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HIV/neuroAIDS biomarkers

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Abstract

HIV infection often causes neurological symptoms including cognitive and motor dysfunction, which have been collectively termed HIV/neuroAIDS. Neuropsychological assessment and clinical symptoms have been the primary diagnostic criteria for HIV/neuroAIDS, even for the mild cognitive and motor disorder, the most prevalent form of HIV/neuroAIDS in the era of combination antiretroviral therapy. Those performance-based assessments and symptoms are generally descriptive and do not have the sensitivity and specificity to monitor the diagnosis, progression, and treatment response of the disease when compared to objective and quantitative laboratory-based biological markers, or biomarkers. In addition, effects of demographics and comorbidities such as substance abuse, psychiatric disease, nutritional deficiencies, and co-infection on HIV/neuroAIDS could be more readily determined using biomarkers than using neuropsychological assessment and clinical symptoms. Thus, there have been great efforts in identification of HIV/neuroAIDS biomarkers over the past two decades. The need for reliable biomarkers of HIV/neuroAIDS is expected to increase as the HIV-infected population ages and their vulnerability to neurodegenerative diseases, particularly Alzheimer's disease increases. Currently, three classes of HIV/neuroAIDS biomarkers are being pursued to establish objective laboratory-based definitions of HIV-associated neurologic injury: cerebrospinal fluid biomarkers, blood biomarkers, and neuroimaging biomarkers. In this review, we will focus on the current knowledge in the field of HIV/neuroAIDS biomarker discovery.

Graphical abstract

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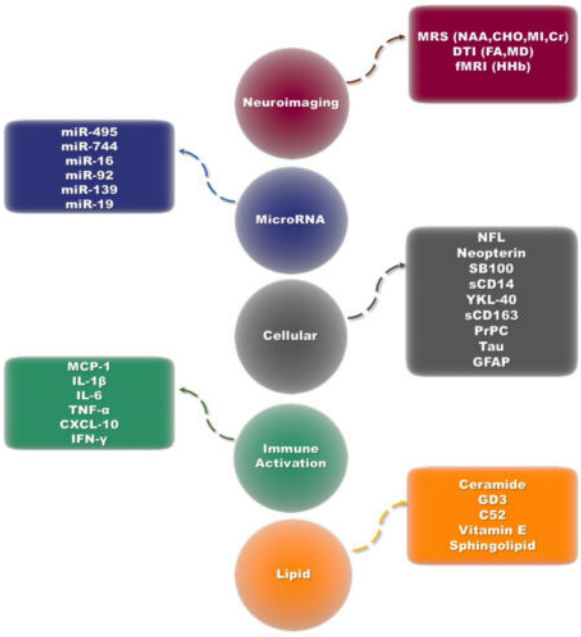

Keywords

HIV/neuroAIDS; Biomarkers; CSF; Blood; Neuroimaging

1. Introduction

Thirty-three million people are estimated to be currently living with HIV worldwide, with over 2.7 million newly infected individuals annually. Within the United States, over one million adults and adolescents may be living with diagnosed or undiagnosed HIV infection, with an estimated 56,000 new HIV infections occurring annually (Hall *et al.*, 2008; UNAIDS, 2015). The introduction of combination antiretroviral therapy (cART) in the mid-1990's led to reduced viral replication, improved immune function and increased life expectancy among HIV-infected individuals (Egger *et al.*, 2002; May *et al.*, 2007; May *et al.*, 2006; Munoz *et al.*, 1997; Pedersen *et al.*, 2015).

