REVIEW

A guide to interpretation of neuroimmunological biomarkers in the combined antiretroviral therapy-era of HIV central nervous system disease

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Abstract: Biomarkers have emerging roles in diagnosis, prognostication, and treatment monitoring in patients with human immunodeficiency virus-related central nervous system disease. Currently, it is unlikely that a single biomarker will be able to fulfill these roles; rather a combination is more likely. In this review, we use a pathogenetic framework to discuss biomarkers derived from both the blood and cerebrospinal fluid. Emphasis is given to those biomarkers that have a more solid evidence base and those that are easily measurable.

Keywords: CSF, biomarker, HAND, HIV, CART

Introduction

The management of human immunodeficiency virus (HIV)-related central nervous system (CNS) disease requires biomarkers to assist diagnosis, prognostication, and assessment of response to treatment. Currently, no single biomarker alone fulfils any one or more of these roles with appropriate sensitivity or specificity. Combinations of markers may be useful in caring for patients with HIV,[1] but much work remains to validate these biomarkers with their clinical correlates.[2] The translation of this work into day-to-day patient care is an ongoing endeavor. A particular challenge is to develop biomarkers that can be used in resource-poor settings, where the majority of disease burden lies. This review aims to outline biomarkers associated with HIV-related CNS disease framing discussion in the context of current understanding of these conditions' neuroimmunological and virological pathogeneses. The clinical phenotype of HIV-associated neurocognitive disorders (HAND) may be changing in treated patients as they age with different risk factors emerging. That acknowledged, this review focuses on the more well-established aspects of HAND.

The biomarkers reviewed are limited to those measured *ex vivo* in bodily fluids; discussion of *in vivo* determined imaging-based biomarkers is beyond the scope of this article.

Definitions and background
HIV-related central nervous system disease

It was realized early in the HIV/AIDS epidemic that the nervous system is peculiarly sensitive to the effects of the virus. However, the majority of published work describing HIV-related neurological disease describes individuals infected with HIV type-1, clade B. Although this viral clade accounts for the majority of HIV cases in resource-rich countries, different HIV-1 clades are prevalent in other parts of the world and in West Africa, HIV type-2 is commonly found.

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HIV can cause CNS dysfunction at the time of acute seroconversion and thereafter, in untreated patients, with increasing frequency mirroring the severity of immunological decline. In the CNS, HIV has a predilection for the deep white matter, basal ganglia, and possibly spinal cord. The neurotropism of the virus results in the end-stage clinical syndromes of dementia and vacuolar myelopathy, respectively. However, it is recognized that a spectrum of neurocognitive syndromes exist of which HIV dementia is the most severe. Abnormalities less severe than dementia are defined either as asymptomatic neurocognitive impairment or mild neurocognitive disorder. With HIV dementia, these syndromes have recently been recategorized under the umbrella term HAND.[3]

The advent of combined antiretroviral therapy (CART) has profoundly affected the incidence and severity of HAND; its impact is pertinent to the study of biomarkers. Prior to CART, the principle diagnostic aim was to distinguish between potentially treatable CNS diseases caused by opportunistic infection from that resulting directly from the virus. Often conditions overlapped resulting in "layering" of pathologies.[4] Since CART it is apparent that although HAND incidence has fallen prevalence has increased, there are fewer new cases of HIV dementia.[5] These epidemiological changes are due to increased longevity of individuals who pre-CART were cognitively impaired; in addition, new cases of milder HAND are recognized in individuals receiving CART who appear to have adequate systemic virological control. It is critically important that studies seeking HAND biomarkers distinguish between individuals with static, albeit severe, impairment from those with active and progressing HAND. Furthermore, since CART, confounds to HAND have changed. In resource-rich countries, the increasing longevity of the HIV-infected cohort has resulted in the increased prominence of factors including hepatitis C coinfection, aging, and the adverse side effects of CART, such as metabolic derangements.[6–8] In addition, it is increasingly recognized that CART regimens containing drugs that achieve therapeutic levels in cerebrospinal fluid (CSF) are associated with better cognitive outcomes.[9] Not all CART regimens have equivalent CNS penetration. This may explain some of the variation in the influence of CART on CSF biomarkers and cognitive outcomes.

The pathogenesis of HIV-related CNS disease is incompletely understood. HIV is thought to enter the brain in infected monocytes soon after infection. Pathologically, activated macrophages and astrocytes, sometimes with multinucleated giant cells, are seen in brain parenchyma, which is named as HIV encephalitis (HIVE). Progression of disease is marked by blood–brain barrier breakdown, rarefaction of white matter, astrocyte apoptosis, dendritic simplification, and neuronal loss. Productive infection occurs in perivascular macrophages and microglia, but restricted nonproductive infection can occur in astrocytes.[10] Potent toxic products arise from viral and cellular gene products. These include the viral proteins as well as proinflammatory cytokines.

