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Relationship of antiretroviral treatment to postmortem brain tissue viral load in human immunodeficiency virus–infected patients

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Human immunodeficiency virus (HIV)-1 invades the central nervous system (CNS) soon after infection and is partially protected there from host immunity and antiretroviral drugs (ARVs). Sanctuary from highly active antiretroviral therapy (HAART) in the CNS could result in ongoing viral replication, promoting the development of drug resistance and neurological disease. Despite the importance of these risks, no previous study has directly assessed HAART's effects on brain tissue viral load (VL). The authors evaluated 61 HIV-infected individuals for whom both histories of HAART treatment and postmortem brain tissue VL measurements were available. Two groups were defined based on HAART use in the 3 months prior to death: HAART(+) subjects had received HAART, and HAART(−) subjects had not received HAART. HIV RNA was quantified in postmortem brain tissue ($\log_{10}$ copies/10 μg total tissue RNA) and antemortem plasma ($\log_{10}$ copies/ml) by reverse transcriptase–polymerase chain reaction (RT-PCR). Brain tissue VLs were significantly lower among HAART(+) subjects compared to HAART(−) subjects (median 2.6 versus 4.1; $P = .0007$). These findings suggest that despite the limited CNS penetration of many antiretroviral medications, HAART is at least partially effective in suppressing CNS viral replication. Because some HAART regimens may be better than others in this regard, regimen selection strategies could be used to impede CNS viral activity, limit neuronal dysfunction, and prevent or treat clinical neurocognitive disorders in HIV-infected patients. Furthermore, such strategies might help to prevent the development of ARV resistance. *Journal of NeuroVirology* (2006) **12,** 100–107.

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Introduction

The demonstration that human immunodeficiency virus (HIV) disease progression can be slowed or reversed by suppression of active viral replication with highly active antiretroviral therapy (HAART) has established active viral replication as a central factor in acquired immunodeficiency syndrome (AIDS) pathogenesis. Reverse transcriptase–polymerase chain reaction (RT-PCR)–based viral load assays currently in widespread clinical use measure active replication of HIV in host target cells, such as CD4 lymphocytes and tissue macrophages, including those in the central nervous system (CNS). Attaining virologic suppression by HAART in the CNS is theoretically important for two reasons. First, inhibiting CNS viral replication should protect against further neural injury directly related to increased viral burden and thereby ameliorate and prevent HIV CNS disease, especially neurocognitive dysfunction. Second, HAART-induced viral suppression in the CNS could forestall compartmentalized evolution of drug resistance. Studies finding relatively poor brain tissue penetration of antiretroviral drugs (ARVs) have raised concerns that the CNS may be a sanctuary where viral replication may persist (Major *et al*, 2000; Pomerantz, 1999; Schrager and D'Souza, 1998). Furthermore, clinical (Dore *et al*, 1999) and neuropathological (Gray *et al*, 2003; Langford *et al*, 2003; Masliah *et al*, 2000; Neuenburg *et al*, 2002) reports have presented indirect evidence that HAART might be less effective in treating CNS disease compared to systemic HIV disease. Even modest degrees of residual CNS viral replication could be neurologically and systemically deleterious.

Evaluating HAART's effectiveness in suppressing CNS viral replication has been difficult because brain tissue viral load (VL) cannot be directly assessed *in vivo*. Surrogates, including cerebrospinal fluid (CSF) VL, have been evaluated instead. Although phylogenetic comparisons of CSF HIV RNA sequences support evolutionary segregation from lymphatic tissue and plasma virus (Wong *et al*, 1997), other lines of evidence suggest that HIV in CSF has multiple sources (Ellis *et al*, 2000; Haas *et al*, 2003). Thus, CSF VL may not be the best indicator of HAART's efficacy in the brain. The purpose of this study was to compare VLs in autopsy-derived brain tissue from HIV-infected patients treated with HAART in close proximity to death [HAART(+)], to those not so treated [HAART(−)]. Two previous studies examined HIV RNA levels in brain tissue (McClernon *et al*, 2001; Wiley *et al*, 1998); however, neither study examined the relationship of brain VL to antemortem HAART treatment. We predicted that poor ARV penetration would result in a lack of differences between brain tissue VLs in these two groups.

