

## **Immediate initiation of antiretroviral therapy is associated with decreased levels of microglial activation in HIV-1 infection**

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## Background

Investigation into the neuropathogenesis of HIV-1 in the central nervous system (CNS) has demonstrated viral penetration into the cerebrospinal fluid (CSF) during acute infection.<sup>1</sup> Efforts to understand the pathogenesis of HIV-associated neurocognitive disorder (HAND) have accelerated in recent years as clinicians have increasingly recognized the burden of this condition on the cognitive functioning of an aging HIV-infected population.<sup>2</sup> Recent work studying neurofilament light chain (NFL), a marker of axonal injury, has shown that such injury is not yet present in acute HIV infection but develops in a subset of individuals later in the disease course, during primary or chronic infection.<sup>3-5</sup> While the specific mechanism of neuronal injury remains incompletely understood, correlations with markers of inflammation suggest that neuroinflammatory processes may be an important underlying process.<sup>4</sup> Such work has also suggested that immediate initiation of antiretroviral therapy might mitigate the development of neuronal injury, while later initiation may lead to improvement, but not necessarily normalization, in CSF markers of such injury.<sup>5</sup>

The glycoprotein molecule YKL-40, also known as human chitinase 3-like 1 (CHI3L1), is a member of the glycosyl hydrolase family that is expressed by inflammatory cells during differentiation; serum levels of YKL-40 are up-regulated in a variety of inflammatory and neoplastic conditions.<sup>6</sup> In the CNS, YKL-40 is a putative marker of microglial activity.<sup>7</sup> While the physiological function of the molecule itself is incompletely understood, numerous efforts are underway to investigate its role in inflammatory disease processes within the CNS, including multiple sclerosis and Alzheimer's disease.<sup>8-11</sup> To our knowledge, there have been no formal clinical studies investigating the value of YKL-40 as a biomarker over the course of HIV infection, although prior work in models of SIV-associated encephalitis have suggested that this molecule may be of prognostic value.<sup>7</sup>

In this study, we sought to characterize CSF YKL-40 levels in two cohorts of HIV-1 infected subjects initiating combination antiretroviral therapy (cART) at different timepoints in the disease course and to correlate this molecule with markers of disease progression, neuroinflammation, and neuronal injury.

## Methods

Subjects were derived from 3 separate groups of Thai individuals enrolled in studies at the Southeast Asia Research Collaboration with Hawaii (SEARCH) research program in Bangkok, Thailand, as previously described.<sup>1</sup> Briefly, participants in RV254/SEARCH 010 (NCT 00796146) had acute HIV infection (AHI) and participants in SEARCH 011 (NCT 00782808) were treatment-naïve individuals with chronic HIV infection (CHI) who met Thai Ministry of Public Health guidelines for treatment initiation. SEARCH 013 enrolled 18 cognitively normal HIV-uninfected Thai controls, who underwent cross-sectional analysis at a single timepoint. All subjects underwent blood and CSF sampling, neuropsychological testing, and proton-magnetic resonance spectroscopy. The specific details of the participants and procedures included in this study have been described in detail previously.<sup>5</sup>

Following baseline assessment, subjects were immediately initiated on cART, which consisted of standard non-nucleoside reverse-transcriptase inhibitor (NNRTI)-based regimens (typically efavirenz, tenofovir, and emtricitabine or an intensified regimen that also included raltegravir and maraviroc) in SEARCH 010 and regimens with efavirenz or nevirapine and a nucleoside reverse-transcriptase inhibitor (NRTI) backbone of lamivudine, zidovudine, tenofovir, or stavudine in SEARCH 011.

CSF sampling procedures involved collection of 10 mL of fluid with lumbar puncture using standard sterile procedures. CSF HIV RNA levels were measured using the Roche Amplicor kit (version 1.5), with a lower limit of detection of 50 copies/mL in plasma and 100 copies/mL in CSF. Standard CSF parameters, including protein and glucose levels and cell count were measured at the time of collection. Blood and CSF neopterin, CSF chemokine (CXC motif) ligand 10 (CXCL10)/interferon gamma-induced protein 10 (IP-10) and CSF chemokine (CC motif) ligand 2 (CCL2)/monocyte chemoattractant protein 1 (MCP-1) were quantified using commercially available assays.<sup>1</sup> CSF neurofilament light chain was measured using a 2-site enzymatic quantitative immunoassay (Uman Diagnostics), as previously described.<sup>5</sup> Proton-MRS and neuropsychological testing procedures have been described in detail elsewhere.<sup>5</sup>

CSF YKL-40 levels were measured by solid phase sandwich ELISA (R&D Systems, Inc.) according to the manufacturer's instructions using one batch of reagents. The measurements were performed by board-certified laboratory technicians who were blinded to clinical data. The intra-assay coefficient of variation was below 5% and all samples were well above the detection limit (3.55 ng/l) and within the linear range of the standard curve. YKL-40 levels were assessed in subjects at baseline, 24 weeks, and 96 weeks after initiation of cART in the AHI group and at baseline and 48 weeks after initiation of cART in the CHI group.

