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Plasma Cystatin C Associates with HIV-Associated Neurocognitive Disorder but is a Poor Diagnostic Marker in Antiretroviral Therapy Treated Individuals

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Abstract

Objective—To examine associations between plasma cystatin C and neurocognitive impairment (NCI), and its performance as a diagnostic marker before and during initial antiretroviral therapy (ART).

Methods—Multivariable logistic regression and generalized estimating equations (GEE) examined associations with NCI, determined by neuropsychological measurements, in participants of a 48-week randomized clinical trial of initial ART. Receiver operator characteristic (ROC) curves examined diagnostic models of NCI.

Results—Cystatin C was associated with NCI before ART (OR 3.4 [95% C.I., 1.2, 9.4] for each 2-fold increase in baseline levels) and during 48 weeks of ART, in models that excluded baseline measurements (OR 3.0 [1.2, 7.8] for each 2-fold increase in time-updated levels). The strength of association increased with more severe impairment using HIV-Associated Neurocognitive Disorder criteria (OR 2.2 [0.8, 6.0] with asymptomatic neurocognitive impairment, and OR 4.0 [1.5, 11.0] with mild neurocognitive disorder or HIV associated dementia vs. no impairment, for each 2-fold increase in time-updated levels), or by Global Deficit Scores (OR 2.6 [1.1, 6.3] with mild impairment, and OR 4.6 [1.1, 18.9] with moderate or severe impairment vs. no impairment).

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Contributions: Drs. Kalayjian, Taiwo and Brown conceived of and led the study. Drs. Kalayjian and Albert analyzed the data. All authors contributed to the writing and editing of the manuscript.

Potential Conflicts of Interest

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Cystatin C performed poorly as a diagnostic marker for NCI, however, with an AUC for the ROC curve of 0.58 at baseline, and 0.54 at week-48.

Conclusions—Higher plasma cystatin C significantly associated with NCI but these levels did not appear to be useful as a diagnostic marker for this condition.

Keywords

cystatin C; neurocognitive impairment; HAND; neuroinflammation; endovascular dysfunction

INTRODUCTION

Neurocognitive impairment (NCI) is common in people with HIV (PWH) (1–3). Suppressive anti-retroviral therapy (ART) is necessary but is not sufficient to either prevent or reverse NCI in many PLWH (4). Mechanisms of neuronal injury may differ according to ART exposure, with more severe impairment occurring in the absence of virologically suppressive ART, but milder cognitive dysfunction may be present despite suppressive ART (5). Associations between cardiovascular risk factors and markers of inflammation implicate contributions by monocyte activation and endovascular dysfunction to the risk of NCI in ART treated individuals (6, 7). Because the diagnosis of NCI requires specialized neurocognitive testing, biological markers would be helpful to establish this diagnosis and/or to measure responses to therapeutic interventions, while also helping to identify potential pathogenic mechanisms.

Cystatin C is a low-molecular-weight protein that is constitutively produced by all nucleated cells that have been investigated, and is freely filtered by glomeruli (8). Cystatin C varies less by age, sex and muscle mass than creatinine and is more sensitive for detecting mild to moderate glomerular filtration decrements. Plasma cystatin C levels predicted cardiovascular events and mortality better than serum creatinine-based GFR estimates in the general population, and was associated with cognitive impairment in selected populations (9–16), including PWH receiving virologically suppressive ART (17).

To further explore cystatin C as a potential biomarker for NCI in PWH, we examined associations with NCI, and its performance as a diagnostic marker in predictive models before and during 48 weeks of initial ART.

METHODS

Study population

Participants in this study were enrolled in a prospective clinical trial of initial ART (AIDS Clinical Trials Group Study A5303) and underwent detailed neurocognitive assessments during the first 48 weeks of therapy (18). A5303 compared neurocognitive performance with initial ART between tenofovir disoproxil fumarate (TDF) 300 mg daily vs. maraviroc (MVC) 150 mg twice daily, each combined with emtricitabine 200 mg and darunavir 800 mg plus ritonavir 100 mg daily (18). Eligible participants were ART naïve and 18 years old with plasma viral loads (pVL) >1,000 copies/mL and R5 tropism by the Trofile® phenotypic assay [Monogram Biosciences, San Francisco, CA, USA]. Individuals with HIV associated

neurological disease as documented by their clinical provider, or whom the site investigator felt could not complete the neurocognitive protocol due to HIV or other illness were excluded. The neurocognitive testing performed in A5303 and the primary results of the study were previously reported (18, 19). Additional information can be found at [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01400412): NCT 01400412.

