Progression to Neuropsychological Impairment in Human Immunodeficiency Virus Infection Predicted by Elevated Cerebrospinal Fluid Levels of Human Immunodeficiency Virus RNA

Ronald J. Ellis, MD, PhD; David J. Moore, BS; Meredith E. Childers, MA; Scott Letendre, MD; J. Allen McCutchan, MD; Tanya Wolfson, MA; Stephen A. Spector, MD; Karen Hsia, PhD; Robert K. Heaton, PhD; Igor Grant, MD; for the HNRC Group

Background: If cerebrospinal fluid (CSF) human immunodeficiency virus (HIV) RNA levels are elevated before the development of neuropsychological (NP) impairment, such an observation would support prospective monitoring of CSF HIV RNA levels as well as therapeutic interventions designed to lower CSF HIV levels.

Objective: To determine whether increased CSF HIV RNA levels at an earlier time predict subsequent progression to NP impairment in HIV-infected subjects.

Methods: We examined 139 subjects in a prospective cohort study. Comprehensive NP, neuromedical, and laboratory evaluations were performed at initial and follow-up visits at least 6 months apart. Human immunodeficiency virus RNA levels in plasma and CSF were measured with a commercially available, polymerase chain reaction–based assay. To assess the robustness of our findings, we analyzed changes in NP performance over time in 2 ways. First, we used masked clinical ratings of global NP performance to identify individuals who were initially NP normal, and then determined, in a similarly blinded fashion, which of these subjects subsequently became NP impaired. Second, in a separate analysis, we assessed change in subjects’ raw scores on each of a series of NP test measures between baseline and follow-up.

Results: Among subjects who were not impaired at the initial visit, higher levels of HIV RNA in CSF significantly predicted progression to global NP impairment at the follow-up evaluation. Cerebrospinal fluid HIV RNA levels outperformed other clinical and laboratory measures in predicting progression to NP impairment. Higher CSF HIV RNA levels were associated with worsening performance on tests of attention, learning, and motor function.

Conclusion: Because elevated CSF HIV RNA levels predict subsequent progression to NP impairment, monitoring of CSF viral load and therapy to reduce CSF HIV RNA levels may be clinically warranted, even if impairment is not identified at the time of lumbar puncture.

Arch Neurol. 2002;59:923-928

The events that lead to clinically evident neuropsychological (NP) impairment in individuals with human immunodeficiency virus (HIV) infection are likely to evolve over time, such that significant neuronal injury may cumulate over periods ranging from months to years. Human immunodeficiency virus replication itself may be an early "trigger," rather than a late proximate cause of neural injury. In previous cross-sectional studies, levels of HIV RNA in cerebrospinal fluid (CSF) were elevated in HIV-infected subjects with NP impairment at later stages of disease.3,4 Such cross-sectional studies do not adequately address the likely substantial delay between exposure of central nervous system (CNS) tissues to replicating HIV and the subsequent onset of NP impairment. In addition, if CSF HIV RNA levels were found to be elevated before the development of NP impairment, a causal link between the two would be supported.

For editorial comment see page 909

If elevated CSF HIV RNA levels are important in the pathogenesis of HIV-associated neurocognitive disorders, then they should predict deterioration in the specific domains of NP ability known to be affected by HIV. Cognitive abilities that are selectively vulnerable in HIV infection include attention and working memory, learning, and motor skills.

In the present study we evaluated 2 hypotheses: first, that individuals with higher CSF HIV RNA levels are more likely to progress to NP impairment, and second, that individuals with higher CSF HIV

Author affiliations are listed at the end of this article.
SUBJECTS AND METHODS

SUBJECTS

Participants were 133 men and 6 women with laboratory-confirmed HIV infection prospectively enrolled in a longitudinal cohort study at the HIV Neurobehavioral Research Center, San Diego, Calif. Subjects underwent comprehensive NP and neuromedical evaluations, as well as lumbar punctures on 2 occasions at least 6 months apart. Subjects with a history of major neurologic or psychiatric disorders were excluded. Subjects were also excluded if neurologic signs, CSF abnormalities, or magnetic resonance imaging studies were consistent with brain opportunistic disease. Informed consent was obtained according to a protocol approved by the institutional human subjects review panel.