Biomarkers

A biomarker is a "characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[11] Biomarkers can be released intermittently or at a constant rate. The latter are "bulk" markers, the value being directly proportional to affected tissue volume or extent of disease process. Repeat measurement of intermittent markers allows calculation of the "area under the curve" that can strengthen associations; the integral providing better correlation with the comparator.[12] However, frequent sampling of bodily fluids such as CSF is not practical.

In the case of HIV-related CNS disease, biomarkers are required to indicate presymptomatic involvement of the CNS, to monitor disease-activity as well as response to treatment, and to assist prognostication. Biomarkers can be categorized according to basic principles of disease evolution; namely inflammation, destruction, and repair processes. To document clinically appreciable changes consequent of HIV-related brain disease can require prolonged periods of observation. Therefore, a key additional aim is to identify biomarkers that correlate well with long-term outcome that could be used as surrogate end points in clinical trials.

Biomarkers and compartmentalization

The study of both HIV-related CNS disease and CNS biomarkers requires appreciation of the barriers between the circulation and the CNS, and the CNS and the intrathecal space. For example, in HIV-infected individuals virus may remain active in the CNS when controlled by medication systemically. Although CSF is considered by many to be a "liquid brain biopsy," in reality it is only a surrogate of the intercellular space of brain parenchyma; however, sampling CSF offers a unique and repeatable opportunity to study CNS metabolism and pathology.[12] Six barriers pertain to the CSF-containing intrathecal space of which the blood–CSF barrier is the most significant.[13] Passage of a biomarker across the barrier is affected by the degree of disruption to the barrier and can vary according to the nature of the biomarker, depending on its physicochemical properties as well as its biological features. For example, once activated

T-lymphocytes can cross the blood–brain barrier. Molecules produced within the CNS that are undetectable, or present in very low concentration in blood, can be measured in CSF alone and CNS origin assumed. However, many molecules, such as immunoglobulin G (IgG), are found in much greater quantities in blood than in CSF despite intrathecal production occurring in disease states. For these molecules calculation of a CSF–blood index, using comparative measurements of a similar sized non-CNS produced molecule, such as albumin, is required to correct for passive transfer across the blood–CSF barrier.

Furthermore, there are anatomical considerations to the distribution of molecules within the intrathecal space: ventricular CSF has lower protein levels than lumbar CSF. Disease processes that have prominent involvement of the meninges typically result in more marked lumbar CSF changes than processes predominantly affecting brain parenchyma alone.

HIV and blood biomarkers

Biomarkers of systemic infection activity in HIV, used clinically for monitoring the disease in resource-rich settings, are CD4 T-lymphocyte count and viral load (HIV-RNA). Viral subtypes and drug resistance profiles can assist in medication selection and prognostication. Blood while easier to obtain than CSF is dissociated from the CNS; CD4 count and viral load can only be an approximate guide to probability of HIV-related CNS disease. Although systemic suppression of viral replication can be achieved, this does not necessarily imply CART efficacy within the CNS. In the post-CART era, HAND is increasingly identified in patients with high CD4 counts and undetectable blood viral loads.[3] This reiterates that CNS compartmentalization necessitates the ability to monitor CNS HIV disease separately to biomarkers of systemic viral replication and immune function.

CNS biomarkers

In this review, individual biomarkers are discussed in accordance to their pathogenic function in HIV-related CNS disease, a format originally used by Brew and Letendre.[14] It divides discussion into biomarkers associated with effector cells of disease and their products, viral and host toxins, and markers of neuronal cell damage. Markers to exclude alternative diagnoses and confounds are also described. It is important to note that all of these biomarkers are affected by opportunistic infections. Many of the biomarkers discussed have little data in the post-CART era. In this context, it is important to categorize patients as to whether they are stable on CART vs drug naive or failing treatment, when interpreting biomarker results. A summary of the biomarkers discussed and their utility with CART, where known, is presented in Table 1. Confounds are discussed in detail in the body of the text.

Effector cells

Lymphocytes

CD4 cell count

CD4 cell count below 200/μL at least in the pre-CART era was a useful indicator for the risk of HAND, especially dementia, paralleling the increased vulnerability to opportunistic infections.[15] It is likely that the increased risk of HIV dementia with fall in CD4 count resulted from worsening of immune control leading to increased viral escape and replication.

As discussed earlier, in CART-treated patients the pattern of disease has changed. HAND is now described in patients with normal or near normal CD4 counts and undetectable viral load.[8] There is a growing evidence base that the nadir, rather than current CD4 count is of greater significance in this group.[5,16]

Beta-2-microglobulin (β2M)

β2M is predominantly a marker of cytotoxic T-lymphocytes in the CNS. It is the invariant light chain of the major histocompatibility class I (MHC-I) molecule. MHC-I is constitutively expressed on all nucleated cells except neurons. It is particularly highly expressed on the surface of lymphocytes. Nonspecifically raised levels of β2M have been described in inflammatory and lymphoproliferative conditions.[17] CSF β2M concentrations correlate with the risk of HAND in patients with advanced HIV.[18] The generalizability of β2M elevation in neurological involvement of HIV-1 Clade C has recently been described.[19] However, it is usually not useful in CART-treated patients.

