HIV protease inhibitor exposure predicts cerebral small vessel disease

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Objective: HIV-associated neurocognitive disorders (HANDs) remain prevalent in patients who receive HAART and may be associated with cumulative exposure to antiretroviral medications and other factors. We proposed that chronic toxic effects of antiretroviral drugs could contribute to cerebral small vessel disease (CSVD), which might be one of the key underpinnings of HAND.

Design: Clinicopathological cross-sectional study of HIV-infected adults in the California NeuroAIDS Tissue Network.

Methods: We employed multivariable logistic regression methods to determine associations between HAART exposure (protease inhibitor-based, nonprotease inhibitor-based, or no HAART) and CSVD occurrence (standard histopathology: moderate/severe, mild, or absent). We also associated HAND (relative to normal cognition) with CSVD, HIV-related neuropathologic changes, older age at death (≥ 50 years), sex, or hepatitis C virus infection.

Results: We found that both mild and moderate/severe CSVD were associated with protease inhibitor-based HAART exposure after adjusting for diabetes mellitus [odds ratio (OR) 2.8 (95% confidence interval, CI 1.03–7.9) and 2.6 (95% CI 1.03–6.7), respectively, $n = 134$]. Moderate/severe CSVD was associated with diabetes after adjusting for HAART exposure [OR 7.4 (95% CI 1.6–70.7), $n = 134$]. Notably, HAND was associated with mild CSVD [OR 4.8 (95% CI 1.1–21.2), $n = 63$], which remained statistically significant after adjusting for vessel mineralization, HIV encephalitis, microglial nodular lesions, white matter lesions, or older age.

Conclusion: Protease inhibitor-based HAART exposure may increase the risk of CSVD and thereby neurocognitive impairment in HIV-infected adults. Apart from the possible direct toxicity to cerebral small vessels, protease inhibitor-based HAART may contribute indirectly to CSVD by inducing metabolic abnormalities.

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Introduction

The benefit of HAART for preventing HIV-associated dementia (HAD), the severest form of HIV-associated neurocognitive disorders (HANDs), is well documented [1]. Nonetheless, the milder forms of HAND, asymptomatic neurocognitive impairment (ANI) and mild neurocognitive disorder (MND), remain prevalent

[2,3]. HAD in the pre-HAART era occurred primarily in late stages of AIDS and was found to correlate with HIV encephalitis denoting robust HIV-1 replication [4,5] and microglial activation [6] with aberrant cytokine expression [7] in the brain. In contrast, HAND in the current HAART era can affect even patients with low plasma viral loads and high CD4$^+$ cell counts [3,8,9], and more variability in the clinical course has been observed [3,10]. Recent autopsy studies suggest that HAND is not necessarily associated with HIV encephalitis [11–13] and may be related to various comorbid factors such as cumulative exposure to antiretroviral medications [14], aging-related cerebrovascular and neurodegenerative changes [15,16], coinfection with hepatitis C virus (HCV), and substance use [17,18].

Previous studies suggest that HIV-infected patients are at increased risk of ischemic cerebrovascular disease, potentially caused by infective vasculitis, brain opportunistic diseases, cardiac embolism, hypercoagulopathy, or HIV infection itself [19–26]. Among a variety of brain vessel diseases found in this context [25], cerebral small vessel disease (CSVD) has been associated with ischemic stroke during life [21] and cerebral infarction at autopsy [23]. In patients receiving HAART, particular antiretroviral drugs may directly cause injury to vessel walls or indirectly induce metabolic abnormalities (e.g. dyslipidemia and insulin resistance) that accelerate the development of atherosclerotic large vessel disease and thereby myocardial infarction [27,28]. Nonetheless, the potential impact of HAART exposure on the development of ischemic cerebrovascular disease, including CSVD, remains controversial [20,21,24,25,29–31]. In a community-based study [32], the presence of punctate white matter lesions (hyperintensities) on MRI was found to be associated with older age and higher SBP moreover, there was a trend toward the direct association between white matter lesions and HAART exposure. Whereas in the general population MRI white matter lesions are thought to represent ischemic lesions caused by CSVD [33], the similar lesions in HIV-infected patients may also reflect foci of HIV-associated or inflammatory white matter injury [34–37]. To our knowledge, in the literature there were no reports that specifically addressed CSVD occurrence in the post-mortem brain in relation to HAART exposure.

We proposed that chronic toxic effects of antiretroviral drugs on the cell components of vessel walls could contribute to CSVD, which might be one of the key underpinnings of HAND. More severe forms of CSVD may lead to neurocognitive impairment via cerebral blood flow restriction sufficient to produce microinfarcts and white matter lesions. In mild CSVD, neurocognitive compromise may be associated with disturbance of cerebrovascular autoregulation (in response to fluctuations in systemic arterial pressure and in blood/tissue gas partial pressure and pH) and deficiency in functional hyperemia (adjustments of regional cerebral blood flow in response to local brain activity), which together could impair new protein synthesis in neurons required for synaptic plasticity and memory formation [38–42].