HIV-1 infects the central nervous system (CNS) and often causes neurological symptoms that include motor and cognitive dysfunction (Etherton *et al.*, 2015; Price *et al.*, 1988), which are collectively called HIV/neuroAIDS. In the era of cART, a more discrete form of CNS dysfunction so-called minor cognitive motor disorder (MCMD) has become more common (Brew, 2009; Cohen and Gongvatana, 2009; Sacktor, 2002; Simoes and Justino, 2015). HIV-associated neuropathologies mainly include widespread reactive astrocytes so-called astrocytosis and other changes in the brain depending on the severity of the diseases (Bell *et al.*, 2006; Brew, 2009; Cohen and Gongvatana, 2009; Del Valle and Pina-Oviedo, 2006; Ellis *et al.*, 2007; Gelman, 2015; Sacktor, 2002). At the cellular level, the primary cell targets for HIV infection are macrophages/microglia and, to a lesser extent, astrocytes (Gorry *et al.*, 1998; Lee *et al.*, 1993; Liu *et al.*, 2004; Luo and He, 2015; Saito *et al.*, 1994; Schweighardt and Atwood, 2001; Tornatore *et al.*, 1994). However, neurons that are mostly

affected in the brain of HIV-infected individuals are rarely infected. Therefore, indirect mechanisms have been proposed for HIV/neuroAIDS pathogenesis; they can generally be grouped into three categories: improper immune activation of macrophages/microglia, soluble factors of both viral and host origins from macrophages/microglia and astrocytes, and astrocyte dysfunction.

Since its recognition more than two decades ago, clinical symptoms, neuropsychological testing, and post-mortem neuropathology have been used for HIV/neuroAIDS diagnosis. Meanwhile, more objective laboratory-based biological markers, so called biomarkers have also been pursued to predict and define HIV/neuroAIDS as well as to monitor treatment response and help therapeutic development for HIV/neuroAIDS. Those biomarkers can be derived from cerebrospinal fluid (CSF) such as beta-2-microglobulin, neopterin, quinolinic acid, and monocyte chemo-attractant protein-1 (MCP-1) (Brew *et al.*, 1990; Brew *et al.*, 1992; Cinque *et al.*, 2005; Heyes *et al.*, 1991; Letendre *et al.*, 1999; Thames *et al.*, 2015), or from peripheral blood such as CD16+ monocytes (Kusdra *et al.*, 2002; Luo *et al.*, 2003; Pulliam *et al.*, 1997; Shiramizu *et al.*, 2005); Neuroimaging markers have also been proposed as HIV/neuroAIDS biomarkers to document changes in the size and structure of brain and metabolites in the context of HIV infection (Cao *et al.*, 2015; Cloak *et al.*, 2004; Cysique *et al.*, 2004; Dousset *et al.*, 1997; Filippi *et al.*, 2001; Ge *et al.*, 2003; Hall *et al.*, 1996; Jakobsen *et al.*, 1989; Lee *et al.*, 2003; Paul *et al.*, 2002; Pomara *et al.*, 2001a; Ragin *et al.*, 2004a; Ragin *et al.*, 2004b; Ragin *et al.*, 2005; Tarasow *et al.*, 2003; Thompson *et al.*, 2006; Thompson *et al.*, 2005; Wu *et al.*, 2006). While not eliminating the need for clinical recognition and perhaps neuropsychological testing to measure severity and given that neuroimaging is expensive and not readily accessible, identification of objective laboratory-based quantitative CSF/blood biomarkers for HIV/neuroAIDS represents a major advance in all aspects of HIV/neuroAIDS.

Most of our knowledge about HIV/neuroAIDS is derived from clade B HIV. However, it should be pointed out that clade C HIV have been shown to be less neurotoxic compared to clade B HIV (Rao *et al.*, 2008). Several factors may have attributed to this difference, and they include mutations in the dicysteine motif of clade C Tat protein (Mishra *et al.*, 2008), weaker chemotactic activity of clade C Tat protein (Gandhi *et al.*, 2009), and lower replication efficiency of clade C HIV in macrophages (Constantino *et al.*, 2011). However, a more recent study has found no significant differences in the incidence of cognitive impairment between clade B and C HIV (de Almeida *et al.*, 2013). This study has attributed lower prevalence of HIV/neuroAIDS complications in regions with higher clade C infection (India and Sub-Saharan Africa) to less intensive study of impairments, presence of a myriad of comorbid disorders of CNS, and application of routine neurological exam instead of standardized neuropsychological assessments. In the review, we will aim to summarize all studies on HIV/neuroAIDS biomarkers.

