Journal of NeuroVirology, 15: 360–370, 2009

ISSN 1355-0284 print / 1538-2443 online
DOI: 10.3109/13550280903131915

informa
healthcare

Cliniconeuropathologic correlates of human immunodeficiency virus in the era of antiretroviral therapy

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The objective of this study was to examine the spectrum of human immunodeficiency virus (HIV) brain pathology and its clinical correlates in the antiretroviral era. We carried out a cross-sectional survey, analyzing prospective clinical and neuropathological data collected by the National NeuroAIDS Tissue Consortium (NNTC), comprising 589 brain samples from individuals with advanced HIV disease collected from 1999 onwards. We assessed gender, ethnicity/race, mode of transmission, age, year of death, nadir CD4, plasma viral load, last antiretroviral regimen, presence of parenchymal HIV brain pathology, HIV-associated neurocognitive disorder, and major depressive disorder. We compared cohort demographic variables with Centers for Disease Control and Prevention US HIV/AIDS statistics and examined associations of parenchymal HIV brain pathology with demographic, clinical, and HIV disease factors. With regard to Centers for Disease Control and Prevention US data, the NNTC was similar in age distribution, but had fewer females and African Americans and more Hispanics and men who have sex with men. Only 22% of the brains examined were neuropathologically normal. Opportunistic infections occurred in 1% to 5% of the cohort. Parenchymal HIV brain pathology was observed in 17.5% of the cohort and was associated with nadir CD4 and plasma viral load. Brains without parenchymal HIV brain pathology often had other noninfectious findings or minimal nondiagnostic abnormalities that were associated with HIV-associated neurocognitive disorder. Clinically, 60% of the cohort reported a lifetime episode of major depressive disorder and 88% had a HIV-associated neurocognitive disorder. No pathological finding correlated with major depressive disorder. Both antiretroviral treatment regimen and elevated plasma HIV viral load were associated with presence of parenchymal HIV brain pathology; however, multivariate analyses suggest a stronger association with plasma viral load. The frequency of HIV brain pathology was lower than previous pre-antiretroviral reports, and was predicted by lower nadir CD4 and higher plasma viral load. Noninfectious pathologies and minimal changes correlated with HIV-associated

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I.P.E. is supported by grants R01MH079881, R41MH079728, R25MH074508, R25MH081482, and P30 MH62512, R24MH59745, and U01MH083506. This publication was also made possible from NIH funding through the NIMH and NINDS Institutes by the following grants: Manhattan HIV Brain Bank (U01MH083501, R24MH59724), Texas NeuroAIDS Research Center (U01MH083507, R24NS45491), National Neurological AIDS Bank (U01MH083500, R24NS38841), California NeuroAIDS Tissue Network (U01MH083506, R24MH59745), Statistics and Data Coordinating Center (U01 MH083545-01). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NNTC or NIH. The authors report no conflicts of interest.

Received 12 November 2008; revised 26 January 2009; re-revised 15 May 2009; accepted 8 June 2009.

neurocognitive disorder, suggesting a shift in pathogenesis from florid HIV replication to other, diverse mechanisms. *Journal of NeuroVirology* (2009) **15**, 360–370.

Keywords: all infections; HIV dementia; risk factors in epidemiology

Introduction

The introduction and widespread use of efficacious antiretroviral therapy has tremendously influenced the natural history of the human immunodeficiency virus (HIV) infection. The frequency of central nervous system (CNS) opportunistic infections, which were accompanied with a high mortality, has decreased dramatically in countries where antiretroviral agents are available (Price and Spudich, 2008). Two clinical neuropsychiatric disorders remain prevalent following the introduction of antiretrovirals, the first is major depressive disorder (Bing *et al*, 2001) and the second is HIV-associated neurocognitive disorder (HAND) (Grant, 2008). Although the frequency of the most severe neurocognitive disorder, HIV-associated dementia, has reduced, there are still persistent milder forms of HIV-associated neurocognitive disorder even in those taking antiretrovirals (Ances and Ellis, 2007). The neuropathologic basis for milder forms of cognitive impairment is unclear, but may indicate that HIV-associated inflammatory and neurodegenerative pathology persists in the brain. Three prior autopsy-based studies with subjects from the antiretroviral era displayed variable percentages (15% to 25%) of HIV-related brain pathologies; in two, HIV encephalitis displayed equal frequency in therapeutic and nontherapeutic eras, in one, its frequency increased (Masliah *et al*, 2000, Morgello *et al*, 2002; Langford *et al*, 2003). In a comprehensive review comparing HIV neuropathology in the pre-antiretroviral and antiretroviral eras, Bell noted that the reported frequency of HIV encephalitis in the antiretroviral era was not much decreased when contrasted to investigations performed prior to the availability of antiretrovirals (Bell, 2004).