The present cross-sectional study was aimed at determining in HIV-infected adults associations between HAART exposure during life and CSVD at autopsy in the California NeuroAIDS Tissue Network (CNTN). We also examined the potential association between CSVD and HAND. We hypothesized that HAART exposure would be associated with higher likelihood of CSVD.

Methods

Study cohort

We assembled 144 HIV-infected autopsy cases that had detailed data on antiretroviral medications and died during 1999–2011 in the CNTN. The University of California San Diego Human Research Protections Program approved the project and all study participants provided written informed consent to participate. The written consent to autopsy was also obtained. There were 120 men (83.3%). The age at death ranged from 26 to 70 years [median 45 years, interquartile range (IQR) 13.3 years]; 47 (32.6%) cases aged at least 50 years (i.e., older age). Eighty-three cases (57.6%) were white, 31 (21.5%) Hispanic, 23 (16.0%) black, five (3.5%) Asian, and two (1.4%) others. The median HIV infection duration (i.e. in each case, the estimated duration of HIV infection from the patient's first awareness to death) was 11.9 years (IQR 8.9 years, $n = 116$). Of 141 cases with available data, 16 (11.3%) had diabetes 41 (29.1%) had systemic arterial hypertension, and 21 (14.9%) had dyslipidemia. Lifetime history of cigarette smoking was present in 45 (32.4%) of 139 cases and that of psychostimulant (primarily methamphetamine) dependence (determined according to either Psychiatric Research Interview for Substance and Mental Disorders [43] or Composite International Diagnostic Interview [44]) in 20 (28.6%) of 70 cases. HCV infection was found in 51 (41.5%) of 123 cases undergoing serological testing. Older age (≥ 50 years) was associated with higher proportions of diabetes, hypertension, and dyslipidemia ($P = 0.046$, 0.01, and 0.045, respectively, Fisher's exact test), but not with those of cigarette smoking, psychostimulant dependence, or HCV infection ($P = 0.6$, 1.0, and 0.1, respectively, Fisher's exact test).

The median post-mortem interval was 13 h (IQR 18.3 h). There were 140 (97.2%) cases with AIDS-defining conditions, three pre-AIDS, and one undetermined. Additionally, the systemic autopsy findings frequently included pneumonia, malignant neoplasms (primarily carcinomas and non-Hodgkin lymphomas), hepatic cirrhosis, atherosclerotic cardiovascular disease, myocardial infarction,

chronic pancreatitis, and sepsis. In the brain, opportunistic infections (i.e. cytomegalovirus infection, cryptococcosis, progressive multifocal leukoencephalopathy, and toxoplasmosis) were found in 32 (22.7%), primary non-Hodgkin lymphomas in four (2.8%), secondary non-Hodgkin lymphomas in five (3.5%), and metastatic carcinomas in two (1.4%) of 141 cases examined.

Antiretroviral treatment

In each autopsy case, individual drug components of the antiretroviral regimen used at the last follow-up visit closest to death were recorded within a median of 10.2 weeks (IQR 27.5 weeks) prior to death. In our study, HAART was defined as regimens containing three or more antiretroviral medications from at least two different drug classes.

Of 144 cases, 64 (44.4%) were on HAART regimens, 11 (7.6%) were on non-HAART regimens, 51 (35.4%) were no longer on antiretroviral treatment, and 18 (12.5%) were antiretroviral naive at their last visit. Of those 64 cases on HAART, 51 (79.7%) received protease inhibitor-based regimens. None of the non-HAART regimens contained protease inhibitors. Accordingly, our association analyses focused on protease inhibitor exposure. The HAART status was categorized into protease inhibitor-based, nonprotease inhibitor-based, and no HAART (i.e., non-HAART regimens, discontinuation of antiretroviral treatment, and being antiretroviral naive).

In the entire follow-up period, the median on-antiretrovirals duration (i.e. in each case, the estimated total duration of exposure to any antiretroviral regimens) was 1.5 years (IQR 5.2 years, $n = 132$). The median off-antiretrovirals duration (i.e. in each case, the HIV infection duration subtracted by the on-antiretrovirals duration) was 10.0 years (IQR 9.3, $n = 106$).

Neurobehavioral assessment

Of 144 cases, 123 (85.4%) underwent comprehensive neuropsychological testing at a median of 25.3 weeks (IQR 35.4 weeks) prior to death. Seven domains of neurocognitive functioning were assessed: information processing speed, attention/working memory, learning, delayed recall, verbal fluency, abstraction/executive functioning, and motor/psychomotor speed, with statistical correction for demographic variables (i.e., age, sex, ethnicity, and education) [45]. Both the number and severity of deficits across the neuropsychological battery were considered. A clinical diagnosis of HAND was made according to standard criteria requiring at least mild impairment of at least two domains of neurocognitive functioning [46]. The neurocognitive diagnoses were distributed as follows: normal cognition ($n = 28$, 22.8% of 123 cases), ANI ($n = 11$, 8.9%), MND ($n = 16$, 13.0%), and HAD ($n = 13$, 10.6%). Also, there were 40 patients (32.5%) having neuropsychological impairment due to other causes (such as severe neurological comorbidities that could not be ruled out as the primary cause of any neurocognitive impairment) and 15 patients (12.2%) whose diagnoses were inconclusive based on insufficient data; these patients were excluded from statistical analysis.