Monocytes

CD14+/CD69+ monocytes

CD14+/CD69+ monocytes particularly the CD14lo/CD69hi subset in peripheral blood appear to be important in HAND pathogenesis, although they may be nonspecifically elevated in a range of infections.[20] The prognostic significance in an asymptomatic population is unclear although CART reduced this subset in 1 study.[21] Recently, levels of HIV-DNA within monocytes measured prior to CART have been demonstrated to correlate with baseline cognitive function as well

Table 1 Summary of biomarkers and their utility in CART-treated patients

Biomarker	Effect of HIV infection	In HAND[a]	Useful in patient on CART[b]
Cerebral spinal fluid			
β2 microglobulin	Increased	Increased	May indicate treatment failure if high
Soluble CD14s	Increased	Increased	Yes
Neopterin	Increased	Increased	May indicate treatment failure if there is further elevation
Quinolinic acid	Increased	Increased	If remains elevated
s100	Increased	Increased	Yes
Interleukins	Increased	IL-1, IL-6, IL10	Unknown
TNF superfamily proteins	Increased	Increased	Yes
Interferons	Increased	Increased	May indicate treatment failure if high
Monocyte chemoattractant protein-1 (MCP-1)/CCL2	Increased	Increased	Yes and may indicate treatment failure if high
Fractalkine	May be increased	Increased	Unknown
TGFβ[c]	Variable	Variable	Unknown
uPAR[d]	Increased	Increased	May indicate treatment failure if high
HIV RNA	May be increased	Variable	Yes
Arachidonic acid metabolites	Increased	Increased	Unknown
Nitric oxide	Increased	Variable	Yes
Platelet activating factor	May be increased	Increased	No
Neurofilament light chain		Increased	May indicate treatment failure if high
Amyloid β1-42		Decreased	Yes
Tau		Increased	Unknown
Sphyngolipids		Increased	Unknown
Protein	Increased	Inconsistent	Unknown
ICAM-1[e]		Increased	Unknown
MMP[f]		Increased	Unknown
Blood			
CD4 count	Important especially if <200	Low count increases risk	Nadir value seems more useful
CD14+/CD69+ monocytes	Increased	Increased	Maybe
HIV DNA	Increased	Increased	Yes
VEGF[g]	Increased	Increased	Yes

Abbreviation: TNF, tumor necrosis factor.

[a]HIV-associated neurocognitive disorder; [b]Combined antiretroviral therapy; [c]Transforming growth factor β; [d]Urokinase plasminogen activator receptor; [e]Intercellular adhesion molecule; [f]Matrix metalloproteinases; [g]Vascular endothelial growth factor.

as that measured after 48 weeks of antiretroviral therapy.[22] Furthermore, monocyte HIV-DNA load of 3.5 $\log_{10}$ or more predicted all cases of HIV dementia. However, these findings await validation in larger, non-Thai, cohorts.

Soluble CD14s (sCD14)

CD14 exists in both soluble and membrane forms, predominantly on human monocytes.[23] It is a coreceptor for lipopolysaccharide and modulates the cell's response to infection. It is released by stimulated monocytes *in vitro*. Elevations in serum concentration have been associated with disease progression in patients with HIV.[24,25] Ryan et al[26] described higher plasma concentrations in cognitively impaired patients on CART compared with cognitively unimpaired patients. In distinction to other markers of macrophage activation in the CNS, sCD14 may derive primarily from trafficking monocytes and perivascular macrophages, rather than from native microglia.[27] Thus, sCD14 levels may indicate differences between individuals of CNS infiltration by immune cells.

If levels of sCD14 correlate with those of CD14+/CD69+ cells, they may prove easier for monitoring as sCD14 can be tested using a simple enzyme-linked immunosorbent assay. Soluble CD14 levels can decrease with CART but, whereas raised levels are nonspecific, such elevated levels may identify patients at risk of subsequent neurological injury.[21,28]

Neopterin

Neopterin serves as a marker for monocytes, macrophages, microglia, and to a lesser extent, astrocytes. It is a product of guanosine triphosphate metabolism that is produced mainly by monocytes, macrophages, microglia, and astrocytes.[29,30] High concentrations have been found in the CSF of patients with both opportunistic CNS infections and HIV dementia. In addition, levels decrease with antiretroviral treatment.[29] This finding has been replicated in HIV-1 clade C.[19] Elevated CSF neopterin correlates with HIV dementia severity.[29] In patients with advanced HIV disease, elevated levels are associated with increased risk of dementia.[31] Furthermore, in 1 study

after 2 years of viral suppression only 55% of patients had normal CSF neopterin.[32] The implications of this finding to the risk of later development of HAND are not known.

