Elevated Cerebrospinal Fluid Neurofilament Light Protein Concentrations Predict the Development of AIDS Dementia Complex

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The light subunit of neurofilament protein (NFL) is a sensitive indicator of central nervous system axonal injury. We retrospectively identified 9 subjects participating in a longitudinal cohort study who developed acquired immunodeficiency syndrome dementia complex (ADC) and who had had a lumbar puncture performed within 2 years before presentation. Elevated cerebrospinal fluid (CSF) NFL concentrations were found in 7 (78%) of the 9 case patients who later developed ADC, compared with 9 (33%) of 27 CD4 cell count–matched HIV-1–infected control subjects. By contrast, no differences were found in CSF HIV-1 RNA or neopterin concentrations between the 2 groups. CSF NFL may prove to be a useful predictive marker for ADC.

In its more-severe form, AIDS dementia complex (ADC) is a devastating complication of HIV-1 infection. Before the introduction of highly active antiretroviral therapy (HAART), ADC developed in ∼20% of HIV-1–infected patients [1] and was accompanied by high morbidity and mortality. Its incidence has substantially decreased in parallel with the decline in opportunistic infections and tumors in areas where HAART has become available [2], and currently dementia develops mainly in patients who do not receive effective treatment.

Despite major advances in diagnostic methods, identification of ADC relies mainly on clinical recognition, at times supplemented by neuropsychological testing and exclusion of other diagnoses, including opportunistic infections in particular. Although the majority of patients with ADC have low CD4 cell counts and high cerebrospinal fluid (CSF) HIV RNA concentrations, these are not specific findings, and it is currently not possible to predict the risk of the development of dementia in an individual patient on the basis of laboratory findings. Although the central nervous system (CNS) is exposed to HIV early during the course of systemic infection, clinically significant cognitive impairment generally occurs much later, providing a relatively large window of opportunity to intervene and prevent its development.

Detection of the light subunit of neurofilament protein (NFL) is a sensitive marker of axonal disruption in the CNS. Its CSF concentration is elevated in a number of neurodegenerative disorders [3]. We have recently reported that CSF NFL concentration is also elevated in patients with ADC and that this increase responds to combination antiretroviral treatment [4]. To explore the utility of this marker as a means of predicting the development of dementia, we analyzed CSF for NFL in patients who had been followed longitudinally with lumbar punctures (LPs) and subsequently developed ADC.

Methods. Since 1985, HIV-infected patients in Göteborg, Sweden, have been included in a longitudinal study that includes serial sampling of CSF, plasma, and serum. LPs are performed in a standardized manner at least annually and more frequently in connection with antiretroviral treatment introduction or cessation. Both asymptomatic and symptomatic patients are included, and, as of December 2006, the cohort included 321 subjects who had undergone 1078 LPs. From this cohort, we retrospectively identified 9 patients (1 woman and 8 men) who had developed ADC during the course of the study and had had a LP performed within 2 years before symptomatic presentation. These patients were chosen as the affected study group. Their CSF samples had all been obtained before 1996, when HAART became available. The ages of the 9 case patients ranged from 32 to 57 years (median, 40.0 years), and their CD4 cell counts ranged from $10^3$ to $400 \times 10^3$ cells/L (median, $127 \times 10^3$ cells/L). Eight of the 9 case patients had received zidovudine and/or didanosine monotherapy either before or at
Figure 1. Time course of changes in salient laboratory findings and AIDS dementia complex (ADC) stage in 9 HIV-1–infected case patients. Shown are sets of 2 graphs for each case patient arranged within 9 panels. The top graph in each panel shows the changes in plasma (P) and cerebrospinal fluid (CSF) HIV-1 RNA and CSF neopterin concentrations, and the bottom graph shows the CSF neurofilament light protein (NFL) concentration and ADC stage. Treatment periods with zidovudine (blue lines) or didanosine (green lines) are indicated in each bottom graph. The dotted lines indicate the upper normal reference value for CSF NFL concentration (250 ng/L). The first 7 panels show case patients with elevated CSF NFL concentrations before ADC presentation, and the last 2 panels show case patients without detectable presymptomatic elevation.
the time of neurological presentation; the ninth was naive to antiretroviral treatment (figure 1). Diagnosis of ADC was established according to criteria of the Centers for Disease Control and Prevention and the American Academy of Neurology AIDS Task Force [5], and the Memorial Sloan-Kettering scale was used to rate its severity [6].