Histopathology

To systematically evaluate pathologic changes, the neuropathologist (V.S.), who was blinded to clinical and autopsy information, reviewed hematoxylin and eosin (H&E)-stained paraffin-embedded formalin-fixed tissue slides from the following brain regions: frontal [Brodmann's area (BA) 8 and BA4], parietal (BA1–3 and BA7), temporal (BA21–22), hippocampus, basal ganglia (anterior and posterior), anterior cingulate and corpus callosum, occipital (BA17–18), hemispheric cerebellum, midbrain, pons, and medulla.

The present study focused on the following vessel pathologic changes. CSVD was defined as concentric intramural hyalinization of small arteries or arterioles in the forebrain white matter, excluding those vessels in the ependymal vicinity. CSVD was graded as absent (normal), mild (partial-thickness involvement), or moderate/severe (full-thickness involvement with or without perivascular space dilatation) [42,47]; in each case, a grade of CSVD was assigned based on the severest lesion (Fig. 1). Vessel
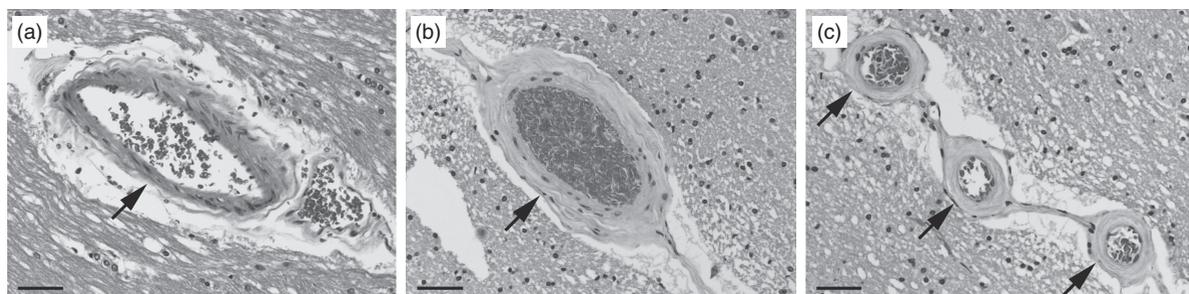

Fig. 1. Histopathology of cerebral small vessel disease in the forebrain white matter of HIV-infected adults. On hematoxylin and eosin staining, cerebral small vessel disease (CSVD) is defined as concentric intramural hyalinization of small arteries or arterioles and graded as absent (normal, with intactness of vascular smooth muscle cells) (a, arrow), mild (partial-thickness involvement) (b, arrow), or moderate/severe (full-thickness involvement) (c, arrows); scale bars 50 μm.

mineralization was defined as intramural deposition of basophilic amorphous material in small and medium-sized arteries [48,49]. Perivascular hemosiderin denoted old perivascular leakage of blood [47,50]. Atherosclerotic large vessel disease was not included because its severity was assessed on gross brain examination by the pathologist at each participating medical center in the CNTN, which was subject to interobserver variation.

We examined the following parenchymal pathologic changes related to HIV infection [51]. HIV encephalitis was defined as multiple foci of microgliosis, multinucleated giant cells, and astrocytosis [52]. Microglial nodular lesions were defined as scattered microglial nodules in the absence of specific causes such as cytomegalovirus infection, HIV encephalitis, and toxoplasmosis. White matter lesions were defined as foci of myelin pallor with macrophage infiltration or astrocytosis in the forebrain in the absence of HIV encephalitis and progressive multifocal leukoencephalopathy. Acute pathologic changes, such as hypoxic-ischemic or hemorrhagic lesions and bacterial microabscesses, were not taken into account, as they were likely associated with conditions occurring in the period close to death.

Statistical analysis

We used nominal and ordinal logistic regression methods to test associations between categorical outcomes and predictors or covariates. For some categories with low frequencies, Firth's penalized-likelihood approximation was employed to achieve reasonable estimates. Following univariable analyses, the multivariable analyses were performed as appropriate. The odds ratio (OR) with its 95% confidence interval (CI) was used to reflect the effect size. The two-tailed P value was considered significant at a threshold of $P < 0.05$. The statistical analyses were performed using R (version 3.0.1, 2013, http://www.r-project.org).

