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Cognition, structural brain changes and systemic inflammation in adolescents living with HIV on antiretroviral therapy

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Abstract

Objective—To investigate the association between neurocognitive impairment, neuroimaging and systemic inflammation in perinatally-infected adolescents living with HIV (PHIV) on ART. Systemic inflammation may be one mechanism driving neurocognitive impairment despite ART, but this has not been investigated in adolescence when the brain is undergoing rapid development.

Setting—Cape Town, South Africa

Methods—Baseline data were drawn from the Cape Town Adolescent Antiretroviral Cohort (CTAAC). PHIV on ART >6 months completed a comprehensive neurocognitive test battery. Diffusion tensor imaging and structural brain magnetic resonance imaging (MRI) was done to determine whole brain fractional anisotropy (FA), mean diffusivity (MD), grey and white matter volumes and cortical thickness. We examined how neurocognitive and neurostructural measures

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Prof Hoare was the principal investigator of this study. She conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.

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Dr Heany carried out the statistical analyses in the manuscript, and approved the final manuscript as submitted

Dr Phillips designed the data collection instruments, and coordinated and supervised data collection and entry, critically reviewed the manuscript, and approved the final manuscript as submitted.

Prof Zar is principal investigator on CTAAC, she critically reviewed the manuscript, and approved the final manuscript as submitted.

Prof Stein, and Prof Myers assisted in study conceptualization, advised on statistical analysis, critically reviewed the manuscript, and approved the final manuscript as submitted.

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were associated with a concurrently measured marker of systemic inflammation, high-sensitive CRP (hs-CRP).

Results—168 PHIV ages 9-12 years (mean CD4 980 cells/μL; 85.3% VL<50 copies/mL) and 43 controls were included in the analysis. PHIV had similar hs-CRP (p=.17) to controls, after participants with hs-CRP >10 were excluded from the analysis. 48% of the PHIV in this analysis have a neurocognitive disorder. Whole brain grey (p=.049) and white matter volumes (p=.044) were lowest in PHIV with a major neurocognitive disorder. Higher MD, was found in PHIV with a major neurocognitive disorder (p=.002). Among PHIV with a neurocognitive disorder, hs-CRP negatively correlated with general intelligence, visual spatial acuity and executive function (all p=<.05). Whole brain MD correlated with higher hs-CRP values (p=<.01) in PHIV.

Conclusion—A marker of systemic inflammation was associated with both neurocognitive impairment and mean diffusivity increases in PHIV.

Keywords

HIV; adolescence; inflammation; neuroimaging; DTI; MRI; neurocognitive; South Africa

Introduction

Neurocognitive impairments despite antiretroviral therapy (ART) are well documented in perinatally-infected adolescents living with HIV(PHIV) [1]. Systemic inflammation may contribute to such impairments, but there are few data to support this hypothesis. PHIV on ART from South Africa have impairment in multiple domains of cognitive functioning, including verbal and full scale IQ, information processing speed, attention, language, finger tapping, processing speed, attention, verbal memory and executive function [1-3]. In addition PHIV have increased risk of regional CNS white matter microstructure alterations, smaller grey matter volumes, reduced cortical surface area and decreased gyrification, when compared to HIV negative controls, suggesting abnormal neurodevelopment in PHIV[4].

There is evidence that C-reactive protein (CRP), a marker of systemic inflammation, is associated with an increased risk for cognitive decline in adults[5]. CRP is able to measure down to concentrations of 3–5 mg/L, whereas high sensitivity C-reactive protein (hs-CRP) measures down to levels around 0.3 mg/L. This improved sensitivity allows hs-CRP to be used to detect low-grade inflammation. Inflammatory mechanisms have also been hypothesized to contribute to the neuropathology leading to late-age cognitive decline and dementia. Some data demonstrate an association of inflammatory markers, including CRP, with these late-age changes in cognitive function[6-8]. However, hs-CRP is non-specific with respect to neuropathology. The Honolulu-Asia Aging Study found a significant relationship between hs-CRP measured at midlife, and Alzheimer's disease and vascular dementia 25 years later[9]. Those with the highest quartile of hs-CRP measured in midlife had significantly more longitudinal cognitive decline than those in the lowest quartile, after adjustment for demographic and cardiovascular risk factors[5].