Quinolinic acid (QUIN)

QUIN is a product of the kynurenine pathway for tryptophan metabolism.[33] It is produced by monocytes in response to interferons, especially interferon γ and HIV proteins among other agents. Notably, QUIN is not only a marker of monocyte activation but also a toxin being an excitotoxic agonist at *N*-methyl D-aspartate (NMDA) receptors. It can also cause cell death through lipid peroxidation and the generation of free radicals.[34] QUIN is at present only measurable using gas chromatography/mass spectroscopy, which severely limits its clinical utility.

Increased CSF QUIN may be seen in opportunistic infections and HIV dementia; in the latter concentrations correlate with dementia severity.[33] One small study showed that elevated CSF concentrations increased the risk of dementia.[35] QUIN cannot cross an intact blood–brain barrier making CSF levels a more specific marker of disease activity within the brain. Thus, raised CSF levels usually indicate an intrathecal process and have been shown to respond rapidly to antiretroviral treatment.[33,36]

Microglia

CSF markers for microglia are currently inferred from those previously discussed with respect to monocytes. There are no specific markers for microglia at present. In pathological studies, the degree of microglial activation is the best correlate for the presence and severity of HAND.[37] Early neuropathological studies used ferritin as a marker for activated microglia but CD68 is now more usually used as a marker.[38] Ferritin is readily measurable in CSF and it is used clinically as a biochemical marker of recent subarachnoid hemorrhage. CSF ferritin levels have been studied in HIV neurological disease but where elevated levels were found these did not show specificity to HIV-mediated CNS disease, as opposed to opportunistic infections.[39] Development of a specific CSF marker of microglial activation would be of significant clinical benefit.

Astrocytes

S-100

S-100 is an acidic calcium binding protein, which exists in dimers containing α and/or β subunits. S100β, which contains two β subunits, is found almost exclusively in astrocytes and elevation in levels are associated with astrocytosis.[40]

CSF S100β levels are elevated in many inflammatory, degenerative, and traumatic CNS conditions. Raised S100β is associated with increased severity of HAND and more rapid progression to dementia.[40] Pathogenically, high levels may lead to neuronal apoptosis.[41] Recently, Woods et al[42] associated elevations in CSF S100β with decreased verb word generation counts and executive functioning independently of current CD4 count, HIV dementia score and CART use. CSF S100β did not correlate with deficits of psychomotor speed or semantic memory.

Glial fibrillary acid protein

Glial fibrillary acid protein is an astrocyte structural protein, the exact function of which is unclear. Although levels have been described to change in neurodegeneration, it does not appear to correlate with HIV-associated CNS disease.

Modulators

Immune cells are the primary targets of HIV. Thus biomarkers concerned with the activation or suppression of the immune system are a rational focus of research. Many immune functions and cellular interactions of the immune system are modulated by cytokines. This diverse group of signaling peptides and glycoproteins is produced by and acts on specific cells, eliciting characteristic effects on growth, mobility, differentiation and function of target cells. These in turn regulate immune and inflammatory responses as well as hematopoiesis, angiogenesis, and healing.[43]

Interleukins

The most studied interleukins are concerned with regulation of inflammation and activation of the immune response and are produced by cells important to the neuropathogenesis of HIV. There are 2 types of helper T-lymphocytes and each type produces a particular group of interleukins with distinct targets. Those produced by T helper 1 (Th1) cells (eg, interleukin-2 [IL-2]) generally activate macrophages, whereas Th2 cell derived (eg, IL-6 and IL-10) factors generally activate B-lymphocytes. Others, such as IL-1, are produced by macrophages as well as other antigen presenting cells and promote inflammation and lymphocyte homing.

There is an ever-increasing number of interleukins but most studies in HAND (the majority predating CART) have focused on 3 interleukins: IL-1, IL-2, and IL-6. Among 6 studies that measured IL-1 in CSF, 4 identified a relationship with HAND, either in adults[44,45] or in children.[46,47] Most of the 9 studies that measured IL-6 in CSF, also identified associations with brain injury, in either adults[44,48–50] or children.[46,47]

In contrast, none of the studies of IL-2 identified associations with neurological disease. In fact, only 3 studies even compared levels of IL-2 or its soluble receptor in paired CSF and blood samples, to a measure of brain injury.[44,51,52] Of the interleukins measured in other CSF studies,[53–55] only IL-10 elevation in CSF in comparison to paired serum samples was associated with HAND by 1 of the 2 largest studies in this series.[56]

IL-1, IL-6, and IL-10 are mainly produced by antigen presenting cells such as macrophages in contrast to IL-2 produced by Th1 cells. This is consistent with the importance of macrophages but not Th1 cells in the neuropathogenesis of HIV. Th2 lymphocytes do produce IL-6 and IL-10 and so may also be involved in neuropathogenesis.

