III International Clinical Virology Symposium and Advances in Vaccines

Buenos Aires, Argentina

Utility of HIV-1 EQ SuperLow Viral load Testing in HIV-associated Cognitive Impairment

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ABSTRACT

Background: Cognitive impairment can occur or persist during antiretroviral therapy (ART). Explanations include comorbidities, neurotoxic ART, persistent neuroinflammation, or persistent HIV replication in central nervous system (CNS). We assessed whether low levels of HIV in cerebrospinal fluid (CSF) were associated with inter-individual differences in ART regimens and neuropsychological (NP) performance using a proprietary HIV-1 EasyQ (EQ) SuperLow viral load (VL) assay by bioMONTR.

Methods: 329 participants (pts) were selected from the CHARTER cohort because they were taking ART, and had HIV RNA levels below 50 c/mL with an ultrasensitive assay (Roche Amplicor) in CSF and blood. Paired CSF and blood plasma (PL) specimens that were obtained within 1 hour of each other were assayed with HIV-1 EQ SuperLow VL assay with LDL of 2 c/mL. To determine the stability of low-level HIV in CSF, a follow-up specimen from 61 pts was assayed (median duration between visits, 7.0 months). Penetration of ART into the CNS was estimated by CNS Penetration-Effectiveness (CPE) ranks. NP performance was summarized by the Global Deficit Score (GDS), a validated method which integrates relevant information about 7 NP performance domains.

Results: Pts were mostly non-white (55%), middle-aged (mean 45 yr) men (76%) with AIDS (75%) who were HCV seronegative (68%). Median duration of the current ART regimen was 15 months. Median CD4 count was 467/μL. By the more sensitive assay, 136 (41%) had detectable HIV in CSF and 216 (66%) had detectable HIV in plasma. Detectable HIV in CSF was associated with worse CPE scores (mean 1.49 vs. 1.70, d = 0.29, p = 0.009) and detectable HIV in PL (71% pts with HIV detected in CSF vs. 62% undetected, p = 0.077). Other demographic or disease characteristics were not found in association. Of this group of pts, 39 (28%) had detectable HIV in CSF but not in PL, had worse global deficit scores (GDS) (0.63 vs. 0.37, p = 0.012), and were particularly likely to have at least moderate global impairment (GDS greater than 0.93, 28% vs. 8%, p = 0.005). Multivariate analyses identified that worse global deficit scores were associated with detectable HIV in CSF but not in PL (b = 0.13, p = 0.007), HCV seropositivity, shorter durations of ART, and ethnicity other than white (model R^2 = 0.29, p < 0.0001). In the subgroup with a follow-up CSF specimen assayed, CSF values became undetectable in 18 (30%) pts although remained detectable in the other 43 (70%). Transition from detectable to undetectable was associated with higher CD4 counts at the second visit (46% of undetectable pts had CD4 > 500 vs. 12% in detectable, p = 0.09), and HCV seropositivity (57% vs. 24%, p = 0.04), but not CPE scores or improved global NP performance.

Conclusions: Despite virologic suppression in CSF and PL by Amplicor v1.5, ART-treated individuals frequently (41%) had low, detectable levels of HIV in CSF using a proprietary HIV-1 EQ SuperLow assay, and this was associated with less penetrant ART. At least a quarter (28%) of individuals had persistent HIV in CSF only, and this was linked to worse neurocognitive functioning. People with HIV may have cognitive impairment as a result of ART that is incompletely effective in CNS and may require HIV-1 testing below 50 copies/ml.

BACKGROUND

Cognitive impairment can occur or persist in HIV+ individuals during antiretroviral therapy (ART). Potential explanations for this include comorbidities (e.g., HCV co-infection, premorbid brain injury), neurotoxic ART, overly sensitive assessment methods, persistent neuroinflammation, or persistent HIV replication. In prior findings, we reported that 42% of 40 CSF specimens that had HIV RNA levels below 50 copies/mL still had detectable HIV when they were assayed with a more sensitive method [1].

The objectives of this study were to confirm this finding using a much large sample size, to compare low levels of HIV in CSF to low levels in blood, and to compare the presence of HIV in CSF to inter-individual differences in clinical, treatment, and neuropsychological (NP) characteristics.

References:

[1]: *Letendre S, McClernon D, Benjamin R, and CHARTER Group. Presence of HIV RNA in Cerebrospinal Fluid that Is Undetectable with the Ultrasensitive Assay. The 14th Conference on Retroviruses and Opportunistic Infections. 25-28 February 2007, Los Angeles, CA. Abstract 369.*

RESULTS

***Figures: 1)** Association of detectable HIV in CSF with lower ART penetration, **2) P**ersistent HIV in CSF (detectable in CSF but not in PL) linked to worse global neurocognitive performance, **3)** Lower neurocognitive improvement in subjects with detectable HIV in CSF in both visits.*

Figure 1.

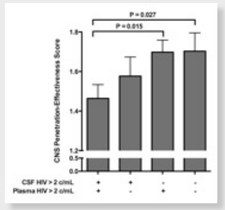


Figure 2.

