Title

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Journal

AIDS, Publish Ahead of Print(&NA;)

ISSN

0269-9370

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Publication Date

2019-06-01

DOI

10.1097/qad.0000000000002192

Peer reviewed

AIDS, Publish Ahead of Print

DOI: 10.1097/QAD.0000000000002192

Effects of Comorbidity Burden and Age on Brain Integrity in HIV

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Conflicts of interest and source funding: C.M. receives royalties from UptoDate and Wolters Kluwer. This work was supported in part by awards from National Institutes of Health (the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) N01 MH2205 and HHSN271201000036C, to I. Grant; R01 MH107345, to R.K. Heaton and S.L. Letendre; and P30 MH62512, to R.K. Heaton; T32AA013525 to RS; and T32DA031098 to LMC). The views expressed in this article are those of the authors and do not reflect the official policy or position of the United States Government.

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ABSTRACT

Objective: The influence of confounding neurocognitive comorbidities in persons living with HIV (PLWH) on neuroimaging has not been systematically evaluated. We determined associations between comorbidity burden and brain integrity and examined the moderating effect of age on these relationships.

Design: Observational, cross-sectional substudy of the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) cohort.

Methods: 288 PLWH (mean age=44.2) underwent structural MRI and MR spectroscopy as well as neurocognitive and neuromedical assessments. Consistent with Frascati criteria for HIV-associated neurocognitive disorders (HAND), neuromedical and neuropsychiatric comorbidity burden was classified as incidental (mild), contributing (moderate), or confounding (severe-exclusionary) to a diagnosis of HAND. Multiple regression modeling predicted neuroimaging outcomes as a function of comorbidity classification, age, and their interaction.

Results: Comorbidity classifications were 176 incidental, 77 contributing, and 35 confounded; groups did not differ in HIV disease characteristics. Relative to incidental and contributing participants, confounded participants had less cortical gray matter and more abnormal white matter and ventricular CSF, alongside more neuroinflammation (choline, myo-inositol) and less neuronal integrity (*N*-acetylaspartate). Older age exacerbated the impact of comorbidity burden: to a greater extent in the confounded group, older age was associated with more abnormal white matter (p=.017), less total white matter (p=.015), and less subcortical gray matter (p=.014).

Conclusions: Neuroimaging in PLWH reveals signatures associated with confounding neurocognitive conditions, emphasizing the importance of evaluating these among individuals with suspected HAND. Older age amplifies subcortical and white matter tissue injury, especially in PLWH with severe comorbidity burden, warranting increased attention to this population as it ages.

Key Words: HIV, comorbidity, aging, brain, MRI, MRS, neurocognitive disorders

INTRODUCTION

Combination antiretroviral therapy (cART) increases life expectancy among people living with HIV (PLWH)[1], with a rising rate of PLWH aged 50 and older[2]. Nevertheless, milder forms of HIV-associated neurocognitive disorders (HAND)[1, 3, 4], neuroimaging abnormalities[5], and comorbidities[6] remain prevalent in the cART-era. Frascati criteria for HAND require that neurocognitive impairment be at least partially attributable to HIV-infection, and provide guidelines for comorbidity review[7]. Clinical judgement is required to determine the extent to which non-HIV-related comorbidities influence interpretation of neurobehavioral assessment, with a severe comorbidity classification precluding a diagnosis of HAND[1, 7].

Given the high prevalence of comorbidities that may compromise CNS integrity among PLWH, some authors have questioned whether reported rates of HAND are inflated[6]. While severe comorbidity burden enhances risk for neurocognitive impairment in PLWH[1, 8], prevalence of HAND in PLWH with minimal-to-moderate comorbidity burden ranges from 19% to 50%[1, 9, 10]. Comorbidities may be particularly damaging among older PLWH, who are at increased risk for HAND[11, 12], brain atrophy[13], and acquisition of age-related comorbidities[14].

Structural MRI and MR spectroscopy (MRS) studies demonstrate HIV-related structural and neurochemical alterations to both cortical and subcortical gray and white matter tissues[5, 15]. We have previously reported on neuroimaging signatures of PLWH without major neurocognitive confounds[16, 17]. However, neuroimaging correlates of Frascati-based comorbidity classifications have not been systematically evaluated. Thus, we examined the impact of three levels of comorbidity burden (i.e., mild, moderate, and severe) on neuroimaging measures of brain integrity, as well as the potential moderating effect of age on these relationships.

