HIV disease duration, but not active brain infection, predicts cortical amyloid beta deposition

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Short title: Beta amyloid and duration of HIV

Abstract

Objective: Abnormal deposition of the antimicrobial peptide amyloid beta (Aβ) is a characteristic of Alzheimer's Disease (AD). The objective of this study was to elucidate risk factors for brain Aβ in a cohort enriched for human immunodeficiency virus (HIV) and other neurotropic pathogens.

Design: Cross-sectional cohort study.

Methods: We examined autopsy brains of 257 donors with a mean age of 52.8 years; 62% were male; and 194 were HIV+ and 63 HIV-. Hyperphosphorylated tau (p-tau) and Aβ were identified in frontal and temporal regions by immunohistochemistry. APOE genotyping was performed. Clinical and neuropathological predictors for Aβ were identified in univariate analyses, and then tested in multivariate regressions.

Results: Cortical Aβ was identified in 32% of the sample, and active brain infection in 27%. Increased odds of Aβ were seen with increasing age and having an APOE ε4 allele; for the overall sample, HIV+ status was protective and brain infection was not a predictor. Within the HIV+ population, predictors for Aβ were duration of HIV disease and APOE alleles, but not age. When HIV disease duration and other HIV parameters were introduced into models for the entire sample, HIV disease duration was equivalent to age as a predictor of Aβ.

Conclusion: We hypothesize that dual aspects of immune suppression and stimulation in HIV, and beneficial survivor effects in older HIV+ individuals, account for HIV+ status decreasing, and HIV duration increasing, odds of Aβ. Importantly, with HIV, disease duration replaces age as an independent risk for Aβ, suggesting HIV-associated accelerated brain senescence.

Key words: HIV, beta amyloid, brain infection

Introduction

The role of neuroinflammation in Alzheimer's Disease (AD) has renewed interest in the potential for neurotropic infection to participate in AD pathogenesis (1; 2; 3; 4; 5). There are concerns that human immunodeficiency virus (HIV) may pre-dispose to accumulation of abnormal proteins characteristic of AD neurodegeneration (6; 7). People with HIV constitute an informative population to explore impacts of neurotropic infection on neurodegeneration: they are routinely monitored for immunologic and virologic status, and the timing and mechanisms by which HIV enters brain and impacts central nervous system (CNS) immunity have been elaborated (8; 9). Thus, unlike other viral infections, the duration, clinical severity, and CNS impacts of HIV disease can be uniformly ascertained and examined in the context of neurodegenerative pathology.

Cortical deposition of amyloid beta (Aβ) is a hallmark of AD, and in some research nosologies, is considered the earliest manifestation of disease, preceding clinical deficits by up to 20 years (10). Aβ peptides have anti-microbial properties, and many infectious agents, including HIV, bias cellular processing of amyloid precursor protein to enhance formation of Aβ in the innate immune response (11; 12). Thus, middle-aged HIV+ populations, harboring a potentially amyloidogenic microbe that stimulates innate immunity, and that cannot be eradicated but can be measured, provide opportunity to examine associations between infection and Aβ deposition. Accordingly, small groups of predominantly middle aged, HIV+ individuals have been examined with positron emission tomography (PET), revealing no evidence of brain amyloid in excess of age-matched HIV- controls (13; 14; 15). However, conventional PET imaging may be insensitive to early stages of amyloid deposition, and neuroimaging lacks the resolution afforded by direct examination of brain tissue by light microscopy (16).

The Manhattan HIV Brain Bank (MHBB) has operated a longitudinal, observational cohort study, brain donation program, and biorepository since 1999. MHBB also conducts brain donation programs with HIV- individuals whose medical and psychiatric morbidities are prevalent in HIV disease. Utilizing densely annotated brain tissues from HIV+ and demographically-matched HIV- donors, we investigated risks for Aβ deposition in mid-frontal and inferior temporal cortex and hippocampus, neuroanatomical regions characteristic of early AD involvement.

Materials and methods

Patient population: Brains were obtained between 1999 and 2019 from MHBB donors. The MHBB operates under oversight of the Icahn School of Medicine at Mount Sinai Institutional Review Board. All brain donations were obtained after approved consent, either from decedents during pre-mortem study via anatomical gift documentation, or from primary next of kin via authorization for autopsy and medical research. At the time of analysis, the

demographic composition of the MHBB was: 61% male, 39% female; 45% black, 30% Hispanic, 24% white; and mean age of 53.0 +/- 11.0 years at study termination. There were no demographic differences (age, sex, race/ethnicity) in the HIV+ and HIV- donor populations for this study.