Results

HAART and brain vessel pathologic changes

Cerebral small vessel disease

Mild CSVD was present in 24.8% and moderate/severe CSVD in 47.4% of 137 cases examined. In univariable logistic regression analyses, protease inhibitor-based HAART (relative to no HAART) was associated with higher likelihood of mild CSVD (relative to absent) [OR 3.1 (95% CI 1.2–8.6), $P = 0.02$], and the similar trend of moderate/severe CSVD approached statistical significance [OR 2.2 (95% CI 0.9–5.6), $P = 0.08$]. Nonprotease inhibitor-based HAART was not significantly associated with either mild or moderate/severe CSVD [OR 1.0 (95% CI 0.1–8.2) and 3.2 (95% CI 0.8–17.9), $P = 1.0$ and 0.1, respectively). Among potential covariates analyzed, older age and diabetes were associated with

moderate/severe CSVD [OR 2.6 (95% CI 1.1–6.6) and 6.6 (95% CI 1.5–61.8), $P = 0.04$ and 0.01, $n = 137$ and 134, respectively]. There were no significant associations between mild CSVD and any of the covariates ($P > 0.4$). Race/ethnicity was not significantly associated with either mild or moderate/severe CSVD (overall $P = 0.1$) (Table 1).

The multivariable analysis built using those variables found to be significant in univariable analyses (Model: CSVD = HAART + older age + diabetes + hypertension, $n = 134$) showed trends toward higher likelihood of mild and moderate/severe CSVD with protease inhibitor-based HAART after adjusting for older age, diabetes, and hypertension [OR 2.7 (95% CI 1.0–7.7) and 2.4 (95% CI 1.0–6.2), $P = 0.05$ and 0.06, respectively]. Nonprotease inhibitor-based HAART was not significantly associated with either mild or moderate/severe CSVD after adjusting for older age, diabetes, and hypertension ($P = 0.9$ and 0.2, respectively). Diabetes was associated with higher likelihood of moderate/severe CSVD after adjusting for HAART, older age, and hypertension [OR 5.7 (95% CI 1.2–55.2), $P = 0.02$], but not with mild CSVD ($P = 0.5$). In contrast, older age was not significantly associated with either mild or moderate/severe CSVD after adjusting for HAART, diabetes, and hypertension ($P = 0.6$ and 0.2, respectively), nor was hypertension after adjusting for HAART, older age, and diabetes ($P = 0.8$, both). Therefore, older age and hypertension were excluded from the multivariable model.

In the multivariable analysis (Model: CSVD = HAART + diabetes, $n = 134$), protease inhibitor-based HAART was associated with higher likelihood of mild and moderate/severe CSVD after adjusting for diabetes [OR 2.8 (95% CI 1.03–7.9) and 2.6 (95% CI 1.03–6.7), respectively, $P = 0.04$, both]. Nonprotease inhibitor-based HAART was not significantly associated with either mild or moderate/severe CSVD after adjusting for diabetes ($P = 1.0$ and 0.1, respectively). Diabetes was associated with higher likelihood of moderate/severe CSVD after adjusting for HAART [OR 7.4 (95% CI 1.6–70.7), $P = 0.007$], but not with mild CSVD ($P = 0.4$).

Vessel mineralization

Vessel mineralization in the globus pallidus was identified in 47 (34.6%) of 136 cases, in seven of which it was also present in other brain regions including the cerebellar white matter, thalamus, hippocampus, dentate gyrus, and cerebral peduncle. The presence of vessel mineralization was not significantly associated with that of CSVD ($\chi^2 = 0.17$, df = 2, $P = 0.9$, χ^2 for independence). In univariable logistic regression analyses, no significant associations were found between vessel mineralization and protease inhibitor-based or nonprotease inhibitor-based HAART ($P = 0.6$ and 0.3, respectively) and the covariates ($P > 0.13$).

Table 1. HAART and covariates in relation to brain vessel pathologic changes.[a]