METHODS

Participants

Participants included 288 English-speaking PLWH from the neuroimaging substudy of the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) investigation[1, 16], drawn from five university centers: Johns Hopkins University (Baltimore, MD, n=50); Icahn School of Medicine at Mount Sinai (New York, NY, n=65); University of California at San Diego (San Diego, CA, n=80); University of Texas Medical Branch (Galveston, TX, n=59); and University of Washington (Seattle, WA, n=34). CHARTER was structured to examine and follow individuals who were as representative as possible of the population of adults living with HIV and receiving primary care at US university-based clinics; as such, potential participants were not excluded on the basis of comorbid conditions that may affect brain structure and function. All participants completed a cross-sectional evaluation consisting of a blood draw, comprehensive neurobehavioral and neuromedical assessments, and scanning protocols for MRS and structural MRI. All scans occurred between May 2004 and August 2007. Procedures were approved by local Institutional Review Boards (IRBs) and all participants provided written informed consent.

Comorbidity Classifications

In accordance with published Frascati criteria for HAND and guidelines for reviewing developmental and neuromedical histories[7], each case was reviewed by a senior clinician (R.K.H.) to determine whether neuromedical and neuropsychiatric comorbidity burden was "incidental" (normal/mild), "contributing" (moderate), or "confounding" (severe) to a diagnosis of HAND[1]. In order to adequately classify comorbidity burden, clinical judgment is required to determine their severity, potential influence on neurocognitive performance and everyday functioning, and timing in relation to the course of HIV disease. Interrater

reliability of the comorbidity classifications was previously assessed using independent ratings for 269 patients from another CHARTER clinician-investigator (D.B.C.)[1]. Seventy-four percent of these independent classifications were identical. Of the original 269 classifications, only 7% changed after discussion and less than 5% resulted in a change in HAND classification (change to or from confounding). Of the 288 participants in this substudy, comorbidity classifications were 61% (n=176) incidental, 27% (n=77) contributing, and 12% (n=35) confounding. Confounded cases consisted of participants with severe comorbidities (e.g., neurodevelopmental, cerebrovascular, systemic medical, and substance-related) that could sufficiently explain all observed neurocognitive or everyday functioning impairments, thus precluding a HAND diagnosis (see Table 1 for frequency of selected comorbidities across classification groups).

Neuromedical Assessment

All participants underwent structured medical and neurological examinations that included a comprehensive history inclusive of medication utilization, and venipuncture. A subset of participants (n=175) underwent a lumbar puncture for CSF collection. Routine clinical chemistry panels, complete blood counts, rapid plasma reagin, hepatitis C virus antibody, and CD4+ T cell enumerations were performed at each site's Clinical Laboratory Improvement Amendments (CLIA)–certified, or CLIA equivalent, medical center laboratory. HIV RNA concentration in plasma and CSF were measured using reverse transcriptase-polymerase chain reaction (Roche Amplicor, v. 1.5, with a lower limit of quantitation 50 copies/ml).

Neurocognitive Assessment

All participants completed a comprehensive neurocognitive test battery, covering seven cognitive domains commonly impacted by HIV-related brain dysfunction. Normative

standards were used to correct for effects of age, education, gender, and race/ethnicity, as appropriate[4]. Individual test scores were converted to demographically-corrected T scores and associated deficit scores within each domain to determine domain-specific impairment[1]. Participants with at least mild impairment in two or more of the seven neurocognitive domains were classified as neurocognitively impaired, in accordance with Frascati criteria[7].

Psychiatric Assessment

Current mood symptoms were assessed using the Beck Depression Inventory II (BDI-II)[18]. The computer-based Composite International Diagnostic Interview (CIDI) was administered to determine DSM-IV diagnoses of current and lifetime substance use and mood disorders[19].

Neuroimaging Assessments

MR imaging was performed on six General Electric 1.5-Tesla scanners at five sites using the same sequences, and scanner performance was annually reviewed in person for quality through review of human phantom data; in addition, because scanner differences (*e.g.,* hardware, software, head coil upgrades) can influence neuroimaging metrics, we included a "scanner" variable in statistical analyses to account for scanner-related effects[20] as in our prior work[16, 21]. Four series were acquired for structural morphometric analysis, including coronal two-dimensional T2- and proton-density (PD)-weighted fast spin echo sequences (section thickness=2.0 mm), and three-dimensional sagittal T1- and PD-weighted spoiled gradient recalled acquisitions (section thickness=1.3 mm)[16, 21]. MRS was performed using a standardized point-resolved spectroscopy protocol (echo time=35ms, repetition time=3000ms)[22].

Multi-channel Structural MRI: As described previously[16, 21], we used the multi-channel dataset in a semi-automated workflow to measure cortical and subcortical gray matter; total cerebral and abnormal (*e.g.*, hyperintense regions on T2-weighted images) white matter; and ventricular and cerebral sulcal CSF, as well as supra-tentorial cranial vault volume to account for individual differences in head size. The workflow includes image inspection for motion and other artifacts, re-slicing to a standard space, intra-subject mutual information registration, bias-correction with nonparametric non-uniformity normalization, removal of non-brain tissue, three-tissue segmentation (gray matter, white matter, and CSF), abnormal white matter designation, and anatomical labeling performed by trained anatomists. This approach includes the identification of regions of cerebral white matter with abnormal MRI signal characteristics; these regions segmented as gray matter, but are anatomically located within the white matter.