Several studies in adults living with HIV, have demonstrated that increased markers of inflammation, including hs-CRP, predict increased mortality during treated HIV infection,

even among those with high current $CD4^+$ T-cell counts[10]. In most of these studies, the mortality associations were much stronger than observed in HIV-uninfected cohorts, suggesting that inflammation was likely to be a more important contributor to morbidity and mortality in the context of HIV infection than it is in the general population[10]. HIV-positive adults, global neurocognitive impairment is associated with elevation of peripheral biomarkers of inflammation[11]. For example patients with cognitive impairment have higher plasma sCD163 and sCD14 than those who were not impaired[12,13].

In the current study we aimed to investigate whether neurocognitive and global neurostructural measures were associated with a concurrently measured marker of systemic inflammation, hs-CRP in the CTAAC cohort.

Methods

A neuroimaging and neurocognitive study was conducted in a subgroup of PHIV enrolled in CTAAC. Routine care providers told all adolescent/caregiver dyads attending one of the 4 recruitment sites, who were in the target age range, about the study. Interested adolescents/caregivers were formally screened, and if eligible and willing to participate, provided with an appointment to attend an enrolment visit 1–2 weeks later at Red Cross War Memorial Children's Hospital. PHIV were recruited primarily from primary care sector health care service from across Cape Town; inclusion criteria were aged 9–12 years, perinatally infected, had been on ART for > 6 months, knew their HIV status and able to provide informed parental consent and participant assent. PHIV were sampled consecutively and were not recruited based on disease complexity or virological suppression/unsuppression. Controls were HIV negative and matched for age and sex. Controls were excluded if they had known pre-existing disease or if informed consent and assent was not obtainable. All youth screened for the control cohort underwent rapid HIV testing prior to enrolment to confirm negative status. HIV exposure in the control group is unknown, however the adolescents selected have similar ethnicity, home language, years of education and annual household income. PHIV were examined clinically including a full neurological examination by a medical officer, and were assessed for neurodevelopmental disorders not attributable to HIV. Exclusion criteria for both groups were: a current or recent medical condition, such as a UTI, respiratory tract infection, diabetes mellitus, epilepsy, tuberculosis; an identified CNS condition such as past or current TB meningitis, encephalitis or bacterial meningitis, documented cerebrovascular accident, lymphoma; a history of head injury with of loss of consciousness, or any radiological evidence of skull fracture; a history of perinatal complications such as hypoxic ischemic encephalopathy or neonatal jaundice, or neurodevelopment disorder not attributed to HIV. Participants were enrolled from August 2013 to April 2015. Eleven adolescents in CTAAC cohort were included in a previous work conducted by our team on a smaller, cross-sectional, mixed treatment cohort in Cape Town. Baseline health and sociodemographic questionnaires were administered to obtain general health information, past history and data on ancestry, language, education and treatment. All study measurements were conducted separately from routine HIV care. Physical examination was done to exclude any current illness. Bloods for CD4, viral load and hs-CRP were drawn concurrently. The Tina-quant CRPHS immunoturbidimetric assay was used for the quantitative determination of hs-CRP in mg/L. Participants were excluded from analysis

if hs-CRP >10, as done in prior studies[14]. Levels of hs-CRP over 10.0 mg/L may signify an additional infection or an inflammatory condition. Ethical approval was obtained from the University of Cape Town's Faculty of Health Sciences research ethics committee.

Neurocognitive assessment

Each participant was assessed using a battery of standardized neurocognitive tests commonly used in paediatric neuropsychological assessment and research in South Africa. Tests were administered in the children's home language. Test instructions were translated and back-translated into isiXhosa, and test administration complied with International Test Commission guidelines[15]. Where possible neurocognitive measures were adjusted for age by using age-adjusted scaled scores in the scoring of the tests. General intellectual functioning was measured using the Wechsler Abbreviated Scale of Intelligence (WASI) [16]. The following tests were used to examine cognitive domains: Fingertip Tapping subtest from the NEPSY-II[17], Grooved Pegboard Test[18]. Subtests from the Wechsler Intelligence Scale for Children (WISC-IV) [19], measured information processing speed, The Rey Complex Figure Test (RCFT) [20], the Boston Naming Test – Short Form-South Africa (BNT-SF-SA) [21],category and phonemic fluency, immediate and delayed recall trials of the RCFT and the Hopkins Verbal Learning Test-Revised (HVLT-R) [22], WISC-IV Digit Span backwards (DS backwards) subtest, the Color Trails Test 2 (CTT2) and the NEPSY-II Inhibition subtests.

