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Cerebrospinal Fluid Biomarkers and HIV-Associated Neurocognitive Disorders in HIV- Infected Individuals in Rakai, Uganda

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Abstract

Objective—In the United States, increased cerebrospinal fluid (CSF) inflammatory cytokines have been observed in antiretroviral therapy (ART)-naïve, HIV-seropositive individuals with HIV-associated neurocognitive disorder (HAND). We characterized the relationship between HAND and CSF biomarker expression in ART-naïve, HIV-seropositive individuals in Rakai, Uganda.

Methods—We analyzed CSF of 78 HIV-seropositive, ART-naïve Ugandan adults for 17 cytokines and 20 neurodegenerative biomarkers via Luminex multiplex assay. These adults underwent neurocognitive assessment to determine their degree of HAND. We compared biomarker concentrations between high and low CD4 groups and across HAND classifications, adjusting for multiple comparisons.

Results—Individuals with CD4<200 cells/μL (N=38) had elevated levels of CSF Interleukin (IL)-2, IL-12, GM-CSF, TNF-α, matrix metalloproteinase (MMP)-1, MMP-7 and S100 calcium-binding protein B (S100B) and lower levels of Amyloid β42. Individuals with CD4 351-500 cells/μL (N=40) had significantly higher CSF levels of Interleukin (IL)-1β, Amyloid β42, and soluble receptor for advanced glycation end products (sRAGE). Increasing levels of S100B, platelet-derived growth factor-AA (PDGF-AA), brain-derived neurotrophic factor (BDNF) and sRAGE were associated with decreased odds of mild neurocognitive disorder (n=22) or HIV-associated dementia (n=15) compared with normal function (n=30) or asymptomatic neurocognitive impairment (n=11). Increased levels of interferon (IFN)-γ were associated with increased odds of

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mild neurocognitive impairment or HIV-associated dementia relative to normal or asymptomatic neurocognitive impairment.

Conclusions—Proinflammatory CSF cytokines, chemokines, and neurodegenerative biomarkers were present in increasing concentrations with advanced immunosuppression, and may play a role in the development of HAND. The presence of select CNS biomarkers may also play a protective role in the development of HAND.

Keywords

HIV-Associated Neurocognitive Disorders; Cytokine Expression; Neurodegenerative Biomarkers; Inflammation; Dementia

Introduction

HIV-associated neurocognitive disorder (HAND) is characterized by cognitive, behavioral, and motor dysfunction. HAND is a clinical spectrum that includes Asymptomatic Neurocognitive Impairment (ANI), Mild Neurocognitive Disorder (MND), and HIV-Associated Dementia (HAD; Antinori, Arendt et al. 2007). Prior to the use of antiretroviral therapy (ART), HIV-associated dementia was prevalent in HIV-seropositive individuals. With increasing access to ART, the prevalence of HAD has decreased, whereas the prevalence of lesser forms of ANI and MND have increased (Clifford and Ances 2013). Up to half of all HIV-seropositive individuals on ART demonstrate some degree of neurocognitive impairment (Clifford and Ances 2013).

HIV infects the central nervous system (CNS) soon after acquisition, causing neuronal damage and loss, prior to the development of neurologic symptoms (Resnick, Berger et al. 1988, Everall, Luthert et al. 1993). HIV-1 infected macrophages and T-cells cross the blood-brain barrier into the CSF, which can infect CNS macrophages and microglia cells. These infected cells within the CNS are thought to cause the subsequent release of chemokines and cytokines that further disrupt the blood brain barrier and encourage invasion by an increasing number of inflammatory cells (Rao, Ruiz et al. 2014).

In this study, we sought to characterize the relationship between CSF inflammation and HAND in HIV-1 seropositive Ugandans by quantifying CSF cytokine and neurodegenerative biomarker concentrations among participants with either moderate or advanced immunosuppression.