Single-Voxel MRS: As described previously[22], three regional voxels were acquired: frontal gray matter (FGM; 20x20x20mm and 64 acquisitions), frontal white matter (FWM; 20x20x20mm and 64 acquisitions), and basal ganglia (BG; 20x20x15mm and 96 acquisitions). MRS concentrations of *N*-acetylaspartate, choline, myo-inositol, and creatine were quantified using LCModel with water suppression[23]. Water suppression allows for the examination of absolute metabolite levels, our primary measures of interest; although ratios to creatine have been commonly reported with the aim to provide standardization across sites and studies, this approach has limitations and there is evidence that HIV infection independently affects creatine levels directly, confounding the interpretation of ratio values and existing findings[as in 22, 24]. Only metabolite estimates for appropriately placed voxels with adequate spectra (standard deviation<21) were used; therefore, sample size varied by MRS region or metabolite. Structural segmentation was used to estimate the proportion of relevant

tissue volume within each MRS voxel (*e.g.*, amount of gray matter in FGM voxel) to control for individual sampling variability.

Statistical Analysis

Comorbidity group differences on demographics, HIV disease characteristics, neurocognitive impairment, and comorbid conditions were examined using ANOVA, Kruskal-Wallis, or Chi-square tests as appropriate. For significant omnibus results, pair-wise comparisons were conducted using Tukey's Honest Significant Difference (HSD) tests for continuous outcomes and Bonferroni-corrections for categorical outcomes.

A series of multivariable linear regression models was used to model each structural MRI and MRS measure as a function of comorbidity group, age, and their interaction, covarying for scanner. Comorbidity status was dummy coded with confounded participants serving as the reference group. Supratentorial cranial volume was included as a covariate in structural MRI analyses to control for individual differences in head size. Similarly, the proportion of relevant tissue volume within each voxel (e.g., amount of gray matter in FGM) was included as a covariate for MRS analyses to control for individual sampling variability. Models lacking a significant comorbidity and age interaction were re-run without interaction terms to estimate the independent effect of age across the whole sample. For models with significant comorbidity group by age interactions, we added individual comorbidities that differed in prevalence across age as covariates to examine potential attenuation of age-related comorbidity group effects. To shed light on findings in the present study, exploratory analyses also examined whether the interactive effects of age and individual comorbid conditions followed the pattern of overall comorbidity group by age interaction effects. Standardized betas are presented to enhance interpretability of comorbidity group and age

effects across imaging outcomes. All analyses were performed using JMP Pro version 12.0.1 (JMP®, Version <*12.0.1*>, SAS Institute Inc., Cary, NC, 1989-2007).

RESULTS

Sample Characteristics

Table 2 provides demographics, HIV disease characteristics, depressive symptoms, and rates of neurocognitive impairment across the three groups. Participants were predominantly male (81%) with a mean age of 44.2 years. Of the 288 study participants, 130 (45%) were neurocognitively impaired and rates of impairment across comorbidity group demonstrated a stair-step pattern (i.e., incidental < contributing < confounding) similar to that of the total CHARTER cohort[1]. As expected, confounded participants reported the most depressive symptoms and had the lowest estimated premorbid verbal IQs and education levels. Similarly, confounded participants had a significantly higher rate of any lifetime substance use disorder (97%) than non-confounded participants (Table 1), although none met criteria for current substance use disorder. Groups did not differ on HIV disease characteristics, with the full sample demonstrating evidence of cART-induced immune reconstitution based on active ARV use (75%) and higher current CD4 counts (median = 459 cells/mm^3) compared to low nadir CD4 counts (median = 152 cells/mm^3) and AIDS diagnoses (67%). 50% of the sample had detectable plasma HIV RNA (≥50 copies/ml) and 31% (175/255) had detectable CSF HIV RNA.

Structural MRI, comorbidity groups, and age

Effect size estimates for comorbidity group, age, and their interaction in regression models for each structural measure are provided in Table 3. Confounded participants exhibited evidence of greater brain abnormality across all structural measures, with

significantly smaller volumes of cortical gray matter (vs. incidental) and more abnormal white matter (vs. incidental and contributing) and ventricular CSF (vs. incidental). Compared to the incidental group, the contributing group displayed significantly smaller volumes of cortical gray matter volumes (β=-0.08, p=0.01) and larger volumes of ventricular CSF (β=0.14, p=0.02) resulting in stair-step patterns of greater structural damage with increasing comorbidity burden.