The variable frequency of HIV encephalitis in autopsy populations underscores another important consideration: how does autopsy neuropathology help to elucidate the clinical phenotypes relevant to diseased populations? The relationship of HIV encephalitis to cognitive impairment is somewhat unclear as earlier studies had indicated some correlation but that HIV encephalitis did not explain all HIV-associated dementia (Anthony and Bell, 2008; Glass *et al*, 1995). More recent investigations have observed that HIV-associated neurocognitive disorder prior to death has 92% specificity and 67% sensitivity in predicting HIV encephalitis at autopsy (Cherner *et al*, 2002), suggesting that, in some populations, HIV encephalitis may underlie the cognitive deficits that are frequently observed in HIV-infected individuals. By contrast, any potential contribution of HIV brain pathology to major depressive disorder has had very little attention, although it has been previously shown that major depressive disorder is associated with gene expression dysregulation (Everall *et al*, 2006).

In order to understand what is happening to primary HIV brain pathology in the antiretroviral era, we have analyzed brain samples derived from the National NeuroAIDS Tissue Consortium (NNTC), supported by the National Institutes of Health. The NNTC, established in 1998, recruits individuals with advanced HIV disease and who consent to an autopsy in order to collect, store, and distribute demographic, prospectively acquired clinical and biological data as well as nervous system tissue samples, cerebrospinal fluid, and blood. As of 2008, more than 2000 HIV-infected patients had been enrolled in NNTC centers and 589 autopsies had been performed. There were two aims to the current study: first to compare the demographic data of the NNTC cohort to the demographics of the cumulative 2001–2005 US population with acquired immunodeficiency syndrome (AIDS) deaths as described by the Centers for Disease Control and Prevention (CDC, 2007) to provide a picture of how representative the NNTC is of the national epidemic; and second to determine the frequency of parenchymal HIV brain pathology, which includes HIV encephalitis, HIV leukoencephalopathy, and microglial nodular encephalitis, and to ascertain if there are particular demographic, biological, and clinical characteristics of individuals with parenchymal HIV brain pathology.

Results

NNTC demographics and comparison to US AIDS epidemic

The demographic characteristics of the NNTC autopsy series as compared to US CDC data are reported in Table 1. We found that females were somewhat underrepresented in the NNTC series ($P<.001$), with 8% more females in the US epidemic. In terms of race/ethnicity, the NNTC cohort had 29% fewer Black Americans and 14% more Hispanics than reported in the US AIDS epidemic ($P<.001$). The composition of the main modes of transmission in the NNTC also differed somewhat from the US AIDS epidemic in having almost 10% more male-to-male sexual encounter, almost 9% less intravenous drug users (IDUs) and 8% fewer heterosexual sex ($P<.001$).

Table 1 Comparison of NNTC with Centers for Disease Control and Prevention AIDS demographics

	NNTC	US AIDS 2001–2005 deaths	Statistical significance (Pearson correlation coefficient)
Age			$P=.13$
<25	0.7%	1.5%	
25–34	9.0%	11.1%	
35–44	39.3%	37.3%	
45–54	33.6%	33.6%	
55–64	14.0%	12.0%	
≥65	3.5%	4.5%	
Sex			$P<.001$
Male	82.8%	74.6%	
Female	17.2%	25.4%	
Race/ethnicity			$P<.001$
White, Not Hispanic	43.3%	30.6%	
Black, Not Hispanic	23.9%	53.3%	
Hispanic	29.2%	15.0%	
Other	3.6%	1.1%	
Mode of transmission			$P<.001$
M2M	45.1%	35.5%	
IDU	23.5%	32.1%	
M2M + IDU	8.6%	7.8%	
Heterosexual	15.0%	23.2%	
Other	7.8%	1.3%	

Note. Comparison of the age distribution, sex, race/ethnicity, and mode of transmission in the NNTC cohort with the data provided by the Centers for Disease Control and Prevention on US AIDS deaths 2001–2005. There were statistically significant differences between the two cohorts with regard to sex, race/ethnicity, and mode of transmission.
IDU = intravenous drug user; M2M = male-to-male sexual encounter.

NNTC demographics and the presence of parenchymal HIV brain pathology

The most frequently observed HIV brain pathology was HIV encephalitis, which was noted in 66 (11%) of cases in the entire cohort, followed by microglial nodular encephalitis and aseptic leptomenningitis, which each affected 29 (5%) cases, and HIV leukoencephalopathy was reported in 14 (2%) cases. Six cases of parenchymal HIV brain pathology had more than one of these diagnoses (five cases had HIV encephalitis with HIV leukoencephalopathy and one case had HIV encephalitis with microglial nodular encephalitis). Parenchymal HIV brain pathology (i.e., HIV encephalitis, leukoencephalopathy, or microglial nodular encephalitis) was present in 17% (103/589) of the cases.

Table 2 displays the demographic features of the study participants. Of the 589 subjects, 392 had further clinical and antiretroviral treatment data collected during the longitudinal follow-up. Parenchymal HIV brain pathology was associated with on-study antiretroviral regimen ($P=.026$), CD4 nadir during follow-up ($P=.003$), and last plasma viral load ($P<.001$); it was not associated with age, sex, race/ethnicity, mode of HIV transmission, duration of infection, status at study entry (alive versus autopsy only), year of death, length of follow-up, neurocognitive impairment, or depression (Table 2). Neuropsychological diagnoses ($n=336$) and major depressive disorder diagnoses ($n=264$) were only available on a subset of cases and did not differ between those with and without parenchymal HIV brain pathology.

