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Research Paper

Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study

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ABSTRACT

Background: Cerebrospinal fluid (CSF) neurofilament light chain protein (NFL) is a sensitive marker of neuronal injury in a variety of neurodegenerative conditions, including the CNS dysfunction injury that is common in untreated HIV infection. However, an important limitation is the requirement for lumbar puncture. For this reason, a sensitive and reliable blood biomarker of CNS injury would represent a welcome advance in both clinical and research settings.
Methods: To explore whether plasma concentrations of NFL might be used to detect CNS injury in HIV infection, an ultrasensitive Single molecule array (Simoa) immunoassay was developed. Using a cross-sectional design, we measured NFL in paired CSF and plasma samples from 121 HIV-infected subjects divided into groups according to stage of their systemic disease, presence of overt HIV-associated dementia (HAD), and after antiretroviral treatment (ART)-induced viral suppression. HIV-negative controls were also examined.
Findings: Plasma and CSF NFL concentrations were very highly correlated ($r = 0.89$, $P < 0.0001$). While NFL was more than 50-fold lower plasma than CSF it was within the quantifiable range of the new plasma assay in all subjects, including the HIV negatives and the HIV positives with normal CSF NFL concentrations. The pattern of NFL changes were almost identical in plasma and CSF, both exhibiting similar age-related increases in concentrations along with highest values in HAD and substantial elevations in ART-naïve neuroasymptomatic subjects with low blood $CD4^+$ T cells.
Interpretation: These results show that plasma NFL may prove a valuable tool to evaluate ongoing CNS injury in HIV infection that may be applied in the clinic and in research settings to assess the presence if active CNS injury. Because CSF NFL is also elevated in a variety of other CNS disorders, sensitive measures of plasma NFL may similarly prove useful in other settings.

1. Introduction

Infection of the central nervous system (CNS) is a nearly universal feature of systemic human immunodeficiency virus-1 (HIV) infection. It develops early in systemic HIV infection (Valcour et al., 2012) and continues throughout its untreated course (Gisslen et al., 1999). While often seemingly innocent, this infection can evolve to a form that is associated with CNS injury, most severely manifesting as HIV-associated dementia (HAD) with high morbidity and mortality (Price et al., 1988). However, infected individuals can also manifest less severe CNS injury that elides detection (Antinori et al., 2007; Heaton et al., 2011). Individuals with HIV infection can suffer CNS dysfunction from a variety of other conditions that can confuse diagnosis. Current research diagnostic classification depends on performance on a neuropsychological test battery which may be difficult to implement and also can be confounded by other conditions and co-morbidities (Antinori et al., 2007). Moreover, in patients with neurocognitive impairment it may be difficult to distinguish ongoing CNS injury related to HIV infection from prior but now static CNS injury, particularly without clear longitudinal observation. In order to more objectively define ongoing CNS injury in

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individual patients and in research settings evaluating the prevalence or incidence of CNS disease or CNS treatment effects, sensitive and reliable objective biomarkers can prove to be of great value.

To date, measurement of neurofilament light chain (NFL) concentrations in the cerebrospinal fluid (CSF) appears the most sensitive and useful biomarker of active CNS injury in HIV infection — showing elevations in not only overt HAD but also in patients with less severe, inapparent or clinically confounded impairment (Jessen Krut et al., 2014; Peterson et al., 2014). However, the need to sample CSF has limited the application of this measurement, particularly in the clinical contexts of screening patients, assessing those who refuse lumbar puncture, or evaluating those who suffer other conditions that obscure evaluation. An assay that could be applied more broadly to cohort studies and clinical trials that do not include lumbar puncture would also be valuable in assessing the prevalence of CNS injury and its response to treatment. Hence, a biomarker requiring only blood sampling rather than lumbar puncture would clearly represent a useful advance in clinical management and research settings.

To address this need we have developed an ultra-sensitive immunoassay for plasma NFL using Single molecule array (Simoa) technology (Rissin et al., 2010). In this study we compare plasma with CSF NFL concentrations of NFL in a cross-sectional study of HIV-infected individuals and HIV-negative controls and show that the plasma analysis using this sensitive method yields results that are concordant with CSF analysis despite the differences of NFL concentrations in the two fluids.

2. Methods

2.1. Study Design and Patients

This was a cross-sectional study using archived blood and CSF samples originated from two cohort studies: one in Gothenburg, Sweden and the second in San Francisco, California. The samples and related background data were all obtained between 1992 and 2014 within the context of research protocols approved by the institutional review boards of the two study sites. All blood and CSF samples were obtained after informed consent of subjects under these IRB-approved protocols.