Ethics Statement

The Institutional Review Board of each study site approved the protocol. All participants provided written informed consent.

Neurocognitive Assessment

Neuropsychological performance was assessed at baseline and weeks 24 and 48 as previously described including: Language/Premorbid skills (WRAT-4 Reading), Verbal Learning (Hopkins Verbal Learning Test Revised learning trials), Attention/Working Memory (WAIS-III Symbol Search, Stroop Word), Speed of Information Processing (Digit Symbol, Trailmaking A, Stroop color naming), Executive Function (Trailmaking B, Stroop Interference, Letter Fluency, Semantic Verbal Fluency), Fine Motor Skills (Grooved Pegboard bilateral), and Verbal Memory (Delayed Recall–HVLt-R, Recognition–HVLt-R) and also included an assessment of activities of daily living (ADL).

These tests were averaged into two summary scores: a total z score and a global deficit score (GDS) (20). Total z scores were computed through the average of the 15 individual test z scores that were computed by subtracting each raw test score from its demographically corrected normative score (adjusted for age, education, gender, and race/ethnicity), then dividing by the normative standard deviation. The resulting z scores varied around zero (the average performance), where positive and negative scores denote better than average, and impaired performance, respectively. The GDS was computed as the average of the individual test deficit scores, where all normal deficit scores were set to 0 and positive scores from 1 to 5 denote increasing impairment (21).

NCI and HAND

Mild NCI was defined as having at least 2 neurocognitive domains with the mean domain GDS ≥ 1 . Impairment also was categorized by conventional HIV-Associated Neurocognitive Disorder (HAND) criteria as: Normal (GDS <1 for all domains, ADL=0); Asymptomatic Neurocognitive Impairment (ANI, GDS ≥ 1 for at least 2 domains, ADL =0); Mild Neurocognitive Disorder (MND, GDS ≥ 1 for at least 2 domains and ADL ≥ 1 , or GDS ≥ 2 for 2 domains and ADL 0–3); HIV Associated Dementia (HAD, GDS ≥ 2 for 2 domains, ADL ≥ 4) (20).

Cystatin C

Plasma cystatin C was measured by solid phase sandwich ELISA (R&D Systems, Minneapolis MN, USA) from stored frozen plasma, with a normal reference range between 0.58–1.00 mg/L in women and 0.62–1.01 mg/L in men (22).

Statistical analyses

Baseline covariates were compared according to the presence or absence of NCI using the Wilcoxon rank-sum test for continuous, and the Pearson chi-square test for categorical variables.

Spearman rank correlations examined associations between variables. Baseline for all covariates was defined as the randomization date. All analyses were as-treated and included only participants who remained on their randomized MVC or TDF component by week-48, who did not have an interruption in treatment for more than 10 weeks and who had available data at baseline, and weeks 24 and 48. Individual test z scores were treated as missing if participants were unable to perform any individual test because of a reason unrelated to HIV associated neurological disease.

Associations between $\log_2$ transformed plasma cystatin C levels with simultaneous NCI outcomes at baseline, and weeks 24 and 48 were examined at by logistic regression. Exploratory variables included: age, sex, race/ethnicity (as Hispanic; black not Hispanic; or white), education status (as <12 vs. 12 years), primary language (as English vs. other), randomized treatment assignment, estimated glomerular filtration rates using the Chronic Kidney Disease Epidemiology Collaboration equation (23), and pVL. Generalized estimating equations (GEE) examined baseline and time-updated (that included baseline, weeks 24 and 48 levels) associations between $\log_2$ transformed plasma cystatin C with either NCI or HAND and assumed an independent covariance structure. Mixed effects linear models similarly examined associations with z score and assumed an autoregressive covariance structure. Adjusting variables in these models included age, sex, race/ethnicity, education status, primary language, randomized treatment assignment and either ART duration or time-updated pVL suppression.