Results

Table 1 lists pertinent demographic and clinical characteristics of the subjects. Thirty subjects (49%) received HAART within 3 months of death and 31 (51%) did not receive HAART within 3 months prior to death. ARV regimens in HAART(+) subjects were as follows: one protease inhibitor (PI) plus two or more nucleoside reverse transcriptase inhibitors

Table 1 Characteristics of cases in the analysis.

	HAART(+) N = 30	*HAART(−) N* = 31	*P*
Year of death[a]	2000 (1999–2001)	2000 (1998–2001)	n.s.
Age at death in years, mean (SD)	42.7 (8.7)	41.0 (6.6)	n.s.
Gender, no. male (percent)	26 (86.7)	26 (83.9)	n.s.
Ethnicity, no. Caucasian (percent)	20 (66.7)	21 (67.7)	n.s.
Cause of death, no. (percent)[b]			n.s.
Fulminant infection	17 (56.7)	14 (51.9)	
Central nervous system OI	3 (10.0)	1 (3.7)	
Cardio/pulmonary	6 (20.0)	8 (29.6)	
Other	4 (13.3)	4 (14.8)	
Diagnosed with AIDS at death, no. (percent)	30 (100.0)	31 (100.0)	n.s.
Last known CD4 count (cells/μl)[a,c]	67 (14–185)	14 (4–128)	.043
Antemortem plasma HIV RNA ($\log_{10}$ copies/ml)[c]	3.8 (2.6–5.4)	5.3 (4.6–5.9)	.036
Neurologically symptomatic, no. (percent)[d]	13 (92.9)	11 (64.7)	n.s.
Days from last confirmed HAART use to death[a]	29 (6–53)	n.a.	
Postmortem interval (hours)[a,c]	24 (18.75–24)	24 (9.5–24)	n.s.

[a]Values are median (interquartile range).
[b]Fulminant infection: bronchopneumonia, pneumonia, tuberculosis, sepsis, small bowel bacterial disease; CNS OI: cytomegalovirus encephalitis, CNS lymphoma, herpes simplex virus encephalitis; cardio/pulmonary: myocardial infarction, respiratory failure, hypertensive vascular collapse, hypotensive shock, pulmonary infarction; other: HIVE, HIV leukoencephalopathy, gastric lymphoma, hepatorenal failure, liver failure, metastatic melanoma.
[c]CD4 count available for 27 HAART(+) and 27 HAART(−) subjects; plasma VL available for 15 HAART(+) and 18 HAART(−) subjects; postmortem interval available for 30 HAART(+) and 30 HAART(−) subjects.
[d]Neurologically symptomatic is defined as neuropsychological impairment within 1 year of death. Data available for 17 HAART(−) and 14 HAART(+) subjects.

(NRTIs) in 15 (50%); one non-nucleoside reverse transcriptase inhibitor (NNRTI) plus two or more NRTIs in five (17%); and other combinations of three or more drugs in 10 (33%). Most HAART(+) subjects ($n = 18$) took regimens that included only one CNS-penetrating drug; 10 subjects' regimens included two penetrating drugs, and 2 subjects' regimens included three penetrating drugs (see materials and methods for listing of penetrating drugs). Most HAART(+) subjects ($n = 20$, 67%) had tried at least three different regimens. Among the HAART(−) subjects, 24 (77%) had received some type of anti roviral treatment (ART) in the past (>3 months prior to death); 11 of these had previously taken a HAART regimen.