The deleterious impact of severe comorbidity burden on brain structure was exacerbated with increasing age (Table 3; Figure 1): compared to the effect of age in the contributing group, older age in the confounded group was associated with more abnormal white matter (β=0.48 vs. β=0.25; p=.017), less total white matter (β=-0.35 vs. β=-0.19; p=.015), and less subcortical gray matter (β=-0.54 vs. β=-0.32; p=.039). Similarly, the negative effect of age on total white matter volume was greater in confounded participants compared to incidental participants (β=-0.54 vs. β=-0.23; p=.006). In contrast, older age was associated with less cortical gray matter for incidental (β=-0.26; p<.001) and contributing (β=-0.19; p<.001) groups, while confounded participants displayed low cortical gray matter volumes at a younger age and demonstrated no further age-related effect (β=-0.04; p=.644). While age did not moderate the effect of comorbidity group on ventricular and sulcal CSF volumes, older age predicted larger ventricular (β=0.29; p<.001) and sulcal CSF volumes (β=0.34; p<.001) independent of comorbidity group.

To examine potential attenuation of age-related comorbidity group effects, we covaried for individual comorbidities that differed in prevalence across age. Increasing age was associated with greater likelihood of hypertension (OR=1.08; *p*<.001), diabetes (OR=1.05; *p*=.039), hyperlipidemia (OR=1.06; *p*=.026), hepatitis C (OR=1.08; *p*<.001), and lifetime alcohol use disorder (OR=1.03; *p*=.040), as well as lower likelihood of lifetime methamphetamine use disorder (OR=0.93; *p*<.001). However, covarying for these age-related

comorbidities did not attenuate any of the aforementioned significant comorbidity group by age interaction effects (all *p*s<.05).

Finally, multivariable regression models were conducted to explore the potential interactive effects of age and specific conditions on imaging outcomes that were significantly predicted by the overall comorbidity group by age interaction analyses. While these analyses are not meant to be definitive association studies, they may shed light on the interpretation of findings within this manuscript and guide future explorations. Significant individual comorbidity by age interaction effects followed similar patterns to those detected in the overall comorbidity group by age interaction analyses. Specifically, the deleterious effect of age on abnormal white matter was significantly stronger in participants with hypertension (β=0.39 vs. β=0.08; p=.047) and hepatitis C co-infection (β=0.39 vs. β=0.08; p=.035). Moreover, older age was associated with less cortical gray matter for participants without a history of special education (β=-0.25; p<.001) or head trauma (β=-0.25; p<.001), yet older age did not relate to cortical gray matter volume for participants with a history of special education (β=-0.00; p=.927) or head trauma (β=-0.09; p=.200).

MRS, comorbidity groups, and age

Significant main effect size estimates of comorbidity group and age for MRS outcomes are presented in Table 4. Comorbidity group was significantly associated with *N*-acetylaspartate and choline in FWM as well as *N*-acetylaspartate in BG (Table 4). Specifically, compared to the incidental group, confounded participants displayed significantly lower levels of *N*-acetylaspartate in FWM and BG as well as a trend toward higher levels of myo-inositol in BG (*p*=.052). Compared to the contributing group, confounded participants displayed lower levels of *N*-acetylaspartate in BG and higher levels of choline in FWM. The contributing group did not significantly differ from the incidental

group for any MRS outcome. Independent of comorbidity group, older participants had lower levels of *N*-acetylaspartate in FWM and higher levels of choline and myo-inositol in both FWM and FGM. No comorbidity group by age interactions were detected for MRS outcomes.

DISCUSSION

The present study examined the cerebral impact of comorbidity burden and age in PLWH enrolled in the multi-site CHARTER study. We have previously reported on the neuroimaging correlates of HAND in PLWH without confounding neurocognitive comorbidities[16, 17]. Unlike most neuroAIDS studies that aim to isolate HIV-specific mechanisms of CNS injury and therefore exclude confounded individuals, we deliberately included confounded individuals as they may represent a particularly vulnerable and substantial subpopulation of PLWH[1]. Our findings indicate that PLWH with comorbidities severe enough to preclude a diagnosis of HAND display greater brain abnormalities than those with minimal-to-moderate comorbidity burdens. Specifically, confounded PLWH demonstrated evidence of lower neuronal integrity and structural volumes as well as more neuroinflammation in cortical, subcortical, and white matter tissues. Importantly, we demonstrate conditional effects of age on the relationship between comorbidity burden and brain integrity such that older age and severe comorbidities synergistically contribute to subcortical and white matter tissue injury. In addition to prior CHARTER studies documenting that confounded PLWH are at greater risk for neurocognitive and everyday functional impairment at baseline[1] as well as neurocognitive decline over an average course of three years[25], our findings demonstrate distinct neurological differences across comorbidity classifications and underscore the importance of including these classifications during HAND-diagnostic decision making.