Clinical annotation: For participants who completed assessments in prospective study, medical illnesses and medications were elucidated by interview and medical record review, and laboratories including CD4 T-cell counts and plasma HIV RNA loads were obtained. Substance use (SUD) and mood disorders (depression, dysthymia, bipolar) were elaborated by psychiatric interview (the Composite International Diagnostic Interview v 2.1 or the Psychiatric Research Interview for Substance and Mental Disorders v 1.9B). For all donors, a review of the electronic medical record was conducted at the time of autopsy, inclusive of medical, psychiatric and social worker assessments. 56% of the sample came from the prospective study (75% of HIV+ donors, and none of the HIV-). For HIV+ donors, the duration of HIV disease, last CD4 T-cell count and plasma HIV load prior to death, and last combination antiretroviral therapy (cART) regimen taken within 2 weeks of demise, were recorded. For all donors, presence or absence of SUD, mood disorder, hepatitis B virus (HBV), hepatitis C virus (HCV), herpes virus (simplex, zoster, or cytomegalovirus), and anti-herpes therapy, were recorded.

Brain processing and analysis: The entire brain was removed at autopsy, and half fixed in 10% phosphate-buffered formalin for routine processing and generation of a minimum of 50 tissue blocks for histologic assessment by a board-certified neuropathologist with expertise in neuro-infectious disorders (SM). Brain pathologies were categorized using standard data dictionaries (17). Neuropathologies recorded for this study included: HIV-associated pathology (HIV leptomeningitis, microglial nodule encephalitis, and leukoencephalopathy), other brain infections (bacterial, mycobacterial, viral, fungal, and protozoan), infarcts, and other focal lesions (tumor, intracranial hemorrhage, contusion, demyelination, optic degeneration, aneurysm, shunt tract). Terminal neuropathologies (anoxic and metabolic changes) were also noted.

Blocks of mid frontal gyrus (primarily Brodman's area 9) and medial temporal lobe with representation of hippocampus, entorhinal- and neo-cortices (over 90% mid-hippocampus) were sectioned at 5 microns and stained with hematoxylin and eosin, Luxol fast blue, and Bielschowsky techniques. Immunohistochemistry was performed on a Ventana Benchmark XT autostainer (Roche Tissue Diagnostics, Tucson, Arizona), utilizing primary antibodies to detect Aβ (clone 4G8, recognizing the Aβ17-24 fragment, Biolegend, San Diego, California) and p-tau (clone AT8, recognizing the ser202/thr205 phosphorylated epitope of tau, Thermo Fisher Scientific, Waltham, MA). Slides were independently reviewed by three neuropathologists (SM, EC, JFC) and scored for the presence and location of Aβ and p-tau. If scores did not agree, differences were adjudicated by consensus.

Double label IHC was manually performed on selected cases using the ImmPRESS Duet Double Staining Polymer Kit (Vector Laboratories, Burlingame, CA). A mixture of primary

antibodies to detect Aβ and either GFAP (Dako #Z0334, Santa Clara, CA), Iba1 (Invitrogen #PA5-27436, Carlsbad, CA), or CD68 (SP 251, Sigma-Aldrich, St. Louis, MO) was used. ImmPACT SG (blue/gray) was applied to detect Aβ, followed by ImmPACT Vector Red to detect GFAP, Iba1, or CD68.

Genotyping: APOE genotyping was performed using DNA extracted from peripheral blood mononuclear cells or brain tissue with the Qiagen DNeasy blood and tissue kit according to manufacturer's instructions (Qiagen USA, Germantown, MD). Taqman Assays-on-demand (Applied Biosystems, Foster City, CA) were used to target rs429358 and rs7412 to determine APOE alleles. Assays were performed in triplicate according to manufacturer's protocols using the Applied Biosystems 7900HT system.