Using data from the test battery, as well as theoretical knowledge about the construct(s) each test is intended to test, we created ten separate composite cognitive domains: general intellectual functioning, attention, working memory, visual memory, verbal memory, language, visual spatial ability, motor coordination, processing speed and executive function[2]. To determine the statistical strength of each cognitive domain, we conducted Cronbach's alpha tests on various combinations of neuropsychological tests to determine which neuropsychological tests had strong inter-relatedness (or internal consistency) within a specific domain. For the Cronbach's alpha tests we only used the total scaled scores of the tests, and/or subtests, for each of the individual neuropsychological tests. Scaled scores were used because they take into account the child's age, sex and the various developmental changes happening in the different age groups. To compute the individual test scaled scores, we used the individual test publisher norms. Where test publisher norms were not available (for example; for the HVLT) we converted the test raw score into a *z*-score. The z-scores were based on the control sample means and standard deviation. The controls were SES, age and sex matched to the group of adolescents living with HIV.

A Cronbach's alpha value of 0.7 was considered high and deemed as an indication of good inter-relatedness between the tests. The Cronbach's alpha tests were only done for domains in which there were more than one neuropsychological test measuring performance in that domain. The domains of language and visual spatial ability, each consisted of only one neuropsychological test from the battery, and thus Cronbach's alpha values are not reported for these two domains. Tests which did not contribute well to the cognitive domains with regards to the Cronbach's alpha value were excluded from the individual composite cognitive domain scores.

Composite cognitive domain scores were calculated by averaging the scores of the all tests that comprised each domain, so that a single score for each domain was determined for each participant[2]. We used the CTAAC control sample (n=44) means and standard deviations to convert all scaled and T-scores of the individual neuropsychological tests into *z*-scores. After available test publisher norms were used to score tests, in addition we converted these into z-scores for the purposes of the setting up the composite cognitive domains scores. The reason for this was to ensure that we were combing and comparing different tests in the same metric system.

South Africa has significant issues with inequalities in SES, education and early childhood development programs (to name but a few of the inequalities in our society). The controls for this study match the PHIV+ sample for age, sex and SES circumstances. Controls from these poor communities often do not have access to high quality education and are often also going to school hungry, tired and with issues stemming from their living environments and are thus not able to perform as well as controls from more affluent areas in South Africa or Cape Town. Hence the importance of generating z-scores based on the control sample means and standard deviation Next, we applied the youth HIV-associated neurocognitive disorder diagnostic criteria [1], based on a DSM V model of neurocognitive disorders, to each participant's neuropsychological profile in order to classify each as having either a major neurocognitive disorder, a minor neurocognitive disorder, or no cognitive impairment. The application of these diagnostic criteria involves drawing on data from both cognitive performance and functional ability. We defined major ND as being cognitive performance of > 2 *SD* below the mean in at least two domains and functional impairment, minor ND as being cognitive performance of > 1 *SD* below the mean in at least two domains and functional impairment, and No ND as being all other cognitive performance. Functional impairment encompassed the following; repeated grades at school or CBCL total competence subscale.

