66)	111 (174)
Hepatitis C[1,7], N (%)							
No	15 (71)	51 (61)	66 (63)	44 (61)	10 (62)	27 (67)	81 (63)
Yes	6 (29)	32 (39)	38 (37)	28 (39)	6 (38)	13 (33)	47 (37)
History of depression[1,7], N (%)							
No	10 (32)	34 (41)	44 (39)	33 (38)	9 (60)	11 (39)	53 (41)
Yes	21 (68)	49 (59)	70 (61)	53 (62)	6 (40)	17 (61)	76 (59)
History of any substance abuse[1,7], N (%)							
No	9 (29)	38 (46)	47 (41)	38 (44)	6 (40)	15 (54)	59 (46)
Yes	22 (71)	45 (54)	67 (59)	48 (56)	9 (60)	13 (46)	70 (54)
Individual substance abuse[1,7], N (%)							
Alcohol[4]	18 (58)	20 (24)	38 (33)	27 (31)	4 (27)	7 (25)	38 (29)
Cannabis	11 (35)	20 (24)	31 (27)	25 (29)	4 (27)	5 (18)	34 (26)
Stimulants	10 (32)	14 (17)	24 (21)	16 (19)	4 (27)	4 (14)	24 (19)
Cocaine	10 (32)	12 (14)	22 (19)	15 (17)	3 (20)	4 (15)	22 (17)
Sedatives	6 (19)	9 (11)	15 (13)	12 (14)	0	3 (11)	15 (12)

Characteristic	Neuropsychological cohort			Neuropathological cohort			
	HAND versus without[1]			HIVE or MGNE versus without[1]			
	No HAND	HAND	All subjects	No HIVE or MGNE	No HIVE with MGNE	HIVE[2]	All subjects
Hallucinogens[9]	6 (19)	4 (5)	10 (9)	7 (8)	1 (7)	2 (7)	10 (8)
Opiates	4 (13)	5 (6)	9 (8)	5 (6)	2 (13)	2 (7)	9 (7)
Other	1 (3)	5 (6)	6 (5)	4 (5)	1 (7)	1 (4)	6 (5)
CNS Vascular Pathology, N (%)							
Focal (territorial) infarction	1 (3)	13 (12)	14 (9)	12 (11)	3 (12)	2 (3)	17 (9)
Hypoxic/ischemic damage[6]	2 (5)	10 (9)	12 (8)	10 (10)	4 (15)	1 (2)	15 (8)
Hemorrhage	2 (5)	1 (1)	3 (2)	2 (2)	1 (4)	4 (6)	7 (4)
CNS Infections, N (%)							
Aseptic Leptomeningitis[6]	3 (8)	8 (7)	11 (7)	5 (5)	5 (19)	2 (3)	12 (6)
Cryptococcus	3 (8)	6 (6)	9 (6)	7 (7)	0	3 (5)	10 (5)
CMV Encephalitis[1]	2 (5)	5 (5)	7 (5)	6 (6)	1 (4)	4 (6)	11 (6)
Bacterial parenchymal infection	1 (3)	1 (1)	2 (1)	2 (2)	0	2 (3)	4 (2)
Toxoplasmosis –active or healed	0	2 (2)	2 (1)	1 (1)	1 (4)	4 (6)	6 (3)
Progressive multifocal leukoencephalopathy	0	1 (1)	1 (1)	1 (1)	0	2 (3)	3 (2)
Tuberculosis	0	1 (1)	1 (1)	1 (1)	0	0	1 (1)
Other infections	2 (5)	2 (2)	4 (3)	3 (3)	0	3 (5)	6 (3)
Other CNS Pathology, N (%)							
Alzheimer Type 2 Gliosis	6 (15)	23 (21)	29 (20)	20 (19)	6 (23)	11 (17)	37 (19)
Leukoencephalopathy	1 (3)	5 (5)	6 (4)	4 (4)	0	4 (6)	8 (4)
Lymphoma	1 (3)	4 (4)	5 (3)	3 (3)	1 (4)	6 (9)	10 (5)
Contusion[7]	1 (3)	0	1 (1)	1 (1)	0	0	1 (1)
Organ Pathology[7]							
Heart weight, mean (SD)	375 (106)	347 (98)	356 (101)	367 (110)	364 (93)	340 (81)	357 (98)
Cardiac, N (%)							
Abnormal	26 (67)	57 (59)	83 (61)	61 (66)	11 (48)	32 (54)	104 (59)

