

# NEUROLOGY

**CSF soluble Fas correlates with the severity of HIV-associated dementia**  
A. Towfighi, R. L. Skolasky, C. St. Hillaire, K. Conant and J. C. McArthur  
*Neurology* 2004;62;654-656

**This information is current as of December 13, 2007**

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://www.neurology.org/cgi/content/full/62/4/654>

*Neurology*® is the official journal of the American Academy of Neurology. Published continuously since 1951, it is now a weekly with 48 issues per year. Copyright © 2004 by AAN Enterprises, Inc. All rights reserved. Print ISSN: 0028-3878. Online ISSN: 1526-632X.



# CSF soluble Fas correlates with the severity of HIV-associated dementia

A. Towfighi, MD; R.L. Skolasky, MA; C. St. Hillaire, BS; K. Conant, MD; and J.C. McArthur, MBBS, MPH

**Abstract**—Soluble Fas (sFas) and soluble Fas ligand (sFasL) are associated with cellular dysfunction and death and are elevated in CSF from patients with HIV dementia (HIV-D). The authors investigated whether these markers correlated with dementia severity and course. sFas and sFasL were measured in 15 highly active antiretroviral therapy (HAART)-naïve HIV-D subjects, 30 HAART-naïve HIV+ controls, and 17 HIV- controls. HIV-D subjects had higher CSF sFas levels than controls. Subjects with moderate/severe dementia had higher CSF sFas levels than those with mild dementia. CSF sFas trended lower in those with progressive dementia.

NEUROLOGY 2004;62:654–656

HIV-associated dementia (HIV-D), a subcortical dementia characterized by motor dysfunction, cognitive decline, and behavioral changes, develops in approximately 15% of patients with AIDS. The clinical progression of HIV-D is highly variable and is slower or even static in individuals treated with highly active antiretroviral therapy (HAART).

The pathogenesis of HIV-D involves astrocyte dysfunction and apoptosis. Astrocytes have important neuroprotective functions.<sup>1</sup> They decrease neurotoxin levels,<sup>2</sup> clear excess excitatory amino acids,<sup>3</sup> produce neurotrophic factors,<sup>4,5</sup> and maintain the blood-brain barrier.<sup>6</sup> Postmortem brain tissue examination reveals that HIV+ patients have more astrocyte apoptosis than HIV- controls.<sup>1</sup> In addition, patients with rapidly progressive HIV-D have more astrocyte apoptosis than those with slowly progressive HIV-D.<sup>1</sup>

Astrocyte apoptosis may occur through the Fas-FasL system. Postmortem brain tissue examination demonstrates that astrocytes upregulate Fas expression in HIV-D.<sup>7</sup> In vitro studies reveal that soluble Fas ligand (sFasL) released from HIV-infected macrophages triggers apoptosis of uninfected astrocytes.<sup>8</sup> HIV+ individuals with neurologic deficits have higher CSF sFas concentrations than HIV- controls.<sup>9</sup> Individuals with HIV-D have higher levels of CSF sFas, sFasL, and serum sFasL than HIV+ nondemented controls.<sup>10</sup>

This study investigates whether CSF and serum sFas and sFasL correlate with dementia severity and dementia progression. If the markers correlate with disease severity, they can be used for following patients longitudinally. If they correlate with disease course, they can serve as predictive markers.

**Methods.** We reviewed patients seen through the Johns Hopkins HIV Neurology program between 1987 and 1995. Three study groups were identified: individuals with HIV-D, HIV+ controls,

and HIV- controls. After informed consent was obtained, CSF and serum samples were collected.

Between 1987 and 1995, 54 individuals were diagnosed with HIV-D at Johns Hopkins. At diagnosis, each patient received a Memorial Sloan Kettering (MSK) clinical severity score, ranging from 0 (normal) to 4 (severe/end stage). Eighteen subjects whose serum or CSF samples were not obtained within 2 months of diagnosis were excluded. The charts of the remaining 36 were reviewed. Subjects with CNS opportunistic infections, neurosyphilis, multiple sclerosis, Parkinson disease, Alzheimer disease, stroke, neoplasm, myopathy, autoimmune disease, lymphoproliferative disease, or history of head trauma were excluded. The final total was 15. Three subjects were naive to antiretrovirals. The remainder received mono or dual NRTI therapy.

Subjects were grouped by dementia severity using MSK scores. The rate of dementia progression was computed using the MSK slope (difference between MSK score at diagnosis and MSK score at next visit divided by total number of months elapsed). The duration between visits ranged from 1 to 8 months (13/15 returned for follow-up within 4 months).

Forty-three HAART-naïve HIV+ controls were identified by the Johns Hopkins HIV Neurology database. Individuals with inflammatory or infectious conditions were excluded, yielding a total of 30. Seventeen of these subjects were on mono or dual antiretroviral therapy.