n (%)	Cerebral small vessel disease			Vessel mineralization		Perivascular hemosiderin	
	Moderate/severe	Mild	Absent	Present	Absent	Present	Absent
Overall	65 (47.4)	34 (24.8)	38 (27.7)	47 (34.6)	89 (65.4)	105 (75.0)	35 (25.0)
HAART status							
Protease inhibitor-based	OR 2.2[d]	OR 3.1*,[d]		OR 0.8		OR 1.2	
	24 (48.0)	17 (34.0)	9 (18.0)	15 (30.6)	34 (69.4)	38 (76.0)	12 (24.0)
Nonprotease inhibitor-based	OR 3.2	OR 1.0		OR 1.9		OR 2.1	
	9 (75.0)	1 (8.3)	2 (16.7)	6 (50.0)	6 (50.0)	11 (84.6)	2 (15.4)
No HAART[b]	32 (42.7)	16 (21.3)	27 (36.0)	26 (34.7)	49 (65.3)	56 (72.7)	21 (27.3)
Older age[c]	OR 2.6*	OR 1.5		OR 1.2		OR 1.6	
Yes	27 (60.0)	10 (22.2)	8 (17.8)	17 (37.8)	28 (62.2)	37 (80.4)	9 (19.6)
No	38 (41.3)	24 (26.1)	30 (32.6)	30 (33.0)	61 (67.0)	68 (72.3)	26 (27.7)
Men	OR 1.0	OR 1.3		OR 1.7		OR 0.8	
Yes	53 (46.9)	29 (25.7)	31 (27.4)	41 (36.6)	71 (63.4)	86 (74.1)	30 (25.9)
No	12 (50.0)	5 (20.8)	7 (29.2)	6 (25.0)	18 (75.0)	19 (79.2)	5 (20.8)
Diabetes mellitus	OR 6.6*,[d]	OR 2.1		OR 0.9		OR 1.0	
Yes	13 (81.3)	2 (12.5)	1 (6.3)	5 (31.3)	11 (68.8)	12 (75.0)	4 (25.0)
No	51 (43.2)	30 (25.4)	37 (31.4)	40 (34.2)	77 (65.8)	90 (74.4)	31 (25.6)
Hypertension	OR 1.6	OR 1.1		OR 0.8		OR 1.3	
Yes	22 (56.4)	8 (20.5)	9 (23.1)	12 (30.8)	27 (69.2)	31 (77.5)	9 (22.5)
No	42 (44.2)	24 (25.3)	29 (30.5)	33 (35.1)	61 (64.9)	71 (73.2)	26 (26.8)
Dyslipidemia	OR 0.9	OR 1.2		OR 0.4		OR 1.1	
Yes	9 (42.9)	6 (28.6)	6 (28.6)	4 (20.0)	16 (80.0)	16 (76.2)	5 (23.8)
No	55 (48.7)	26 (23.0)	32 (28.3)	41 (36.3)	72 (63.7)	86 (74.1)	30 (25.9)
Cigarette smoking	OR 1.2	OR 1.1		OR 0.6		OR 0.8	
Yes	22 (48.9)	11 (24.4)	12 (26.7)	11 (25.0)	33 (75.0)	32 (71.1)	13 (28.9)
No	40 (46.0)	21 (24.1)	26 (29.9)	33 (37.9)	54 (62.1)	69 (76.7)	21 (23.3)
Psychostimulant dependence	OR 0.4	OR 0.7		OR 0.7		OR 1.8	
Yes	6 (33.3)	6 (33.3)	6 (33.3)	5 (27.8)	13 (72.2)	16 (84.2)	3 (15.8)
No	24 (51.1)	14 (29.8)	9 (19.1)	17 (36.2)	30 (63.8)	36 (75.0)	12 (25.0)
Hepatitis C virus infection	OR 1.9	OR 0.8		OR 0.9		OR 1.2	
Yes	27 (56.3)	9 (18.8)	12 (25.0)	16 (32.7)	33 (67.3)	39 (78.0)	11 (22.0)
No	26 (38.2)	20 (29.4)	22 (32.4)	23 (34.8)	43 (65.2)	52 (75.4)	17 (24.6)

CSVD, cerebral small vessel disease; OR, odds ratio.
[a]Odds ratios were calculated by using univariable logistic regression methods in relation to 'Absent' outcomes (in columns) and 'No' predictors/covariates (in rows).
[b]Included in the 'No HAART' category were patients who received non-HAART regimens or no longer received antiretroviral treatment at their last follow-up visit closest to death, and those who had never received antiretroviral treatment.
[c]Age at death at least 50 years.
[d]In the multivariable logistic regression analysis (Model: CSVD = HAART + Diabetes mellitus), both mild and moderate/severe CSVD were associated with protease inhibitor-based HAART exposure after adjusting for diabetes (OR 2.8 and 2.6, respectively, $P = 0.04$, both). Moderate/severe CSVD was associated with diabetes after adjusting for HAART exposure (OR 7.4, $P = 0.007$).
*Two-tailed $P < 0.05$.

Perivascular hemosiderin
Of 140 cases examined, perivascular hemosiderin was found in 105 (75%). In univariable logistic regression analyses, no significant associations were found between perivascular hemosiderin and protease inhibitor-based or nonprotease inhibitor-based HAART ($P = 0.7$ and 0.4, respectively) and the covariates ($P > 0.29$).

HAART and brain parenchymal pathologic changes
Of 141 cases examined, HIV encephalitis was present in 22 (15.6%), microglial nodular lesions in 12 (8.5%), and white matter lesions in 17 (12.1%). In univariable logistic regression analyses, there were no significant associations between each of the parenchymal pathologic changes and protease inhibitor-based or nonprotease inhibitor-based HAART ($P > 0.23$ and > 0.34, respectively). Among potential covariates analyzed, older age was associated with lower likelihood of HIV encephalitis [OR 0.3 (95% CI 0.1−0.9), $P = 0.03$, $n = 141$], which remained significant after adjusting for HAART ($P = 0.049$); there was no significant interaction effect between older age and HAART ($P = 0.8$). No other significant associations were found between the parenchymal pathologic changes and any of the covariates ($P > 0.08$) (Table 2).