Consistent with greater HAART exposure in the HAART(+) group, their antemortem plasma VLs were significantly lower (median [interquartile range (IQR)] 3.8 [2.6–5.4]; $n = 18$) than in the HAART(−) group (5.3 [4.6–5.9]; $n = 15$; $P = .0357$). Antemortem plasma was collected a median of 81 days before death (IQR: 51–131). In keeping with their advanced disease stage and incomplete response to ART, only six subjects (15%) showed virologic suppression in plasma prior to death (plasma HIV RNA <400 copies/ml); of these six, four (67%) were in the HAART(+) group.

Frontal cortex VLs were significantly lower in HAART(+) as compared to HAART(−) subjects (median [IQR] 2.6 [1.7–3.7] versus 4.1 [3.0–4.7] $\log_{10}$ copies per 10 μg total RNA; Wilcoxon $P = .0007$) (Figure 1). Brain VLs were detectable in 24 (77%) subjects in the HAART(+) group and all of the HAART(−) subjects (Fisher's exact $P = .0047$). Although brain tissue VL measurements in white matter, basal ganglia, and hippocampus were available for fewer cases, HAART-related differences were similarly significant for these brain regions (data not shown).

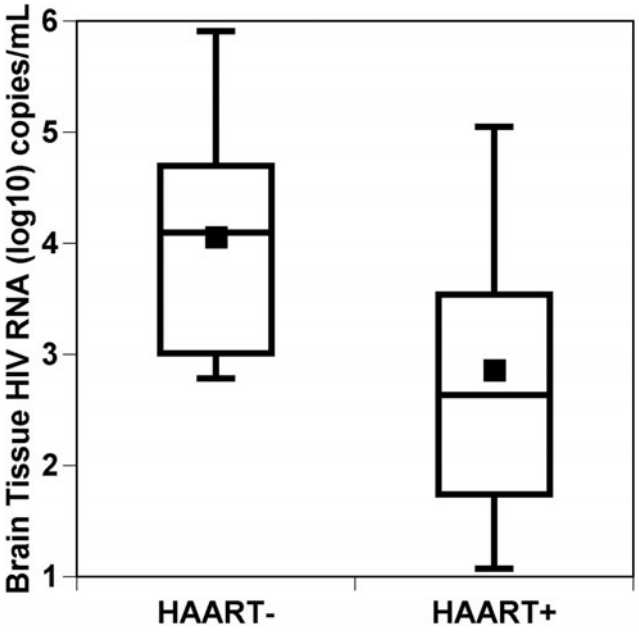

Figure 1 Postmortem frontal cortex viral load according to whether subjects did [HAART(+)] or did not [HAART(−)] receive combination antiretroviral therapy during the 3 months prior to death. Box-and-whisker plots indicate medians (center lines), means (squares), interquartile ranges (boxes) and 5 and 95 percentiles (whiskers).

HAART(+) subjects taking only one penetrating drug ($n = 18$) had slightly higher brain VL (2.9 [2.0–3.5]) than those who took two or more penetrating drugs ($n = 12$), (2.3 [1.7–4.8]), but this difference was not statistically significant ($P = .6394$). Two of the six subjects showing virologic suppression in plasma prior to death had >100 (2.0 $\log_{10}$) copies in brain tissue; the first subject (2.8 $\log_{10}$ copies) discontinued HAART 69 days before death; the second (4.2 $\log_{10}$ copies) discontinued HAART 1 day before death.

Of the 61 subjects, 31 (51%; 14 HAART[+] and 17 HAART[−]) had antemortem neuropsychological (NP) testing within 1 year of death. The HAART(+) and HAART(−) groups did not differ in the frequency of NP impairment (Table 1).

As expected, plasma and brain VLs were correlated (Rho = 0.48, $P = .0055$). To determine if the relationship between HAART and brain VL was simply mediated by a reduction in plasma VL, regression analysis was performed. In a multivariate model, both antemortem plasma VL ($F = 4.2$, $P = .0487$), and HAART treatment ($F = 8.1$, $P = .0082$) were significant independent predictors of postmortem frontal cortex VL. Furthermore, the interaction term was not significant ($P = .1323$), indicating that the relationship between HAART and brain VL was not dependent on plasma HIV RNA.