Characteristic	Neuropsychological cohort			Neuropathological cohort			
	HAND versus without[1]			HIVE or MGNE versus without[1]			
	No HAND	HAND	All subjects	No HIVE or MGNE	No HIVE with MGNE	HIVE[2]	All subjects
Left ventricle hypertrophy	11 (28)	19 (19)	30 (22)	23 (24)	4 (16)	9 (16)	36 (20)
Myocardial infarction[4]	9 (24)	1 (1)	10 (8)	10 (11)	1 (4)	1 (2)	12 (7)
Other pathology	14 (35)	37 (37)	51 (37)	38 (39)	7 (28)	16 (27)	61 (34)
Kidney abnormal, N (%)	24 (62)	57 (60)	81 (60)	59 (63)	18 (82)	34 (60)	111 (65)
Liver abnormal, N (%)	25 (63)	73 (73)	98 (70)	72 (73)	15 (60)	35 (60)	122 (67)
Pulmonary system abnormal, N (%)	32 (80)	82 (85)	114 (83)	78 (82)	20 (83)	48 (80)	146 (82)
Gastrointestinal system abnormal, N (%)	9 (24)	22 (34)	31 (30)	16 (24)	5 (23)	16 (35)	37 (27)

[1] Definitions
HAND: HIV-associated neurocognitive disorders diagnosed at any time
HIVE: Human Immunodeficiency Virus Encephalitis
MGNE: Microglial nodule encephalitis
HAART: Highly active antiretroviral therapy; defined as being active within one year of death
Hepatitis C: Defined as any laboratory evidence of Hep C infection
Substance abuse/Depression: Defined as any current/past diagnosis of abuse or depression on PRISM/CIDI assessment
CMV: Cytomegalovirus

[2] Five subjects in this group also had MGNE

[3] p<.01 across both the HAND and HIVE/MGNE groups

[4] p<.01 between the two HAND groups only

[5] p<.01 across the HIVE/MGNE groups

[6] p<.05 across the HIVE/MGNE groups

[7] Sample size within these groups or subgroups are smaller than overall sample size due to the availability of data

[8] Below 50 copies/ml or 400 copies/ml depending on the assay performed

[9] p<.05 between the two HAND groups only

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Table 2

Spearman correlation between log brain HIV- RNA and normalized neuropsychological T scores

	All subjects			Subjects with HIVE[1]			Subjects without HIVE		
Neuropsychological domain	**N[2]**	**Rho**	**P-value**	**N**	**Rho**	**P-value**	**N**	**Rho**	**P-value**
Abstract Executive Functioning[5]	128	−0.237	**0.0071**[3]	29	−0.370	0.0480	99	−0.052	0.6108
Speed of Information Processing	134	−0.249	**0.0038**[3]	32	−0.322	0.0722	102	−0.152	0.1284
Attention Working Memory[5]	127	−0.229	**0.0095**[3]	28	−0.439	**0.0193**[3]	99	−0.080	0.4322
Learning	134	−0.368	**0.0001**[3]	32	−0.463	**0.0076**[3]	102	−0.271	**0.0059**[3]
Memory	133	−0.287	**0.0008**[3]	30	−0.432	**0.0171**[3]	103	−0.203	0.0400
Verbal Fluency	132	−0.243	**0.0051**[3]	31	−0.367	0.0432	101	−0.119	0.2352
Motor	126	−0.182	**0.0412**[3]	28	−0.154	0.4340	98	−0.051	0.6169
Composite	119	−0.290	**0.0014**[4]	24	−0.389	0.0627	95	−0.204	**0.0469**[4]

[1] HIVE, HIV encephalitis

[2] N values vary according to the tests completed. A subject must complete 10 out of 14 tests to receive a composite score.

[3] Significant at alpha=0.05 after applying Benjamini-Hochberg correction for multiple comparisons

[4] Significant at alpha=0.05. The composite score is not subject to multiple comparison assumptions

[5] Significant at alpha=0.05 for comparison between Rho for subjects with HIVE and subjects without HIVE