METHODS

Study Participants

In 2014-2016, the Rakai Health Sciences Program (RHSP) in Rakai District, Uganda recruited a prospective cohort of 400 HIV-seropositive individuals into a neurocognitive study. Participants were selected from both the RHSP's open longitudinal Rakai Community Cohort Study (RCCS) (Wawer, Sewankambo et al. 1999, Grabowski, Lessler et al. 2014) and HIV clinics within the RCCS's geographic catchment area. Inclusion criteria were: HIV-seropositive; ART naïve; >20 years of age; meeting CD4 cell count criteria; willing and able

to provide written informed consent; currently residing in the District; and intending to stay in Rakai for ≥2 years. CD4 criteria were either advanced (CD4 <200 cells/μL, n = 200) or moderate (CD4 351-500 cells/μL, n = 200) immunosuppression. Persons with a previous history of CNS opportunistic infections were excluded. Potential participants underwent a clinical assessment at enrollment to identify and exclude persons with possible comorbid conditions including malaria, syphilis, hypertension, diabetes, cardiovascular disease, or thyroid conditions that may influence neurocognitive function.

Participants were separately consented, in writing, to receive an optional lumbar puncture performed in outpatient clinics at enrollment. We measured 17 CSF cytokines/chemokines on 78 specimens via multiplex profiling with the Luminex platform (Human 17-Plex Panel, Bio-Rad, Hercules, CA). An additional 20 biomarkers of neurodegenerative breakdown products (Milliplex Catalog: HMMP1-55K-03; HMMP2-55K-05; HNDG4MAG-36K-05; HND1MAG-39K-07; HNDG3MAG-36K-10) were measured on 68 of these 78 specimens with sufficient volume (EMD Millipore, Chicago, IL). Undetectable levels of biomarkers were set at 50% of the manufacturer's lower limit of detection.

Neurocognitive Analysis

At study enrollment, all participants underwent a comprehensive neurocognitive assessment to determine HAND classification. The degree of HAND was determined using the Frascati Criteria (Antinori, Arendt et al. 2007) evaluating neurological and functional symptoms, neurological exam, and test results, compared to age and education matched HIV-seronegative normative neuropsychological data obtained in Kampala, Uganda (Sacktor, Wong et al. 2005). A neurological symptom questionnaire was administered to assess memory, gait and limb coordination, depression, anxiety, psychosis, extremity numbness/tingling, seizure, headache, and neck stiffness. The neurocognitive exam consisted of testing in the following domains: verbal memory, motor, and psychomotor speed (International HIV Dementia Scale), attention span (Digit Span Forward and Backward), gross motor skills (Timed Gait), fine motor skills (Grooved Pegboard, Finger Tapping), psychomotor speed (Symbol Digit Modalities Test, Color Trails 1), verbal memory and learning (WHO-UCLA Auditory Verbal Learning Test learning and delayed recall), executive functioning (Color Trials 2), language fluency (Semantic Verbal Fluency) and depression screening (Center for Epidemiologic Studies Depression Scale). A peripheral neuropathy screening history and brief screening exam evaluated HIV-sensory neuropathy. The Karnofsky functional performance, Instrumental Activities of Daily Living, and Patient Assessment of Function Inventory assessed disability and functional status (**Supplemental Table 1**).

Statistical Analysis

CSF biomarkers were expressed as medians and interquartile ranges (IQR), and categorized as detectable or undetectable based on manufacturer's published detection limits. Wilcoxon rank-sum tests evaluated differences in CSF biomarker concentrations across CD4 group (low: CD4 <200 cells/μL; high: CD4 351-500 cells/μL) and HAND classification (normal or asymptomatic versus mild neurologic disorder or HIV-associated dementia, and the four HAND classifications separately). Cuzick's nonparametric test for trend assessed trends in cytokine concentrations across the four HAND stages. We additionally evaluated differences

in detectable versus undetectable CSF biomarker concentrations via X^2 or Fisher's exact tests when >20% of measured biomarkers were below the limit of detection. Results were evaluated using a two-sided alpha of 0·05 with adjustment for multiple comparisons using the Holm-Bonferroni method.

We estimated odds ratios for the association between HAND classification (normal or asymptomatic versus mild neurocognitive disorder or HIV-associated dementia) and $\log_2$ transformed individual CSF biomarker concentrations via logistic regression using a robust variance estimator with adjustment for CD4 count and age. The above analyses were conducted in Stata/IC 13·1 (StataCorp LP, College Station, TX).