Neuropathology and antiretrovirals

The influence of antiretroviral regimen was analyzed in several ways in order to account for the complexity of the data, such as that attributable to regimen changes over time, estimated neuroeffectiveness of antiretrovirals (Letendre, 2007), differences in study entry relative to disease stage, variable durations of observation, mode of transmission, and CD4 increase (defined as the difference between reported nadir and observed highest CD4 count). The on-study antiretroviral regimen for a study participant was defined to include all drugs recorded as part of the current or past regimen, at one or more of the study visits.

Univariate analysis

The presence of parenchymal HIV brain pathology differed by the type of on-study antiretroviral regimen ($P=.026$; Table 2), from 27% (34/124) among those not taking antiretrovirals, to 22% (25/112) for those with exposure to a protease inhibitor and nucleoside inhibitor regimen, 17% (4/24) for those exposed only to nucleoside reverse transcriptase inhibitors, 12% (10/85) for those exposed to a triple-class regimen, and 11% (5/47) for those on a non-nucleoside reverse transcriptase inhibitor and nucleoside reverse transcriptase inhibitors. Those with parenchymal HIV brain pathology had significantly fewer drugs in the cumulative regimen as compared to those without ($P=.020$); however, a larger proportion of individuals with parenchymal HIV brain pathology had received no antiretroviral treatment during the study period ($P=.014$).

Table 2 NNTC cohort demographics with and without HIV brain pathology

	Overall ($N=589$)	Parenchymal HIV brain pathology ($N=103$)	No parenchymal HIV brain pathology ($N=486$)	P value
Age at death				.65 (WRT)
median (IQR)	45 (39, 51)	44 (39, 53)	45 (39, 51)	
Sex (M, F)				.62 (FET)
Females	101 (17%)	24 (23%)	77 (16%)	
Males	488 (83%)	79 (77%)	409 (84%)	
Race/ethnicity				.83 (FET)
White	255 (43%)	43 (42%)	212 (44%)	
Black	141 (24%)	24 (23%)	117 (24%)	
Hispanic	172 (29%)	31 (30%)	141 (29%)	
Unknown/other	21 (4%)	5 (5%)	16 (3%)	
Mode of transmission				.58 (FET)
IDU	165 (28%)	28 (27%)	137 (28%)	
M2M	232 (39%)	43 (41%)	189 (39%)	
Heterosexual	77 (13%)	10 (10%)	67 (14%)	
Blood prod rec.	20 (3%)	6 (6%)	14 (3%)	
Other	20 (3%)	6 (6%)	14 (3%)	
Unknown	75 (13%)	12 (11%)	63 (13%)	
Duration of infection (years)				
median (IQR)	11 (7, 15)	11 (6, 14)	11 (7, 15)	.67 (WRT)
Alive at study entry				.24 (FET)
Yes	405 (69%)	76 (74%)	329 (68%)	
No (autopsy only)	184 (31%)	27 (26%)	157 (32%)	
Year of death (1998–2007)				.89 (WRT)
median (IQR)	2002 (2000, 2004)	2002 (2000, 2004)	2002 (2000, 2005)	
Length of study follow-up (months)				.10 (WRT)
(N = 405, excludes autopsy only patients)				
median (IQR)	3 (0, 15)	8 (0, 20)	2 (0, 15)	
CD4 Nadir during follow-up ($N=395$)				.003 (WRT)
Median (IQR)	37 (7,142)	15 (5, 68)	48 (8, 148)	
Last log viral load within 6 mo. Of death (N = 371)				
Median (IQR)	4.5 (2.6, 5.4)	5.3 (4.3, 5.8)	4.2 (≤2.6, 5.2)	<.001 (GWRT)
On study ARV regimens ($N=392$)				.026 (FET)
PI/NRTI	112 (29%)	25 (32%)	87 (28%)	
PI/NNRTI/NRTI	85 (22%)	10 (13%)	75 (24%)	
NNRTI/NRTI	47 (12%)	5 (6%)	42 (13%)	
NRTI only	24 (6%)	4 (5%)	20 (6%)	
None	124 (32%)	34 (44%)	90 (29%)	
Neurocognitive impairment ($N=336$)				.71 (FET)
NP–normal	41 (12%)	5 (8%)	36 (13%)	
Asymptomatic NPI	18 (5%)	4 (6%)	14 (5%)	
Minor NCD	71 (21%)	16 (24%)	55 (20%)	
HAD	75 (22%)	16 (24%)	59 (22%)	
NPI–other	131 (39%)	25 (38%)	106 (39%)	
Depression ($N=264$)				.35 (FET)
MDD during study	88 (33%)	13 (25%)	75 (35%)	
MDD in the past	72 (27%)	14 (27%)	58 (27%)	
No MDD	96 (40%)	24 (48%)	80 (38%)	

Note. A summary of the demographic variables for the groups with and without HIV brain pathology. These include age at death, sex, race/ethnicity, mode of transmission, duration of infection, alive at study entry, year of death and length of study follow-up, CD4 nadir during follow-up, last viral load within 6 months of death, on study antiretroviral regimen, presence of neurocognitive impairment, and presence of major depressive disorder. Of these variables, only the on study antiretroviral regimen was significantly different between the two groups.