NP TESTING, CLINICAL RATINGS, AND PROGRESSION TO NP IMPAIRMENT

Each participant completed a comprehensive NP test battery that assessed 8 major neurocognitive ability areas (Table 1). In accordance with the recommendations of the National Institute of Mental Health Workshop on Neuropsychological Assessment Approaches in HIV Infection, clinical ratings were based on multiple NP tests within each ability area performed by a senior neuropsychologist (R.K.H.). Briefly, clinical ratings were assigned by means of age-, education-, sex-, and (when available) ethnicity-corrected T scores (all measures normalized to a mean of 50 and an SD of 10). Performance on a specific test was considered impaired when the T test score was more than 1 SD below the mean (T<40). An “impaired” clinical rating for each ability area was assigned when a participant performed below this cutoff point on 2 or more NP tests within an ability area. A global rating of “impaired” was assigned to participants who were rated as impaired in 2 or more separate ability areas. Thus, focal, isolated deficits would not warrant a rating of global impairment. Previous work1,3,6 has shown that these clinical ratings are reliable and sensitive to brain dysfunction of diverse causes. Participants were categorized into 4 groups according to their neurocognitive performance at the 2 study visits. Subjects in the first group (NL-NL) were rated as globally normal on NP testing at both the initial and follow-up visits. Those in the second group (IMP-IMP) were impaired at both visits. Subjects in the third group (NL-IMP) were normal at the initial visit and worsened to become NP impaired at the follow-up visit, while those in the fourth group (IMP-NL) improved to become normal at the follow-up visit. Only subjects who were NP normal at the initial visit were considered at risk to progress to NP impairment at the second visit.

The individual tests of learning, attention and working memory, and motor abilities used in this study are noted briefly by cognitive domain (Table 1). More detailed discussions of these may be found elsewhere.7,10

ADDITIONAL CLINICAL EVALUATIONS

Each subject underwent a comprehensive neuromedical evaluation that included medical and medication use history, neurologic and general physical examinations, and laboratory studies including CD4 lymphocyte counts, routine hematology and chemistry, and brain magnetic resonance imaging. Subjects were classified by means of the Centers for Disease Control and Prevention HIV Disease Classification.11 Participants were also classified by antiretroviral (ARV) medication treatment status in 2 ways: (1) taking ARVs or not taking ARVs at the initial visit and (2) new ARVs or no new ARVs at the follow-up visit. Five

RESULTS

DEMOGRAPHIC, CLINICAL, AND LABORATORY CHARACTERISTICS AND FOLLOW-UP INTERVALS

Table 2 summarizes these data. The 139 subjects included 133 men and 6 women, with an average age of 33.8 years (SD, 7.2), and an average education of 13.6 (1.8) years. Ninety-eight (71%) were white, 20 (14%) were African American, 12 (9%) were Hispanic, 4 (3%) were of other ethnicities, and 5 (4%) did not report their racial background. The median CD4 count at baseline was 378/µL (interquartile range [IQR], 238-523/µL); 34 subjects (24%) had CD4 lymphocyte counts less than 200/µL. Eighty subjects (58%) were taking ARVs at the initial visit, and 52 (37%) changed ARVs between the initial visit and follow-up. Initial study visits occurred between February 1, 1990, and April 30, 1998. The median interval between the initial study visit and the follow-up visit was 1.1 years (IQR, 1.0-1.8).

GLOBAL NP IMPAIRMENT RATINGS

At the initial visit, 94 subjects (68% of the total) were rated as globally NP normal, and 45 (32%) were impaired. Of the 94 NP normal subjects, 18 (19%) were impaired at the follow-up visit (the NL-IMP subgroup), and 76 (81%) remained normal (the NL-NL subgroup). Of the 45 initially impaired subjects, 31 (69%) remained impaired (IMP-IMP) and 14 (31%) improved to normal (IMP-NL). These 4 subgroups did not differ significantly with respect to age, education, or medical characteristics (Table 2).