The primary statistical endpoint for this study was the CSF YKL-40 level before and after initiation of cART. We used the Mann-Whitney U test and the Kruskal-Wallis test with post-hoc testing corrected with Dunn multiple comparison to compare values between groups. Nonparametric correlations between measured parameters used Spearman rank correlation coefficients; linear regression was also conducted for illustrative purposes. Analyses were performed with SPSS (version 19.0) and GraphPad Prism (version 5.0d) software.

All subjects provided written informed consent and the protocols were approved by the Chulalongkorn Hospital Institutional Review Board and the equivalent bodies at each of the collaborating institutions.

## **Results**

### *Characteristics of participants at baseline*

Data were available for 33 subjects with AHI and 34 subjects with CHI. Baseline characteristics of these participants are displayed in Table 1. Overall, the AHI group had a higher proportion of male subjects, and participants in this group had higher CD4<sup>+</sup> T lymphocyte counts and higher HIV viral loads in the plasma and CSF. The CSF of subjects with CHI had a more inflammatory profile, with elevated CSF white blood cells,

CSF CXCL10/IP-10, and elevated CSF neurofilament light chain, as described in a previous study.<sup>5</sup>

At baseline, subjects with chronic untreated HIV infection had higher CSF YKL-40 levels compared with those with AHI and HIV-uninfected controls ( $p=0.01$  and  $p=0.04$ , respectively). There was no difference in CSF YKL-40 level between AHI subjects at baseline and normal Thai controls (Figure 1a).

#### *Characteristics of participants at follow-up on suppressive cART*

At follow-up, plasma HIV viral loads were suppressed in 23/25 (92%) of AHI subjects; two subjects had low-level viremia (64 and 74 copies/mL). All subjects for whom data were available were suppressed in the CSF. At the follow-up timepoint in the CHI group, all subjects had suppressed plasma viral loads. One subject had a CSF viral load of 400 copies/mL.

Median CSF YKL-40 levels measured at follow-up timepoints (24 weeks in the acute infection group and 48 weeks in the chronic infection group) decreased in both groups. Still, however, there remained a difference in median YKL-40 levels between AHI and CHI subjects ( $p=0.003$ ); at follow-up there was no longer a difference between the CHI group and HIV-uninfected controls (Figure 1b).

When analysis was limited to subjects with serial YKL-40 measurements ( $n=20$  in the AHI group and  $n=10$  in the CHI group), there were absolute decreases in median YKL-40 measurements in both AHI and CHI groups. This trend approached significance in the AHI group ( $p=0.09$ ) at 24-week follow-up, and there remained no evidence of increased YKL-40 at 96-week follow-up, although the sample size was small ( $n=8$ ). While the median CSF YKL-40 level decreased at 48-week follow-up in the CHI group, no statistically significant difference was detected, although again the sample size was small ( $n=10$ ).

#### *Correlates of CSF YKL-40*

There were no correlations between CSF YKL-40 and standard parameters of HIV infection, such as CD4+ T lymphocyte count, plasma viral load, or CSF viral load, in either group at either time point.

At baseline, there was a trend toward a correlation between CSF YKL-40 and CXCL10/IP-10 in subjects with acute HIV infection (Figure 1c). Correlations between YKL-40 and neopterin, CXCL10/IP-10, and neurofilament light chain were not identified in the acute infection group at either 24- or 96-week follow-up after treatment initiation.

In subjects with chronic HIV infection, CSF YKL-40 correlated with CSF neopterin, CXCL10/IP-10, and neurofilament light chain at baseline before treatment (Figure 1c). Correlations between YKL-40 and neopterin, CXCL10/IP-10, and neurofilament light chain were not identified at 48-week follow-up in the chronic infection group, although the sample size was limited ( $n=10$ ).

No correlations were identified between YKL-40 and proton-MRS neuroimaging markers or markers of neuropsychological performance in either the acute or chronic HIV infection group at either time point (data not shown).