cART-era volumetric studies report cortical and subcortical gray matter atrophy, white matter volume loss and microstructural abnormalities, and more abnormal white matter in PLWH compared to seronegative controls[5, 15, 26, 27]. Two recent meta-analyses from O'Connor and colleagues provide further evidence for HIV-related neurostructural damage, with the most reliable and prominent effects occurring in total brain volume, gray matter volume, and CSF volume[28, 29]. The observation that confounded PLWH had greater structural brain abnormalities than incidental and contributing PLWH is not surprising and validates the relevance of comorbidities to brain integrity. The source of variability in cortical, subcortical, and white matter volumes across comorbidity classifications is likely multifactorial. By definition, confounded individuals exhibit higher rates of non-HIV-related comorbidities, including neurodevelopmental, cerebrovascular, and systemic medical conditions. Cardiometabolic disorders (e.g., hypertension, diabetes mellitus) and hepatitis C co-infection are examples of conditions among confounded individuals that are known to contribute to CNS dysfunction among PLWH as well as the general population[30-36]. Furthermore, these conditions increase with age and may therefore help explain why older confounded PLWH demonstrate the strongest evidence of brain injury. Our individual comorbidity by age interaction analyses suggest a role for hypertension and hepatitis C in driving our age-related findings, as the deleterious effect of age on abnormal white matter was strongest in participants with these comorbid conditions. However, our comorbidity group by age findings remained significant after adjusting for individual conditions that were associated with age, which suggests that a confounded classification reflects broad vulnerability to age-related brain injury that may not be fully explained by any single comorbid condition. More work is needed to determine whether the brain abnormalities present in older, confounded PLWH are more strongly linked to comorbidities of aging or "legacy" effects of conditions acquired during younger age.

Contrary to expectations, younger confounded PLWH displayed similarly low cortical gray matter volumes relative to older confounded PLWH, whereas older age predicted lower cortical volumes among non-confounded PLWH. Examination of model estimates (Table 3) and visual inspection of simple slopes (Figure 1, panel D) suggest that the predicted cortical volume of a 35-year old confounded PLWH closely matches that of a non-confounded PLWH in their early 50's. One potential explanation for this finding is the presence of non-age-related conditions that impact cortical structure among confounded participants, including learning disabilities[37], neurotrauma[38], and substance-related complications[39]. Notably, age did not significantly predict cortical gray matter in patients with low estimated premorbid IQ or head trauma, suggesting that the low volumes of cortical gray matter observed among younger confounded participants may reflect longstanding differences in structural brain integrity as well as acquisition of acute brain insults.

Notably, a stair-step pattern was observed by which increasing comorbidity burden was associated with smaller volumes of cortical gray matter, accompanied by larger volumes of ventricular CSF. However, no other significant structural nor neurochemical differences were detected between contributing and incidental participants, suggesting that moderate comorbidity burden is preferentially sensitive to our measure of cortical gray matter. Relative to the incidental group, the smaller cortical volume detected in the contributing group may confer risk of neurocognitive dysfunction as we observed significantly higher rates of neurocognitive impairment among contributing (52%) versus incidental (35%) participants. Although we observed the highest prevalence of neurocognitive impairment in confounded participants (83%), confounded participants displayed widespread neuroanatomical alterations that could also explain the presence of neurocognitive deficits. Furthermore, while the confounded group displayed distinct patterns of age-related effects on brain structure compared to non-confounded groups, the effect of age was similar between the incidental and

contributing groups. The striking pattern of CNS injury in confounded PLWH, compared to the similar neuroanatomical profiles of incidental and contributing participants, does not indicate comorbidity group differences in underlying HIV-related neurotoxicity; however, the unique impact of confounding neurocognitive comorbidities on brain integrity aligns with the Frascati comorbidity classification distinction between confounded and non-confounded PLWH.

Confounded participants also exhibited patterns of neurometabolic dysfunction in FWM and BG compared to incidental and contributing participants. Our results demonstrating lower levels of *N*-acetylaspartate (FWM and BG) and higher levels of choline (FWM) and myo-inositol (BG) in confounded PLWH parallel past studies, in which confounding conditions were considered exclusionary, reporting that cART-era PLWH experience reductions in neuronal integrity with accompanying elevations in neuroinflammation across cortical and subcortical regions[40-42]. Investigating the independent and interactive effects of HIV status, cardiovascular risk, and age on MRS outcomes, Cysique et al.[43] reported that acute cardiovascular events predicted lower *N*-acetylaspartate and higher myo-inositol in the posterior cingulate cortex, yet these effects were not moderated by age. Although older age independently predicted lower *N*-acetylaspartate (FWM) and higher choline and myo-inositol (FWM and FGM) in our sample, we similarly did not detect any moderating effects of age on the association between comorbidity burden and MRS outcomes. In addition to enhanced cerebrovascular risk, our results may be partly explained by greater prevalence of substance use, systemic medical conditions (e.g., hepatitis c co-infection), and head trauma among confounded participants; all aforementioned comorbidities have been linked to neurochemical abnormalities and neurocognitive impairment in HIV-infection[39, 44, 45].