ARV = antiretroviral; IDU = intravenous drug user; M2M = male-to-male sexual encounter; IQR = interquartile range; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-NRTI; PI = protease inhibitor; NP = neuropsychological; NPI = neuropsychologically impaired; MCMD = minor cognitive motor disorder; HAD = HIV-associated dementia; MDD = major depressive disorder.

Multivariate analysis of parenchymal HIV brain pathology

An initial analysis including all 392 patients and predicting the presence or absence of parenchymal HIV brain pathology revealed that, when controlling for antiretroviral class exposure, neither the length of follow-up ($P=.093$), the number of drugs taken ($P=.81$), or the CNS penetration-effectiveness rank ($P=.13$) were statistically significant.

In a subset analysis ($N=222$) for which the nadir CD4 and last plasma viral load were available, after

controlling for last plasma viral load ($P<.001$), antiretroviral exposure was no longer significant ($P=.38$). The subgroup of $N=222$ subjects was comparable with the overall sample in terms of frequency of parenchymal HIV brain pathology, antiretroviral exposure, age, mode of transmission, number of drugs in regimen, and penetration score. However, the subgroup differed significantly in race/ethnicity from those not included (fewer African American subjects, 19% versus 24% respectively; $P=.027$), gender (fewer females, 14% versus 18%; $P=.012$), and length of follow-up (shorter follow-up, 8 versus 9 months median; $P=.030$). A further subset analysis including CD4 increase ($N=89$) revealed a similar consistent effect of viral load.

The interaction of parenchymal HIV brain pathology, antiretroviral exposure, and viral load is shown in Figure 1. In examining the range of these receiver operator characteristic curves (ROCs) (less than 2.83, between 2.84 and 4.69, and above 4.70), the higher the blood HIV RNA level at the last clinic visit prior to death, the larger the proportion having parenchymal HIV brain pathology. Of note, the proportion of individuals who took non-nucleoside reverse transcriptase inhibitor–containing antiretroviral regimens and had parenchymal HIV brain pathology was small across all HIV RNA strata. These small proportions may have limited the power of our analyses to distinguish the relationships between HIV RNA levels, non-nucleoside reverse transcriptase inhibitor use, and parenchymal HIV brain pathology.

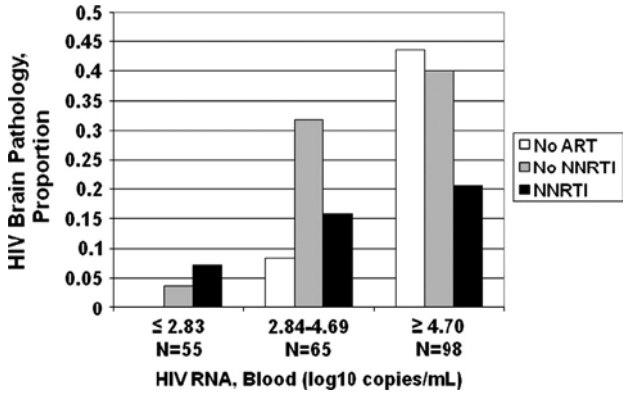

Figure 1 This figure Demonstrates the frequency of HIV brain pathology depending on viral load for those taking and not taking non-nucleoside reverse transcriptase inhibitors. HIV RNA thresholds used in this figure were selected using receiver operator characteristic curves (ROC). The calculated ROC of the log viral load for those individuals not receiving antiretroviral at last study visit was 4.7, whereas for those on antiretroviral it was 2.83. Subjects with a log viral load less than 2.83 ($n=55$) had a frequency of HIV brain pathology of 4.8%, those individuals with a log viral load between 2.84 and 4.69 ($n=65$) had an 18.5% of HIV brain pathology, whereas those whose log viral load was above 4.70 ($n=98$) suffered a 34.7% frequency of HIV brain pathology.

Neuropathological findings in the NNTC cohort

As shown in Table 3, 17.5% (103/589) had parenchymal HIV brain pathology. Only 22% (127/589) of brains were reported on neuropathological examination to be normal. Some of the most frequent co-occurring opportunistic infections were cytomegalovirus (CMV) encephalitis, cryptococcus, and progressive multifocal leukoencephalopathy (PML). None of the observed opportunistic infections were more common among those with or without parenchymal HIV brain pathology. Findings that did differ between the parenchymal HIV brain pathology and no parenchymal HIV brain pathology groups were as follows: (1) cerebral lymphoma was more common among those with parenchymal HIV brain pathology; (2) parenchymal hemorrhage was was significantly more common in the group with parenchymal HIV brain pathology; (3) other noninfectious pathology was less common in the parenchymal HIV brain pathology; and (4) minimal nondiagnostic abnormalities were also less frequently observed in the parenchymal HIV brain pathology group. Other pathological findings did not differ between the parenchymal HIV brain pathology and no-parenchymal HIV brain pathology groups. Of note, it was observed that 12% of the heterosexual and 15% of the intravenous drug user transmission groups had focal territorial infarcts, whereas only 4% of the male-to-male sexual encounter had such a pathological finding ($P=.001$); no other vascular pathology varied between transmission groups.