Statistical procedures: Presence or absence of cortical Aβ deposition was modeled as a categorical variable in multivariate (nominal) logistic regression. Initially, univariate analyses to predict amyloid were conducted to determine candidates for multivariate models; factors with association at $p<0.1000$ were entered into logistic regression, and odds ratios and confidence intervals calculated. Additional modeling used $\chi 2$, Fisher's exact test, or analysis of variance to investigate associations between HIV status and duration of HIV infection on clinical and neuropathologic factors. Analyses were generated using JMP version 9.0 for Macintosh, and SAS/STAT Version 9.4 of the SAS System for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Population characteristics and clinical predictors of A β: Clinical characteristics of the overall population, and analyses for associations with extracellular A β deposition in frontal and/or temporal gray matter are presented in table 1. The average age of the sample was 52.8 years, with a range of 21 to 86 years. The population was predominantly black and Hispanic, with slightly more men than women. The distribution of APOE genotypes was: 0.4% ε 2/ ε 2; 13.6% ε 2/ ε 3; 4.7% ε 2/ ε 4; 56.0% ε 3/ ε 3; 23.0% ε 3/ ε 4; and 2.3% ε 4/ ε 4; thus, allelic frequencies (9.5% ε 2, 74.3% ε 3, 16.1% ε 4) were consistent with other racially heterogeneous New York City populations (18). 30% had one or more ε 4 alleles, and 19% had one or more ε 2 alleles.

Age, sex, HIV status, hypertension, diabetes, and both APOE alleles had significant or trend-level association with A β deposition in univariate analyses. Age, HIV status, and APOE ε4 remained significant predictors of A β when entered into nominal logistic regression, with r2 = 0.1724, $\chi 2$ = 55.76, $p<0.0001$ for the overall model. Odds ratios (OR) and 95% confidence intervals (CI) for significant predictors were: for age, 1.07 [1.04, 1.11] for every year change, $\chi 2$ = 19.57; for APOE ε4, 3.97 [2.10, 7.69], $\chi 2$ = 17.40; and for HIV+ status, 0.47 [0.23, 0.92], $\chi 2$ = 4.87.

Within HIV analysis for clinical predictors of A β: As HIV was a protective factor for amyloid deposition, we next examined clinical predictors of A β within this group. Variables

assessed included demographic factors, HIV-specific indices of immunologic/virologic status, presence and type of cART at death, and factors with significantly different prevalence in HIV+ and HIV- individuals. These factors included SUD (present in 77% of HIV+ and 43% of HIV-, $\chi2 = 26.35$, p<0.0001), mood disorders (68% of HIV+, 11% of HIV-, $\chi2 = 62.33$, p<0.0001), HBV (50% of HIV+, 22% of HIV-, $\chi2 = 14.96$, p=0.0001), and herpesvirus infection (46% of HIV+, 19% of HIV-, $\chi2 = 14.86$, p=0.0001).

Within the HIV+ population, significant or trend-level univariate associations with A β were seen for age, sex, APOE alleles, HIV disease duration, diabetes, hypertension, plasma HIV load, cART, and CD4 T-cell count (table 2). While many variables were related, variable inflation factors were all under 5. When entered into nominal logistic regression to predict Aβ, the overall model was significant with r2 = 0.2381, $\chi2 = 50.60$, p<0.0001. Predictors that remained significant were APOE ε4 and ε2 alleles, and duration of HIV disease; age and other immunovirologic variables were not significant, although sex and undetectable viral load remained at trend level (p values 0.0626 and 0.0777, respectively). Odds ratios and 95% CI for significant predictors were: HIV duration, 1.07 [1.01, 1.31] for every year change, $\chi2 = 4.60$; APOE ε4, 4.76 [2.07, 11.63], $\chi2 = 12.77$; and APOE ε2, 0.23 [0.05, 0.84], $\chi2 = 4.08$.

With the complex relationship of HIV status (protective in the overall population for Aβ) and HIV disease duration (longer duration a risk for Aβ in the HIV population), we wished to further explore these variables in the entire population. Accordingly, HIV- individuals were coded as "0" years duration HIV, as having undetectable viral loads, and as not being on cART; then, HIV status, age, sex, APOE alleles, HIV duration, HIV load, and cART were entered into nominal logistic regression to predict Aβ for the entire population. The model was significant (p<0.0001) with r2 = 0.1932, and $\chi2 = 58.91$. Significant predictors for amyloid were: HIV status (OR 0.19, CI [0.034, 0.92], $\chi2 = 4.02$, p=0.0449), age (OR 1.04, CI [1.01, 1.08], $\chi2 = 7.06$, p=0.0079), sex (OR for males 0.51, CI [0.27, 0.97], $\chi2 =4.34$, p=0.0373), APOE ε4 (OR 3.95, CI [2.02, 7.98], $\chi2 =15.44$, p<0.0001), APOE ε2 (OR 0.39, CI [0.14, 0.94], $\chi2 =3.98$, p=0.0462), and HIV disease duration (OR 1.07, CI [1.01, 1.14], $\chi2 =4.66$, p=0.0309); again, while having HIV+ status was protective, longer duration HIV disease constituted an independent risk for Aβ.