Receiver operator curve (ROC) performance characteristics including the area under the curve (C-statistic) examined univariate logistic regression models of NCI with simultaneous cystatin C levels at baseline and week-48. All statistical tests were two-sided with significance defined as $P < 0.05$ and were not adjusted for multiple comparisons. We used SAS statistical software 9.4 (Cary, NC, USA), or R version 3.3.2 (The R Foundation for Statistical Computing Platform).

Results

Neurocognitive performance as determined by GDS and z score was previously reported and mainly improved during initial ART in the 230 participants of this trial (19). Participants with more severe baseline impairment had greater improvements and there were no significant differences in neurocognitive performance between the MVC and TDF arms.

Among the 225 participants with complete data, only 51 of the 102 (50%) participants with baseline impairment remained impaired at week-48, including 17 of 28 (61%) with ANI, 31 of 69 (45%) with MND, and 3 of 5 (60%) with HAD. By contrast, 13 of the 123 (11%) participants without NCI at baseline were subsequently impaired by week-48, including 6 with ANI, and 7 with MND.

Plasma cystatin C levels at baseline and weeks 24 and 48 were available in 217 participants. White participants had higher baseline plasma cystatin C levels than non-whites ($P<0.0001$; Appendix Figure 1). Baseline levels were positively associated with age ($r=0.18$, $P=0.008$) and pVL ($r=0.32$, $P<0.0001$), and negatively associated with estimated GFR ($r=-0.31$, $P<0.0001$).

Mean plasma cystatin C levels declined by 0.08 mg/L (-8.6%), and 0.09 mg/L (-9.6%) from baseline to week-24, and -48, respectively. Larger 48-week declines were associated with higher baseline pVL ($r=0.26$, $P=0.0001$) but the magnitude of decline was not associated with age, race/ethnicity, randomized treatment assignment, baseline GFR or baseline GDS (data not shown).

Correlates of NCI by Logistic Regression

The baseline univariate correlates of NCI included: less than a high school education (OR 6.9 [95% C.I., 1.9, 24.3]; Supplemental Table 1), race/ethnicity other than white (OR 3.9 [2.1, 7.4] for black participants; OR 2.9 [1.4, 5.9] for Hispanic participants) or a primary language other than English (OR 2.7 [1.1, 6.5]). Plasma cystatin C significantly associated with simultaneous NCI in separate, multivariable logistic regression models adjusted for race/ethnicity, education status and randomized treatment assignment, at baseline (OR 3.8 [1.1, 12.6], $P=0.03$ for each 2-fold cystatin C increase) and at week-24 (OR 4.3 [1.2, 16.1], $P=0.03$) but not at week-48 (OR 2.9 [0.8, 10.5], $P=0.11$). Although the effect size for this association was larger for white, compared to non-white participants (OR: 5.2 vs. 2.9, respectively), race/ethnicity did not modify this association ($P=0.59$ for the interaction with race/ethnicity).

Correlates of NCI & HAND by Repeated Measures Analyses

Baseline and time-updated cystatin C levels significantly associated with increased NCI risk by adjusted GEE models (OR 3.4 [1.2, 9.4], $P=0.02$ or 3.2 [1.3, 7.5], $P=0.009$ for every 2-fold cystatin C increase in baseline or time-updated cystatin C levels, respectively; Supplemental Table 2, Models 1&2). The strength of association increased with more severe impairment by HAND criteria (OR 2.2 [0.8, 6.0], $P=0.13$ with ANI, and OR 4.0 [1.5, 11.0], $P=0.008$ with MND or HAD vs. no impairment, for every 2-fold increase in time-updated levels) and by GDS score (OR 2.6 [1.1, 6.3], $P=0.03$ with mild impairment, and OR 4.6 [1.1, 18.9], $P=0.04$ with moderate or severe impairment vs. no impairment; Supplemental Table 2, Models 6–9).