Thirty-one HIV- controls were identified by the Johns Hopkins Neurology database. Those with inflammatory or infectious conditions were excluded, yielding a total of 17.

sFas and sFasL concentrations were measured by ELISA (OncoGene, Boston, MA) following the manufacturer's instructions. Three to six samples from each group were run in duplicate for quality control.

Baseline demographics were analyzed using nonparametric analysis of variance. Differences in marker levels were analyzed using the nonparametric Mann-Whitney test. Correlations between markers and other parameters were determined by Spearman rank correlation analysis. All statistical analyses were performed using SAS v8.1 (SAS Institute, Cary, NC).

**Results.** The median age of the HIV- controls, HIV+ controls, and HIV-D subjects was 50.5, 32, and 42 years. The ratio of women to men in the HIV- controls, HIV+ controls, and HIV-D subjects was 11:6, 1:29, and 2:13. The median CD4+ cell count was 433 cells/mm<sup>3</sup> for the HIV+ control group and 93 cells/mm<sup>3</sup> for the HIV-D group. At diagnosis, nine subjects had mild, five had moderate, and

From the Department of Neurology and Epidemiology, Johns Hopkins University, Baltimore, MD.

Supported by NS 26643 (J.C.M.), RR00522 (GCRC JHU), NS 35619 (Leon Epstein).

Received May 23, 2002. Accepted in final form October 14, 2003.

Address correspondence and reprint requests to Dr. Justin McArthur, Johns Hopkins Hospital, 600 N. Wolfe St. Meyer 6-109, Baltimore, MD 21287; e-mail: jm@jhmi.edu

**Table** Levels of sFas and sFasL among different study groups

Groups	Median (IQR*) concentration			
	CSF sFas,† U/mL	CSF sFasL, ng/mL	Serum sFas,† U/mL	Serum sFasL, ng/mL
HIV- control, n = 17	0.131 (0.113, 0.161)	0.230 (0.221, 0.244)	6.470 (5.403, 8.826)	0.977 (0.843, 1.241)
HIV+ control, n = 30	0.153 (0.140, 0.183)	0.284 (0.211, 0.416)	8.486 (7.279, 9.330)	0.991 (0.790, 1.359)
HIV-D, n = 15	0.215‡ (0.155, 0.411)	0.268 (0.187, 0.581)	9.159 (7.446, 11.881)	0.892 (0.393, 1.192)
Mild HIV-D, n = 8	0.210 (0.162, 0.342)	0.358 (0.152, 0.675)	9.159 (8.223, 11.253)	1.040 (0.355, 1.235)
Moderate/severe HIV-D, n = 7	0.327§ (0.255, 0.752)	0.268 (0.211, 0.576)	9.473 (6.613, 12.143)	0.688 (0.431, 1.146)
HIV-D improver, n = 4	0.232 (0.174, 0.495)	0.411 (0.221, 0.609)	12.157 (6.613, 14.252)	1.138 (0.227, 1.363)
HIV-D progressor, n = 3	0.173 (0.150, 0.411)	0.166 (0.110, 0.769)	8.992 (8.223, 11.253)	0.932 (0.355, 1.235)
HIV-D stable, n = 7	0.213 (0.155, 0.555)	0.306 (0.187, 0.581)	9.325 (7.565, 11.619)	0.851 (0.524, 1.148)

\* IQR = interquartile range: 25th, 75th percentile.

† 1 Unit = amount of sFas produced by 10,000 cells (Oncogene Research Products, Boston, MA).

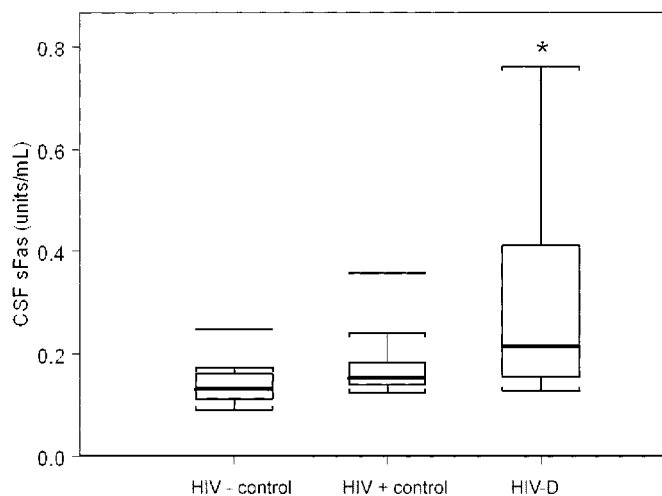
‡ CSF sFas is significantly elevated in patients with HIV-D compared with HIV+ controls and HIV- controls ( $\chi^2_1 = 5.92, p = 0.015$  and  $\chi^2_1 = 6.01, p = 0.014$ ).