2. CSF biomarkers for HIV/neuroAIDS

CSF is the optimal medium for monitoring the alterations in the CNS environment due to its proximity to brain parenchyma (Mattsson, 2011). The enrichment of CNS proteins in this biofluid makes it superior to blood in studies of neurodegenerative disorders. The relatively

simple, cost-effective, and routine sampling in clinics is considered the great advantage of CSF over neuroimaging markers which require highly specialized instruments (Blennow and Zetterberg, 2015).

Alterations in CSF frequently occur throughout the course of systemic HIV infection (McArthur *et al.*, 1988). In the pre-cART era, detection of viral RNA in the CSF was an indicator of presence and severity of HIV-associated neurocognitive disorders (HAND) (Fox, 2013). This association however no longer exists in the cART era since both neurocognitively unimpaired and HIV/neuroAIDS patients have been reported to have a low level of CSF viral RNA while replication of the virus has been undetectable in blood. Although there is debate on the nature of this CSF viral escape, a plausible explanation is ongoing replication in the CNS (Dahl *et al.*, 2014). In the cART era, the quest for the identification of objective markers has shifted towards the CSF-secreted products by the CNS cells that are specifically damaged by HIV and its toxic products.

Most of the CSF biomarkers that have been studied to date can be grouped into one of the three categories: viral, immune-related, and neural markers. Studies of CSF HIV can be quantitative measurement of virus, most commonly assessing viral RNA and the CSF viral load, and qualitative characterization of CSF virus, including genotypic and functional properties. Despite the fact that HIV infection is the pre-requisite for HIV/neuroAIDS (Spudich *et al.*, 2005) and the fact that HIV exhibit CNS compartmentalization with respect to blood virus (Harrington *et al.*, 2005; Power *et al.*, 1995; Strain *et al.*, 2005), none of these has so far proven to be predictive of CNS injury. On the other hand, the relationship of intrathecal immunoactivation to CNS injury was recognized early in the epidemic, several CSF immunologic markers were found at elevated levels during CNS infection of HIV before introduction of assays for HIV RNA (Brew *et al.*, 1990; Brew *et al.*, 1992). Among the biomarkers of immune activation that have been studied most extensively are beta-2-microglobulin, neopterin, quinolinic acid, and MCP-1 (Brew *et al.*, 1990; Brew *et al.*, 1992; Cinque *et al.*, 2005; Heyes *et al.*, 1991; Letendre *et al.*, 1999). None of these has entered mainstream clinical practice related to the diagnosis of HIV/neuroAIDS. Use of brain-specific biomarkers to document neurodegeneration has been pursued in a variety of neurologic diseases, but until more recently, not with the same enthusiasm in HIV/neuroAIDS. Candidate biomarkers in this category include molecular products of neurons, astrocytes, oligodendrocytes, and microglia. There are two general issues with these biomarkers. The first relates to their individual sensitivity to detect ongoing brain injury in the setting of HIV/neuroAIDS. This needs to be established for each biomarker. The second relates to the intrinsic non-specificity of these biomarkers as a group. Thus, virtually by definition, many types of injury can result in release of neural biomarkers into CSF (Norgren *et al.*, 2003). However, this limitation becomes less critical if one uses this class of biomarkers as indicators of active injury rather than to define the type of injury. In that context, sensitive neural biomarkers should have a critical and useful role. To date, several CSF biomarkers of cellular injury have been studied in the context of HIV/neuroAIDS; we will discuss them based on the type of the cell involved and the significance and correlation of those biomarkers with HIV-induced injury.

2.1 Cellular biomarkers

The consequence of HIV invasion of the CNS is neuronal damage in the form of synaptodendritic loss and neuron death (Rao *et al.*, 2014). Identifying sensitive surrogate biomarkers of HIV-induced neuronal damage in the CSF is of great importance in staging of HIV/neuroAIDS, response to therapy, and uncovering other mechanisms of injury to neurons. We have summarized the available cellular HIV/neuroAIDS biomarkers in the order of their significance along with their detection methods, strengths, and limitation (Table 1).