Twenty-seven untreated HIV-1–infected patients (7 women and 20 men) without neurological symptoms were then selected as cross-sectional control subjects after being matched for CD4 cell counts with the affected subjects. These individuals were selected from a cohort of neurologically asymptomatic patients from 4 centers in Göteborg, Sweden; Milan, Italy; San Francisco, California; and Sydney, Australia [4]. The 3 control subjects with CD4 cell counts closest to that of each case patient were included. The ages of these control subjects ranged from 23 to 66 years (median, 38.0 years), and their CD4 cell counts ranged from \(9 \times 10^5\) to \(430 \times 10^5\) cells/L (median, \(120 \times 10^5\) cells/L). The difference in CD4 cell count between the control subjects and the case patients was a median of \(7 \times 10^5\) cells/L (range, \(0 \times 10^5\)–\(70 \times 10^5\) cells/L). None of the control subjects developed dementia during a follow-up period of at least 24 months, although a majority (20/27) started HAART within 2 years of clinical presentation. The median (IQR) CD4 cell count was 67 (10–120) cells/L, with a range of 23 to 66 years (median, 38.0 years), and their CD4 cell counts were included. The ages of these control subjects ranged from 32 to 60 years (median, 45.5 years).

All CSF samples were obtained within the context of research studies approved by ethics committees at each site, and aliquots of CSF were stored after centrifugation at \(-70^\circ\text{C}\) until the time of the study. Concentrations of NFL in CSF were analyzed using an ELISA that has been described elsewhere [3]. The upper normal reference value at the laboratory is 250 ng/L for those below the age of 60 years and 380 ng/L for those below the age of 70 years.

HIV-1 RNA was quantified in cell-free CSF and plasma by use of the Roche Amplicor Monitor assay (versions 1.0 and 1.5; Hoffman La-Roche). Neopterin was measured by use of a commercially available radio immunoassay (Henningtest Neopterin; BRAHMS), which has a normal reference value of \(\leq 4.3\) nmol/L in CSF [7]. Routine assessments also included CSF white blood cell count and peripheral-blood CD4 cell count determinations.

Nonparametric methods were used for group descriptors (median and interquartile range [IQR]) and comparisons. Independent samples were compared using the Mann-Whitney U test. The Kruskal-Wallis test was used when \(> 2\) unpaired groups were compared.

**Results.** Seven of the 9 case patients with ADC had elevated CSF NFL concentrations during the 24 months before presentation (figure 1), compared with 9 (33%) of the 27 HIV-1–infected control subjects. This indicates a sensitivity of 78% and a specificity of 67% for an elevated CSF NFL in predicting ADC under current circumstances. For the 2 case patients (17 and 47) without elevated CSF NFL concentrations, the samples were obtained >12 months before ADC presentation, and they both exhibited high CSF NFL concentrations at the time of presentation with overt dementia. The median (IQR) CSF NFL concentration was 954 (429–1550) ng/L in the case patients, compared with 146 (<125–422) ng/L in the CD4 cell count–matched HIV-1–infected control subjects (\(P < .01\); figure 2) and <125 (<125–137) ng/L in the HIV-negative control subjects (data not shown). All 30 HIV-negative control subjects had CSF NFL concentrations <250 ng/L.

Although CSF HIV-1 RNA and CSF neopterin levels were elevated in the case patients with ADC, they were also high in the HIV-1–infected control subjects; indeed, no significant differences were found in these measurements between the case patients and the HIV-1–infected control subjects (figure 2). The median (IQR) CSF HIV-1 RNA level was 3.53 (2.26–5.11) log\(_{10}\) copies/mL in the case patients, compared with 3.86 (3.10–4.30) log\(_{10}\) copies/mL in the HIV-1–infected control subjects.
log₁₀ copies/mL in control subjects, and the median (IQR) CSF neopterin concentrations were 23.8 (19.5–39.3) and 22.0 (13.7–32.8) nmol/L, respectively.