Histological patterns of Aβ: We examined patterns of Aβ deposition, to determine if distribution differed between HIV+ and HIV- individuals. The HIV+ group was split into short and long duration HIV for this analysis, to aid in understanding the complex HIV risk associations for Aβ. The grouping of HIV+ individuals was based on the median (15 years) for HIV duration, which was an inflection point for Aβ deposition (figure 1).

The following patterns of Aβ were recorded (figure 2): extracellular deposits in gray matter (used for analyses described above; the majority were diffuse "lake-like" or "fleecy" accumulations with variable size, and in 22 patients, also dense plaque cores) (19); extracellular deposition in the sub-pial molecular layer (a subset of gray matter Aβ, recorded because of the superficial gliosis commonly encountered with infectious processes that circulate through cerebrospinal fluid); deposits in white matter (recorded because of historical

observations of WM amyloid precursor protein accumulations in untreated disease); cerebral congophilic angiopathy (CAA); and sharply-demarcated staining within glial processes. In a subset of cases, double IHC staining for Aβ and either GFAP, CD68, or Iba1 revealed that glial deposits were in variable proportions of astrocytes and microglial cells (figure 2). Intraneuronal Aβ staining, although previously reported in HIV (20), was not analyzed, as it showed predictable neuroanatomic specificity (for example, it was present routinely in lateral geniculate nucleus regardless of HIV status), as well as batch staining effects.

Participants who were HIV- and those with long duration HIV were older than those with short duration HIV; accordingly, in all patterns or distributions except white matter deposition, both HIV- and HIV long duration groups showed greater frequencies of Aβ in pairwise comparisons (table 3).   Individuals with long duration HIV and those who were HIV- showed trend level differences in age (Student's t test p=0.0676); there was no difference in frequency of cortical Aβ deposition or CAA between these two groups. Individuals with long duration HIV had more white matter and less glial Aβ than the HIV- group at trend level (white matter amyloid, $\chi2$=3.348 p =0.0673; glial amyloid, $\chi2$=2.751 p=0.0972). Thus, there was a suggestion that HIV might alter the relationship between glia and amyloid in both gray and white matter, consistent with concepts of altered microglial and astroglial biology in HIV infection; however, this is only speculative, as significance was only at trend level.

Other neuropathologies and Aβ: There was a high rate of significant neuropathologies in this autopsy sample, with 72% of brains displaying abnormalities, inclusive of terminal anoxic and metabolic changes. The frequency of infectious brain pathologies was higher in HIV+ (34%) than HIV− (6.4%); 24% of the HIV+ sample had active HIV and 14% other infections. Brain infection had trend level significance in association with Aβ, driven by HIV pathology (p values for Aβ association with: any brain infection 0.0951; active brain HIV 0.0777; other brain infection 0.6789). The direction of association was negative; that is, with infection there was less Aβ deposition. Similarly, other focal brain pathologies were more common in HIV+ (45%) than HIV- (21%), with negative trend-level associations for Aβ (p = 0.0862). Neuronal accumulations of p-tau were seen with similar frequency in HIV+ (60%) and HIV- (68%); this was the only histology demonstrating a significant positive association with Aβ (p<0.0001). When all pathologies were introduced into multivariate analysis, only neuronal p-tau remained a significant predictor of Aβ. Neuronal p-tau was roughly twice as frequent as Aβ in the entire sample. Only 17 (6.6%) of 257 brains had Aβ deposition in the absence of neuronal p-tau; 14 (82%) of these were from HIV+ donors.

Finally, for the entire sample, we added neuronal p-tau to clinical predictors of Aβ (age, HIV status, sex, APOE alleles, with duration of HIV, viral load, and cART set at the null level for HIV-). The model was significant (p<0.0001) with r2 = 0.2212 and $\chi2$ = 67.448. HIV status (OR 0.18, CI [0.03, 0.90], p=0.0417), male sex (OR 0.46, CI [0.24, 0.87], p=0.0181), both APOE ε2 (OR 0.37, CI [0.13, 0.89], p=0.0353) and ε4 (OR 4.90, CI [2.40, 10.49], p<0.0001) alleles, the duration of HIV disease (OR 1.08 for every year, CI [1.02, 1.15], p=0.0131), and

neuronal p-tau (OR 3.31, CI [1.47, 7.75], p=0.0045) were significant predictors; age was not a significant predictor of A β.