2.1.1 Neurofilament proteins (NF)—Subunits of the NF have been proposed as suitable biomarkers of neuronal damage in multiple neurodegenerative disorders (Skillbäck *et al.*, 2014). These are neuron-specific intermediate filaments with roles in structural support for axons and are classified based upon the molecular weight of their core chains as light, medium or heavy NF. Elevated levels of neurofilament proteins, especially light NF, have been found in the CSF following damage to the axons in Alzheimer's (Zetterberg *et al.*, 2015), multiple sclerosis (MS) (Gresle *et al.*, 2011), and amyotrophic lateral sclerosis (ALS) (Norgren *et al.*, 2003).

In the context of HIV infection, elevation in CSF light NF has been reported by several groups. Highest levels of CSF NFL are observed in HIV-associated dementia (HAD), decreasing with cART and resurging following the interruption of treatment (Abdulle *et al.*, 2007). In primary HIV infection, chronic neuro-asymptomatic infection, and HAD, CSF light NF seems to be the most sensitive CSF biomarker when compared to other CSF biomarkers such as soluble amyloid precursor protein (sAPP) and total tau protein (t-Tau) (Jessen Krut *et al.*, 2014). CSF light NF has been shown to be positively correlated to plasma viral load, and negatively with CD4 cell count, suggesting a link between neuronal injury and systemic HIV infection (McGuire *et al.*, 2015). Direct association of monocyte activation with neuronal injury at all stages of HAND has also been noted because of the significant correlation of CSF light NF with monocyte activation markers, soluble CD163 (sCD163) and soluble CD14 (sCD14) (McGuire *et al.*, 2015). A recent study indicates lack of elevation in CSF light NF in acute HIV infection, confirming light NF as a suitable marker of neuronal injury supported by the fact that this injury occurs later in the disease (Peluso *et al.*, 2015).

2.1.2 Amyloid precursor protein (APP)—APP is a membrane spanning protein with neuroprotective roles; it is ubiquitously expressed in neuronal cells. Secretase cleavage products of this precursor protein however have been linked to a variety of neurodegenerative disorders. β-amyloid 42 (Aβ42) is the product of APP cleavage by β-secretase, and its aggregation is considered a major contributor to Alzheimer's pathology (Nhan *et al.*, 2015).

The inflammatory cascade initiated by HIV invasion of the CNS and some of the viral products are capable of altering amyloid metabolism. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) secreted by HIV-infected microglia have been shown to induce elevated APP transcription and cleavage. MCP-1 secreted by HIV-infected and activated

astrocytes increases extracellular Aβ42 levels. HIV Tat protein, a neurotoxic protein of HIV-1, has been shown to inhibit neprilysin, the proteolytic enzyme that prevents Aβ42 aggregation, leading to the elevation of Aβ42 and soluble forms of APP and altered metabolism of Aβ42 and APP (Liu *et al.*, 2000; Ortega and Ances, 2014).

The effect of HIV infection of the CNS on amyloid levels has been studied for both soluble APP forms (sAPPα/β), and Aβ42 deposits in the brain. The consistent finding seems to be a decrease in soluble forms of CSF APP with HIV/neuroAIDS, but there are discrepancies in reports regarding Aβ42 deposition. Among these studies, one reports reduced sAPPα/β in HAD but no change in primary HIV infection (Peterson *et al.*, 2014). Another study reports a decrease only in CSF Aβ42 of HIV/neuroAIDS patients compared to HIV-infected individuals without cognitive impairment (Clifford *et al.*, 2009); and the third study finds reductions in both CSF sAPPα/β and Aβ42 of HAD patients (Krut *et al.*, 2013). Reductions in soluble forms of CSF APP could be explained by an increase in deposition of Aβ42 in neurons; and one study confirms this relationship in early infection with HIV (Peluso *et al.*, 2013), and another suggests that increase in Aβ42 deposition is concomitant with increased frequency of detection in plasma of Aβ42 in HIV-related cognitive impairments (Mothapo *et al.*, 2015). Two other studies however report no change in Aβ42 deposition in the context of HIV/neuroAIDS (Ances *et al.*, 2012a; Steinbrink *et al.*, 2013). An explanation for the discrepancies among studies of Aβ42 deposition in HIV/neuroAIDS patients would be the age difference of the cohorts studied. The reports with increased amyloid deposition within cognitively impaired HIV patients happen to have more middle-aged and older cohorts, while studies that have reported no change in deposition seem to have recruited younger cognitively impaired HIV patients. It is conceivable to conclude that sAPP reduction in CSF serves as a suitable biomarker for HAND while aging has to be considered when amyloid deposition is being studied.