Brain vessel or parenchymal pathologic changes and HIV-associated neurocognitive disorders
In univariable logistic regression analyses, mild CSVD (relative to absent) was associated with higher likelihood of HAND (relative to normal cognition) [OR 4.8 (95% CI 1.1−21.2), $P = 0.04$, $n = 63$]. In multivariable analyses (Model: HAND = CSVD + single predictor/covariate), the direct association between mild CSVD and HAND remained significant after adjusting for vessel

Table 2. HAART and covariates in relation to brain parenchymal pathologic changes.[a]

n (%)	HIV encephalitis		Microglial nodular lesions		White matter lesions	
	Present	Absent	Present	Absent	Present	Absent
Overall	22 (15.6)	119 (84.4)	12 (8.5)	129 (91.5)	17 (12.1)	124 (87.9)
HAART status						
Protease inhibitor-based	OR 1.0		OR 1.6		OR 1.8	
	8 (15.7)	43 (84.3)	6 (11.8)	45 (88.2)	9 (17.6)	42 (82.4)
Nonprotease inhibitor-based	OR 1.0		OR 0.4		OR 0.3	
	2 (15.4)	11 (84.6)	0 (0.0)	13 (100.0)	0 (0.0)	13 (100.0)
No HAART[b]	12 (15.6)	65 (84.4)	6 (7.8)	71 (92.2)	8 (10.4)	69 (89.6)
Older age[c]	OR 0.3*		OR 0.2		OR 2.0	
Yes	3 (6.5)	43 (93.5)	1 (2.2)	45 (97.8)	8 (17.4)	38 (82.6)
No	19 (20.0)	76 (80.0)	11 (11.6)	84 (88.4)	9 (9.5)	86 (90.5)
Men	OR 2.3		OR 2.4		OR 1.6	
Yes	20 (17.1)	97 (82.9)	11 (9.4)	106 (90.6)	15 (12.8)	102 (87.2)
No	2 (8.3)	22 (91.7)	1 (4.2)	23 (95.8)	2 (8.3)	22 (91.7)
Diabetes mellitus	OR 0.4		OR 0.7		OR 0.5	
Yes	1 (6.3)	15 (93.8)	1 (6.3)	15 (93.8)	1 (6.3)	15 (93.8)
No	19 (15.6)	103 (84.4)	11 (9.0)	111 (91.0)	15 (12.3)	107 (87.7)
Hypertension	OR 0.8		OR 1.3		OR 1.1	
Yes	5 (12.5)	35 (87.5)	4 (10.0)	36 (90.0)	5 (12.5)	35 (87.5)
No	15 (15.3)	83 (84.7)	8 (8.2)	90 (91.8)	11 (11.2)	87 (88.8)
Dyslipidemia	OR 1.5		OR 1.1		OR 0.8	
Yes	4 (19.0)	17 (81.0)	2 (9.5)	19 (90.5)	2 (9.5)	19 (90.5)
No	16 (13.7)	101 (86.3)	10 (8.5)	107 (91.5)	14 (12.0)	103 (88.0)
Cigarette smoking	OR 0.9		OR 0.4		OR 0.9	
Yes	6 (13.3)	39 (86.7)	2 (4.4)	43 (95.6)	5 (11.1)	40 (88.9)
No	14 (15.4)	77 (84.6)	10 (11.0)	81 (89.0)	11 (12.1)	80 (87.9)
Psychostimulant dependence	OR 0.8		OR 1.3		OR 1.1	
Yes	2 (10.5)	17 (89.5)	1 (5.3)	18 (94.7)	3 (15.8)	16 (84.2)
No	6 (12.5)	42 (87.5)	2 (4.2)	46 (95.8)	7 (14.6)	41 (85.4)
Hepatitis C virus infection	OR 0.8		OR 0.3		OR 0.4	
Yes	8 (16.0)	42 (84.0)	2 (4.0)	48 (96.0)	3 (6.0)	47 (94.0)
No	13 (18.6)	57 (81.4)	9 (12.9)	61 (87.1)	10 (14.3)	60 (85.7)

OR, odds ratio.
[a]Odds ratios were calculated by using univariable logistic regression methods in relation to 'Absent' outcomes (in columns) and 'No' predictors/covariates (in rows).
[b]Included in the 'No HAART' category were patients who received non-HAART regimens or no longer received antiretroviral treatment at their last follow-up visit closest to death, and those who had never received antiretroviral treatment.
[c]Age at death at least 50 years.
*Two-tailed $P < 0.05$.

mineralization [OR 6.7 (95% CI 1.3−34.0), $P = 0.02$, $n = 61$], HIV encephalitis [OR 4.6 (95% CI 1.03−20.6), $P = 0.045$, $n = 63$], microglial nodular lesions [OR 5.1 (95% CI 1.1−22.7), $P = 0.03$, $n = 63$], white matter lesions [OR 4.8 (95% CI 1.1−21.3), $P = 0.04$, $n = 63$], or older age [OR 4.8 (95% CI 1.1−21.2), $P = 0.04$, $n = 63$]. The interaction effects of CSVD and each of the pathologic changes (i.e. vessel mineralization, HIV encephalitis, and white matter lesions) or older age on HAND were not statistically significant ($P > 0.58$) and thereby were not included in the above multivariable models. For microglial nodular lesions, the data were insufficient for interaction analysis (Table 3).