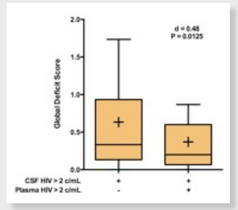
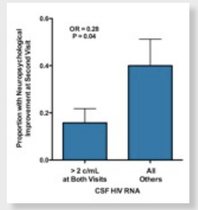


Figure 3.



The modified version of NucliSens EasyQ (EQ) assay detected the presence of detectable HIV in 136 specimens of CSF (41%) and in 216 of PL (66%).

Detectable HIV in CSF was associated with worse CPE scores (1.5 vs 1.7; p=0.009) and a lower proportion of confounding comorbitidies (8% vs 17%; p=0.04).

Detectable HIV in PL was observed in 97 (71%) participants from the group of detectable subjects in CSF, and this occurred in 119 (61%) subjects from the group of participants with undetectable HIV in CSF (p=0.07).

A total of 39 (28%) individuals showed detectable HIV in CSF and undetectable in PL. This was associated with worse global neurocognitive performance (0.63 vs 0.37; p=0.012) and a predisposition to show at least moderate global impairment (GDS > 0.93: 28% vs 8%; p=0.005).

From the 61 subjects with a follow-up visit, CSF values became undetectable in 18 (30%) pts and remained detectable in the other 43 (70%).

With regard to ART regimens, when some subgroups were compared according to the presence of HIV detectability in CSF, greatest differences appeared in the comparison between individuals taking ABV vs TFV (30% vs 48%; p=0.02) and 3TC vs FTC (33% vs 41%; p=0.003), and moderate differences between NVP vs EFV (29% vs 45%; p=0.07) and LPV vs ATV (42% vs 48%; p=0.49).

***Table.** Demographic, clinical and neurocognitive characteristics of the study sample.*

	All Subjects (N = 329)	HIV in CSF > 2 copies/mL (n = 136)	HIV in CSF ≤ 2 copies/mL (n = 193)	P value
Age (Years)	45	44	45	0.30
Gender (Women)	24%	25%	23%	0.55
Ethnicity (White)	45%	47%	44%	0.45
Global Rating, Mean	4.6	4.4	4.7	0.11
Global Impairment	39%	33%	43%	0.08
Confounding Comorbidities	14%	8%	17%	**0.04**
AIDS	73%	68%	76%	0.11
Current CD4 Count	480	491	451	0.07
Nadir CD4 Count	135	147	128	0.82
HCV Seropositive	30%	25%	32%	0.17
Months Current Regimen	15	18	13	0.59
< 95% Doses / 4 Days	9.7%	7%	11%	0.21
CPE Rank, Mean	1.6	1.5	1.7	**0.009**

Data expressed as medians, except when specified.

CONCLUSIONS

- ✓ **ART-treated individuals frequently have low levels of HIV in CSF using a proprietary HIV-1 EQ SuperLow assay despite reaching virologic suppression with Amplicor v1.5.**
- ✓ **HIV in CSF is associated with less penetrant ART.**
- ✓ **28% of individuals appear to have HIV that persists solely within the CNS (detectable HIV in CSF but not in blood) and these individuals present worse cognitive performance than those who appear to have a systemic source of HIV.**
- ✓ **People living with HIV may have cognitive impairment as a result of ART that is incompletely effective in the CNS.**
- ✓ **The HIV-1 EQ SuperLow assay represents a useful tool in HIV associated neurocognitive dysfunction management.**

METHODS

Participants: CHARTER is a North American cohort study based at 6 sites: Baltimore, Galveston, New York, St. Louis, San Diego, and Seattle. This analysis included 329 HIV-infected individuals who completed standardized assessments, had successful lumbar punctures, were taking ART, had HIV RNA levels below 50 c/mL (Roche Amplicor) in CSF and blood, and had at least 2 mL of specimen in storage at -70C.

Neuropsychological Testing: All participants completed a battery of neuropsychological tests that have demonstrated sensitivity to HIV infection, including measures of working memory, information processing speed, learning and memory, motor skills, verbal fluency, and executive functions. These data were summarized using the Global Rating methodology, which accounts for both the number and severity of deficits evident in an individual's neuropsychological profile (range = 1-9, with higher scores indicating greater levels of impairment) and global impairment ratings.

Laboratory Procedures: HIV RNA were measured in plasma and CSF (Roche Amplicor® Ultrasensitive v.1.5). 2 mL CSF specimens were then assayed with the proprietary HIV-1 EQ SuperLow assay capable of quantifying as low as 2 c/mL. This validated assay incorporates NASBA for amplification and molecular beacons for detection.

Statistical Analysis: Univariate analyses were performed using Fisher's Exact tests for categorical variables, t-tests for normally distributed continuous variables, or Wilcoxon rank sum tests for skewed continuous variables that were not improved with transformation.

***Supported by NIH contract N01 MH22005 (CHARTER; PI: I. Grant).** Participating sites include: Johns Hopkins University (J. McArthur); Mt. Sinai School of Medicine (S. Morgello & D. Simpson); University of California, San Diego (J.A. McCutchan); University of Texas Medical Branch, Galveston (B. Gelman); University of Washington, Seattle (A. Collier & C. Marra); Washington University, St. Louis (D. Clifford).*