Finally, we fit Lasso penalized logistic regression models using the PROC HPGENSELECT package in SAS/STAT 14·1 (SAS Institute Inc., Cary, NC) to evaluate the relationship between HAND classification and CSF biomarkers using three biomarker groupings: 1) cytokines and chemokines, 2) neurocognitive biomarkers, and 3) all available biomarkers. Bias-corrected Akaike information criterion informed model variable selection.

Human Subjects

The study, including the optional lumbar puncture, were reviewed and approved by institutional review boards in Uganda (Research and Ethics Committee, Uganda Virus Research Institute; Ugandan National Council for Science and Technology) and in the United States (Western Institutional Review Board, Johns Hopkins University). At study initiation, Ugandan Ministry and WHO guidelines indicated ART initiation at a CD4 cell count of <200; during the enrollment period, this criterion was increased to CD4 <350. Free ART was offered to all individuals who presented at the RCCS or RHSP clinics and met ART initiation criteria in either period. Enrollment into the neurocognitive study of individuals with CD4 cell counts <200 and subsequently <350 was conducted among newly enrolled RCCS participants and new clinic attendees who had not yet initiated ART; all were strongly encouraged by the neurocognitive study team to do so without delay.

RESULTS

Of the overall cohort, 200 participants had advanced immunosuppression (CD4<200 cells/μL), and 200 participants had moderate immunosuppression (CD4 351-500 cells/μL). In all, 198 participants consented to and successfully underwent the optional lumbar puncture, of whom 79 (40%) were in the CD4< 200 group and 119 (60%) had CD4 >350 at enrollment. For this study, we randomly selected 38 with CD4 <200 cells/μL and 40 with CD4 of 351-500 cells/μL for the CSF analysis. Participants with advanced immunosuppression (CD4 <200 cells/μL) had a median CD4 count of 136 cells/μL (IQR 80-171), median age of 34 (IQR 30-41) years, and 34% were women (**Table 1**). Participants with moderate immunosuppression (CD4 351–500 cells/μL) had a median CD4 count of 443 cells/μL (IQR 397-476), median age of 37 (IQR 27-40) years, and 53% were women. Participants with CD4 <200 cells/μL more often had a Karnofsky performance score of ≤90 (71%) versus participants with CD4 351–500 cells/μL (35%; p=0·001). Of the 78 participants, 38% (30/78) were classified without HAND and as having normal neurocognitive function, 14% (11/78) ANI, 28% (22/78) MND, and 19% (15/78) HAD.

Biomarker Analysis

Overall CSF biomarker concentrations are presented in **Supplemental Table 2**. In univariate analyses, participants with CD4 <200 cells/μL compared to participants with CD4 351-500 cells/μL had higher concentrations of IL-2, IL-12, GM-CSF, TNF-α, matrix metalloproteinase (MMP)-1, MMP-7, and S100B with Holm-Bonferroni-adjustment (**Table 2**). Participants with CD4 351–500 cells/μL had higher concentrations of IL-1β, Amyloid β42, and sRAGE compared to participants with CD4 <200 cells/μL.

Logistic regression, adjusting for CD4 count and participant age, indicated that each one-unit increase in $\log_2$ CSF S100B concentrations was associated with decreased odds of MND or HAD compared with normal or ANI participants (OR=0·50, 95%CI: 0·28-0·89, p=0·02), as was each one unit increase in PDGF-AA concentrations (OR=0·46, 95%CI: 0·22-0·97, p=0·04) (**Table 3**). Conversely, increasing concentrations of IFN-γ were associated with increased odds of MND/HAD compared with none/ANI or asymptomatic participants (OR=1·16, 95%CI: 1·00-1·36, p=0·05, per each one-unit increase in $\log_2$ IFN-γ). Finally, participants with MND/HAD were less likely to have detectable levels of BDNF (OR=0·18, 95%CI: 0·04-0·95, p=0·04) or sRAGE (OR=0·11, 95%CI: 0·01-0·97, p=0·05) than none/ANI. With an alternative statistical analysis using multivariable Lasso logistic regression models, S100B was consistently selected based on minimized bias-corrected Akaike information criterion values, with penalized odds ratios ranging from 0·84 to 0·87 per 2-fold increase, implying a decreased risk of MND/HAD relative to none/ANI. We did not observe any significant trends in biomarker concentration by each of the four HAND categories, although sample sizes were small.