Tumor necrosis factor (TNF) superfamily proteins

The TNF family of molecules is involved in immune regulation and inflammation.[57,58] Receptors for TNF and soluble molecules such as Fas and CD30 are considered closely related proteins.[59–62] The prototype of this group is TNF-α which is produced by macrophages and microglia and is central to several pathogenic processes.[63] In HIV disease, it can upregulate HIV replication and TNF-α mRNA levels are elevated in the brain tissue of HAND patients.[64–66]

Most studies that have identified TNF-α in CSF have also identified markers of brain tissue injury, HIV-RNA in CSF, and focal CNS damage.[45,49,64,67–72] Positive studies that have reported on TNF superfamily proteins in CSF to date have identified links with HAND. The endogenous regulation of TNF-α makes it difficult to measure in body fluids. Strong evidence exists that TNF-α,[69] sTNFRs,[73] and sFas[74] can be detected in body fluids despite antiretroviral therapy, supporting that these proteins might be useful biomarkers of ongoing neuroinflammation in treated individuals.

Interferons and the molecules they induce

Interferons are cytokines and are grouped by their immunomodulation functions and target receptors. They are classified into type-1 (IFN α, β, ω, and κ) and type-2 (IFN γ). In the brain, astrocytes and microglia produce mainly IFNα. Endogenous IFNα may help protect the brain from viral infections but exposure to high concentrations or for prolonged periods may damage the brain. Transgenic mice that overproduce IFNα in astrocytes have a high incidence of severe neuropathology, with intractable seizures and early death.[75] The expression of IFNα is elevated in HIVE and correlates with the severity of ante mortem cognitive impairment. Recently, in a mouse model of HIVE, the use of IFNα neutralizing antibodies led to a decrease in cognitive deficits but its potential role in humans remains to be investigated.[76]

All 3 studies of IFNα in CSF have shown an association between higher levels and increased risk of HAND.[48,77,78] Two of these also linked elevated IFNα and higher HIV-RNA levels in CSF[48,77] consistent with ineffective antiviral activity. Three of 4 studies of CSF IFNγ demonstrated higher levels in HIV infection independent of cognition,[51,67,79] the fourth was not able to identify IFNγ in CSF.[44] Only Nolting et al[67] demonstrated increased IFNγ (with IL-1α, IL-15, and TNF-α) in HAND patients compared with HIV-infected nondemented controls; although notably all the other studies were published prior to the advent of CART.

IP-10, an interferon-inducible protein has been reported in 5 CSF studies. Three have compared IP-10 levels with HIV-RNA levels in CSF and found statistically significant correlations.[73,80,81] Gisolf et al[73] identified that IP-10 was elevated in some patients in spite of apparent HIV-RNA control, similar to their findings with sTNFR-II, whereas Probasco et al[81] have found IP-10 is not significantly elevated in HIV controllers or those with suppressed viral load on treatment. Two studies compared IP-10 and brain injury, identifying both links between higher levels and adverse neurological outcomes.[53,82]

Chemokines

Monocyte chemoattractant protein-1 (MCP-1)

MCP-1, which is also known as CCL2, is a chemokine associated with chemotaxis of monocytes across the blood–brain barrier;[83] HIV and QUIN induce its expression by astrocytes.[84,85] Given the critical role that monocytes are thought to play in HAND pathogenesis, factors promoting monocyte CNS ingress are potentially useful biomarkers. Human studies have shown upregulated MCP-1 expression on macrophages in patients dying with HIVE.[86] Eight of 9 studies have shown a link between higher MCP-1 levels in CSF and worse outcomes.[55,69,84,87–91] Importantly, it has been particularly studied in the post-CART era. A recent study has demonstrated that "elite controllers," who are HIV-infected individuals who have viral loads <50 copies/mL without CART, have CSF MCP-1 levels similar to CART-treated and noninfected individuals.[81]

Other CC chemokines

A smaller number of studies have compared neurological outcomes and levels of other CC chemokines: MIP-1α,

MIP-1β, and RANTES. These chemokines bind to CCR5, the receptor commonly used for HIV entry into microglia and lymphocytes.[92] They are implicated in HIV neuropathogenesis through their identification in HIVE brain tissue and tissue from a simian HAND model.[93–96] Their elevation has not been consistently demonstrated in published CSF studies[55,97] and particularly in treated individuals it has been difficult to detect these chemokines.[53]

Fractalkine

Fractalkine is a chemokine that binds to CX3CR1 appears important in reducing neurotoxicity associated with activated microglia.[98] Two studies have demonstrated nonspecific elevations in CSF fractalkine in HIV-positive patients with neurological sequelae including dementia.[99,100] This is not immediately reconciled with *in vitro* data suggesting fractalkine is neuroprotective. Elevated levels may suggest a host attempt at neuroprotection. The Magnetic Resonance Spectroscopy consortium group demonstrated that lower CSF fractalkine levels were associated with lower neuronal pattern scores on proton magnetic resonance spectroscopy.[101]

Transforming growth factor beta (TGFβ)

TGFβ contributes to down regulation of T-lymphocyte and macrophage activation, modulation of proinflammatory cytokines, and protection against neuronal excitotoxicity secondary to HIV infection.[102] In HIV CNS disease, TGFβ is produced by CD8 lymphocytes, astrocytes, and microglia. CSF TGFβ concentrations are elevated in mild HAND but undetectable in more severe disease.[48,103] The influence of CART and the prognostic significance of TGFβ are not known.