PREDICTING PROGRESSION TO GLOBAL NP IMPAIRMENT

We performed analyses to determine whether elevated CSF HIV RNA levels at an earlier time were associated with a higher risk of progression to NP impairment. For these analyses, only subjects rated NP normal at the initial visit (n=94) were considered to be at risk for progressing to NP impairment. The follow-up interval for subjects who became impaired did not differ significantly from that of subjects who remained normal (median [IQR]: NL-IMP, 1.2 [1.0-2.9]; NL-NL, 1.1 [0.98-1.7]; P=.10, Wilcoxon test). We compared the predictive
power of HIV RNA levels in CSF with those in plasma. In separate logistic regression analyses, higher CSF HIV RNA levels were significantly associated with the later development of global NP impairment ($\chi^2 = 6.0; P = .01$). By comparison, higher plasma HIV RNA levels showed a statistical trend toward significance ($\chi^2 = 3.1; P = .08$). When both plasma and CSF levels were entered simultaneously into the logistic regression, the $\chi^2$ (P values) for CSF and plasma were 3.4 (.06) and 0.54 (.46), respectively, and the model $\chi^2$ was 8.1 ($P = .02$). Because these comparisons of the predictive power of HIV RNA levels were confounded by the different assay sensitivities (detection limits) used for plasma and CSF, we repeated the analysis applying the less sensitive (higher) cutoff (400 copies per milliliter) to both plasma and CSF levels. In this analysis, higher CSF HIV RNA values at baseline were still associated with subsequent neurocognitive impairment ($\chi^2 = 3.8, P = .05$).

To quantify the increase in risk associated with higher CSF HIV RNA levels, we compared the rates at which NP impairment developed in the 61 subjects (65%) with CSF HIV RNA levels of 200 copies or more per milliliter, with those of the 33 subjects (35%) with CSF HIV RNA levels less than 200 copies per milliliter. This cutoff (200 copies/mL) was chosen on the basis of data from a previous study.1 Subjects with higher CSF HIV RNA levels were significantly more likely to become impaired than those with lower CSF HIV RNA levels (26% vs 6%; odds ratio, 5.5; 95% confidence interval, 1.4-36; $P = .03$). We considered the possibility that subjects with higher CSF HIV RNA levels were more likely to be classified as having “borderline normal” NP functioning at the initial visit. This is important because such subjects would be predicted to be more likely to cross the threshold to become “mildly impaired” at a subsequent visit than subjects who had normal (not borderline) NP performance at baseline. Indeed, we found that progression to NP impairment was more common among subjects who were borderline at baseline than among those who were normal, not borderline (12/42 vs 6/52; $P = .06$). Nevertheless, when both variables were entered simultaneously into a logistic regression, CSF HIV RNA significantly predicted progression to NP impairment ($\chi^2 = 4.5; P = .03$), while borderline NP status did not ($\chi^2 = 2.1; P = .15$).

To assess further the specificity of the association between CSF HIV RNA levels and neurocognitive impairment, and to evaluate other potential predictors of incident NP impairment, we performed similar logistic regression analyses using other subject characteristics measured at the initial visit. In contrast to CSF HIV RNA levels, future impairment was not predicted by CD4 counts.
at the initial visit ($\chi^2 = 1.79; P = .17$), initial Centers for Disease Control and Prevention disease stage classification ($\chi^2 = 3.57; P = .17$), ARVs at the initial visit ($\chi^2 = 0.39; P = .53$), or changes in ARVs at follow-up ($\chi^2 = 0.07; P = .79$). In a stepwise logistic regression analysis adjusting for baseline NP performance, future impairment was associated with higher CSF HIV RNA levels, but no other markers significantly improved the model's predictive ability (CD4 count, $\chi^2 = 1.42; P = .23$; Centers for Disease Control and Prevention disease stage classification, $\chi^2 = 2.0; P = .37$; age in years, $\chi^2 = 0.25; P = .62$; education, $\chi^2 = 0.0; P = .99$; initial ARV status, $\chi^2 = 0.49; P = .78$).

Cerebrospinal fluid HIV RNA levels at the subjects' second visit were significantly correlated with levels at the first visit (Spearman $\rho = 0.48, P < .001$). Among subjects who became NP impaired, CSF HIV RNA levels were higher at the second visit (median [IQR], 3.3 [2.1–4.0] vs 2.6 [1.7–3.4] log copies/mL), as well as at the first, implying that persistence of increased CSF viral load impacted poorly on neurocognitive status.