We considered the possibility that damage to the blood-brain barrier (BBB), common among late stage HIV-infected individuals, might allow better penetration of ARVs into the CNS and therefore greater reduction of brain VL. Accordingly, we grouped subjects who were and were not likely to have BBB breakdown. Opportunistic infections (OIs) likely to cause BBB breakdown were present in 15 subjects. These OIs included cytomegalovirus (CMV), *Mycobacterium avium* complex (MAC), metastatic melanoma, non-Hodgkin's lymphoma (NHL), sepsis, tuberculosis meningititis, and toxoplasmosis. One subject had evidence of possible BBB breakdown due to chronic obstructive pulmonary disease requiring ventilator support. To determine whether BBB breakdown was responsible for the overall relationship between the HAART(+) and HAART(−) we analyzed the relationship between HAART use and brain VL in a subgroup of individuals who did not have conditions likely to result in BBB breakdown. In this subgroup analysis, brain VL was significantly lower in the HAART(+) group compared to the HAART(−) group ($P = .0077$). Additionally, we found that among HAART users, brain VL did not differ between subjects who were and were not likely to have BBB breakdown ($P = .5689$).

Findings of classical HIV encephalitis (HIVE) such as multinucleated giant cells and microglial nodules were present in 37 of the 61 cases (60.7%). Fifteen (50%) of the HAART(+) subjects had evidence of HIVE at autopsy compared to 22 (71%) of subjects in the HAART(−) group. Viral load in the frontal cortex was higher in brains with HIVE than in those without HIVE (median 4.1 [3.0–5.1] versus 2.8 [2.2–3.1]; $P = .0006$), regardless of HAART use. The relationship between HIVE and brain VL persisted even after adjusting for HAART use ($F = 7.2$, $P = .0074$).

Discussion

The goal of this study was to ascertain whether HAART effectively suppresses HIV viral replication in brain tissue as determined by measuring postmortem frontal cortex VL in AIDS patients at autopsy. Because collection of brain tissue from humans is restricted to sampling from autopsies, and because previous studies have demonstrated that withdrawal of ART leads to return of viral replication in the plasma within 3 months (Fagard *et al*, 2003), we limited our consideration of ART to the period within 3 months of death. Despite previously published evidence that the penetration of ARVs into brain tissue may not be sufficient to control viral replication in infected CNS target cells (Huisman *et al*, 2000; Wu and Nie, 1998), we found that VL in brain tissue collected at autopsy was significantly reduced in HIV-infected individuals who had received HAART within 3 months of death compared to those not receiving HAART during this period. Our findings imply that HAART substantially inhibited CNS viral replication.

One possible explanation for this is that damage to the blood-brain barrier, common among HIV-infected individuals, might allow better penetration of ARVs into the CNS (Petito and Cash, 1992; Power *et al*, 1993; Smith *et al*, 1990). To address this possibility, we evaluated a subgroup of our subjects in whom BBB breakdown was likely to be highest by virtue of having CNS opportunistic conditions such as cryptococcal meningitis or primary CNS lymphoma. Antemortem HAART was associated with lower brain VLs even in subjects who did not have conditions likely to result in BBB breakdown. Furthermore, among those on HAART within 3 months of death, those with conditions potentially associated with BBB breakdown did not have lower brain VLs than those subjects without.

Although the results of this paper primarily focus on frontal cortex viral load, we evaluated potential differences in the efficacy of ART in lowering VL in other brain regions (white matter, hippocampus, and basal ganglia), which might reflect differences in regional ARV drug penetration or regional susceptibility to HIV infection. We found that ART was similarly effective in lowering VL in these regions, suggesting that regional differences in drug penetration or susceptibility are not present or are not important to lower VL regionally.