The Child Behavior Checklist caregiver rated version (CBCL) [23] was used to assess functional impairment and is one of the most widely used and psychometrically sound measures for assessing child behavioral and emotional problems and psychopathology [24]. This 113-item instrument provides parent-reported information on the child's overall competencies and problems, as well as internalizing and externalizing behaviours. For each item, the parent is asked to rate how accurately a given statement (e.g., "Acts too young for his/her age") describes the child, with response options ranging from 0 (*not true*) through 1 (*somewhat or sometimes true*) to 2 (*very true or often true*). The CBCL was scored using the author developed ABESA scoring software for CBCL. The software makes use of multi-cultural norms for scoring. The CBCL was completed by the adolescent's primary caregiver. In some cases, this was the biological mother and in others another relative who was primarily responsible, and with whom they lived. We did not include adolescents living in group homes in the CTAAC neuro-sub study. The primary caregiver's depressive symptomatology was measured by the CES-D and appropriate referrals were made as necessary.

Image acquisition

Structural and Diffusion weighted imaging was performed at the Cape Universities Brain Imaging Centre on a 3T Siemens Allegra scanner within 7 days of neurocognitive assessment. For the diffusion-weighted imaging a single-channel transmit-receive head coil was used with the following parameters: TR = 8800ms, TE = 88ms, field-of-view of 220mm, 1.8 x 1.8 x 2.0 mm^3 image resolution, 65 slices, 0% distance factor and 2x Generalized Autocalibrating Partial Parallel Acquisition (GRAPPA) acceleration. Images were acquired in an axial orientation with 30 gradient directions at b = 1000mm/s^2, and 3 directions with b = 0mm/s^2. The acquisition was repeated 3 times to allow for redundancy in data. A multi-echo Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) T1-weighted image was acquired with the following parameters: FOV = 256 x 256mm, TR = 2530ms, TE = 1.53/3.21/4.89/6.57ms, TI = 1100ms, flip angle = 7°, 144 slices, in-plane resolution = 1.3 x 1.0mm^2 and slice thickness of 1.0mm[4].

DTI Pre-processing

Diffusion weighted images from individual participants were co-registered to their corresponding b=0 image in order to correct for eddy current distortions and movement artifacts within FMRIB Software Library (FSL) 5.0.1 and imported into MATLAB R2013b for processing. This entailed the affine registration to the average b= 0mm/s^2 image of the first acquisition. For each of the acquisitions, outlier data points were determined by calculating the Z-values at the 25^{th} and 75^{th} percentile of the registered diffusion image. Any data points that were 3 SD from the mean were excluded. The corrected images were exported to FSL 5.0.1 after correction. In FSL 5.0.1 images underwent brain extraction in the Brain Extraction Toolbox (BET) to remove any non-brain tissue and fit a linear tensor model to produce fractional anisotropy (FA) and mean diffusivity (MD) maps.

Fractional anisotropy images were analysed with the Tract-based Spatial Statistics (TBSS) [25]. Each participant's FA was registered to a study-specific target. This target was determined by registering each participant to every other participant. The mean square displacement coefficient of each image was calculated and the participant with the lowest mean displacement was chosen as a representative target for the group. After registration to the study-specific target, each image was then up-sampled to Montreal Neurological Institute (MNI) space, taking into account the previous transformation parameters. An average FA was created and thinned to produce a mean FA skeleton with a threshold of 0.2. This skeleton is representative of the centres of white matter tracts common to the group. Registration and skeleton projection were also applied to the MD images as described above. For the calculation of whole brain MD and FA, the 48 white matter regions, defined by the JHU-atlas[26] were extracted from the average FA and MD of every subject's registered TBSS skeleton. A mean was calculated, which consisted of the mean FA or MD per region weighted by the ROI voxel size, in order to calculate an aggregate score of the whole brain.

Freesurfer pre-processing

T1-weighted images were processed with Freesurfer V5.3 on the Lengau cluster at the Centre for High Performance Computing (CHPC), Rosebank, Cape Town, South Africa. The pipeline has been described previously by Fischl et al. [27]. T1-weighted images were

normalized, bias-field corrected for intracranial volume and skull-stripped. Inner and outer cortical surfaces were modelled as triangular tessellation. Cortical thickness measurements were obtained by calculating the distance (in mm) between pial and grey-white matter surfaces at each vertex location. The vertex data was normalized to the “fsaverage” template included with Freesurfer by utilizing a curvature matching technique. Cortical grey matter and white matter volume was calculated as the product of surface area and cortical thickness and corrected for intracranial volume by utilizing the proportion method of correction[28].