## Discussion

This study is the first description of the dynamics of CSF YKL-40 in HIV-infected individuals. In recent years, there has been growing interest in the role of YKL-40 as a marker of inflammation;<sup>6</sup> in the CNS, efforts have been made to determine whether it may be used as a biomarker of active neuroinflammation or disease progression in disorders such as Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, and traumatic brain injury.<sup>8-13</sup> CSF YKL-40 was initially studied in the context of HIV-1 infection in a pigtail macaque model, where it was shown to be a predictive biomarker of SIV encephalitis. In that study, there was also a mention of a correlation between increased CSF HIV viral loads and elevated YKL-40, although data were not displayed.<sup>7</sup>

HIV replication in the brain primarily targets macrophages, and the infection itself results in immune activation of infected and non-infected glial cells such as microglia and astrocytes.<sup>14</sup> This study showed that elevated CSF YKL-40 levels are present in chronic but not acute HIV infection, supporting the hypothesis that microglial activation may be important in the neuropathogenesis of HIV-1, but suggesting that this is a progressive rather than immediate process.

CSF YKL-40 never became elevated in AHI subjects who initiated cART immediately after diagnosis. Early cART initiation might therefore reduce microglial activation and by extension prevent or mitigate downstream neuronal injury. This result parallels findings regarding CSF neurofilament light chain, a marker of neuronal injury, which also remains unchanged from baseline in subjects initiating treatment immediately upon diagnosis.<sup>5</sup>

The initiation of cART resulted in improvement in the CSF concentrations of YKL-40 in untreated individuals with chronic HIV infection, suggesting a role for cART in reducing the neuroinflammatory cascade. This finding is consistent with prior work demonstrating reduction in neurofilament light chain, which correlated with the inflammatory marker neopterin at the follow-up timepoint in this group.<sup>5</sup> Notably, YKL-40, like neurofilament light chain in the earlier study, remained elevated in CHI subjects compared with AHI subjects at follow-up, suggesting that some of this neuroinflammation is slow to reverse or is irreversible. We also note the trend toward decreased YKL-40 in AHI subjects at 24-week follow-up compared with baseline levels. This result may suggest that cART initiation can have an anti-inflammatory effect even when levels are not initially elevated. The clinical significance of this effect requires further evaluation.

CSF YKL-40 correlated with CXCL10/IP-10 and neopterin, both markers of monocyte activity, at baseline in CHI subjects; in AHI subjects it demonstrated a trend toward correlation with CXCL10/IP-10 alone. CXCL10/IP-10 was significantly elevated in the CHI group compared with the AHI group.<sup>5</sup> Overall, this pattern is consistent with YKL-40 as a marker of activity within the monocyte lineage and suggests that activity within this lineage is part of HIV neuropathogenesis. CSF YKL-40 also correlated with CSF neurofilament light chain in chronic HIV infection, and the dynamics of change in these markers following treatment initiation appear to be similar,<sup>5</sup> suggesting that microglial activation might be one of the mechanisms underlying neuronal injury in chronic untreated infection.

### *Limitations*

This study has several limitations, including a relatively small sample size and limited longitudinal CSF data. Potential biases include selection bias in enrolling lower-risk or more-adherent subjects or unknown biases related to attrition in both AHI and CHI groups. There are no established benchmarks for YKL-40 levels in southeast Asian populations, but our use of an HIV-uninfected control cohort does provide some basis for comparison.

### *Implications*

Overall, our results characterize YKL-40 in the CSF of HIV-infected subjects for the first time, provide further evidence for a benefit of early initiation of antiretroviral therapy in these subjects, and suggest a mechanism through which cART might confer neuroprotective effects. In the context of recent work suggesting that damage to the immune system may occur early in HIV infection<sup>15</sup> and that there exists an overall benefit for immediate initiation of antiretroviral therapy in all HIV-infected individuals regardless of CD4 count,<sup>16</sup> our work provides further specific information on the mechanism of the benefit of early treatment initiation within the CNS.