Associations with cystatin C also remained significant in models that only included measurements while participants received ART (OR 3.0 [1.2, 7.8], $P=0.02$), and in models that only included measurements when participants were simultaneously suppressed to <50 copies of HIV-RNA/mL (OR 2.8 [1.1, 7.5], $P=0.03$, Supplementary Table 2, Models 4&5).

Time on ART protected against NCI in these models (OR 0.5 [0.4, 0.8], $P=0.001$ per 48 weeks of ART). In a separate model, time-updated pVL suppression was similarly protective as, and interchangeable with time on ART (OR 0.4 [0.2, 1.1], $P=0.08$ for pVL suppression to $<1,000$ copies but ≥ 50 copies HIV-RNA/mL, and OR 0.7 [0.5, 0.9], $P=0.008$ for suppression to <50 copies vs. $\geq 1,000$ copies HIV-RNA/mL; Supplemental Table 2, Model 3).

Baseline and time-updated cystatin C levels associated with z score, a continuous measure of cognitive function, in adjusted longitudinal models (z score increased by: 0.25 [0.09, 0.41], $P=0.003$ or 0.12 [0.002, 0.24], $P=0.046$ for each 2-fold increase in baseline or time-updated cystatin C level, respectively) but changes in cystatin C did not associate with changes in cognitive performance as determined by either z scores or HAND categories (data not shown).

ROC Analyses for HAND at Baseline and Week-48

Cystatin C performed poorly as a diagnostic marker for NCI despite these robust associations. In a univariate model of NCI with simultaneous plasma levels, the C-statistic was 0.58 at baseline, and 0.54 at week-48, and there were no discernable, clinically useful cutoffs (Figure 2A&B). Using a previously suggested cutoff of 0.75 mg/L (16), the sensitivity, specificity and accuracy for this diagnosis at or above this threshold was 72%, 27% and 37%, respectively.

DISCUSSION

In this randomized clinical trial of initial ART, we observed robust associations between cystatin C with cognitive impairment both before starting ART, and during 48 weeks of viralogically suppressive ART independent of participant demographics, education status, primary language, ART duration, or the randomized treatment assignment. The strength of association increased with increasing severity of cognitive impairment, but changes in cystatin C levels during therapy did not associate with changes in cognitive function, and cystatin C did not appear to be a useful diagnostic marker for NCI.

Higher plasma cystatin C levels associated with increased all-cause mortality, cardiovascular events and heart failure in many large studies within of the general population (11, 12, 14, 15). Higher cystatin C also associated with worse cognitive function in prospective cohorts of community dwelling elderly individuals (ages 65 years or 70 to 79 years) (13, 16) and in individuals with Parkinson's disease (9, 10). Cystatin C associated with increased risk of subclinical brain infarcts or cerebral small vessel disease in two community based cross-sectional studies of elderly adults in the US and in Japan (24, 25), and with increased risk of ischemic and hemorrhagic stroke in a cross-sectional study from China (26).

Among older PWH who were viralogically suppressed on ART (age 50 years, pVL <50 copies HIV-RNA/mL), higher plasma cystatin C levels previously associated with a trend toward increased odds of NCI, in a cross-sectional analysis of a single-center study from the United States (17). Cystatin C levels were moderately discriminatory as a diagnostic marker for NCI in that study, wherein levels of at least 0.75 mg/L corresponded with a sensitivity of 61% and a specificity of 66% for NCI. In an analysis of eight plasma or cerebrospinal fluid protein biomarkers linked to NCI, recursive partitioning models accurately classified participants by their prognosis for cognitive stability or improvement but they performed poorly as diagnostic models (27).

The pathways underlying the association of cystatin C and NCI were not directly evaluated in our study. However, higher cystatin C levels have been shown to associate with markers of

inflammation and coagulation, including C reactive protein and plasminogen (28, 29). Among PWH, higher cystatin C independently associated with carotid intima-media thickness and epicardial fat content, and with elevated inflammation markers (IL-6 and soluble TNF receptors I & II), and soluble vascular cell adhesion molecule-1, a marker of endothelial activation (30). Also in PWH, cystatin C-based estimated GFR was less accurate than creatinine-based estimates, when compared to measured GFR (31, 32), where bias with the former associated with T-cell activation, pVL suppression, ART exposure and HCV infection (32). These correlations with inflammation and endovascular activation point to neuroinflammation and endovascular dysfunction as possible explanations for the observed associations between cystatin C and NCI. Our findings also describe cognitive benefits in association with viral suppressive ART that was also previously observed in some but not all studies (3, 6, 33).