Discussion

Abnormal accumulations of Aβ and p-tau are central to current nosologies of AD, and essential to its histologic diagnosis (21). Clinical manifestations of AD begin an exponential rise in the 7th decade, and many models of pathogenesis presume accumulation of abnormal proteins predates symptom onset by one to two decades (10; 22). Some models postulate amyloid deposition is the earliest biochemical lesion, preceding neuronal degeneration (10; 23). Thus, disease initiation and amyloid deposition might occur in the 5th and 6th decades of life, rendering middle-aged populations ideal for studying factors that increase risks for abnormal proteostasis. At age 50, 10% or fewer individuals demonstrate brain amyloid by PET imaging, dependent on APOE genotype (24; 25). However, neuroimaging is less sensitive than light microscopy, and the earliest stages of brain Aβ accumulation are missed in PET studies (16). Our autopsy population, with a mean age of 52.8 years, is well suited to investigating risks for the earliest stages of Aβ deposition. We examined neuroanatomical regions typical of early A β and p-tau accumulation in AD. The finding that 32% of our population had parenchymal Aβ is consistent with the premise that this early biochemical lesion occurs in middle age. 72% of our sample died at 60 years of age or younger; of this subgroup, Aβ was present in 26%.

Neuronal p-tau was present in 62% of our sample, suggesting Aβ deposition is not the earliest event in AD pathogenesis, although it is unclear what aspects of either amyloid or p-tau contribute to future development of AD. These proteins cannot be reliably placed on a timeline for developing AD, and may represent "benign" accumulations of aging, or indicators of other disorders such as traumatic brain injury. Morphologically, most parenchymal amyloid in our cohort was diffuse, and there is no human data to suggest diffuse amyloid transforms into neuritic plaque. Many cognitively normal individuals demonstrate amyloid on PET, and neuronal p-tau in individuals coming to autopsy in the 2nd and 3rd decade is well documented (25; 26). However, when Aβ is detected by PET, future risk for incident dementia increases (27). It is unclear if this predictive power applies in the context of a chronic, neurotropic infection with immunologic consequence, such as HIV.

HIV has a bivalent relationship to systemic immunity: while untreated disease results in immunosuppression, under cART there may be immune stimulation (28). Magnetic resonance spectroscopy (MRS) demonstrates HIV-associated neuroinflammation in all disease stages, with and without therapy, albeit waning over time with cART (29; 30). Thus, the duration of HIV's CNS impact can be estimated through ascertaining a date of initial diagnosis, and disease severity can be measured by CD4 counts and viral loads. The ability to assess a longitudinal burden of HIV stands in contrast to other neurotropic viruses implicated in AD, where point-in-time estimates of viral activity are apparent only with symptomatic activation or in autopsy brain tissue. For example, herpesviruses are implicated in AD due to brain virus detection in proximity to amyloid, and identification of brain anti-microbial

molecular signatures with elevated viral transcripts (5; 4). Many non-herpes microbes have been similarly implicated, raising questions about causal inferences (5; 4; 31; 3).

While our autopsy study is cross-sectional, it is advantaged by having measures of HIV duration and activity, allowing examination of these factors in concert with traditional risks for Aβ. In our cohort, traditional risk factors behaved in the expected manner: increasing age, positive APOE ε4 status, and female sex increased risk for Aβ, and APOE ε2 was protective. Furthermore, another characteristic of neurodegeneration – neuronal p-tau – also constituted an independent risk. It has been observed that the most important genetic risk for Aβ, APOE ε4, also conveys risk for p-tau (32). The association of both neurodegenerative proteins may reflect a common biological senescence with shared genetic susceptibility.

An important aspect of our study was the complex relationship between HIV status, infection duration, age, and Aβ. Within the HIV+ sample, longer HIV duration predicted Aβ, and undetectable viral load was a trend-level positive predictor. This is partially consistent with a previous autopsy study, where higher plasma HIV loads predicted lower likelihood of Aβ (7). This prior within-HIV study also demonstrated association of Aβ with increasing age, but did not examine HIV duration. The fact that HIV duration replaced age as a predictor in our study is striking, as it is widely accepted that age is the strongest risk for neurodegeneration (33). Age is a surrogate for unclear biological processes, as multiple genetic and environmental factors contribute to senescence. One study examining the brain methylome observed that longer duration HIV correlated with epigenetic evidence of accelerated aging (34). Thus, HIV duration may be a critical variable in advancing brain biologic age, and our study suggests disease duration replaces chronologic age as the pertinent time-dependent variable for predicting deposition of proteins associated with senescence and neurodegeneration.