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	<b>Acute HIV Infection (n= 33)</b>	<b>Chronic HIV Infection (n=34)</b>	<b>HIV-Uninfected (n=18)</b>	<b>p-value (acute vs chronic)</b>
Age (years)	29 (24-37)	34 (29-36)	33 (27-39)	0.150
% Male	94	41	50	< 0.001
CD4 Count (cells/uL)	401 (318-568)	228 (146-342)	-	<0.001
Log Plasma HIV	5.5 (4.9-6.3)	4.8 (4.4-5.3)	-	0.002
Log CSF HIV	3.1 (1.7-4.3)	4.1 (3.7-4.8)	-	0.006
Estimated Time Infected*	18 (13-24) days	3.7 (0.9-6.4) years	-	-
CSF WBC (cells/uL)	0 (0-3)	3 (2-9)	0 (0-0)	0.003
CSF CXCL10/IP-10 (pg/mL)	539 (229-748)	833 (566-1011)	-	0.006
CSF Neopterin (nmol/L)	7.7 (4.7-13.5)	9.3 (7.0-13.0)	2.6 (1.9-2.9)	0.381
CSF Neurofilament (ng/L)	243 (204-333)	327 (251-568)	299 (210-337)	0.002
Typical cART Regimens	NNRTI-based cART +/- RAL/MVC	NNRTI-based cART	-	-

Table 1. Comparison of baseline data at week 0 pre-cART initiation for acute HIV infection, chronic HIV infection, and HIV-uninfected participants. Note that duration of infection for chronic group participants is time since diagnosis, which is subject to recall bias. WBC, white blood cells; CXCL10, chemokine CXC motif ligand 10; IP-10, interferon gamma induced protein 10; cART, combination antiretroviral therapy; NNRTI, non-nucleoside reverse-transcriptase inhibitor.



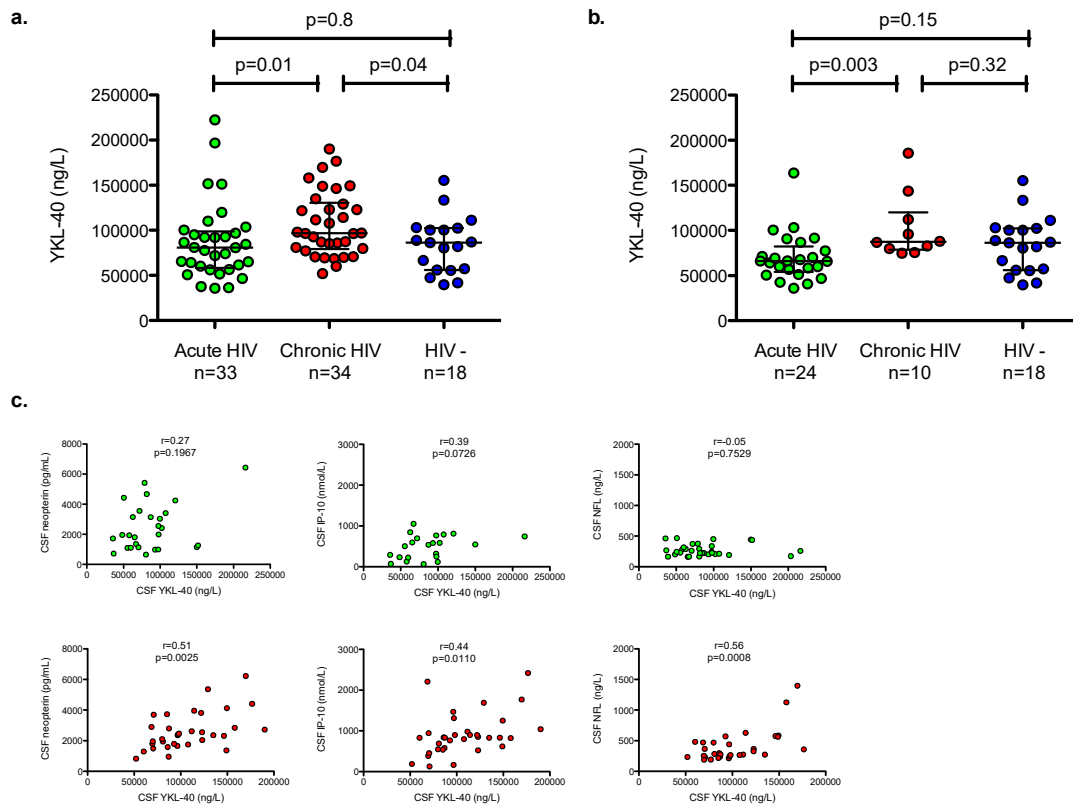


Figure 1. (a) Baseline YKL-40 levels in subjects with acute HIV infection (AHI), chronic HIV infection (CHI), and HIV-uninfected controls. (b) Levels of YKL-40 in subjects with AHI at 24-week follow-up, CHI at 48-week follow-up, and HIV-uninfected controls. (c) Baseline (pre-treatment) association between CSF YKL and CSF neopterin (left), CSF CXCL10/IP10 (middle), and CSF neurofilament light chain (right) in subjects with AHI (top row) and CHI (bottom row).