### RELATIONSHIPS BETWEEN CSF HIV RNA LEVELS AND PERFORMANCE ON SPECIFIC NP TESTS

We hypothesized that elevated CSF HIV RNA levels would predict declines in performance in the specific ability areas known to be selectively affected in individuals with HIV neurocognitive disorders. These areas were attention and working memory, learning, and motor skills. By contrast, we expected that CSF HIV RNA levels would not predict changes in performance in other ability areas. Since any decline from previous performance was of interest, we included all subjects in this analysis, even those rated as globally NP impaired at the initial visit. The outcome of interest was change in raw score from the initial visit to follow-up on each NP test. In all cases, initial test performance was a predictor of both subsequent test performance and change from initial testing to follow-up. Therefore, we adjusted all regression models for baseline performance. To account for the possibility that longer follow-up intervals might be associated with greater declines in performance, we evaluated time as an initial predictor in all of the regression models. Because none of the models showed time to be a significant predictor, it was removed from the models presented in Table 3.

The direction and strength of the association between CSF HIV RNA levels and decline in NP test performance is reflected in the partial $\beta$ coefficients, and associated $F$, $\Delta R^2$, and $P$ values for these models. Table 3 lists these values for each of the separate regressions of CSF HIV RNA levels on NP test performance. Higher CSF HIV RNA levels at baseline significantly ($P < .01$) predicted declines in performance on the following tests: Story Learning ($P < .001$), Wechsler Adult Intelligence Scale–Revised Digit Span ($P = .003$), and Grooved Pegboard, nondominant hand ($P = .007$). In separate regressions examining whether baseline CSF HIV RNA levels predicted declines in Finger Tapping, nondominant hand (a measure of motor skills), and the Category Test (a measure of abstraction), we found trends in the predicted direction that did not meet the prespecified level of statistical significance. Although CSF HIV RNA levels were predictive of decline in performance on some measures of learning, attention, and motor skills, CSF HIV RNA levels were not related to declines in other NP domains (Table 3). Specifically, CSF HIV RNA levels were not significantly related to change in NP test performance on any measures of verbal, complex perceptual motor, memory, abstraction and executive functioning, or sensory abilities. This pattern of NP performance suggests that elevated CSF HIV RNA levels are related to changes in the specific NP abilities typically associated with HIV and do not represent a more generalized diffuse decline.

### COMMENT

We found that, among subjects who were initially NP normal, elevated CSF HIV RNA levels at an initial study visit significantly predicted progression to NP impairment after a median follow-up of approximately 1 year. Elevated plasma HIV RNA levels showed a weaker, nonsignificant relationship to incident NP impairment. These findings are consistent with the hypothesis that increased CSF viral burden in some patients may trigger a neurodegenerative process that results in HIV-associated neurocognitive disorders.

A previous study contrasts with ours in showing that elevated plasma HIV RNA levels were associated with incident dementia. Differences in study methods, such as our study's examination of CSF HIV RNA levels and the inclusion of baseline performance, and the specific NP battery used may account for the different findings. Our hypothesis that increased CSF viral burden may trigger subsequent neurocognitive decline is supported by our observation that elevated CSF HIV RNA levels predicted deterioration in the areas of learning, attention and working memory, and motor function. Previous work has shown that these are the abilities most likely to be impaired in subjects with HIV neurocognitive disorders. 3

Elevated CSF HIV RNA levels in this study were associated with worsening performance on some, but not all, of the NP tests predicted to be sensitive to neurocognitive changes in HIV. An example is the Figure Memory

---

**Table 1. Neuropsychological Tests Grouped by Domain**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learning</td>
<td>Story Learning (points/trials)</td>
</tr>
<tr>
<td></td>
<td>Figure Learning (points/trials)</td>
</tr>
<tr>
<td>Attention/working memory</td>
<td>WAIS-R Digit Span</td>
</tr>
<tr>
<td></td>
<td>WAIS-R Arithmetic</td>
</tr>
<tr>
<td></td>
<td>WAIS-R Block Design</td>
</tr>
<tr>
<td></td>
<td>WAIS-R Digit Symbol</td>
</tr>
<tr>
<td>Motor</td>
<td>Pegs Time (nondominant hand),</td>
</tr>
<tr>
<td></td>
<td>Pegs Time (dominant hand),</td>
</tr>
<tr>
<td></td>
<td>Finger Tapping (nondominant hand),</td>
</tr>
<tr>
<td></td>
<td>Finger Tapping (dominant hand),</td>
</tr>
<tr>
<td>Sensory</td>
<td>Sensory Perceptual Examination</td>
</tr>
<tr>
<td></td>
<td>Sensory Abstract/executive functioning</td>
</tr>
</tbody>
</table>