Urokinase plasminogen activator receptor (uPAR)

Urokinase plasminogen activator receptor (uPAR) and its ligand uPA have an important role in cell migration and extracellular proteolysis. It is elevated in brain tissue in all opportunistic infections in HIV-positive patients, except cryptococcosis, and it is also elevated in HAND.[104] Raised CSF soluble uPAR levels are seen in HAND and decline significantly with CART.[105]

Toxins
Viral
HIV-RNA viral load

HIV-RNA viral load is a marker of active viral replication; however, plasma HIV-RNA levels have limited utility as a biomarker for HAND. CSF HIV-RNA is also nonspecific with levels raised in asymptomatic patients as well as those with opportunistic infections and HAND.[106–108] In CART-naive patients with HAND, the severity of disease is correlated with CSF HIV-RNA levels[106,109] and falls with antiretroviral treatment.[110] In those with HAND receiving CART, CSF viral load does not predict progression.[69] Whereas, in cognitively asymptomatic subjects on CART, an elevated CSF viral load (>200 copies/mL) is reported to predict progression of cognitive impairment at a median of 1 year.[111] The risk of HAND in "elite" viral controllers is not known although in a recent study of such patients no evidence of intrathecal inflammation was found on CSF examination.[81]

Although uncommon, in CART-treated patients HAND can still develop despite an undetectable HIV-RNA level in the CSF.[69,112,113] There are many proposed mechanisms for this. First, that this indicates HAND that has not fully responded to CART with residual permanently damaged brain tissue, so-called inactive HAND.[112] Second, that clinically apparent neurological deficit is driven by a coexistent illness, such as Hepatitis C, rather than HIV.[114,115] Third, that HIV initiates the process, which then becomes autonomous of the virus.[69] Finally, some individuals develop an immune reconstitution disorder after initiation of CART, leading to clinical decline in neurological function, as a consequence of increased CNS immune activation.[116]

HIV-DNA

HIV-DNA results from reverse transcription of viral RNA and can be integrated into chromosomal DNA. Plasma levels reflect latent infection and appear to correlate with HAND, being elevated in CART-treated and untreated patients with clinical HAND.[117,118] Data regarding CSF HIV-DNA have not been reported. This may be due to technical difficulties in measurement due to low cell counts in CSF. However, as discussed earlier, HIV-DNA measured in peripheral monocytes appears more strongly associated with HAND and might be a useful biomarker.

HIV proteins

HIV proteins that are encoded on the virus's genome include gp120, Nef, Tat, gp41, and VPR. *In vitro* toxicity to neural cells has been demonstrated; often through the proteins' actions on cell signaling pathways, or through generation of other toxic factors.[119] HIV proteins have proved difficult to measure in CSF, consequently limiting their use as biomarkers.

Host toxins

Host toxins include arachidonic acid metabolites, nitric oxide, and platelet activating factor (PAF) as well as

previously discussed neurotoxins: QUIN, S100β, interferons, interleukins, and TNF-α.

Arachidonic acid metabolites

Arachidonic acid metabolites, which include prostaglandins, are the product of cyclooxygenase metabolism of lipids rich in arachidonic acid. Levels were significantly correlated with the presence and severity of HAND, as well as with β2M and neopterin, in studies before the introduction of CART. The effect of antiretroviral drugs and the significance of elevated concentrations with regards to direct disease causation is not known.[120]

Nitric oxide

Nitric oxide is considered to be an important neurotoxin in HAND, where it is predominantly produced by macrophages and microglia. CSF concentrations of nitric oxide metabolites are raised in opportunistic CNS infections associated with HIV,[121] which may result from damage to the blood–brain barrier.[122] Pre-CART, no increase in CSF levels of nitric oxide and its metabolites were noted in HAND.[123] However, a more contemporary study has demonstrated increased CSF nitric oxide and 3-nitrotyrosine modified proteins among those who subsequently develop cognitive decline or have used intravenous illicit drugs.[124] In HAND brain tissue, there is evidence of increased activity of nitric oxide's associated enzyme. The discrepancy between these 2 studies could be due to differences between the cohorts in proportion of active neurodegeneration cases or frequency of intravenous drug use.