HIV-associated neurocogntive disorder and parenchymal HIV brain pathology

HIV-associated neurocognitive disorder did not relate significantly to the presence or absence of parenchymal HIV brain pathology, but it was noted that there were statistically significant correlations with Alzheimer's type II gliosis and minimal nondiagnostic abnormality. The relationship of Alzheimer's type II gliosis to the HIV-associated neurocognitive disorder spectrum was just significant when neuropsychological impairment-other was included ($P=.050$), and it became more significant when neuropsychological impairment-other was excluded ($P=.027$). Whereas the frequency of Alzheimer's type II gliosis ranged from 8% to 11% in the NP–normal, asymptomatic neuropsychological impairment, and minor cognitive motor disorder groups, it was 25% in the cases with HIV-associated dementia. By contrast, the minimal nondiagnostic brain abnormality was only significantly related to the HIV-associated neurocognitive disorder spectrum of disorders if neuropsychological impairment–other was included ($P<.001$) where it appeared to occur less often in the NP–normal and neuropsychological impairment–other groups, while having almost double the frequency in the asymptomatic

Table 3 Clinical neuropathological findings in the NNTC cohort

	Overall ($N=589$)	HIV brain pathology ($N=103$)	No HIV brain pathology ($N=486$)	P-value
Normal brain	127/589 (22%)	0/103 (0%)	127/486 (26%)	
Opportunistic infections				
CMV encephalitis	25 (4%)	7 (7%)	18 (4%)	0.18 FET
Cryptococcus	23 (4%)	4 (4%)	19 (4%)	1.0 FET
PML	28 (5%)	3 (3%)	25 (5%)	0.45 FET
Bacterial leptomeningitis	7 (1%)	0 (0%)	7 (1%)	0.61 FET
Bacterial Parenchymal Infection	7 (1%)	1 (1%)	6 (1%)	1.0 FET
Toxoplasmosis–active	8 (1%)	2 (2%)	6 (1%)	0.63 FET
Toxoplasmosis–healed	6 (1%)	3 (3%)	3 (1%)	0.07 FET
Tuberculosis	6 (1%)	0 (0%)	6 (1%)	0.6 FET
Other infectious pathologies	32 (5%)	4 (4%)	28 (6%)	0.63 FET
Neoplasia				
Cerebral lymphoma	30 (5%)	10 (10%)	20 (4%)	**0.026 FET**
Vascular pathology				
Focal (territorial) infarction	49 (8%)	5 (5%)	44 (9%)	0.24 FET
Hemorrhage—dura or leptomeninges	18 (3%)	3 (3%)	44 (3%)	1.0 FET
Hemorrhage parenchymal	26 (4%)	10 (10%)	17 (3%)	**0.031 FET**
Other histological findings				
Aseptic leptomeningitis	29 (5%)	9 (9%)	20 (4%)	0.07 FET
Anoxic-ischemic encephalopathy	27 (5%)	6 (6%)	21 (4%)	0.45 FET
Alzheimer type II gliosis	88 (15%)	17 (17%)	71 (15%)	0.65 FET
Other Noninfectious pathology	115 (20%)	10 (10%)	105 (22%)	**0.006 FET**
Minimal Nondiagnostic abnormality	69 (12%)	1 (1%)	68 (14%)	**<0.001 FET**

Note. Describes the opportunistic infections, cerebral lymphoma, and other histological findings noted in the NNTC cohort. The percentages in the HIV brain pathology and no HIV brain pathology columns refer to the percentage of that group with the particular lesion listed.
CMV = cytomegalovirus; PML = progressive multifocal leukoencephalopathy (Data in bold is abbreviation).

neuropsychological impairment, minor cognitive motor disorder, and HIV-associated dementia groups. There was no association between Alzheimer's type II gliosis or minimal nondiagnostic abnormality with major depressive disorder.

Last recorded antiretroviral regimen

In an alternative analysis, the last recorded antiretroviral regimen at the study visit prior to death did not correlate with the presence of parenchymal HIV brain pathology ($P=.098$), Alzheimer type II gliosis ($P=.085$), other noninfectious pathology ($P=.87$), minimal nondiagnostic abnormalities ($P=.67$), or clinically with either major depressive disorder during the study ($P=.97$) or past major depressive disorder ($P=.082$).

Discussion

In this study of 589 brain samples, NNTC-standardized clinical neuropathology examination was correlated with prospectively acquired uniform clinical data obtained during life. We noted that in the entire cohort only 22% of the brains were neuropathologically normal. Parenchymal, as opposed to meningeal, HIV brain pathology (i.e., HIVencephalitis, HIV leukoencephalopathy, and microglial nodular encephalitis) was observed in 17% of the cases. Parenchymal HIV brain pathology appeared to be predicted by only two clinical variables: a lower CD4 nadir during the study period and a higher log plasma viral load within 6 months of death.