Another aging change is the development of dysregulated immunity with a pro-inflammatory bias, termed immunosenescence (35). In treated HIV disease, elevated inflammatory markers may occur after years of cART, including mediators of innate immunity, such as IFNα and TNFα (36). Relevant to neurodegeneration, some literature suggests autoimmune diseases characterized by innate immunity confer increased risk for AD, and that exposure to anti-TNFα agents mitigates risk (37; 38). Increased risk for Aβ in long duration HIV may be predicated on persistent immune stimulation, with duration of inflammation more critical to amyloid production than chronologic age. This hypothesis could explain enhanced risk for Aβ with undetectable viral load, as control of viremia is a predicate for restored immune function.

However, accelerated senescence is seemingly counter to the protective effect of HIV status on amyloid in the entire sample. Explanations for this may be two-fold. The majority (72%) of younger, HIV+ individuals with shorter duration disease had CD4 counts <200 cells/mm3 and detectable viral loads; thus, immunocompromise may have decreased risk. In the older, longer duration HIV group, survivor bias may have pertained, with factors like advantageous health behaviors mitigating risk. While a prior American autopsy study described increased

risk for amyloid with HIV, its HIV- controls were a historical sample from Germany, with sampling of brain regions not typical for early stages of Aβ deposition (39; 26). Differences in Aβ frequency (29.3% of HIV+, 25.8% of historical HIV-) may have reflected demography, geographic location, and neuroanatomical sites examined. In contrast, our study used a contemporaneous HIV- group enriched for HIV-associated comorbidities, with uniform neuroanatomic sampling.

Finally, Aβ was not associated with active brain infection in our study. In animal models demonstrating infectious associations, therapy lessens Aβ deposition (11). 77% of our HIV+ population was on cART at death, and 25% of the overall population received anti-herpetic medication; it is possible that therapies were mitigating factors.

There are limitations to our study. Amyloid was assessed with an antibody to the Aβ17-24 fragment, which recognizes longer peptides in the beta secretase pathway; while abnormal, these may not be fibrillar. As an autopsy study, donors had serious medical morbidity, and findings might not pertain to healthier individuals. Regardless, the intriguing relationships between HIV and Aβ in our population support the need for further investigation into the immunologic and epigenetic bases of this association, as well as neuroimaging confirmation of the impacts of disease duration in healthier HIV+ populations.

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Figure 1. Numbers of HIV+ individuals with (red bars) or without (blue bars) cortical Aβ deposition, displayed by the number of years HIV disease duration. The median of the distribution for HIV duration is 15 years, denoted by a dotted line. The frequency of Aβ deposition demonstrates a significant increase at and above the median.
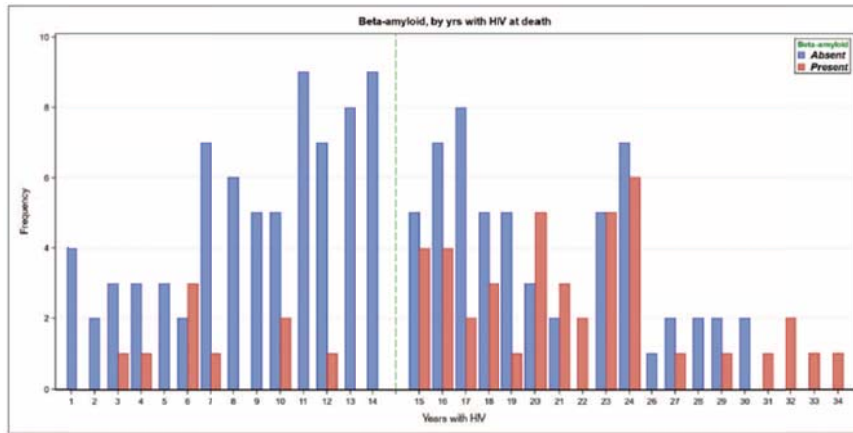