In addition, total grey matter volume, white matter volume and mean cortical thickness were exported to SPSS 25.0 for correlation analyses. Mean cortical thickness was determined by weighting each cortical region by the surface area and thickness per region and calculating the aggregate for the whole brain.

Statistical analysis

Statistical analyses were performed using SPSS 25 software (IBM, Armonk, NY, USA). Demographic data, laboratory tests and neurocognitive domains were analysed using independent sample T tests for continuous variables and Chi-squared tests for categorical variables.

First, differences in hs-CRP between the PHIV and control groups were assessed using T tests (Table 1). Participants with hs-CRP>10 were removed from the analysis. Differences in cognitive functioning were also compared between PHIV and control groups (Table 2). Second, associations between hs-CRP and 10 cognitive function domains were tested using bivariate correlations. This was done in the PHIV and control groups separately, as well as in the combined group. Additionally, the PHIV group was stratified according to neurocognitive disorder classification (Table 3). For the purposes of creating a binary variable for impaired vs not impaired, minor and major NCD were combined. Third, bivariate correlations were run between hs-CRP and whole brain structural values. Again, this was done separately for the PHIV and control groups, and then combined. Differences across neurocognitive impairment groups with hs-CRP and whole brain structure were also assessed. A Bonferroni correction for multiple comparisons was used.

Results

204 PHIV infected and 44 HC were enrolled. Participants with hs-CRP>10 were excluded from the analysis. One control participant and 26 PHIV had hs-CRP>10. An additional 10 participants did not have a recorded hs-CRP result at the time of testing. 168 PHIV and 43 controls were included in the analysis. PHIV had similar hs-CRP (p=.17) to controls, after participants with hs-CRP >10 were excluded from the analysis. Demographic characteristics were similar between PHIV and controls (Table 1). Adolescents living with HIV, however, were more likely to have repeated grades at school. Most HIV-infected adolescents were on first line ART (67%), with a mean CD4 count of 980 cells/mm^3, median viral load of <LOD (level of detection) copies/mL and mean age of initiation of ART of 3.48 years. The range of age of initiation was 0.25yrs - 10.98yrs.

There were significant differences between the PHIV and control groups in the following domains: general intellectual functioning, executive functioning, working memory, verbal memory, visual memory, language and processing speed. There were no significant differences in the domain of motor coordination, attention and visual spatial ability. In addition the PHIV group had significantly lower scores on the CBCL functional competence subscale (p=.017). See Table 2 for details. 87 PHIV participants had no neurocognitive disorder, 72 had a minor neurocognitive disorder and 9 a major neurocognitive disorder. 48% (n=81) of the PHIV in this analysis have a neurocognitive disorder (minor and major combined), and 20% (n=9) of the control participants have a mild neurocognitive disorder. None of the control group have a major neurocognitive disorder. The sample size of PHIV with major NCD is very small (n=9), and therefore difficult to establish reliable phenotypes with such few participants, however small number major NCD in PHIV post ART are consistent with previous study of a different cohort of PHIV and controls in Cape Town[1].

Associations between hs-CRP and cognitive function

In PHIV with a neurocognitive disorder, three cognitive domains were significantly negatively associated with hs-CRP, namely general intelligence, visual spatial acuity, and executive function (all p=<.05) see Table 3. The controls and PHIV without a NCD, had no significant correlations between hs-CRP and cognitive functioning domains.

Associations between hs-CRP and whole brain structure (FA, MD, grey and white matter volume, and cortical thickness)

In the PHIV, and PHIV and control groups combined, whole brain mean MD is positively correlated with hs-CRP (p=<.01). In the control group, there were no associations between hs-CRP and whole brain mean values (i.e. FA,MD, total grey matter, total white matter, mean cortical thickness, etc.). See Table 4

Neurocognitive disorder classification had no significant associations with hs-CRP. However, neurocognitive disorder classification was associated with significantly smaller grey and white matter volumes, with PHIV with a major neurocognitive disorder having the lowest volumes (p=.006 and p=.013). Cortical thickness was highest in PHIV with no neurocognitive disorder (p=.031). Additionally MD was significantly higher in PHIV with a neurocognitive disorder (p=<.001).