DISCUSSION

In this study of HIV-seropositive, ART-naïve individuals, we found those with CD4 <200 cells/μL expressed higher CSF levels of the proinflammatory cytokines IL-2, IL-12, GM-CSF, TNF-α and neurodegenerative biomarkers MMP-1 and MMP-7. The increased presence of inflammatory cytokines as well as neurodegenerative biomarkers in the CSF of HIV-seropositive, ART-naïve individuals with CD4 <200 cells/μL belies the highly inflammatory process involved in HIV disease progression. Given the link between HIV disease progression and HAND, these increased CSF cytokines and neurodegenerative biomarkers provide insight into the pathophysiology of HAND. Previous studies have demonstrated higher CSF levels of GM-CSF, TNF-α, MMP-1 and MMP-7 in individuals with HIV-associated dementia and encephalitis as compared with individuals without dementia (Perrella, Guerriero et al. 1992, Nottet, Persidsky et al. 1996, Conant, McArthur et al. 1999).

We have also demonstrated that HIV-seropositive, ART-naïve individuals with CD4 351–500 cells/μL have elevated levels of IL-1β compared with individuals with CD4 <200 cells/μL. Increased IL-1β expression is implicated in Alzheimer's disease pathogenesis through increased production and reduced clearance of amyloid-β (Aβ) peptides (peptides of 36-42 amino acids) (Xu and Ikezu 2009). The presence of IFN-γ functions as a co-stimulatory cytokine alongside IL-1β to augment the production of amyloid-β peptides from astrocytes (Blasko, Veerhuis et al. 2000). We observed decreased concentrations of CSF amyloid-β42

among those with CD4 <200 cells/μL as compared with CD4 of 351-500 cells/μL. Previous studies reported lower CSF amyloid-β42 concentrations among individuals with AIDS dementia complex at levels similar to those observed in Alzheimer's disease (Brew, Pemberton et al. 2005). Brain tissue from HIV-seropositive individuals on ART has shown significantly increased amyloid-β peptide deposits in the frontal cortex and to a lesser degree in the hippocampus and basal ganglia on autopsy (Green, Masliah et al. 2005).

We also observed that in HIV-seropositive individuals with CD4 <200 cells/μL, higher CSF concentrations of S100 calcium-binding protein B (S100B) were associated with a significantly decreased odds of mild neurocognitive disorder or HIV-associated dementia. S100B concentrates in astrocytes and oligodendrocytes and is involved in the regulation of intracellular processes. Released by astrocytes and oligodendrocytes into the extracellular space, S100B has been found to have concentration dependent roles in neuronal health (Steiner, Bogerts et al. 2011). At low extracellular concentrations, S100B promotes neural survival, while high concentrations of S100B can promote neuronal apoptosis and pro-inflammatory cytokine production (Steiner, Bogerts et al. 2011). Therefore, high CSF concentrations of S100B may likely indicate glial injury and death with release of intracellular stores (Stanley, Mrak et al. 1994). In vitro studies have also shown that astrocyte-derived S100B induces the overexpression of amyloid precursor protein (Li, Wang et al. 1998), and in HIV-seropositive patients receiving ART, those with higher S100B levels had a more rapid progression to dementia (Pemberton LA1 and BJ. 2001). In fact, HIV-seropositive individuals with elevated S100B levels have increased expression of amyloid precursor protein and the presence of neurofibrillary tangles and neuritic plaques, which suggests a neurodegenerative process similar to that of Alzheimer's disease (Stanley, Mrak et al. 1994).

Our findings suggest high CSF S100B concentrations, as seen in individuals with CD4 <200 cells/μL, may cause neurotoxicity and induce overexpression of amyloid precursor protein. This then may cause subsequent deposition of CSF amyloid β42 into neuritic and senile plaques. As neuroinflammation and neurodegeneration continue, individuals develop worsening degrees of neurocognitive impairment and dementia, resulting in lower CSF levels of both S100B and amyloid-β peptides. Similar results have been reported with higher levels of CSF S100B detected in mild-to-moderate Alzheimer's disease than compared to those with advanced Alzheimer's disease (Peskind, Griffin et al. 2001).