§ Individuals with moderate/severe dementia have significantly higher levels of CSF sFas than individuals with mild dementia ( $\chi^2_1 = 6.47, p = 0.011$ ).

HIV-D HIV-associated dementia.

one had severe dementia. Of the 14 who returned for follow-up, four improved, seven were stable, and three progressed.

CSF sFas was detectable in 13/15 HIV-D subjects, 30/30 HIV+ controls, and 15/16 HIV- controls. CSF sFas was significantly elevated in HIV-D subjects compared with HIV+ and HIV- controls ( $p = 0.015$  and  $p = 0.014$ ) (table, figure 1). There was no correlation between CD4 count and CSF sFas levels in HIV-D subjects ( $p = 0.323$ ). In the



**Figure 1.** Concentrations of CSF soluble Fas (sFas) are plotted for the three study groups: HIV- controls, HIV+ controls, and HIV dementia (HIV-D) subjects. The box indicates interquartile range (IQR), the solid line within the box represents the median, and the vertical bars indicate a measure of spread equal to 1.5 times the IQR (upper and lower fences). Single horizontal lines represent outliers.

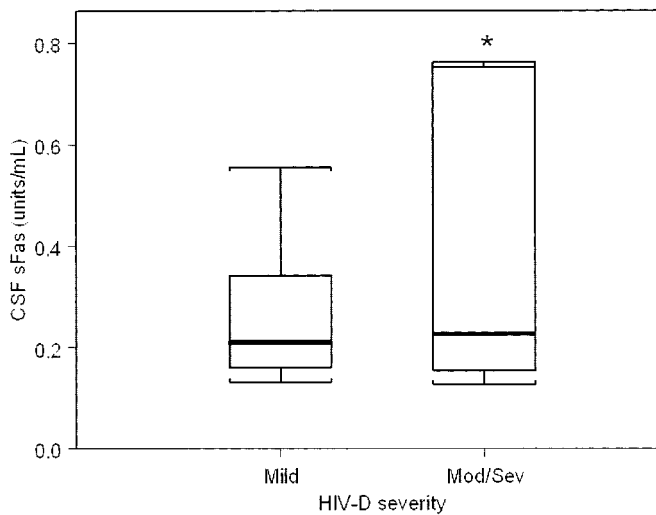
\*Subjects with HIV-D have higher CSF sFas levels than HIV+ controls and HIV- controls ( $\chi^2_1 = 5.92, p = 0.015$  and  $\chi^2_1 = 6.01, p = 0.014$ ).

HIV+ controls, serum and CSF sFas correlated with each other ( $p = 0.030$ ), whereas in HIV-D subjects, they did not ( $p = 0.375$ ), suggesting that CSF sFas elevation in HIV-D is not merely secondary to elevated serum sFas.

CSF sFasL was detectable in all subjects except one HIV- control. Serum sFas and sFasL were detectable in all subjects. There were no significant differences between the groups for CSF sFasL, serum sFas, or serum sFasL (see the table). Subjects with moderate/severe dementia had higher levels of CSF sFas than those with mild dementia ( $p = 0.011$ ) (see the table, figure 2). They also had lower CD4+ cell counts than those with mild dementia ( $p = 0.023$ ). CSF sFasL trended lower in subjects with more severe dementia (see the table). Subjects with progressive dementia trended toward lower CSF sFas and sFasL levels than those who improved or remained static (see the table).

There was no correlation between CSF white blood cell count and CSF sFas or sFasL levels, suggesting that CSF sFas elevation was not due to pleocytosis. There was no correlation between marker levels and antiretroviral use. There was no correlation between dementia progression and length between visits.

**Discussion.** This study showed that CSF sFas is significantly elevated in subjects with HIV-D compared with HIV+ and HIV- controls, corroborating prior studies.<sup>9,10</sup> In addition, CSF sFas was significantly higher in subjects categorized in the pre-HAART era with moderate/severe dementia compared with those with mild dementia. Elevated CSF sFas in the later stages of HIV-D suggests astrocyte activation, Fas expression, astrocyte apoptosis, and ultimately neuronal dysfunction. CSF sFas unexpectedly trended lower in subjects with progressive dementia. The difference, however, was insignificant. CSF sFasL trended lower in both severe and progressive dementia. Possible explanations are that



**Figure 2.** The levels of CSF soluble Fas (sFas) were compared between two groups: mild dementia (Memorial Sloan Kettering [MSK] 0.5 or 1) and moderate/severe dementia (MSK 2 or 3). The box indicates interquartile range (IQR), the solid line within the box represents the median, and the vertical bars indicate a measure of spread equal to 1.5 times the IQR (upper and lower fences). \*Subjects with moderate/severe dementia have higher CSF sFas levels than those with mild dementia ( $\chi^2_1 = 6.47$ ,  $p = 0.011$ ).

sFasL was bound to Fas (and therefore not measured by our assay) or there was less metalloproteinase cleavage of FasL into sFasL in severe or progressive dementia.