Although clinical and research records indicated that most patients in the HAART(+) group indeed took their prescribed antiretroviral medications, it is possible that some did not maintain adequate adherence to their regimen. This would result in a conservative bias—i.e., a failure to show lower VL in the HAART(+) group—because subjects not taking their HAART regimens adequately would be expected to show incomplete viral suppression. Thus, our finding that viral loads were substantially different between the two groups, although they did not differ with respect to other clinical variables, strongly supports the notion that HAART exposure differed between the two groups.

The concept of "CNS viral escape" (Wendel and McArthur, 2003) entails high CNS viral replication even when plasma VL is low. In the present study, we identified a subset of six subjects who had achieved plasma virologic suppression on HAART prior to death. Two of the six HAART(+) subjects (33%) had measurable VL in brain tissue at autopsy. One of the two remained on HAART until the day before death, and had detectable brain tissue VL despite undetectable plasma VL. This case implies that restricted penetration of ARVs may permit residual brain viral replication in some subjects.

To our knowledge this is the first study that directly assesses brain tissue HIV RNA (VL) in autopsied patients who were documented to receive HAART treatment in close proximity to death. Two previous studies examined HIV RNA levels in brain tissue (McClernon et al, 2001; Wiley *et al*, 1998); however, neither study examined the relationship of brain VL to antemortem HAART. Other prior studies done in the setting of treatment with just one or two drugs, such as zidovudine monotherapy and didanosine/zalcitabine dual therapy, rather than highly active combination therapies, or HAART, are less relevant, since mono- and dual therapies are no longer used and HAART is now the standard of care in the United States and Europe.

Our findings are consistent with prior, less direct evidence that HAART provides some degree of inhibition of CNS viral replication. For instance, Gray *et al* (1991) observed that pathological findings of HIVE were less severe in patients who had taken zidovudine during life compared to those who had not received this drug. Additionally, HAART has been shown to suppress CSF VL effectively in many cases (Ellis *et al*, 2000; Foudraine *et al*, 1998; Staprans *et al,* 1999). However, the relationship between VL in brain and CSF is still a subject of debate (Tyler and McArthur, 2002).

Decreased brain VLs were observed in HAART(+) subjects even though many of these patients demonstrated ongoing virologic failure (elevated plasma VL >400 copies/ml) prior to death. Virologic failure may have been due to poor absorption, inadequate

adherence, or acquired resistance to prescribed ARVs. Furthermore, perceived clinical futility often led providers to discontinue HAART days or weeks prior to death. Resurgence of viral replication during this treatment-free period would tend to reduce our ability to find an effect of HAART on brain VL. These considerations imply that the magnitude of HAART's effect observed in this study near the end of life may be an underestimate of HAART's true effect in the CNS during successful therapy.

Because the goal of this study was to determine whether HAART can contribute to decreased VL in brain, we measured VL using an RT-PCR method that quantifies retroviral genomic RNA that is present in intact virions within infected cells, or as free (non–cell-associated) virus. Intact virions are important for stimulating host immune responses and may mediate direct neurotoxicity. We did not evaluate other forms of retroviral nucleic acids, such as integrated proviral DNA or unintegrated, circular 2-LTR provirus. In addition, we did not directly assess concentrations of viral proteins such as gp120 and Tat. Previous studies have evaluated these other indices of viral infection and transcriptional activity. For example, proviral DNA is readily extracted from brain tissue and has been previously measured (Chun *et al*, 1997). Outside the CNS, the majority of proviral DNA is considered replication incompetent, existing as either partially or completely reverse-transcribed, unintegrated DNA (Chun *et al*, 1997). However, no human data exist to describe the proportion of HIV DNA that is replication competent in the CNS. Because CNS target cells differ from those in the periphery, the relative proportions of productive and nonproductive cellular infection may be substantially different. There are conflicting data on the role of nonproductive infection in neuropathogenesis (Saito *et al*, 1994; Wiley *et al*, 1996). Thus, no consensus exists on the relative importance of various forms of viral nucleic acids, and this is an area requiring further investigation.