**Discussion.** CSF NFL concentration was elevated before the clinical presentation of ADC in 7 of the 9 case patients we could identify. In contrast, although both CSF viral load and neopterin levels were also elevated, they did not distinguish these patients from a group of CD4 cell count–matched HIV-1–infected control subjects. This suggests that NFL may be a useful marker in distinguishing who will progress to the development of neurological deterioration.

ADC remains an important consequence of HIV-1 infection. Although its incidence has substantially decreased since the introduction of HAART [2], its prevalence has increased because more treated patients are surviving [8]. Additionally, less-severe forms of ADC—now encompassed under the terms “minor cognitive-motor disorder” and “asymptomatic cognitive impairment”—have been singled out as an increasingly important problem [9]. Additionally, antiretroviral treatment may also result in a different, milder form of CNS disease [10]. These complexities have increased the difficulty in distinguishing HIV-induced CNS disease from other neurological disorders in HIV-infected individuals, especially in patients with equivocal or mild symptoms and signs. In these settings, a laboratory marker that could identify patients who are indeed experiencing active brain injury or who are at enhanced risk of progressing to overt ADC would be very valuable, for both clinical trials and clinical practice.

CSF NFL is the first marker that seems to answer this need, although further studies are needed to fully define its sensitivity. Conflicting results for CSF HIV RNA level as a predictor of neurologic deterioration have been presented [11, 12]. However, in contrast to NFL [4], high CSF viral loads are commonly found in asymptomatic patients who are not receiving antiretroviral treatment [13]. Likewise, high CSF levels of such immunoactivation markers as neopterin and β2-microglobulin are frequently found during the asymptomatic stage of HIV infection [13]. Although high CSF β2-microglobulin concentrations have been linked to an increased risk of the development of ADC in patients with low CD4 cell counts [14], this risk applies to the patient group as a whole and is of limited value with respect to an individual patient. In the present study, neither CSF HIV-1 RNA nor CSF neopterin levels distinguished those who went on to develop ADC from those who did not.

In an earlier cross-sectional study, we found that NFL concentrations were elevated in the CSF in the majority of patients with a clinical diagnosis of ADC, with higher levels in those with more-severe disease. We also noted that CSF NFL concentrations decreased after the initiation of effective treatment [4]. Additionally, we observed that a minority of subjects without neurological symptoms or signs also had elevated CSF NFL levels. This was more common in subjects with lower peripheral-blood CD4 cell counts. Our best interpretation of this finding is that these individuals suffered subclinical brain injury related to HIV infection. The present results support this and additionally suggest that these individuals are more susceptible to developing progressive ADC.

We also have found that many patients with CNS opportunistic infections or tumors have elevated CSF NFL levels [4]. This is, of course, understandable and is consistent with their ongoing brain injury. Diagnostically, it also emphasizes that finding NFL is not specific and must be interpreted in the context of clinical presentation and other clinical and laboratory information. None of the patients in this study had or developed any CNS opportunistic infection during the study follow-up.

The neurofilament is a major structural element of the neuron and is found most conspicuously in larger neurons and their myelinated axons. It is composed of a triplet protein, of which the light subunit is an essential component of the neurofilament core. It maintains the axonal caliber and plays a crucial role in the structural and functional integrity of axons and in their capacity to rapidly conduct nerve impulses [15]. NFL measured in CSF is a sensitive marker of axonal damage in a number of conditions in which injured axons leak neurofilament protein [3].

The present study clearly indicates the capacity of CSF NFL to distinguish HIV-infected patients at high risk of developing ADC from patients with a similar degree of immunodeficiency but a lower risk. In fact, the predictive capacity of CSF NFL may have been underestimated, because a majority of the control subjects with elevated NFL concentrations started antiretroviral treatment soon after sampling, possibly preventing eventual cognitive deterioration. However, although unlikely, it could not, in contrast, be ruled out that the patients in the control group with normal CSF NFL concentrations might have developed dementia if HAART had not been initiated, which would result in overestimation. Although the retrospective design of this study limits our conclusions, a prospective study would present an ethical dilemma and would be impossible to perform without treatment intervention.

Although the clinical use of CSF NFL needs to be elucidated further, the present and earlier studies suggest that it holds promise as both a predictor and a diagnostic tool for HIV-related CNS injury. It also holds promise as a tool for following neurological treatment effects during HIV-1 infection.

**References**