There were limitations to this study. First, the groups were not matched with respect to demographics or CD4 cell count. It is difficult to match subjects by CD4 count because HIV-D inherently affects those in the later stages of AIDS. Second, although elevated CSF sFas in HIV-D suggests sFas involvement in HIV-D pathogenesis, a causal relationship and the precise mechanisms involved remain unknown. Third, the study was not powered to show significant differences in CSF sFas levels among the progressors, improvers, and stable subjects.

Our small study size was due to our use of purposefully stringent exclusion criteria. We eliminated all subjects with conditions associated with elevated sFas or sFasL. This may explain why other studies found sFasL elevation in HIV-D subjects whereas our study did not. sFasL could be a nonspecific marker, reflecting macrophage activation, that is elevated in a variety of conditions affecting patients with late stage AIDS. In addition, we excluded all subjects with HAART exposure.

One can speculate about the role of Fas-associated astrocyte apoptosis in HIV-D. A potential model is as follows: during HIV infection, reactive astrocytes express Fas. When cells expressing FasL (CD4+ cells, monocytes, and macrophages) enter the CNS, FasL binds to Fas on astrocytes and induces cellular dysfunction or death. Because astrocytes serve several neuroprotective functions, their dysfunction results in neuronal injury. This model suggests that Fas signaling is important in the pathogenesis of HIV-D; therefore, this pathway is a potential target in treating HIV-D.

## References

1. Thompson KA, McArthur JC, Wesselingh SL. Correlation between disease progression and astrocyte apoptosis in HIV-associated dementia. *Ann Neurol* 2001;49:745-752.
2. Giulian D, Li J, Bartel S, Broker J, Li X, Kirkpatrick JB. Cell-surface morphology identifies microglia as a distinct class of mononuclear phagocyte. *J Neurosci* 1995;15:7712-7726.
3. Nicholls D, Attwell D. The release and uptake of excitatory amino acids. *Trends Pharmacol Sci* 1990;11:462-468.
4. Wesselingh SL, Power C, Glass J, et al. Intracerebral cytokine messenger RNA expression in acquired immunodeficiency syndrome dementia. *Ann Neurol* 1993;33:576-582.
5. Griffin DE, Wesselingh SL, McArthur JC. Elevated central nervous system prostaglandins in HIV-associated dementia. *Ann Neurol* 1994;35:592-597.
6. Brack-Werner R. Astrocytes. HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS* 1999;13:1-22.
7. Elovaara I, Sabri F, Gray F, Alafuzoff I, Chiodi F. Upregulated expression of Fas and Fas ligand in brain through the spectrum of HIV-1 infection. *Acta Neuropathol (Berl)* 1999;98:355-362.
8. Aquaro S, Panti S, Caroleo MC, et al. Primary macrophages infected by human immunodeficiency virus trigger CD95-mediated apoptosis of uninfected astrocytes. *J Leukoc Biol* 2000;68:429-435.
9. Sporer B, Koedel U, Goebel FD, Pfister HW. Increased levels of soluble Fas receptor and Fas ligand in the cerebrospinal fluid of HIV-infected patients. *AIDS Res Hum Retroviruses* 2000;16:221-226.
10. Sabri F, De Milito A, Pirskanen R, et al. Elevated levels of soluble Fas and Fas ligand in cerebrospinal fluid of patients with AIDS dementia complex. *J Neuroimmunol* 2001;114:197-206.

**CSF soluble Fas correlates with the severity of HIV-associated dementia**  
A. Towfighi, R. L. Skolasky, C. St. Hillaire, K. Conant and J. C. McArthur  
*Neurology* 2004;62;654-656

**This information is current as of December 13, 2007**

**Updated Information  
& Services**

including high-resolution figures, can be found at:  
<http://www.neurology.org/cgi/content/full/62/4/654>

**Subspecialty Collections**

This article, along with others on similar topics, appears in the following collection(s):

**HIV**

<http://www.neurology.org/cgi/collection/hiv> **HIV dementia**  
[http://www.neurology.org/cgi/collection/hiv\\_dementia](http://www.neurology.org/cgi/collection/hiv_dementia)

**Permissions & Licensing**

Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:

<http://www.neurology.org/misc/Permissions.shtml>

**Reprints**

Information about ordering reprints can be found online:

<http://www.neurology.org/misc/reprints.shtml>