Figure 2. Patterns of Aβ deposition. a. Diffuse amyloid plaques of varying sizes in cerebral cortex, with and without paravascular locations (Aβ immunostain with diaminobenzidine chromogen and hematoxylin counterstain, original magnification 100X, scale bar = 100um). b. A dense core plaque (Aβ immunostain with diaminobenzidine chromogen and hematoxylin counterstain, original magnification 400X, scale bar = 25um). c. Subpial, diffuse deposits of amyloid (Aβ immunostain with diaminobenzidine chromogen and hematoxylin counterstain, original magnification 200X, scale bar = 50um). d. Cerebral congophilic angiopathy, with adjacent diffuse plaques and glial Aβ (Aβ immunostain with diaminobenzidine chromogen and hematoxylin counterstain, original magnification 600X, scale bar = 16um).   e. Aβ plaque (gray) and reactive astrocytes identified with immunohistochemical stain for glial fibrillary acidic protein (GFAP) (red). Aβ is seen within astrocyte cytoplasm (representative arrow). (Immunohistochemical stain for Aβ and GFAP with ImmPACT SG and ImmPACT Vector Red chromogens, original magnification 600X, scale bar = 16um). f. Aβ plaque (gray) and reactive microglia identified with immunohistochemical stain for Iba1 (red). Aβ is seen within microglial cell cytoplasm (representative arrow). (Immunohistochemical stain for Aβ and Iba1 with ImmPACT SG and ImmPACT Vector Red chromogens, original magnification 600X, scale bar = 16um).
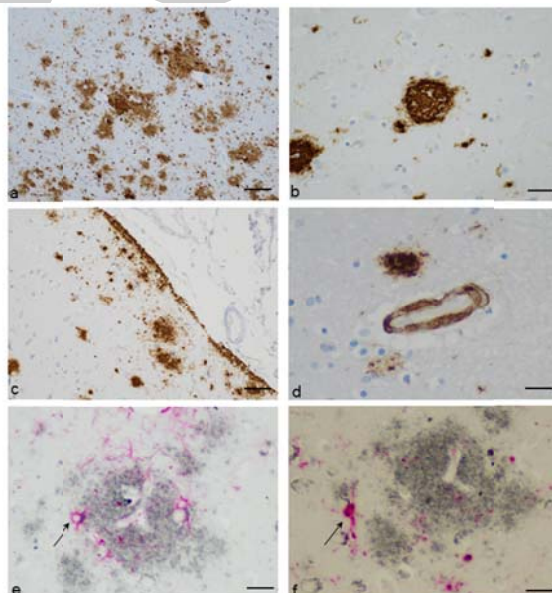
Table 1. Clinical characteristics of the overall study population, and analyses for associations with Aβ deposition

	Total sample (n=257)	Aβ absent (n=174)	Aβ present (n=83)	Association with Aβ	
				Univariate, P value	Multivariate[1], P value, (OR; 95% CI)
Mean age (SD)	52.8 (12.1)	50.3 (12.4)	58.0 (9.8)	<0.0001	<0.0001 (1.07; 1.04,1.11)
Male gender, n (%)	160 (62%)	116 (67%)	44 (53%)	0.0356	
Race/ethnicity, n (%)				0.8512	
Black	113 (44%)	77 (44%)	36 (43%)		
Hispanic	86 (33%)	60 (34%)	26 (31%)		
White	51 (20%)	32 (18%)	19 (23%)		
Other	7 (3%)	5 (3%)	2 (2%)		
HIV positive, n (%)	194 (75%)	139 (80%)	55 (66%)	0.0187	0.0274 (0.47; 0.23,0.92)
APOE ε4, n (%)	77 (30%)	40 (23%)	37 (45%)	0.0005	<0.0001 (3.97; 2.10,7.69)
APOE ε2, n (%)	48 (19%)	38 (22%)	10 (12%)	0.0633	
HCV, n (%)	120 (47%)	78 (45%)	42 (51%)	0.3859	
HBV, n(%)	111 (43%)	79 (45%)	32 (39%)	0.3006	
Any viral hepatitis, n (%)	166 (65%)	111 (64%)	55 (66%)	0.6985	
Any herpes infection, n (%)	102 (40%)	70 (40%)	32 (39%)	0.7974	
On herpes therapy, n (%)	65 (25%)	45 (26%)	20 (24%)	0.7608	
Hypertension	119 (46%)	73 (42%)	46 (55%)	0.0429	
Diabetes mellitus	68 (26%)	37 (21%)	31 (37%)	0.0063	

[1] Statistically significant (p<0.05) terms shown.