Platelet activating factor

PAF is produced by infected or activated monocytes. Although it has many actions in HIV-associated diseases there is convincing evidence that its neurotoxicity is at least in part mediated by NMDA receptor activation.[125–128] PAF levels are nonspecifically elevated in HAND. *In vitro* tests suggest that CART regimens may have anti-PAF effects[129] and a PAF antagonist has shown promising results in a mouse model of HIVE.[130] However, a case-controlled study of HIV-positive patients starting CART showed no significant change in CSF PAF levels over 4 months of treatment.[131]

Target cell
Neuron
Neurofilament light chain (NFL)

NFL is an essential part of the core of the triplet protein neurofilament. Neurofilament is a major structural protein of neurons and is found mostly in large myelinated neurons.[132] Its main function is to maintain axonal caliber. NFL is significantly raised in HAND and levels rise with CART interruption.[133,134] In a longitudinal study, a group of patients who subsequently developed HAND had raised NFL levels on CSF taken up 2 years prior in comparison to CD4-matched controls.[135] This suggests elevated NFL may be a preclinical indicator of risk of HAND in the next 2 years. NFL levels decrease in the majority of patients treated with CART and this is paralleled by a clinical improvement in severe HAND.[136] Neurofilament heavy chain, which *in vitro* is more stable than NFL, is elevated in a variety of peripheral and CNS pathologies characterized by neuronal injury including multiple sclerosis and the Guillain-Barré syndrome.[137–139] Thus far measurement of CSF neurofilament heavy chain as a biomarker for HAND has not been reported.

Amyloid and Tau amyloid precursor proteins

Amyloid precursor protein are ubiquitously expressed transmembrane proteins with pathogenic significance. When cleaved by α-secretase a soluble form sAPPα is made. Soluble APPα does not form part of the amyloid generation pathway. By contrast if cleaved by β-secretase the soluble form sAPPβ is made which can be further metabolized to Aβ peptides including $A\beta_{1-42}$ which are the major constituents of amyloid plaques.[140] Tau is a structural neuronal protein. There are 2 dominant forms that can be measured: total tau (t-tau) and phosphorylated tau (p-tau). Both nonspecifically represent neuronal damage, though p-tau is more often elevated in patients with Alzheimer disease (AD).[141]

Aging populations are associated with an increased incidence of dementia. Thus as the HIV-infected cohort ages, it is increasingly important to distinguish between HAND and AD. Aβ42 is lower in HAND patients in comparison to both HIV-positive nondementia patients and HIV-negative patients with dementia.[142,143] In the Brew et al[142] cohort, not on CART, p-tau was increased in HIV dementia in comparison to both AD patients and normal controls. By contrast, in the Clifford et al[143] cohort, on CART, t-tau and p-tau were increased in the AD patients but were decreased in the HIV positive – whether with normal or impaired cognition.[143] The reason for the differences between the 2 studies p-tau levels is unclear although it may suggest that treatment with CART decreases t-tau and p-tau, and this is perhaps an indicator of the role of CART with respect to HAND prevention. A recent retrospective study showed decreased sAPP α and β in patients with both HAND and opportunistic CNS infection distinguishing them from HIV-positive asymptomatic controls and AD

patients.[140] The relationship between NFL and tau elevation is not clear although they are both markers of damage to neurons with NFL predominantly indicating damage to large myelinated axons.

Sphingomyelin and ceramide

Sphingomyelin and ceramide are intracellular membrane sphingolipids that are particularly high in concentration in myelin. However, sphingolipids originating from peripheral myelinated axons in nerve roots released into CSF can confound assessment of CNS myelin release. Their presence in the CSF is evidence of cell destruction. In a study of 48 patients, elevated levels were found to correlate with cognitive decline and clinically stable dementia.[144] The same group found increased levels of vitamin E and triglyceride C52 in HAND patients with active cognitive decline. These antioxidants are lowered in AD and aging and are thought to be neuroprotective. This may suggest an attempt by the host to oppose the neurodegeneration of HAND. Elevated sphingolipid was associated with inactive HIV dementia, whereas elevated ceramide with active disease.

Endothelial cells/blood–brain barrier
CSF proteins and their indices

Albumin and IgG are large proteins found in low concentration in the CSF of healthy individuals with an intact blood–CSF barrier. Elevated levels of CSF albumin, and to a lesser extent CSF protein, indicate impairment of the blood–CSF barrier. IgG can be elevated in CSF because of blood–CSF barrier impairment or secondary to intrathecal synthesis. Intrathecal synthesis of IgG is a common finding in HIV patients with or without neurological disease or opportunistic infections.[145] The albumin ratio, a quantitative measure of blood–CSF barrier impairment, is reported in HIV to be elevated; however, links with HIV neurological disease have been inconsistent.[131,146–148] Pre-CART studies typically demonstrated an abnormal albumin ratio, which was associated with decline in CD4 count. Abdulle et al[145] studied the change in albumin ratio and IgG index in neurologically asymptomatic individuals starting CART (median CD4 count 185 cells/μL) finding no significant difference in either parameter after 2 years therapy. In this series, although 56% had abnormal IgG index prior to CART, only 5% had an abnormal albumin ratio. Therefore, the lack of significant change in albumin ratio with treatment is more likely a function of earlier initiation of therapy resulting in fewer patients at entry having demonstrable blood–CSF barrier dysfunction than lack of response to therapy. One study found strong associations between CSF total protein levels and cognitive improvements after changes in antiretroviral medications.[74] However, until there are further corroborating data, CSF total protein cannot be considered a reliable marker of HAND, as HAND may occur seemingly with an intact blood–CSF barrier.