2.1.3 Protease resistant protein, cellular isoform (PrPC)—PrPC is a GPI-anchored protein found in the lipid raft domains of plasma membrane in neurons and astrocytes (Coleman and Hill, 2015). PrPC is the non-pathological isoform of the prion protein and is involved in adhesion and signaling, transmigration of leukocytes across the endothelium, and neuroprotection. In prion diseases however, this normal host protein is converted to PrPSc, the transmissible agent of prion diseases which causes neurotoxicity (Bujdoso *et al.*, 2015). Prion-like, misfolded protein transmission is a common feature of neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson, and Huntington.

PrPC has been shown to be significantly increased in the CNS of HIV patients with neurocognitive impairments and also in SIV-infected macaques with encephalitis. The soluble form of this protein (sPrPC) is released into CSF and its levels have been shown to be correlated with the compromised CNS (Roberts *et al.*, 2010). Increased monocyte influx into the CNS due to HIV infection seems to be involved in PrPC elevation and release in the CSF, since PrPC levels are correlated with increased MCP-1 in CSF, but are independent of viral load and CD4 count (Megra *et al.*, 2013). Anti-HIV properties of PrPC are another reason for making this protein a valuable biomarker of CNS infection. PrPC has been shown to bind viral RNA, inhibiting its translation, and depleting PrPC favors HIV-1 replication (Alais *et al.*, 2012).

2.1.4 Tau protein—Tau is a microtubule-associated protein involved in the formation and stabilization of microtubules and movement of organelles along the axons and dendrites. Phosphorylation of tau occurs following inflammation, and its hyper-phosphorylated form (p-Tau) detaches from microtubules, destabilizing axons and leading to self-assembly of neurofibrillary tangles (Calcagno *et al.*, 2015). Hyper-phosphorylated Tau detected in tangles is a hallmark of Alzheimer's disease and detection of elevated total tau (t-Tau) in CSF is considered a valuable biomarker in the diagnosis of AD (Peterson *et al.*, 2014). Epidemiological studies on Tau levels in HIV/neuroAIDS patients however have been controversial; some reports find no correlations while others show elevated total Tau (t-Tau), p-Tau or both in cognitively impaired HIV patients. Of the 12 total studies in the literature that investigated Tau levels in CSF of HIV/neuroAIDS patients, four recent studies show elevations of t-Tau (Gisslén *et al.*, 2009; Peterson *et al.*, 2014; Smith *et al.*, 2014; Steinbrink *et al.*, 2013). Three early studies also show increases in CSF levels of p-Tau in HIV/neuroAIDS (Anthony *et al.*, 2006; Cohen *et al.*, 2015; Patrick *et al.*, 2011), and two other studies show increase in both t-Tau and p-Tau (Brew *et al.*, 2005; Calcagno *et al.*, 2015). The other three studies report no change in the levels of t-Tau or p-Tau in HIV/neuroAIDS patients (Clifford *et al.*, 2009; Green *et al.*, 2000; McNamara *et al.*, 2015). Since Tau, unlike amyloid plaques, is detected in brains of the majority of normally aged older adults, it is necessary to consider the age of the patient population while using this protein as a biomarker for HIV/neuroAIDS. Total Tau seems to be more closely correlated with the severity of cognitive impairment and therefore a more accurate biomarker for staging of HIV/neuroAIDS in younger patients.