Blood and CSF were included from 7 defined HIV-infected subject groups (n = 121) as outlined in previous studies (Jessen Krut et al., 2014; Peterson et al., 2014): early or 'primary' HIV infection (PHI, defined as within the first twelve months after initial HIV-1 infection) (Spudich et al., 2011); four groups of chronically HIV-infected subject volunteers without a diagnosis of HAD and who were not being evaluated or treated for overt neurological symptoms or signs when recruited, designated as neuroasymptomatic (NA) and divided by blood $CD4^+$ T cell counts into those with >350, 200–349, 50–199, and CD4 < 50 cells/μL; and a group presenting with clinically overt HAD, most commonly of subacute onset. All of these subjects were either naïve to treatment or off treatment for at least 6 months at the time of sampling. Also included was a group of treated HIV-infected subjects with >1 year of plasma virus suppression to below 50 copies/mL of HIV RNA (ART). A group of uninfected (HIV-neg) volunteer subjects (n = 19), confirmed by serological testing at study visit, were recruited from the San Francisco community for research assessments to provide comparison data for the HIV-infected subjects. Background clinical, laboratory and demographic data for each subject group are summarized in Table 1.

2.2. Blood and CSF Sampling

Blood and CSF were obtained according to standard protocols as previously described (Price et al., 2001; Gisslen et al., 2007). CSF was immediately subjected to low-speed centrifugation to remove cells, aliquoted and stored within 1 h of collection at ≤−70 °C until the time of the neuronal biomarker assays. Blood was collected in EDTA tubes and plasma was aliquoted and stored in parallel with CSF for later batch assays.

2.3. Background Laboratory Methods

We explored correlations with background HIV clinical biomarkers including: blood $CD4^+$ T cell counts: CSF and blood HIV RNA concentrations; CSF white blood cell (WBC) counts; CSF:serum albumin ratio as an indicator of blood–brain barrier permeability; and blood and CSF neopterin, a marker of macrophage and microglial activation (Hagberg et al., 2010).

HIV RNA levels were measured in cell-free CSF and plasma at each site using the Cobas TaqMan RealTime HIV-1 (version 1 or 2; Hoffmann-La Roche, Basel, Switzerland), or the Abbott RealTime HIV-1 assay (Abbot Laboratories, Abbot Park, IL, USA). All recorded viral loads that were below the lower limit of quantitation (40 copies/mL) were standardized to a defined 'floor' value of 20 copies/mL for descriptive purposes. Blood and CSF neopterin were analyzed using a commercially available immunoassay (BRAHMS, Hennigsdorf, Germany), with an upper normal reference value of 8.8 nmol/L in blood and 5.8 nmol/L in CSF (Hagberg et al., 2010). Each study visit included assessments by local clinical laboratories using routine methods to measure CSF white blood cell (WBC) count, CSF and blood albumin, and blood $CD4^+$ and $CD8^+$ T lymphocyte counts by flow cytometry.

2.4. Clinical Evaluations

All HIV-infected subjects and controls underwent routine clinical bedside screening for symptoms or signs of CNS opportunistic infections or other conditions that could impact CSF or blood biomarker concentrations. Diagnosis of HAD was based on clinicians' assessment at presentation which was characteristically subacute, and met American Academy of Neurology criteria (Anon., 1991). Many of these subjects were studied before publication of the more formal Frascati criteria (Antinori et al., 2007) and were diagnosed with AIDS dementia complex (ADC) stages 1–4 (Price and Brew, 1988) but met the functional criteria for the Frascati diagnosis of HAD without the requisite extensive formal neuropsychological assessment.

Table 1
Background Subject Characteristics.

Groups	N	Age	Plasma HIV-RNA	CSF HIV-RNA	Blood $CD4^+$ T cells
		Median years (IQR)	Median Log_{10} (IQR)	Median Log_{10} (IQR)	Median cells/mL (IQR)
HIV negative (HIV-neg)	19	34 (29–48)	NA	NA	763 (686–993)
Primary HIV Infection (PHI)	13	36 (30–46)	4.70 (4.11–5.64)	2.73 (1.96–3.94)	539 (340–761)
Neuroasymtpomatic HIV (NA)					
CD4 > 350	19	41 (35–43)	4.08 (3.30–4.44)	3.23 (2.45–3.68)	490 (402–582)
CD4 200–349	17	48 (36–56)	4.91 (4.30–5.31)	4.28 (3.44–4.68)	240 (215–305)
CD4 50–199	19	44 (37–56)	4.97 (4.44–5.25)	3.96 (3.53–4.56)	125 (100–170)
CD4 < 50	20	41 (36–49)	5.40 (4.54–5.88)	3.10 (2.30–3.81)	22 (10–39)
HIV-associated dementia (HAD)	11	43 (38–56)	5.49 (4.83–5.83)	4.34 (2.59–5.31)	60 (30–144)
HIV, treated-suppressed (ART)	22	44 (38–52)	1.30 (1.30–1.30)	1.30 (1.30–1.30)	544 (275–725)