Vascular endothelial growth factor (VEGF)

VEGF is a potent angiogenic and mitogenic peptide. There is 1 study of serum and CSF VEGF that showed elevations in only serum levels in patients with HAND. Even after viral suppression with CART the VEGF levels, decreased but were increased higher than those seen in patients without HAND.[149] This may suggest a role as a serial marker to monitor for improvement post-CART introduction.

Intercellular adhesion molecules (IACM)

HIV gp120 and proinflammatory cytokines can upregulate the expression of adhesion molecules, including ICAM-1 on the luminal surface of brain microvascular endothelial cells.[150] The soluble form sICAM-1 has been studied in CSF. The levels of HIV-positive subjects is higher than noninfected controls without neuroinflammatory disease[151] but lower than subjects with other causes of meningeal inflammation.[152]

Matrix metalloproteinases (MMPs)

MMPs are a family of neutral proteases that are important to normal development and have been implicated in many pathological processes. In the CNS, they can degrade components of the basal lamina leading to blood–brain barrier disruption.[153] MMP-9 has been shown to be elevated in HIV-positive subjects with neurological deficits or CNS opportunistic infections.[154–156]

Proteomics

Recent combined mass spectrometry and 2D Western blot analysis of the CSF protein content in patients with and without cognitive impairment has sought to define a "protein fingerprint" of HIV-associated brain disease.[157] Laspuir[158] identified several candidate biomarkers that are linked to immune signaling and macrophage function in the CNS in their Puerto Rican cohort of predominantly Hispanic women with HAND. Validation in large cohorts remains essential especially as each study has identified different combinations of proteins, although cystatin appeared in both groups' works. Profiles may differ depending on stage and activity of HAND and duration on CART.

Biomarkers to exclude other disease

Several simple blood biomarkers are important in the exclusion of mimics of HAND.

Vitamin B12, red cell folate, and thyroid function

Vitamin B12, red cell folate, and thyroid function are commonly assayed in patients with dementia. They are appropriate to check in HAND patients as B12 and folate deficiency can mimic dementia as well as being associated with myelopathy and neuropathy. Hypothyroidism can on occasions resemble HAND, especially with respect to psychomotor slowing.

CSF leucocyte count

CSF leucocyte count can be useful to indicate causes other than HAND. If the CSF leucocyte count is above 50 cells/μL HIV alone is unlikely, especially when the CD4 count is below 200 cells/μL and additional conditions such as cryptococcal meningitis should be considered. The differential can be useful in guiding further investigation. For example, a polymorphonuclear pleocytosis raises the possibility of cytomegalovirus encephalitis.

Conclusion

As discussed, the number of biomarkers that show promise in indicating aspects of HIV-related neuroinflammation and treatment response is large. However, at present no one marker can be used to predict the presence of HAND or prognosis following treatment. Some markers have greater utility in treatment monitoring, whereas others may assist in prognostication. It has been proposed that a combination of markers be used in ongoing trials.[1,2] This approach would combine indicators of neuronal damage (eg, NFL), HIV activity (eg, HIV-RNA), and neuroinflammation (eg, MCP-1) to create a combined picture of ongoing CNS activity and disease risk.

What biomarkers can the clinician usefully use at present to guide management? This depends upon whether the patient is receiving CART at the time of assessment. In patients who are CART-naive, CD4 count ($>$200 cells/μL), and undetectable CSF viral load ($<$50 copies/μL) are useful as they would be unusual for HAND. Among CART-treated patients, nadir CD4 count is a more useful biomarker. CSF protein is elevated in HIV dementia, which may aid distinction from other neurodegenerative diseases but does not aid exclusion of opportunistic infections.

As the HIV-positive population ages the distinction between HAND and other dementias will become increasingly important and challenging. The amyloid and tau family of proteins may be particularly useful in this group. Markers of repair and inactive disease are also needed, particularly to aid identification of individuals who might benefit from "neuro-intensification" of their CART regimen.

The ideal sole HAND biomarker to replace the combinatorial approach required at present depending on clinical circumstance, should be sensitive (identifying individuals in an asymptomatic state) but have disease specificity, be a "bulk" marker, be present in significant concentration in blood (or be measurable noninvasively), show a response to treatment, and be easily and cost-effectively measured. The search for such a biomarker continues.

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Disclosure

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