2.1.5 Human cartilage glycoprotein 39 (YKL-40)—YKL-40 is a secreted glycoprotein expressed in several cell types with functions in growth stimulation, adhesion, and migration. Up-regulated levels of YKL-40 have been reported in several inflammatory diseases such as ulcerative colitis, Crohn's disease, rheumatoid arthritis, as well as in cancer (Craig-Schapiro *et al.*, 2010). YKL-40 in both plasma and CSF has been shown to increase in AD and both YKL-40 and Aβ-42 have been proposed as a potential prognostic biomarker for pre-clinical AD (Craig-Schapiro *et al.*, 2010). Previous studies have only detected YKL-40 expression in macrophages/microglia, but not in neurons and astrocytes in the context of HIV infection (Bonneh-Barkay *et al.*, 2008). Nevertheless, differential up-regulation of CSF YKL-40 has been reported in SIV-infected pigtailed macaques that had developed encephalitis, and have been correlated with increases in the CSF viral load (Beck *et al.*, 2015). These studies have attributed the elevated YKL-40 expression to productive HIV and SIV infections of microglia (Kolson, 2008). More recent reports however have found increased detection of YKL-40 in activated astrocytes and perivascular macrophages in the context of multiple sclerosis and lentiviral encephalitis (Bonneh-Barkay *et al.*, 2010). The induction of YKL-40 in astrocytes begins immediately following activation and continues for approximately 12 days. CSF levels of YKL-40 have also been positively correlated with IL-1β and TNF-α (Bonneh-Barkay *et al.*, 2012). These properties make YKL-40 a valuable biomarker of acute neuroinflammation. But it is important to note that most of the studies have focused on SIV models; therefore, further investigation of the usefulness of this biomarker for HIV/neuroAIDS is needed.

2.1.6 Calcium binding protein B (S100B)—S100B is a small acidic protein mainly expressed in astrocytes in the CNS and has important roles in cellular energy metabolism, cytoskeletal rearrangement, proliferation, and differentiation (Rothermundt *et al.*, 2003). S100B secretion is elevated in the CSF in various neurodegenerative, inflammatory, and psychiatric disorders including AD, dementia, stroke, subarachnoid hemorrhage, ischemia, and brain trauma. The elevation is indicative of tissue damage or blood brain barrier disruption (Rohlwink and Figaji, 2014). In HIV infection, patients with advanced neurocognitive problems have been shown to have higher levels of CSF S100B (Pemberton and Brew, 2001). In normal physiological concentrations, S100B has been shown to have trophic effects on neurons, but following injury and rise in concentration, this protein could exacerbate the damage. Increased S100B secretion also induces interleukin-6 (IL-6) secretion in CSF, which has been shown to contribute to neuroinflammation and further damage (Piazza *et al.*, 2013). S100B also functions as an inhibitor of glial fibrillary acidic protein (GFAP), the structural intermediate filament (IF) exclusive to astrocytes, by inhibiting the phosphorylation and assembly of GFAP (Sorci *et al.*, 1998). Elevated concentrations of S100B along with IL-1β have been shown to induce gliosis, disrupt neurite growth, and cause calcium mediated neuronal loss in HAD (Stanley *et al.*, 1994). Considering the specificity of this protein to astrocyte dysfunction and blood brain barrier disruption, S100B could be a useful biomarker of early CNS damage and BBB penetration in the context of HIV/neuroAIDS.