By their very nature, autopsy studies tend to be biased and the NNTC cohort may be no exception, especially as it targets individuals who have advanced HIV disease and who agree to an autopsy. However, previous HIV-related autopsy studies will also suffer the same inherent bias and we have attempted to address this issue in the current study by comparing the demographics of the NNTC to the demographics listed in the Centers for Disease Control and Prevention reported AIDS death for 2001 to 2005. In comparison to the notified 13,511 AIDS deaths in the United States from 2001 to 2005 in the Centers for Disease Control and Prevention report (CDC, 2007), the NNTC cohort accounted for 4.4% of these deaths. The 4.4% of cases in the NNTC cohort is a similar percentage to the 6.6% of notified AIDS deaths in the Davies *et al* (Davies *et al*, 1997) UK epidemiological study. The NNTC had a similar age range of deaths but differed significantly in terms of gender, race/ethnicity, and mode of transmission. Although we cannot conclude that the NNTC cohort is statistically the same as the US AIDS epidemic data for 2001–2005, we note that, apart from the fewer African Americans in the NNTC, the other differences percentagewise were small. Thus, with

regard to neuroepidemiology of HIV in the United States in the antiretroviral era, considering that a large cliniconeuropathological HIV study may not occur again soon, this data set may be the most representative assessment of what is happening in the brains of individuals with advanced HIV disease. For comparison to previous published autopsy studies, our frequency of 11% for HIV encephalitis is lower than the 25% reported by Davies *et al* (Davies *et al*, 1997), Masliah *et al* (Masliah *et al*, 2000), and others (Budka, 1991; Budka *et al*, 1991), but more comparable to series with greater representation of minorities (Morgello *et al*, 2002). In addition, we were not able to replicate the 5-fold increase in HIV encephalitis in IDUs in the Davies *et al* study (Davies *et al*, 1997), which were mainly represented by injecting heroin users in Edinburgh as previously described (Bell *et al*, 1996). In the NNTC cohort, the frequency of opportunistic infections and cerebral lymphoma was far less than reported by Davies *et al* in the United Kingdom in the pre-antiretroviral era. For example, in the NNTC, we observed that 4% had cytomegalovirus brain infection and 5% had cerebral lymphoma, whereas in the Davies *et al* (Davies *et al*, 1997) study, these two diseases were 18% and 19%, respectively. By contrast, vascular pathology was similar in the two studies, 3% to 8% of the NNTC cohort and 5% in the UK study.

With regard to the potential effects of antiretroviral regimen on parenchymal HIV brain pathology, we were hampered by not having detailed information on the duration of treatment or dosage with particular antiretrovirals apart from the medication regimen recorded at study visit. When assessing the on-study antiretroviral regimen, we noted that individuals who were untreated prior to death or who had taken a protease inhibitor and a nucleoside reverse transcriptase inhibitor had a similar prevalence of parenchymal HIV brain pathology, consistent with previously published estimates. However, the risk was more than halved when the antiretroviral exposure included a non-nucleoside reverse transcriptase inhibitor and a nucleoside reverse transcriptase inhibitor, with or without a protease inhibitor. This finding was not replicated in a multivariate analysis that reported that last log plasma viral load and nadir CD4 were the significant predictors of parenchymal HIV brain pathology. Because HIV RNA levels in blood were measured at the last time prior to death, whereas the class of antiretroviral exposure was cumulative over the study period, it is possible that univariate observation of a specific effect of non-nucleoside reverse transcriptase inhibitor exposure on parenchymal HIV brain pathology may be mediated via viral load. However, as shown in Figure 1, those individuals taking non-nucleoside reverse transcriptase inhibitor as compared to those not taking non-nucleoside reverse transcriptase inhibitor had lower proportions of parenchymal HIV brain pathology at all levels of last log plasma viral load. Despite the overall large sample size for a clinically well-characterized HIV autopsy study, we were still not able to conclude whether the effect of non-nucleoside reverse transcriptase inhibitors was mediated by a reduction in blood viral load, by a more specific reduction in cerebrospinal fluid (CSF) viral load, or by another unclear mechanism. Although our study was not powered to distinguish the effect of individual non-nucleoside reverse transcriptase inhibitors, animal studies indicate that nevirapine can accumulate in the CNS at levels higher than other antiretrovirals (Gibbs *et al*, 2006). Non-nucleoside reverse transcriptase inhibitors are indicated as effective in treating HIV-related neurocognitive disorders (Arendt, 2002), are proposed to penetrate into the brain more readily than protease inhibitors (Thomas, 2004), and may result in HIV RNA levels in CSF that are less frequently detectable compared with other antiretroviral regimens (Letendre *et al*, 2008).