The cellular targets of HIV infection in the CNS may critically influence responses to HAART. Macrophages and microglia, rather than CD4+ T lymphocytes, are the principal cell population supporting viral replication in the CNS, and the molecular mechanisms of viral replication in these cells are unique (Rohr *et al*, 2003). Thus, infected macrophages may live for months or years rather than a few days, and they produce virus in a more continuous fashion, rather than sporadic or "burst" production. Finally, macrophages and microglia typically harbor the virus in multivesicular bodies in a stable, potentially infectious form for long periods of time. These considerations suggest that suppression of viral replication in the brain might require much longer periods of treatment than lymphoid tissues. Despite these factors, we found marked HAART-associated reductions of VL in brain tissue.

Previously reported phylogenetic data suggest that in untreated HIV infection, the brain is continually reseeded by virus from peripheral blood via trafficking of infected macrophages (Gartner, 2000; Shapshak *et al*, 1999). Thus, in untreated HIV, the proportion of virus derived from native, productively-infected, non-trafficking cells such as microglia may be small. However, when HIV replication in the periphery is inhibited by HAART, viral trafficking also would be expected to diminish, making local sources of replication (CNS) relatively more important, particularly if HAART does not reach these populations. Although most, if not all, virus in the brain probably originally derives from blood via trafficking macrophages, ongoing "cryptic" replication in the CNS might be important. We found that plasma VL prior to death was reduced, although not completely suppressed, in subjects who received HAART within the 3 months prior to death. Therefore, the reduced brain VL observed in the HAART(+) group could be due to reduced trafficking rather than inhibition of viral replication in the brain. However, a multivariate analysis indicated that HAART was associated with reduced brain viral replication independent of plasma VL. This suggests that HAART directly affects CNS viral replication, over and above any additional indirect effects mediated via suppression of systemic viral replication, as reflected in trafficking.

Several limitations of this study should be recognized. First, the resistance patterns of HIV in plasma and CNS of our patients were not measured. Resistance to ARVs may have lessened the impact of HAART on brain VL. Second, most HAART(−) patients had been prescribed HAART earlier in their courses of disease. HAART may have been discontinued in these patients because they were "treatment failures"—meaning that they were not able to attain sustained virologic suppression because of poor adherence, adverse side effects, or resistance. In these patients, the CNS may have played a role in selection for and propagation of resistance mutations and ensuing failure to suppress HIV systemically. Consequently, our findings do not exclude the possibility that in some patients restricted ARV penetration allows for residual viral replication.

Finally, our ability to examine the effects of CNS penetration on VL was limited by the fact that relatively few HAART(+) patients (11/30) took two or more CNS-penetrating ARVs, which are expected to be more effective in reducing brain VL. In fact, we found no significant difference in brain VL reduction comparing subjects taking two or more CNS-penetrating drugs compared to those taking one or none. Recent reports suggest that drug regimens containing three or more penetrating ARVs are more effective in inhibiting viral replication in CSF.(De Luca *et al*, 2002; Letendre *et al*, 2004). Studies of brain VL in individuals taking these regimens may find similar results.

The findings reported here have important implications. Recent evidence suggests that viral proteins synthesized during active viral replication mediate

neuronal injury and apoptosis (Lipton, 1992; Nath, 2002; Xu *et al*, 2004). Furthermore, replicative brain infection may provoke neuronal injury indirectly by driving the production of host inflammatory mediators (Masliah *et al*, 2000). Thus, inhibition of viral replication by CNS-penetrating drugs is likely to be crucial for treating HIV-related brain disease. Future studies should address which agents or combinations optimally suppress viral replication in brain tissue. Ultimately, strategies for optimizing CNS viral inhibition may be important for treating HIV neurocognitive disorders and might help to prevent the development of ARV resistance.