Notable strengths of the present study include both cross-sectional and longitudinal analyses with uniform, detailed neurocognitive testing, within the context of a multicenter randomized clinical trial before and after initial ART. By incorporating variability within individuals over time, longitudinal analyses have the potential to better account for contributions by unmeasured factors between individuals than do cross-sectional analyses (34). Although we cannot exclude a practice effect due to repeated neuropsychological testing of participants in this study, it is unlikely that any such effect would differ according to factors that might also influence cystatin C. Renal function was preserved in all participants such that only 4 participants had a single estimated GFR measurement below 60 ml/min*1.73m² (range 56.05 to 59.31 ml/min*1.73m²) during the 48 weeks of this study. Moreover, those with worse baseline neurocognitive performance had greater improvement during the study. This suggests that the observed changes in cognitive performance were driven by factors other than practice effect since neurocognitively impaired individuals generally have poorer learning abilities and less practice effects.

The strong associations between education status and NCI in the present study are consistent with previous studies in both the general population and among PWH (6, 35, 36). We also observed substantial disparities in cognitive performance by race/ethnicity that is consistent with many studies (6, 33, 37). It is possible that inadequacies of normative data for non-white participants may have contributed these disparities. Notably, 17 (13%) non-white participants, but only 1 (1%) white participant in the present study had less than a high school education, suggesting that confounding by education also may contribute to these differences. Furthermore, we did not adjust for non-English dominant language, or the degree of bilingualism, thus introducing an additional source of potential bias.

In conclusion, the significant independent associations that we observed between cystatin C, an important marker of kidney function and cardiovascular disease risk, and NCI lend additional support for the importance of neuroinflammation and endovascular dysfunction in cognitive impairment, both before and after initiating ART. Despite these robust associations, however, cystatin C levels did not appear to be a useful diagnostic marker for this condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:
Boxplots of plasma cystatin C levels by week and according to Global Development Scores (GDS) as normal (GDS=0), mildly impaired (GDS=1), or moderately to severely impaired (GDS = 2) in black or Hispanic, and white participants.

Figure 2: Receiver Operator Curves (ROC) for baseline (**A**) and week-48 (**B**) univariate logistic regression models of NCI with plasma cystatin C. AUC=area under the ROC curve.

TABLE 1:

Baseline characteristics according to presence or absence of neurocognitive impairment.

	Normal Cognitive Testing n=126	NCI Present n=104	<i>P</i>
Age yrs. <i>med (IQR)</i>	33 (26, 43)	35 (28, 44)	0.37
Sex			0.11
Male <i>n (%)</i>	118 (94)	91 (88)	
Race/Ethnicity <i>n (%)</i>			<.0001
White	73 (58)	29 (28)	
Black, not Hispanic	28 (22)	44 (42)	
Hispanic	23 (18)	27 (26)	
Other	2 (2)	4 (4)	
Primary Language <i>n (%)</i>			0.03
English	118 (94)	88 (85)	
Spanish or Other	8 (6)	16 (15)	
Education yrs <i>med (IQR)</i>	14.3 (13.0, 16.0)	14.0 (12.0, 16.0)	0.001
CD4 cells/ μ L <i>med (IQR)</i>	389 (316, 493)	386 (276, 541)	0.82
Log₁₀ HIV-RNA copies/mL <i>med (IQR)</i>	4.5 (4.0, 5.0)	4.5 (4.0, 5.0)	0.83
GFR [†] mL/min*1.73 m ² <i>med (IQR)</i>	107.5 (94.5, 122.6)	111.8 (98.7, 120.9)	0.45
Plasma cystatin C mg/L <i>med (IQR)</i>	0.89 (0.82, 1.03)	0.94 (0.85, 1.04)	0.14

[†] GFR=estimated glomerular filtration rate