We also considered the potential relationship between neuropathology and major depressive disorder. There was no identified pathological variable that predicted major depressive disorder. This may be because the major depressive disorder in the setting of HIV is not associated with morphological pathological changes but rather gene expression dysregulation (Everall *et al*, 2006), or it may be that there is not specific HIV-associated organic major depressive disorder (Judd *et al*, 2005). To date, our understanding of the elevated rates of major depressive disorder in the HIV-infected population (Bing *et al*, 2001) and the underlying pathophysiology is very rudimentary.

There was also no observed relationship between parenchymal HIV brain pathology and HIV-associated neurocognitive disorder, which is in contrast to earlier publications demonstrating the predictive power of HIV-associated neurocognitive disorder for HIV encephalitis (Cherner *et al*, 2002). Some of the discrepancies may be explained by differences in cohort demographic composition and the introduction of antiretrovirals, and as stated earlier, by the increasing prevalence of co-morbid medical illnesses in HIV-infected populations. Additional difficulty of finding an association may be related to the fact that majority of individuals in the advanced disease NNTC cohort (82%) had documented HIV-associated neurocognitive disorder (Grant, 2008) as compared to a estimates of approximately 30% in a clinical HIV population (Carey *et al*, 2004). HIV-associated neurocognitive disorder was, however, associated with Alzheimer type II gliosis and minimal nondiagnostic abnormalities, but these findings were based on a small subsample. These observations may be added to the known neuropathological changes noted in the advanced disease population with HIV-associated neurocognitive disorder where there can be a combination of irreversible brain changes such as

neuronal loss (Everall *et al*, 1991) and synaptodendritic damage (Everall *et al*, 1999; Masliah *et al*, 1997; Moore *et al*, 2006). The association of Alzheimer type II gliosis raises the possibility that with efficacious treatment of HIV, other, non–HIV-related pathologies may be responsible for the observed clinical phenotype (cognitive impairment), and that great care must be rendered in the clinical assignment of deficits to HIV. As individuals with HIV infection live longer lives, comorbid medical conditions will achieve greater prominence in HIV-infected populations and correlations of clinical phenotypes with autopsy data may assist in understanding which pathogenetic mechanisms are most relevant to the diverse populations under study.

In summary, we have noted a decline in the frequency of parenchymal HIV brain pathology even in the NNTC cohort with advanced HIV disease and high levels of varying degrees of HIV-associated neurocognitive disorder. Both a lower nadir CD4 and a higher log plasma viral load within six months of death predict parenchymal HIV brain pathology at autopsy. Non-nucleoside reverse transcriptase inhibitors may have a specific protective effect against parenchymal HIV brain pathology but this may equally be mediated via reduction in viral load. Neuropathologically, parenchymal HIV brain pathology was associated with parenchymal hemorrhage, other noninfectious pathology, and cerebral lymphoma. Not mode of transmission, age, or gender was associated with parenchymal HIV brain pathology. In those cases without parenchymal HIV brain pathology, there was an excess of nonspecific histological findings. Neither HIV-associated neurocognitive disorder nor major depressive disorder was associated with parenchymal HIV brain pathology, but HIV-associated neurocognitive disorder was associated with Alzheimer type II gliosis and minimal nondiagnostic abnormalities. As the NNTC cohort continues to increase in size, observations such as the potential effect of antiretrovirals on HIV brain pathology, both parenchymal and meningeal, and the contribution of comorbid medical illness to cognitive impairments, may be resolved.

Materials and methods

Demographic, clinical, and biological information for the 589 HIV-infected autopsy cases, collected since 1999, were made available through the NNTC National Coordinating Office for the four participating sites: Texas NeuroAIDS Research Center (TNRC) at the University of Texas Medical Branch, Galveston; National Neurological AIDS Bank (NNAB) at the University of California, Los Angles; California NeuroAIDS Tissue Network (CNTN) at the University of California, San Diego; and Manhattan HIV Brain Bank (MHBB) at The Mount Sinai Medical Center, New York City. Patient enrollment, data collection, tissue sampling protocols, and diagnostic criteria have been described previously (Morgello *et al*, 2001). All demographic and clinical information is collected prospectively during life when subjects complete in-person study visits. In order to maintain standardized diagnostic criteria and uniform categorization of study subjects among NNTC sites, quality assurance (QA) protocols have been established in all the major disciplines involved in the study (neuropsychology, neurology, psychiatry, and neuropathology). The neuropathology QA program consisted of a peer-review process and histological categorization of the diagnostic material according to a new system established by NNTC (Morgello *et al*, 2001). The neuropathologists from the participating NNTC sites hold quarterly conference calls to discuss cases with significant diagnostic variation from site to site.

The demographic information

The demographic information included age at death, gender, ethnicity (Hispanic/Latino or not), race (white, black/African-American, Asian, native Alaskan/American Indian, native Hawaiian/Pacific islander), and mode of exposure. The duration of the infection was calculated using the reported date of HIV diagnosis and the date of the death. The mode of exposure was identified as male-to-male sexual encounter, intravenous drug users, heterosexual sexual transmission, blood product recipient, other, and unknown mode of transmission. For subjects with more than one modes of transmission, the allocation was made to the first in the following priority order: intravenous drug user < blood products recipient < male-to-male sexual encounter < heterosexual < other < unknown.