Discussion

Our study found 48% of the PHIV in this analysis to have a neurocognitive disorder. In addition whole brain grey, white matter volumes and cortical thickness were lowest in PHIV with a neurocognitive disorder. Higher MD, was found in PHIV with a major neurocognitive disorder. hs-CRP negatively correlating with neurocognitive domains including general intelligence, visual spatial acuity, and executive function among PHIV with a neurocognitive disorder. Neuroimaging studies found whole brain MD increased with higher hs-CRP values. Higher MD could be suggestive of inflammation and myelin loss. There were no associations in the control group between hs-CRP and global brain structural measures and neurocognitive function.

Examining whole brain measures by neurocognitive disorder diagnosis is unique to this study. Whole brain grey, white matter volumes and cortical thickness were lowest in PHIV with a neurocognitive disorder. Higher MD, was found in PHIV with a major neurocognitive disorder. These neuroimaging findings provide support for the use of youth HIV-associated neurocognitive disorder diagnostic criteria[1], based on a DSM V model of neurocognitive disorders. We have previously reported on associations of greater brain volumes with better general intellectual functioning in PHIV[4]. Previous studies with HIV infected youth have found worse cognitive performance correlated with smaller regional volumes [29]. Higher MD has also been associated with lower intellectual functioning[30]. HIV infected children with a clinical diagnosis of HIV associated encephalopathy (HIVE) have greater damage to neuronal microstructure when compared to both ART naïve and ART treated HIV infected children[31]. This pattern of smaller gray/white matter volumes and poor white matter integrity suggests that pediatric HIV infection may influence brain development and underlie cognitive impairment seen in this population. These findings suggest that regardless of ART, adolescents in the CTAAC cohort are at a risk for CNS disease[3].

The association of peripheral inflammatory markers with adolescent's cognitive function has received minimal attention in the literature to date. The role of neuroinflammation as a major contributor of HIV related brain injury has been examined in studies where peripheral systemic inflammatory markers are used as indicators of neuroinflammation[32,33]. The state of the science is based on adult participants. In the last few years, the inflammatory response in the systemic circulation has been recognized as a key driver of HIV pathogenesis, both in the periphery and in the CNS. In the CNS of adults living with HIV, there is considerable evidence that this inflammatory response drives the development of neurocognitive disorders or worsens it, possibly independently of viral replication[32]. In neuroimaging studies of adults living with HIV, plasma inflammatory biomarkers were strongly associated with adverse alterations in brain structure and function[34]. Plasma cytokines were significantly higher in adults living with HIV, with cognitive impairment than than those with no cognitive impairment[35]. In the current study there were no associations in the control group between hs-CRP and global structural brain measures and neurocognitive function. The lack of associations observed in the control population may be due to the small numbers in the control group. Indeed, given the values of hs-CRP are similar in both groups, the effects being observed may not be HIV related. Or these findings may suggest that despite similar hs-CRP levels between the groups, that systemic inflammation may be a more important negative contributor to cognition in PHIV. A recent study supports this finding with greater variability in CRP predicting lower executive function, attention/working memory, and psychomotor speed in HIV infected, but only learning in HIV negative women[36]. Studies in adults HIV+ have demonstrated that increased hs-CRP, predict increased mortality during treated HIV infection, even among those with high current $CD4^{+\cdot}$ Mortality associations were much stronger than observed in HIV-uninfected cohorts, suggesting that inflammation was likely to be a more important contributor to morbidity and mortality in the context of HIV infection than it is in the general population[10].