Interestingly, we also found that increased CSF concentrations of platelet derived growth factor-AA (PDGF-AA) and brain-derived neurotrophic factor (BDNF) were associated with decreased odds of developing mild neurocognitive disorder or HIV-associated dementia. In HIV infection, PDGF has been demonstrated to be neuroprotective against neural toxicity mediated through HIV cellular and viral products, including HIV-Tat and envelope glycoprotein GP120 (Yao, Bethel-Brown et al. 2014). BDNF measurements taken from the frontal cortex, hippocampus, and caudate regions of individuals with HIV-associated dementia expressed less BDNF, as compared with individuals without dementia (Bachis, Avdoshina et al. 2012).

Lastly, increased CSF soluble receptor for advanced glycation end products (sRAGE) was associated with decreased odds of having mild neurocognitive disorder or HIV-associated dementia. The receptor for advanced glycation end products (RAGE) is expressed on neural cells (Brett, Schmidt et al. 1993) and binds to advanced glycation end products, amyloid-β peptides, amyloid A, and amphoterin (HMGB-1), leading to chronic inflammation, vasculopathy, Alzheimer's disease, or amyloidosis (Bierhaus, Humpert et al. 2005). Soluble RAGE (sRAGE) is a truncated isoform of RAGE that has been shown to suppresses RAGE-mediated disease pathogenesis (Park, Yeon et al. 2004) by blocking the transport of amyloid-β peptide transport across the blood-brain barrier (Deane, Du Yan et al. 2003, Bachis, Avdoshina et al. 2012). Lower sRAGE plasma concentrations occur in Alzheimer's disease and vascular dementia as compared with cognitively healthy controls, suggesting a protective role of sRAGE against developing neurologic disease (Emanuele, D'Angelo et al. 2005).

Limitations of this study included our cross-sectional study design, thus being unable to decipher cause versus effect over longitudinal time. An additional limitation is that neurological test results were compared with test results from HIV-seronegative norms from urban Kampala, rather than rural Rakai, Uganda and therefore may have lead to misclassification of neurologic impairment. Also, we did not have CSF cytokine and neurodegenerative biomarker data from HIV-seronegative individuals to serve as a comparison group. Future studies should examine the effect of HIV subtype on neuroinflammation.

Conclusion

In our cohort of ART-naïve, HIV-seropositive individuals from Rakai, Uganda we found CNS inflammation to be present in individuals who are severely immunosuppressed. In individuals with mild neurocognitive disorder or HIV-associated dementia, CNS inflammation may contribute in the development of neurological impairment. A better understanding of the interplay between HIV infection and the immune response and resulting neurodegeneration is crucial to our understanding into the pathogenesis of HIV-associated neurocognitive disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Demographic Data

Variable	<200 CD4 cells/μL				350-500 CD4 cells/μL				
	Normal & Asymptomatic	**Mild Neurocognitive Disorder**	**HIV-Associated Dementia**	**Overall**	**Normal & Asymptomatic**	**Mild Neurocognitive Disorder**	**HIV-Associated Dementia**	**Overall**	***P*-value**
N	19 *	11	8	38	22 †	11	7	40	
Percent distribution	50%	29%	21%	100%	55%	27.5%	17.5%	100%	0·87
Median Age	33 (30-37)	38 (26-42)	37 (30-42)	34 (30-41)	37 (27-40)	32 (27-38)	39 (29-45)	37 (27-40)	0·87
Women	8 (42%)	1 (9%)	4 (50%)	13 (34%)	9 (41%)	6 (55%)	6 (86%)	21 (53%)	0·10
Karnofsky Score ≤90	13 (68%)	8 (73%)	6 (75%)	27 (71%)	7 (32%)	3 (27%)	4 (57%)	14 (35%)	<0·01
Median CD4 cells/μL	105 (58-160)	150 (86-184)	151 (135-171)	136 (80-171)	460 (413-476)	426 (380-486)	402 (375-450)	443 (397-476)	N/A

All data presented as median (IQR) or n (%); Asymptomatic = Asymptomatic Neurocognitive Impairment;

P-values based on Kruskal-Wallis and Chi-square comparisons of patients across CD4 groups, and are not stratified by HAND classification.

* N=19 (Normal=12 & Asymptomatic=7)

† N=22 (Normal=18 & Asymptomatic=4)

Table 2

Median (IQR) or Proportion Detectable CSF Biomarkers in HIV-seropositive Adults in Uganda by CD4 Count.