The apparent association between moderate/severe CSVD (relative to absent) and higher likelihood of HAND (relative to normal cognition) did not reach statistical significance in either univariable ($P = 0.4$) or multivariable ($P > 0.36$) analyses. There were no significant associations between HAND and the pathologic changes or covariates other than CSVD, in either univariable ($P > 0.4$) or multivariable analyses after adjusting for CSVD ($P > 0.3$).

Potential influence of HIV infection, on-antiretroviral or off-antiretroviral duration

In univariable logistic regression analyses, HIV encephalitis was associated with longer off-antiretrovirals duration [OR 1.1 (95% CI 1.02−1.3) per 1-year increase in duration, $P = 0.02$, $n = 103$], but not with either HIV infection or on-antiretrovirals duration ($P = 0.2$, both). The HIV infection duration was not significantly associated with mild or moderate/severe CSVD, vessel mineralization, perivascular hemosiderin, microglial nodular lesions, or white matter lesions ($P > 0.2$), nor was the on-antiretrovirals ($P > 0.26$) or off-antiretrovirals ($P > 0.14$) duration. The likelihood of HAND (relative to normal cognition) was lower in association with longer on-antiretrovirals duration [OR 0.8 (95% CI 0.7−0.97) per 1-year increase in duration, $P = 0.02$, $n = 60$], but not associated with either HIV infection ($P = 0.9$) or off-antiretrovirals ($P = 0.2$) duration.

Table 3. Brain vessel or parenchymal pathologic changes and covariates in relation to HIV-associated neurocognitive disorders.

n (%)	HAND	Normal cognition	Odds ratio (OR)[a]
Overall[b]	40 (58.8)	28 (41.2)	
Cerebral small vessel disease (CSVD)			
Moderate/severe	16 (57.1)	12 (42.9)	1.7
Mild	15 (78.9)	4 (21.1)	4.8*
Absent	7 (43.8)	9 (56.3)	
Vessel mineralization			
Present	14 (66.7)	7 (33.3)	1.4
Absent	24 (58.5)	17 (41.5)	
Perivascular hemosiderin			
Present	30 (58.8)	21 (41.2)	0.8
Absent	9 (64.3)	5 (35.7)	
HIV encephalitis			
Present	10 (66.7)	5 (33.3)	1.4
Absent	30 (58.8)	21 (41.2)	
Microglial nodular lesions			
Present	3 (60.0)	2 (40.0)	1.0
Absent	37 (60.7)	24 (39.3)	
White matter lesions			
Present	8 (66.7)	4 (33.3)	1.4
Absent	32 (59.3)	22 (40.7)	
Older age at death (≥50 years)			
Yes	10 (58.8)	7 (41.2)	1.0
No	30 (58.8)	21 (41.2)	
Men			
Yes	32 (59.3)	22 (40.7)	1.1
No	8 (57.1)	6 (42.9)	
Hepatitis C virus infection			
Yes	14 (63.6)	8 (36.4)	1.5
No	21 (53.8)	18 (46.2)	

HANDs, HIV-associated neurocognitive disorders.
[a]Odds ratios were calculated by using univariable logistic regression methods in relation to 'Normal cognition' outcomes (in columns) and 'Absent' predictors or 'No' covariates (in rows).
[b]In 123 HIV-infected patients who underwent comprehensive neuropsychological testing, the neurocognitive diagnoses were distributed as follows: normal cognition ($n = 28$, 22.8%), asymptomatic neurocognitive impairment ($n = 11$, 8.9%), mild neurocognitive disorder ($n = 16$, 13.0%), and HIV-associated dementia ($n = 13$, 10.6%). Also, there were 40 patients (32.5%) having neuropsychological impairment because of other causes (such as severe neurological comorbidities that could not be ruled out as the primary cause of any neurocognitive impairment) and 15 patients (12.2%) whose diagnoses were inconclusive based on insufficient data; these patients were excluded from the statistical analysis of HAND.
*Two-tailed $P < 0.05$.

Discussion

Generally, CSVD is associated with diabetes, hypertension, and aging [42]. In our cohort of HIV-infected adults, we found that moderate/severe CSVD was associated particularly with diabetes. In order to determine associations between CSVD and neurocognitive functioning, we considered only CSVD found within the forebrain. Additionally, to avoid a diagnostic challenge in differentiating CSVD from cerebral amyloid angiopathy, primarily affecting vessels in the leptomeninges and neocortex [53], on H&E-stained brain sections, only CSVD found in the white matter was taken into account.

Of chief importance, we found direct associations between protease inhibitor-based HAART exposure and higher likelihood of both mild and moderate/severe CSVD after statistically adjusting for diabetes. Although the mechanisms remain unclear, it is possible that in the context of HIV infection, some drug components of protease inhibitor-based HAART may be toxic to the cellular components of cerebral small vessels, such as vascular endothelial cells [54,55] and smooth muscle cells, leading to vessel wall degeneration (i.e. CSVD). It is also possible that some drugs in protease inhibitor-based HAART contribute indirectly to CSVD by inducing metabolic abnormalities such as dyslipidemia and insulin resistance, as they have been implicated in the premature development of atherosclerotic large vessel disease [27,28]. Nonetheless, in our study, there was no significant association between dyslipidemia and CSVD. Diabetes was found to be associated with moderate/severe CSVD even after statistically adjusting for HAART exposure, older age, and hypertension.