Table 2. Clinical characteristics of the HIV+ study population, and analyses for associations with Aβ deposition

	HIV pos (n=194)	Aβ absent (n=139)	Aβ present (n=55)	P value for association with Aβ	
				Univariate, P value	Multivariate[1], P value, (OR; 95% CI)
Mean age (SD)	52.6 (0.9)	50.6 (11.1)	57.7 (9.4)	<0.0001	
Male gender, n (%)	121 (62%)	93 (67%)	28 (51%)	0.0396	
SUD (any), n (%)	150 (77%)	109 (78%)	41 (75%)	0.5620	
Depression spectrum disorder[1], n (%)	131 (68%)	95 (68%)	36 (65%)	0.6985	
APOE ε4, n (%)	59 (30%)	32 (23%)	27 (49%)	0.0005	0.0004 (4.76; 2.07,11.63)
APOE ε2, n (%)	37 (19%)	31 (22%)	6 (11%)	0.0748	0.0434 (0.23; 0.05,0.84)
HBV, n (%)	97 (50%)	73 (53%)	24 (44%)	0.2658	
Any herpes infection, n (%)	90 (46%)	62 (45%)	28 (51%)	0.4279	
Hypertension	84 (43%)	55 (40%)	29 (53%)	0.0955	
Diabetes mellitus	42 (22%)	24 (17%)	18 (33%)	0.0184	
cART at death[2], n (%)	148 (77%)	101 (73%)	47 (85%)	0.0634	
Protease Inhibitor in cART regimen, n (%)	84 (57% of treated)	59 (58% of treated)	25 (53% of treated)	0.5506	
INI* in cART regimen, n (%)	38 (26% of treated)	22 (22% of treated)	16 (34% of treated)	0.1120	
NNRTI** in cART regimen, n(%)	50 (34% of treated)	36 (36% of treated)	14 (30% of treated)	0.4832	
Mean duration HIV[3] (SD)	15.5 (7.5)	14.0 (7.0)	19.2 (7.5)	<0.0001	0.0321 (1.07; 1.01,1.31)
Log Plasma VL[4], Median (Q1, Q3)	1.93 [1.69, 4.81]	2.34 [1.69,5.06]	1.69 [1.69,2.59]	0.0064	
Undetectable VL[4], n (%)	92 (48%)	57 (41%)	35 (64%)	0.0050	
CD4 count[1], Median (Q1, Q3)	156 (56,394)	133 (35,298)	188 (103,537)	0.0014	
CD4>200 cells/mm3[1], n (%)	82 (43%)	55 (40%)	27 (49%)	0.2274	

*INI: Integrase inhibitor; **NNRTI: non-nucleoside reverse transcriptase inhibitor

[1] Statistically significant (p<0.05) terms shown

[2] Data not available for 2 cases

[3] Data not available for 1 case

[4] Data not available for 14 cases (10 Aβ absent, 4 Aβ present)

[5] Data not available for 3 cases

Table 3. Histologic patterns of Aβ accumulation in HIV-, HIV+ short duration, and HIV+ long duration participants

	HIV negative (n=63)	HIV duration<15 (n=82)	HIV duration>=15 (n=98)	P-value
Mean Age[1,2,3] (SD)	53.3 (14.9)	46.6 (9.8)	56.8 (9.9)	<0.0001
Gray matter Aβ[1,2], n (%)	28 (44%)	9 (11%)	42 (43%)	<0.0001
White matter Aβ[3] , n (%)	2 (3.2%)	4 (4.9%)	11 (11.2%)	0.1516
Aβ at pia limitans[1,2] (molecular layer), n (%)	8 (12.7%)	1 (1.2%)	16 (16.3%)	0.0009
Glial Aβ[1,2,3], n (%)	38 (60%)	20 (24%)	46 (47%)	<0.0001
Congophilic angiopathy[1,2], n (%)	6 (9.5%)	1 (1.2%)	9 (9.2%)	0.0344
Frontal cortex Aβ[1,2], n (%)	25 (40%)	8 (10%)	40 (41%)	<0.0001
Temporal cortex Aβ[1,2] n (%)	15 (24%)	5 (6.1%)	27 (28%)	0.0003

Duration of HIV disease data not available for 14 individuals. Analyses by Fisher's exact test with exception of age, which used simple ANOVA.

[1] Pairwise comparison between HIV- and short duration HIV+ significant (p<0.05)

[2] Pairwise comparison between long duration HIV+ and short duration HIV+ significant (P<0.05)

[3] Pairwise comparison between HIV- and long duration HIV+ at trend level (P<0.10)