CSF Biomarker	CD4 <200 cells/μL, N=38 Median in pg/mL (IQR) or n (%) detectable	CD4 350-500 cells/μL, N=40 Median in pg/mL (IQR) or n (%) detectable	P-value	Adjusted P-value[#]
IL-1β	0·93 (0·93-1·06)	1·10 (1·01-1·26)	<0·001	**0·03**
IL-2	36 (95%)	12 (30%)	<0·001	**<0·01**
IL-4	18 (47%)	10 (25%)	0·06	>0·99
IL-5	18 (47%)	5 (13%)	<0·01	0·09
IL-6	13·1 (9·7-19·78)	9·645 (5·87-16·95)	0·01	0·29
IL-7	2·3 (1·8-3·5)	1·4 (<0·25-3·5)	0·02	0·48
IL-12	38 (100%)	16 (40%)	<0·001	**<0·01**
MCP-1	1,490 (1,036-2,101)	854 (440-2,305)	0·03	0·67
G-CSF	5·7 (2·8-7·2)	8·1 (4·8-17·2)	0·05	0·92
GM-CSF	176 (145-235)	40 (31-50)	<0·001	**<0·01**
IFN-γ	13 (34%)	23 (58%)	0·05	0·95
TNF-α	33 (87%)	20 (50%)	<0·01	**0·03**
MMP-1	5·98 (4·9-7·1)[*]	≤1·5 (≤1·5-2·89)[†]	<0·001	**<0·01**
MMP-3	173 (52-425)[*]	90 (23-198)[†]	0·07	>0·99
MMP-7	30 (79%)[*]	3 (10%)[†]	<0·001	**<0·01**
MMP-10	11·1 (8·3-15)[*]	7·0 (2·5-13·2)[†]	0·03	0·71
sVCAM-1	21652 (14066-44122)[*]	13,574 (7,873-26,403)[†]	0·02	0·40
Amyloid β42	843 (378-1,938)[*]	4,262 (1,648-8,118)[†]	<0·001	**<0·01**
sRAGE	0 (0%)[*]	8 (27%)[†]	<0·01	**0·03**
S100B	1,026 (770-1,338)[*]	590 (325-957)[†]	<0·01	**0·03**
PDGF-AA	5·52 [4·63, 6·35][*]	5·09 [3·58, 7·34][†]	0·43	>0·99
BDNF	4 (11%)[*]	4 (13%)[†]	0·72	>0·99

[*] N=38

[†] N=30. P-values by Wilcoxon rank-sum test or X^2 test, e.g. IL-2

[#] Multiple comparison p-value adjustment calculated using the Holm-Bonferroni method.

Table 3

CSF Biomarkers in HIV-seropositive Adults in Uganda Stratified by HAND Classification

CSF Biomarker	Normal & Asymptomatic Neurocognitive Impairment N=32	Mild Neurocognitive Disorder & HIV-associated Dementia N=36	Odds Ratio, (95% CI)	P-Value *
INF-γ	≤1·1 (≤1·1-19·4) †	4·2 (≤1·1-51·6) §	1·16 (1·00-1·36)	0·05
S100B	1101 (699-1348)	775 (418-1033)	0·50 (0·28-0·89)	0·02
PDGF-AA	5·9 (4·7-6·9)	5·0 (3·9-6·5)	0·46 (0·22-0·97)	0·04
BDNF detectable	6 (19%)	2 (6%)	0·18 (0·04-0·95)	0·04
sRAGE detectable	6 (19%)	2 (6%)	0·11 (0·01-0·97)	0·05

INF-γ = interferon-γ; S100B = S100 calcium-binding protein B; PDGF-AA = Platelet Derived Growth Factor-AA; BDNF = Brain-derived neurotrophic factor; sRAGE = soluble receptor for advanced glycation end products.

Median values in pg/mL with interquartile range (IQR) or n (%) detectable.

* Age and $CD4^+$ T-cell count-adjusted logistic regression of $\log_2$ CSF biomarker and Mild Neurocognitive Disorder or HIV-associated Dementia versus normal or asymptomatic neurocognitive impairment. Odds Ratio represents the change in odds per one unit increase in increase in CSF biomarker, or, for BDNF and sRAGE, odds ratio if detectable.

† N=41

§ N=37.