Our analyses comparing comorbidity groups on HIV disease and treatment characteristics suggest that the observed impact of comorbidity burden on imaging outcomes is not primarily attributable to group differences in systemic HIV disease severity. All three comorbidity groups displayed similar patterns of cART usage, immune recovery (e.g., difference between nadir and current CD4), and plasma and CSF viral load levels. These results are consistent with the larger baseline CHARTER study in which comorbidity groups did not differ on most HIV-related neuromedical parameters, with the exception of lower nadir CD4 counts and more AIDS diagnoses in confounded participants compared to incidental participants[1]. Given that prior studies have demonstrated strong associations between increasing HIV disease severity (e.g., higher viral load and lower nadir CD4) and smaller brain volume[16, 21, 46], as well as smaller effects of HIV serostatus on brain volume in cART-era cohorts[29], the deleterious effect of increasing comorbidity burden on brain integrity is noteworthy in the absence of group differences in HIV disease burden and treatment characteristics. In the overall CHARTER sample, lower nadir CD4 counts, AIDS diagnosis, and viral suppression on active cART use were related to higher rates of neurocognitive impairment among incidental participants only[1]. Whether similar conditional effects of HIV disease parameters and comorbidity burden hold in the context of neuroimaging remains unknown and is worthy of future investigation.

We acknowledge several limitations to this study. Although our results highlight the clinical relevance of comorbidity classifications among PLWH, we cannot empirically determine the specificity of our findings to PLWH given the absence of a demographically-comparable control group. Next, our global volumetric MRI measures may be less informative with regard to specific mechanisms of neural injury compared to newer MRI techniques that assess cerebral connectivity (e.g., functional MRI, diffusion tensor imaging), however, our estimates of cortical and subcortical gray matter as well as abnormal and total

white matter permit the quantification of broad neuroanatomical features that are sensitive to effects of both HIV and aging[16, 21, 47, 48]. The cross-sectional and associative nature of our study makes it difficult to disentangle the interactive effects of aging and comorbidity burden from longstanding differences in brain integrity, a topic which could be more adequately addressed with a longitudinal design. Prior longitudinal studies have examined the interaction between HIV serostatus and age/time on neuroimaging outcomes, with some reporting HIV-related accelerated aging in selective cortical tissues[13] and others failing to detect interactions between HIV and aging[49]. Previously published longitudinal neurocognitive findings in CHARTER revealed that a confounded comorbidity classification independently predicted incident neurocognitive decline, suggesting that comorbidity burden influences the stability of CNS function across time[25]. However, we are not aware of any studies that have examined the role of comorbidity burden on longitudinal changes in brain integrity in PLWH. Nevertheless, our inclusive approach to comorbidity classifications demonstrates robust sensitivity to neurological dysfunction, consistent with other methods of quantifying age-related acquisition of multi-system damage (e.g., VACS index[50], frailty index[51]). Finally, although we detected a number of significant differences between confounded and non-confounded PLWH, the relatively small number of confounded participants hinders our ability to conduct more sophisticated models with additional covariates and interaction terms.

Taken together, our findings lend further support to the inclusion of Frascati comorbidity classifications during HAND diagnostic decision-making. Those with severe comorbidity burden exhibit the most extensive evidence of abnormal neurocognitive performance and abnormal neuroimaging findings, despite having similar profiles of HIV disease severity to those with minimal/mild-to-moderate comorbidity burden. In contrast, the neuroimaging profiles of participants with minimal/mild comorbidity burden were mostly comparable to those with moderate comorbidity burden. Our results suggest that the presence

of severe comorbid conditions contributes to neurological dysfunction above and beyond the effects of HIV, thus highlighting the need to identify and account for such conditions during clinical evaluation. Furthermore, confounded PLWH may be particularly vulnerable to age-related subcortical gray and white matter tissue injury, warranting increased clinical monitoring to this population as it ages. Given the rising prevalence of older PLWH, clinical efforts aimed at reducing the frequency (e.g., exercise) and severity (e.g., medication adherence) of comorbid conditions may mitigate the public health costs associated with treating HIV-related neurological dysfunction.

ACKNOWLEDGMENTS

This work was supported in part by awards from National Institutes of Health (the CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) N01 MH2205 and HHSN271201000036C, to I. Grant; R01 MH107345, to R.K. Heaton and S.L. Letendre; and P30 MH62512, to R. Heaton; T32AA013525 to RS; and T32DA031098 to LMC). The views expressed in this article are those of the authors and do not reflect the official policy or position of the United States Government. We gratefully acknowledge all individuals who participated in CHARTER studies, as well as all CHARTER study investigators*.