---

*WAIS-R indicates Wechsler Adult Intelligence Scale–Revised; PASAT, Paced Auditory Serial Addition Test.*

---

©2002 American Medical Association. All rights reserved.
Although poor story learning performance was related to elevated CSF RNA levels at the initial visit, figure learning performance did not show the same pattern. Several factors may explain these findings. First, individual NP tests differ substantially in their sensitivity to impairment. For example, “ceiling” and “floor” effects may limit a test’s ability to demonstrate change. The Figure Memory Test has a more restricted range of scores than the Story Memory Test; thus, measurement effects may have limited our ability to detect neurocognitive changes with this measure. Second, individual NP tests do not represent pure measures of specific NP abilities. For example, performance on the Figure Memory Test depends heavily on visuoperceptual and visuoconstructive abilities in addition to learning abilities. It is possible that changes in visuoperceptual abilities depend on...
factors other than the status of HIV infection in the CNS, such as current medication use or level of effort.

Not all subjects with initially elevated HIV RNA levels progressed to NP impairment. Various factors may protect some individuals, or increase risk in others, and virus-host interactions may be particularly important in this regard. Thus, neurodegeneration may require not only the presence of HIV in the CNS, but also the development of a specific host CNS immune response. Alternatively, mechanisms such as increased secretion of fibroblast growth factor may protect the host from the development of CNS disease. An important goal of future studies should be to determine combinations of virus and host factors that predict the development of, or decreased susceptibility to, HIV-induced CNS disease.

Our findings have implications for future research and clinical practice. The observation that neurocognitive decline was associated with HIV RNA levels in CSF, but not plasma, suggests that CSF viral load measurements may be important in assessing the risk of future neurocognitive decline in HIV-infected individuals. In at-risk subjects, interventions targeted at lowering both plasma and CSF viral load may be clinically warranted. Our findings also support the inclusion of NP tests that assess learning, attention and working memory, and motor function in batteries used to evaluate neurocognitive function in HIV-infected individuals.

Accepted for publication November 2, 2001.

From the Department of Neurosciences (Dr Ellis and Ms Childers), HIV Neurobehavioral Research Center (HNRC) (Drs Ellis, Letendre, McCutchan, Heaton, and Grant, Mr Moore, and Ms Childers and Wolfson), and Departments of Psychiatry (Mr Moore and Drs Heaton and Grant), Medicine (Drs Letendre and McCutchan), and Pediatrics (Drs Spector and Hsia), University of California, San Diego, California; San Diego State University/UCSD Joint Doctoral Program in Clinical Psychology (Mr Moore); and Veterans Affairs Healthcare System (Dr Grant). San Diego.

The San Diego HNRC Group is affiliated with the University of California, San Diego, the Naval Hospital, San Diego, and the San Diego Veterans Affairs Healthcare System. A list of the investigators appears on this page.

Author contributions: Study concept and design (Drs Ellis, Spector, and Grant); acquisition of data (Drs Ellis, Letendre, McCutchan, Spector, Hsia, and Heaton; Mr Moore; and Ms Childers); analysis and interpretation of data (Drs Ellis, Letendre, McCutchan, Spector, and Heaton; Mr Moore; and Ms Childers and Wolfson); drafting of the manuscript (Dr Ellis and Mr Moore); critical revision of the manuscript for important intellectual content (Drs Ellis, Letendre, McCutchan, Spector, Hsia, Heaton, and Grant; Mr Moore; and Ms Childers and Wolfson); statistical expertise (Mr Moore and Ms Wolfson); obtained funding (Drs Ellis, Letendre, McCutchan, Spector, and Grant); administrative, technical, or material support (Drs Ellis, Letendre, McCutchan, Hsia, Heaton, and Grant and Ms Childers); study supervision (Dr Ellis).

The HIV Neurobehavioral Research Center is supported by center award P30 MH62512-01 from the National Institute of Mental Health, Bethesda, Md. Dr Ellis is supported by grant RO1 MH58076 from the National Institute of Mental Health.

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, or the US government.

Corresponding author and reprints: Ronald J. Ellis, MD, PhD, HIV Neurobehavioral Research Center, University of California, San Diego, 150 W Washington St, 2nd Floor, San Diego, CA 92103 (e-mail: roellis@ucsd.edu).

REFERENCES