Materials and methods

Subjects
Subjects included in the study consisted of a convenience sample assembled by the HIV Neurobehavioral Research Center (HNRC) and the California NeuroAIDS Tissue Network (CNTN). A major function of the HNRC/CNTN involves prospectively gathering antemortem and postmortem information for purposes of clinical–neuropathological correlation. The study was approved by the local Institutional Review Board and informed consent was obtained from all subjects. Subjects eligible for inclusion were enrolled consecutively between January 1, 1994, and December 31, 2002. Three subjects died before HAART became available and the remaining 58 were alive after HAART became available. To be included in analyses, cases were required to have both ART information and postmortem frontal cortex gray matter available for tissue VL measurement ($N = 61$) (Figure 2). Subjects were excluded if no ART information could be ascertained because at the end of life, the patients were cared for at home or in facilities that did not record treatment information. Antemortem plasma VL measurements also were available for some subjects (Figure 2).

Ascertainment of antiretroviral therapy (ART)
Information on ARV regimens was abstracted from research study visit records and from contacts with medical providers. HAART regimens were those that included three or more concurrently administered ARVs, including HIV protease inhibitors, nucleoside reverse transcriptase inhibitors, and non-nucleoside RTIs. HIV entry inhibitors were not available at the time of this study. Subjects in the HAART(+) group were those who had been prescribed a HAART regimen by their primary-care providers and for whom research and clinical records indicated adequate adherence to the regimen within 3 months of death.

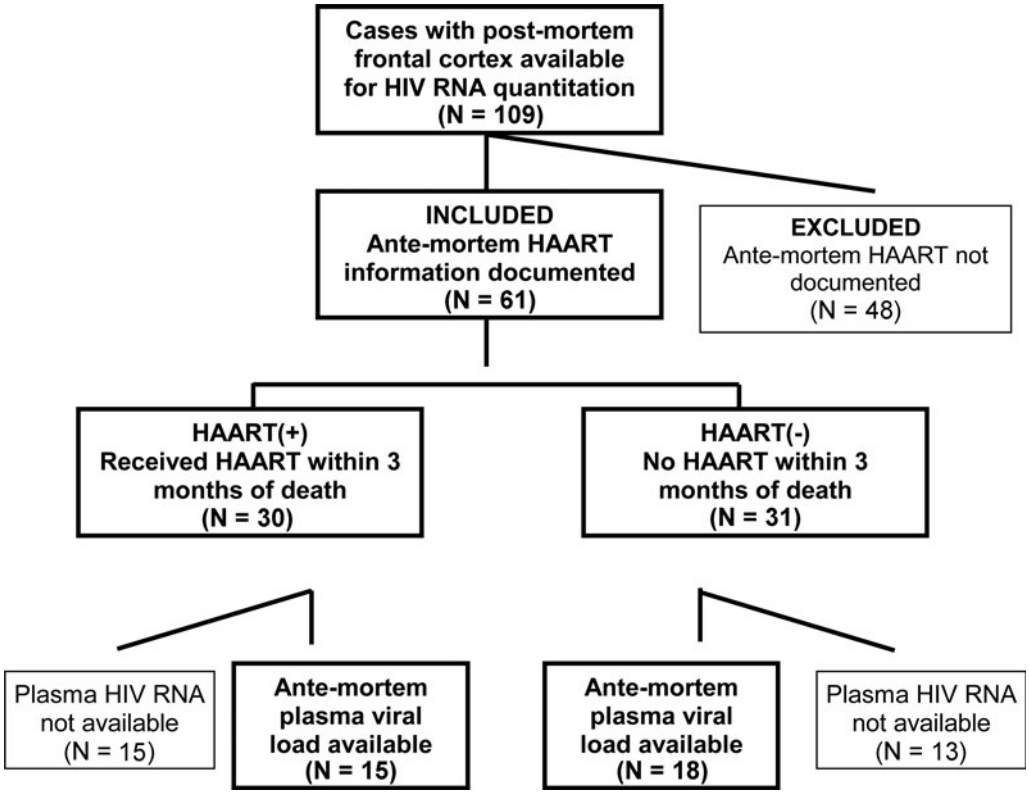

Figure 2 Breakdown of cases analyzed in this study (see text). Primary analyses were based upon a comparison of subjects in the HAART(+) and HAART(−) groups. Secondary analyses compared the subsets of subjects with available data on antemortem plasma viral load.