Limitations of this study should be emphasized. First, the design is cross sectional, limiting causal inferences. However, longitudinal follow-up is underway to better understand the

relationship between HIV and hs-CRP on brain remodeling typically seen in later adolescence. In addition, what hs-CRP means in the aetiology of neurocognitive disorders in PHIV is unclear. Hs-CRP may not be an aetiological mechanism, but rather a marker of a mechanism. Second, the control group is small in comparison to the participant group, therefore the lack of associations observed in the control population may be due to these small numbers and lack of power. Indeed, given the values of hs-CRP are similar in both groups, the effects being observed may not be HIV related. Prevalence of HIV exposure in the control group is unknown. However, our controls represent adolescents from similar demographic backgrounds. Third, only one peripheral serum inflammatory marker was used. Hs-CRP is a very non-specific inflammatory marker, and cognitive function test scores are influenced by many other factors. Fourth, although other congenital infections and incidental CNS abnormalities were excluded as far as possible on history, clinical examination and on clinical review of the MRI scans, it remains a possibility that there may be some overlapping effects of undiagnosed conditions such as congenital CMV. HIV-infected children and adolescents continue to have the largest gaps in treatment in South Africa[37]. All children should receive ART as early as possible, preferable from birth irrespective of clinical stage or immunological status. However, context-specific differences in treatment guidelines and access to ART over the past two decades have resulted in great variability in use of ART among perinatally infected adolescents living with HIV. The range of age of ART initiation in the CTAAC cohort is very wide, from 0.25yrs to 10.98yrs, unfortunately reason for initiation of treatment was often unclear. For example initiation of ART late may have been due to late diagnosis, a poorly-resourced local clinic or to temporary resilience to immunosuppression.

In addition we do not have Nadir CD4 count data.

Conclusion

Adolescence represents a period marked by extensive changes in both brain structure and function. PHIV has a significant negative impact on cognitive function across multiple domains, likely to have detrimental effect on schooling and future employment opportunities. Therefore, identification of markers of physiological health, such as inflammatory markers, associated with adolescent cognitive function has important implications for not simply adolescent's cognitive health, but cognitive function throughout the life span.

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Conflicts of Interest and Source of Funding:

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Table 1:

Baseline demographic and clinical characteristics of CTAAC cohort

Variable	PHIV (N = 168)	Controls (N = 43)	P
Age in years: mean (SD)	10.80 (0.89)	10.70 (0.99)	.49
Gender: male/female	81/87	18/24	.53
Home language: isiXhosa/ Other	150/18	40/2	.24
Years of education: mean (SD)	4.23 (1.11)	4.35 (1.34)	.56
Repeated grades: YES/NO	97(58%)/71(42%)	17(39%)/25(61%)	**.05**
hs-CRP	1.52(1.63)	1.14(1.48)	.17
ART regimen: first/second/third line	113/44/11		
Duration of ART: mean years (SD)	7.08 (2.41)		
Age of initiation: mean years (SD)	3.48 (2.52)		
Viral load (copies/mL): median(IQR); >50	0(40)		
Detectable/Undetectable viral load	30(18%)/138(82%)		
CD4 count (cells/mm3): mean (SD)	980 (503)		

P values are provided for T tests in the case of interval data, and Chi square in the case of nominal data. hs-CRP= highly sensitive c-reactive protein; ART=antiretroviral therapy. 30 patients have detectable VL, i.e. over 50 copies/mL.

Table 2:

Summary of baseline cognitive domain measurements and CBCL subscale ‘functional competence’ score.

Variable	PHIV (N = 168)	Controls (N = 43)	T	P
Cognitive domains: Z score means (SD)				
General intellectual function	−.47 (.78)	.00 (.80)	3.46	**.001**
Attention	−.27 (.84)	.00 (.93)	1.90	.059
Motor coordination	.07 (.99)	.02 (.91)	−0.29	.776
Visual memory	−.46 (.87)	.01 (.98)	3.09	**.002**
Verbal memory	−.53 (1.29)	−.00 (.85)	3.25	**.002**
Working memory	−.42 (.61)	.02 (.62)	4.22	**<.001**
Language	−.35 (1.07)	.01 (1.01)	2.00	**.048**
Visual spatial acuity	−.29 (.87)	.02 (1.01)	1.96	.052
Processing speed	−.50 (.66)	.02 (.67)	4.57	**<.001**
Executive function	−.41 (.67)	.01 (.61)	3.82	**<.001**
CBCL functional competence	38.1(7.7)	41.3(8.1)	−2.4	**.017**

Figures represent Z score means and (SD)

Table 3.