*The CNS HIV Anti-Retroviral Therapy Effects Research was supported by awards N01 MH22005, HHSN271201000036C and HHSN271201000030C from the National Institutes of Health. The CNS HIV Anti-Retroviral Therapy Effects Research (CHARTER) group is affiliated with Johns Hopkins University; the Icahn School of Medicine at Mount Sinai; University of California, San Diego; University of Texas, Galveston; University of Washington, Seattle; Washington University, St. Louis; and is headquartered at the University of California, San Diego and includes: Director: Igor Grant, M.D.; Co-Directors: Scott L. Letendre, M.D., Ronald J. Ellis, M.D., Ph.D., Thomas D. Marcotte, Ph.D.; Center Manager: Donald Franklin, Jr.; Neuromedical Component: Ronald J. Ellis, M.D., Ph.D. (P.I.),

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Author contributions: R.S., R.K.H., L.M.C., and C.F. conceptualized and designed the study. R.S. conducted statistical analyses and wrote the manuscript draft. A.C. contributed to neuroimaging data processing and interpretation. All authors contributed to data interpretation, revised the manuscript for intellectual content, and approved the final manuscript.

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Table 1 Comorbid condition rates by comorbidity group.

	A. Incidental, *n* = 176	B. Contributing, *n* = 77	C. Confounded, *n* = 35	Pair-wise comparisons[a]
Low estimated premorbid IQ[b]	17 (9.7%)	17 (22.1%)	11 (31.4%)	A < B, C
Special education[c]	6 (3.4%)	7 (9.1%)	9 (25.7%)	A < C
Other school problems	10 (5.7%)	29 (37.7%)	17 (48.6%)	A < B, C
Head trauma[d]	10 (5.7%)	23 (29.9%)	18 (51.4%)	A < B, C
Cerebrovascular events[e]	0 (0%)	0 (0%)	7 (20%)	A, B < C
Epilepsy	0 (0%)	0 (0%)	1 (2.9%)	
Other seizure history	1 (0.6%)	9 (11.7%)	6 (17.1%)	A < B, C
Systemic medical disease[f]	48 (27.3%)	42 (55.3%)	29 (82.9%)	A < B < C
CNS opportunistic disease	7 (4%)	4 (5.2%)	1 (2.9%)	
Lifetime major depression	99 (56.3%)	45 (58.4%)	22 (62.9%)	
Current major depression	18 (10.2%)	7 (9.2%)	3 (8.6%)	
Psychotic disorder[g]	3 (1.7%)	11 (14.3%)	4 (11.4%)	
[0,1-5]Lifetime substance use disorders				
Any substance	132 (75%)	55 (71.4%)	34 (97.1%)	A, B < C
Alcohol	103 (58.5%)	34 (44.2%)	26 (74.3%)	
Cannabis	49 (27.8%)	21 (27.3%)	13 (37.1%)	
Cocaine	73 (41.5%)	36 (46.8%)	24 (68.6%)	A < C

Opioid	25 (14.2%)	20 (26%)	8 (22.9%)	
Methamphetamine	31 (17.6%)	13 (16.9%)	6 (17.1%)	
Hallucinogen	11 (6.3%)	3 (3.9%)	2 (5.7%)	
Sedative	15 (8.5%)	5 (6.5%)	5 (14.3%)	
Inhalant	6 (3.4%)	2 (2.6%)	2 (5.7%)	
Other	7 (4%)	2 (2.6%)	2 (5.7%)	
Substance overdose with complications[h]	2 (1.1%)	10 (13%)	7 (20%)	A < B, C
Recent substance use[i]	21 (11.9%)	28 (36.4%)	8 (22.9%)	A < B
Total comorbid conditions, *m* (SD)	3.0 (2.00)	4.7 (2.31)	6.5 (2.16)	A < B < C

[a]All reported pair-wise differences are significant after Bonferroni-adjustment ($\alpha = 0.05/3 = 0.0167$).
[b]Wide Range Achievement Test reading subtest, 3rd edition less than 80.
[c]Special tutoring or grade retention.
[d]Traumatic head injury with loss of consciousness or other neurologic sequelae.
[e]Range from TIAs to completed strokes.
[f]Potentially significant medical comorbidity (e.g., diabetes mellitus, myocardial infarction, hepatitis C infection).
[g]Schizophrenia or bipolar disorder.
[h]Overdose requiring cardiopulmonary resuscitation or hospitalization.
[i]Positive breathalyzer or urine toxicology for psychoactive substances on day of testing.

Table 2 Demographic and clinical characteristics by comorbidity group.