HAART(−) subjects were those for whom records documented that no ARVs were taken within 3 months of death or for those who took only one or two such drugs. The 3-month cutoff was chosen empirically, based on findings that, among individuals with chronic HIV infection who underwent open-ended treatment interruption, 80% had rebounded to plasma VL > 5000 copies/ml by 2 months and 100% by 3 months (Papasavvas *et al*, 2004).

For secondary analyses, CNS-penetrating ARVs were defined as those with CSF concentrations (defined as the median concentration in human CSF from published studies and drug manufacturer literature) that exceeded the susceptibility cut off as defined by the median 50% inhibitory concentration (IC_{50}) in the ViroLogic PhenoSense assay (Heilek-Snyder and Bean, 2002). Specifically, CNS-penetrating ARVs were stavudine, zidovudine, abacavir, efavirenz, nevirapine, and indinavir. The number of CSF-penetrating ARVs in each subject's regimen was counted for use in analyses.

Viral load determination

Total RNA was extracted from samples (approximately 200 mg each) of frozen frontal cortical gray matter, subcortical white matter, basal ganglia gray matter, and hippocampus by using the TRI reagent (Molecular Research Center, Cincinnati, OH). RNA was verified for integrity in an agarose gel by inspecting 18S and 28S ribosomal RNA bands; β-actin expression was used as an internal loading control (data not shown). Approximately 100 to 200 μg of total RNA were obtained, of which 20 μg were precipitated with 3M sodium acetate in 100% ethanol. Pellets were resuspended in RNase-free water and HIV RNA was quantified by RT-PCR using the Amplicor (Roche, Basel, Switzerland) ultrasensitive assay (limit of detection 50 or 1.70 $\log_{10}$ copies), and expressed as $\log_{10}$ copies per 10 μg total RNA. HIV RNA in antemortem plasma ($\log_{10}$ copies/ml) was quantified by using the Amplicor standard assay (limit of detection 400 or 2.6 $\log_{10}$ copies), as was the standard in clinical practice at the time of this study.

Neuropathological evaluation

Alternating thin sections of brain tissue were evaluated using conventional light microscopy after staining with hematoxylin-eosin and Luxol fast blue. To screen for HIV-related viral infections of the CNS such as Epstein-Barr virus (EBV), cytomegalovirus, JC virus (JCV), herpes simplex virus (HSV), human herpes virus (HHV), or varicella-zoster virus (VZV), genomic DNA was extracted and amplified in 35 cycles (93°C for 30 s, 50°C for 30 s, 72°C for 1 min) with a final extension at 72°C for 5 min. Primer sequences for EBV, CMV, JCV, and HSV 1 and 2, HHV 6 and 8, and VZV were obtained from published data as previously described (Langford *et al*, 2002). The quality of extracted DNA was assessed by amplification of the glutaraldehyde 3-phosphate dehydrogenase gene. Controls for PCR detection sensitivity included samples from AIDS patients with confirmed EBV, CMV, JCV, HSV, HHV, and VZV infections. Samples from non-HIV patients without evidence of OIs or brain alterations were used as negative controls.

Statistical analyses

All VLs were $\log_{10}$ transformed prior to analysis. Because the distribution of VL values in brain tissue samples and plasma deviated significantly from normal, group comparisons were made using the Wilcoxon method. Correlations between VLs were tested using Spearman's Rho. Group differences in gender, ethnicity, and AIDS diagnosis were assessed using contingency table analyses.

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