Correlations between hs-CRP and cognitive domains in PHIV with and without a neurocognitive disorder (NCD)

	All subjects (n=211)	**Controls (n=43)**	**PHIV (n=168)**	**PHIVs without NCD (n=87)**	**PHIVs with NCD (n=81)**
hs-CRP x General intelligence	−.08(.22)	−.02(.89)	−.08(.33)	.14(.19)	−.33(**<.01**)
hs-CRP x Attention	−.03(.70)	−.19(.21)	−.03(.70)	−.01(.98)	.09(.46)
hs-CRP x Motor coordination	−.03(.71)	.20(.20)	−.08(.33)	−.02(.82)	−.12(.29)
hs-CRP x Visual memory	−.05(.45)	−.07(.68)	−.03(.74)	.04(.69)	−.08(.50)
hs-CRP x Verbal memory	−.09(.21)	.07(.65)	−.10(.22)	−.01(.95)	−.16(.16)
hs-CRP x Language	−.11(.10)	−.07(.65)	−.11(.16)	−.12(.27)	−.09(.43)
hs-CRP x Visual spatial ability	−.12(.08)	−.06(.67)	−.12(.11)	.02(.85)	−.24(**.03**)
hs-CRP x Processing speed	−.11(.11)	−.15(.35)	−.07(.35)	.08(.44)	−.18(.10)
hs-CRP x Working memory	−.05(.44)	−.19(.22)	−.01(.92)	.07(.50)	−.02(.85)
hs-CRP x Executive function	−.11(.12)	−.06(.71)	−.10(.22)	.04(.74)	−.22(**.04**)

Pearson's r correlation values are provided with p values in the brackets.

Table 4:

Correlations between hs-CRP and mean whole brain structural values

Whole Brain Means	Correlation with hs-CRP Pearson's r (p)
In PHIVs and controls combined	
Grey matter volume	−.04 (.56)
White matter volume	−.05 (.47)
Cortical Thickness	−.02 (.81)
Mean FA	.03 (.72)
Mean MD	.23 (**<.01**)
In Controls	
Grey matter volume	−.21 (.17)
White matter volume	−.15 (.34)
Cortical Thickness	−.22 (.17)
Mean FA	.19 (.40)
Mean MD	−.26 (.25)
In PHIVs	
Grey matter volume	<.01 (.96)
White matter volume	−.02 (.76)
Cortical Thickness	.03 (.70)
Mean FA	.04 (.70)
Mean MD	.28 (<.01)

For each correlation, a bivariate Pearson's correlation r value is given with the p value in brackets. FA=factional anisotropy; MD=mean diffusion.

Table 5:

Differences in hs-CRP and whole brain structures based on neurocognitive disorder classification in PHIV and controls

	No neurocognitive impairment	Minor neurocognitive disorder	Major neurocognitive disorder	F	P
	Mean(SD)	Mean(SD)	Mean(SD)		
PHIV	N=87	N=72	N=9		
hs-CRP	1.5(1.6)	1.6(1.6)	1.6(2.4)	0.11	.899
Grey matter volume	662508(60163)	634747(96093)	609063(80090)	301	**.049**
White matter volume	381535(50367)	361414(63317)	341955(52931)	302	**.044**
Mean cortical thickness	2.68(0.12)	2.62(0.14)	2.66(0.12)	3.6	**.031**
Mean FA	0.292(0.03)	0.294(0.03)	0.262(0.03)	2.0	.137
Mean MD	8.60(0.4)E-4	8.60(0.5)E-4	9.48(1.3)E-4	6.4	**.002**
Controls	N=34	N=9			
hs-CRP	1.1(1.6)	1.1(0.9)		na	**na** *
Grey matter volume	657226(75773)	660035(92703)		na	**na**
White matter volume	378837(52509)	378755(67450)		na	**na**
Mean cortical thickness	2.65(0.12)	2.65(0.14)		na	**na**
Mean FA	0.30(0.02)	0.31(0.03)		na	**na**
Mean MD	8.65(0.4)E-4	8.38(0.5)E-4		na	**na**

hs-CRP= highly sensitive c-reactive protein; FA=fractional anisotropy; MD=mean diffusion.

*Sample sizes for the control sample were too small in this stratification to do ANOVA tests