The clinical significance of CSVD in our study was substantiated by the finding that mild CSVD was associated with HAND even after statistically adjusting for vessel mineralization, HIV encephalitis, microglial nodular lesions, white matter lesions, or older age. Nonetheless, with moderate/severe CSVD, the apparent trend toward higher likelihood of HAND did not reach statistical significance. It is possible that a subset of neurocognitively impaired HIV-infected patients who concurrently had neurological or imaging evidence of cerebrovascular disease were not diagnosed with HAND by definitional criteria, but were instead included, together with those having other neurological comorbidities (e.g. history of moderate or severe head injury, history of learning disability, brain opportunistic diseases, and severe substance use disorders), in a category of neuropsychological impairment due to other causes [46].

Vessel mineralization found primarily in the globus pallidus was consistently reported in pre-HAART AIDS autopsy cohorts [48,49]. In contrast to CSVD, there was no significant association between vessel mineralization and HAART exposure in our cohort. Additionally, the presence of vessel mineralization was not significantly associated with that of CSVD. It is possible that vessel mineralization is pathophysiologically different from CSVD, in which the former may be mediated by the HIV-related effects on vessel walls in a brain region-specific manner, as HIV may directly infect vascular smooth muscle cells [56].

Our study did not find that HAART exposure was associated with lower likelihood of HIV encephalitis. It is possible that a subset of HIV-infected patients was failing antiretroviral treatment (e.g. drug resistance and compliance issues), was consequently in advanced stages of immune suppression, developed HIV encephalitis, and

died shortly afterward. Accordingly, HIV encephalitis was found at autopsy with no significant association with HAART status. In contrast, it may be that those patients who were able to longer maintain the benefit of HAART were less likely to develop HIV encephalitis, lived to older age, and eventually died with pathologic entities other than HIV encephalitis. In support of this scenario, we found lower likelihood of HIV encephalitis in patients who died at older age, even after statistically adjusting for HAART exposure.

In our study, there was no significant association between HAND and HIV encephalitis, in agreement with other recent autopsy studies [11,13]. Patients could develop HAND, particularly ANI and MND, even when their systemic HIV infection and immune function remain under control [3,8,9], whereas HIV encephalitis is characteristically associated with advanced immune suppression. In other words, a significant proportion of HAND is associated with pathophysiological sequences or pathologic changes in the brain other than HIV encephalitis. It is likely that CSVD associated with protease inhibitor-based HAART exposure could occur before advanced immune suppression takes place. We found that mild CSVD was associated with HAND.

We did not find any significant association between nonprotease inhibitor-based HAART exposure and higher likelihood of CSVD. However, we could not entirely exclude the possible effect of nonprotease inhibitor-based HAART on the development of CSVD, given that the number of cases in this HAART category was relatively small.

Our study analyzed the effects of HAART regimens, but not individual drugs because of sample size issues. Nonetheless, we were aware that different antiretroviral drugs even in the same class might carry differential degrees of toxicity on cerebral small vessels. This matter in question can be addressed in future studies using experimental animals [57] and in-vitro cell systems [54]. Also, the toxic effects of antiretroviral drugs might vary with the duration of drug use and drug metabolism. We found the estimated total duration of exposure to any antiretroviral regimens in the entire follow-up period was not significantly associated with either mild or moderate/severe CSVD. Unfortunately, available data on the duration of each antiretroviral regimen used were not sufficient for analysis.

Among HIV-infected patients studied, there might be significant variations in the duration of HIV infection and systemic viral burden, which might influence the development of CSVD as some investigators proposed that HIV might directly infect vascular smooth muscle cells [56]. In our study, the estimated duration of HIV infection was not significantly associated with either mild or moderate/severe CSVD. Data on plasma viral load were not sufficient for analysis. Additionally, whether HIV infection per se conferred a greater risk of CSVD was not addressed in our study because the number of HIV-seronegative autopsy cases was insufficient for analysis.

In conclusion, our clinicopathological study of HIV-infected adults in the CNTN revealed direct associations between protease inhibitor-based HAART exposure and higher likelihood of both mild and moderate/severe CSVD after statistically adjusting for diabetes. Of clinical significance, mild CSVD was associated with HAND. These findings suggest that protease inhibitor-based HAART exposure increases the risk of CSVD and thereby neurocognitive impairment. Some drug components of protease inhibitor-based HAART may be toxic to vascular endothelium and smooth muscle cells, leading to vessel wall degeneration. Further studies on molecular mechanisms of antiretroviral toxicity to the cell components of cerebral vessels and the identification of potential biomarkers for CSVD in HIV-infected patients are of particular interest. For instance, specific antiretroviral drugs or drug combinations may affect the integrity and function of cerebral vessels by inducing premature senescence of vascular endothelium and smooth muscle cells [54,58].

Acknowledgements

Conflicts of interest

There were no conflicts of interest.

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