	A. Incidental, *n* = 176	B. Contributing, *n* = 77	C. Confounded, *n* = 35	*P*	Pair-wise comparisons[a]
[0,1-6]Demographics					
Age (years)	43.6 (8.00)	45.3 (7.37)	45 (7.05)	0.24	
Education (years)	13.3 (2.34)	12.7 (2.42)	11.5 (3.11)	<0.001	A > C
Estimated premorbid IQ[b]	97.2 (12.34)	91.8 (15.66)	84.8 (20.15)	<0.001	A > B > C
Sex (male)	147 (84%)	58 (75%)	27 (77%)	0.28	
Ethnicity				0.23	
White	79 (45%)	28 (36%)	10 (29%)		
Black	78 (44%)	35 (45%)	21 (60%)		
Hispanic	17 (10%)	11 (14%)	4 (11%)		
Other	2 (1%)	3 (4%)	0 (0%)		
[0,1-6]Neurobehavioral					
Neurocognitive impairment	61 (35%)	40 (52%)	29 (83%)	<0.001	A < B < C
BDI-II	8 [4–17.75]	8 [4–17]	15 [8–23]	0.04	A < C
[0,1-6]HIV disease characteristics					
AIDS diagnosis	117 (66%)	53 (69%)	23 (66%)	0.92	
Estimated duration of infection (years)	10.7 (6.01)	11.7 (6.38)	12.7 (5.78)	0.13	
Nadir $CD4^+$ cell count	155 [30.5–299]	120 [20.5–255.5]	158 [25–240]	0.48	
Current $CD4^+$	449.5 [291–	463 [268.25–	461 [274–826]	0.21	

cell count	620.5]	627.25]			
ARV status				0.20	
Currently using	133 (76%)	60 (78%)	24 (69%)		
Never used	17 (10%)	10 (13%)	2 (6%)		
Past use	26 (15%)	7 (9%)	9 (26%)		
[0,1-6] Plasma viral load					
Cells/ml (log)	1.70 [1.70–4.00]	1.75 [1.70–3.63]	1.84 [1.70–4.18]	0.96	
Detectable	89 (51%)	37 (48%)	17 (50%)	0.90	
[0,1-6] CSF viral load[c]					
Cells/ml (log)	1.70 [1.70–2.30]	1.70 [1.70–1.84]	1.70 [1.70–3.06]	0.23	
Detectable	49 (31%)	18 (26%)	13 (43%)	0.26	

Values are presented as mean (SD), median [IQR], or *N* (%). ARV, antiretroviral therapy; BDI-II, Beck-Depression Inventory-II total score.

[a]Pair-wise comparisons were examined using Tukey's H.S.D. ($\alpha = 0.05$) for continuous outcomes and Bonferroni adjustments ($\alpha = 0.05/3 = 0.0167$) for dichotomous outcomes.

[b]Wide Range Achievement Test reading subtest, 3rd edition.

[c]$N = 175$.

Table 3 Standardized beta effect size estimates for structural MRI measures.

Predictor	Cortical gray	Subcortical gray	Abnormal white	Total white	Ventricular CSF	Sulcal CSF
Incidental[a]	**0.16***	0.14	**–0.26****	0.02	**–0.27****	–0.14
Contributing[a]	0.07	0.04	**–0.20*****	0.04	–0.11	–0.06
Age[b]	–0.04	**–0.54***	**0.48****	**–0.35****	**0.42*****	**0.43****
Incidental × age	**–0.18*****	0.20	–0.26	**0.31****	–0.12	–0.06
Contributing × age	–0.07	**0.22*****	**–0.23*****	**0.16*****	–0.05	–0.08

Reported values are standardized regression coefficients for each parameter of interest in multivariable regression analyses predicting structural MRI measures. Significant comorbidity × age interaction plots are presented in Fig. 1.

[a]Compared with confounded. Estimates fixed at average age.

[b]Represents effect of age for confounded group only.

$^{*}P < 0.001$.

$^{**}P < 0.01$.

$^{***}P < 0.05$.

Table 4 Standardized beta effect size estimates for select magnetic resonance spectroscopy measures.

	[0,2-3]Basal ganglia		[0,4-6]Frontal white matter			[0,7-8]Frontal gray matter	
Predictor	NAA	Myoinositol	NAA	Choline	Myoinositol	Choline	Myoinositol
Incidental[a]	**0.27***	–0.19**	**0.23****	–0.14	0.04	–0.11	0.05
Contributing[a]	**0.26***	–0.11	0.17*	–**0.19*****	0.06	–0.13	0.12
Age	–0.09	0.08	–**0.16***	**0.15*****	**0.21******	**0.14*****	**0.13*****

No significant comorbidity and age interactions were detected. Reported values are standardized regression coefficients for each parameter of interest in multivariable regression analyses predicting MRS measures. Sample sizes: Basal ganglia *N*-acetylaspartate ($n = 248$) and myoinositol ($n = 240$); frontal white matter *N*-acetylaspartate ($n = 265$), choline ($n = 264$), and myoinositol ($n = 252$); frontal gray matter choline ($n = 274$) and myoinositol ($n = 271$). MRS, magnetic resonance spectroscopy; NAA, *N*-acetylaspartate.

[a]Compared with confounded.

$^{*}P < 0.01$.

$^{**}P < 0.10$.

$^{***}P < 0.05$.

$^{****}P < 0.001$.

FIGURE LEGEND

Figure 1

Title: Age moderates the relationships between comorbidity burden and brain structure.

Caption: Interaction between age and comorbidity group predicting (A) abnormal white matter, (B) total white matter, (C) subcortical gray matter, and (D) cortical gray matter. All structural volumes are log-transformed.