Clinical information

Clinical evaluation encompassed the DSM IV criteria for neurobehavioral and psychiatric disorders, drug abuse, and routine diagnoses of other CNS-related opportunistic infections and lymphoma. The methods of neurocognitive evaluation have been described previously (Woods *et al*, 2004). These included evaluation by neuropsychological battery of tests, including the learning, memory, attention, speed of information processing, abstraction, and verbal and motor skills. HIV-associated neurocognitive disorder diagnoses were assigned based on a comprehensive neuropsychological and neuromedical evaluations categorized as the absence of neurocognitive disorder (neuropsychologically normal), asymptomatic neuropsychological impairment, minor cognitive motor disorder, HIV-associated dementia, and neuropsychological impairment due to factors other than HIV or of unknown cause (neuropsychological impairment–other). Substance use and psychiatric data were obtained by the Psychiatric Research Interview for Substance and Mental Disorders or the Composite

International Diagnostic Interview (Morgello *et al*, 2006; Wittchen *et al*, 1991). Major depressive disorder was classified as either an episode of major depressive disorder during study period, an episode of major depressive disorder prior to entering study, or no major depressive disorder. With regard to biomarker information, the most recent data from the study visit prior to death for the plasma CD4 count and log plasma viral load was included in the analysis. Nadir CD4 was defined as the lowest CD4 recorded during the study period or available to the study at baseline.

Antiretroviral therapy
The information regarding antiretroviral therapy was obtained by professional interview or chart review. Therapies were categorized by the general class of drugs in the regimen: protease inhibitor; nucleoside reverse transcriptase inhibitor, or non-nucleoside reverse transcriptase inhibitor. For the current study, we assessed both the cumulative combinations of all on study antiretroviral regimens, as well as the final reported regimen at the last study visit prior to death.

Clinical neuropathological findings
The clinical neuropathological diagnoses were made by board certified pathologists at each participating site. As stated above there are QA protocols to ensure standardization of diagnoses. The histological findings were classified into the following groups:

1. Parenchymal HIV-associated brain pathology: This category included postmortem brains with a diagnosis of HIV encephalitis, HIV leukoencephalopathy, microglial nodular encephalitis, diffuse poliodystrophy, and aseptic leptomeningitis. Diagnostic criteria for HIV encephalitis were defined according to the 1991 consensus report (Budka *et al*, 1991), including multiple disseminated foci of microgliosis, astrocytosis, and presence of multinucleated giant cells and can include immunohistochemistry for HIV p24 antigen (Morgello *et al,* 2001). HIV leukoencephalopathy was defined as myelin loss, microgliosis, and astrocytosis with or without giant cells and absence of marked inflammatory infiltrate. With respect to spectrum of HIV pathology in the CNS tissue (Budka *et al*, 1991), microglial nodular encephalitis was included in HIV brain pathology if no secondary cause such as cytomegalovirus could be identified. In our analysis, we grouped parenchymal HIV brain pathology (HIV encephalitis, leukoencephalopathy, and microglial nodular encephalitis) together for demographic and clinical correlations analysis. The rational for this approach was that the group was mainly comprised of HIV encephalitis and there were too few cases of either HIV leukoencephalopathy or microglial nodular encephalitis to allow their separate analysis. Similarly, we only undertook preliminary analysis of aseptic leptomenningitis because of the small number of cases with this condition. Of note diffuse poliodystrophy was not recorded in this cohort.
2. No parenchymal HIV-associated brain pathology: This group was the HIV-infected cases without HIV brain pathology: as defined for group 1.
3. Secondary brain pathology: This group consisted of opportunistic infections and other pathologies, such as non-Hodgkin's lymphoma, that occurred secondarily to immune suppression as a result of HIV infection, such as cytomegalovirus, cryptococcus, and progressive multifocal leukoencephalopathy.
4. Other histological findings: In this category were vascular pathology, Alzheimer type II gliosis, other noninfectious pathology, and minimal non-diagnostic abnormality.

Statistical analysis
The summary of the demographic characteristics was reported as the median and interquartile range (IQR) for the continuous variables, and as group percentages for the categorical variables. The comparison with the larger US HIV/AIDS epidemic 2001–2005 was based on the CDC and Prevention HIV/AIDS Surveillance Report (CDC, 2007), and it used Pearson's chi-square test (Table 1). The association of the demographic features (Table 2) and of the clinical neuropathological findings (Table 3) with parenchymal HIV brain pathology was studied using Fisher's exact test for categorical variables, and with Wilcoxon's rank-sum test for the continuous variables. Plasma HIV-1 RNA $\log_{10}$ viral load was compared between groups using the Gehan-Wilcoxon rank test, in order to account for censoring below the limit of detection of the assay. The association of the antiretroviral exposure and parenchymal HIV brain pathology was further analyzed with univariate and multivariate logistic regression. In the multivariate analysis, the variables significant at the .15 level in the univariate analysis were included. An automatic stepwise model selection procedure using the Akaike information criterion was used, followed by likelihood-ratio testing.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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