2.1.7 Neopterin—Neopterin is a pteridine and a metabolite of guanosine triphosphate catabolism; it is synthesized in macrophages and microglia following stimulation with interferon γ (IFN-γ). Increased neopterin production and secretion in CSF have been noted in viral, bacterial, and parasitic infections, autoimmune disorders, cancer, and neurocognitive impairments. For this reason, neopterin is considered an excellent intrathecal biomarker of macrophage and microglial activation in the CNS (Fuchs *et al.*, 1988). Neopterin is elevated in CSF of untreated HIV patients (Fuchs *et al.*, 1989). Its CSF concentrations initially drop with cART, but following a short plateau, bounce back and persist at higher levels throughout the course of infection despite continued treatment. Highest levels of CSF neopterin have been associated with advanced neurological impairment in HAD, while neuro-asymptomatic HIV patients do not demonstrate such elevation (Griffin *et al.*, 1991). Positive correlation between concentrations of CSF neopterin and NFL has been reported in HAND, indicating association of axonal degeneration with macrophage activation (Abdulle *et al.*, 2007). Neopterin is a well-studied biomarker of immune activation; it can be helpful in staging and therapeutic monitoring of HIV/neuroAIDS, because of its microglial specificity, initial drop following cART, and continued high levels through cART therapy.

2.1.8 Soluble CD163 (sCD163)—sCD163 is the secreted form of the scavenger receptor for hemoglobin; it is expressed on monocytes and macrophages and has roles in endothelial cell adhesion and dampening of systemic inflammation. Several pro- and anti-inflammatory mediators regulate the expression of sCD163; for instance, TNF-α and IFN-γ inhibit its expression while IL-6 and interleukin-10 (IL-10) induce it. sCD163 has been used as a biomarker for systemic monocyte activation in coronary artery disease, rheumatoid arthritis,

Gaucher lysosomal storage disease, and cancer (Onofre *et al.*, 2009). In SIV-infected pigtailed macaques, plasma sCD163 is significantly higher in individuals with encephalitis compared to those without encephalitis (Beck *et al.*, 2015). In humans, plasma levels of sCD163 but not CSF levels are shown to be elevated in both early and chronic infection with HIV. Plasma levels have also been correlated with viral load and associated with monocyte expansion following infection (Burdo *et al.*, 2011). Higher levels of sCD163 in plasma have been reported in HIV patients with more severe neurological impairments. These levels decreased with effective cART, but did not return to the pre-infection baseline levels, indicating persistent immune activation (Burdo *et al.*, 2013). Tracking studies on CD163+ monocytes and macrophages have shown that these cells accumulated in the perivascular regions of brain in HIV encephalitis, and that their numbers are correlated with plasma HIV load (Fischer-Smith *et al.*, 2008).

Despite its solubility and release in plasma, sCD163 levels have not been found to be significantly increased in the CSF of HIV/neuroAIDS patients. This might be due to phenotypic change in monocytes following transmigration and establishment in the perivascular regions. Plasma levels however have been useful in staging, as they indicate an initial drop following cART but present at higher levels during the course of infection. Overall, sCD163 can be useful in staging and therapeutic monitoring of HIV/neuroAIDS in a panel along with other CSF and blood biomarkers.

2.1.9 Soluble CD14 (sCD14)—sCD14 is the soluble form of the monocyte lipopolysaccharide (LPS) receptor which is cleaved and released from the membrane following the activation of monocytes (McGuire *et al.*, 2015). Low concentrations of sCD14 are sufficient to confer LPS responsiveness to non-CD14-expressing cells. Soluble CD14 has also been shown to be present in human milk and linked to the regulation of microbial growth in infant gut (Funda *et al.*, 2001).

Both plasma and CSF levels of sCD14 have been shown to be elevated in HIV infection regardless of cART status and correlated with the severity of neurocognitive impairment; HIV patients with lower test scores in attention and learning had higher sCD14 (Lyons *et al.*, 2011). HIV patients with cognitive impairments showed elevated plasma sCD14, which was correlated with neuropsychological test scores and neuroimaging biomarkers of HIV/neuroAIDS. These levels have also been found to be elevated in HIV patients with cerebral atrophy compared to those without atrophy (Ryan *et al.*, 2001). Plasma levels of sCD14 have been shown to independently predict mortality in HIV infection; patients with highest plasma levels of sCD14 have six times higher risk of death. This is due to the association of chronic HIV infection with intestinal permeability which increases the risk for bacterial sepsis (Sandler *et al.*, 2011). Altogether, soluble CD14 is a useful plasma and CSF biomarker in HIV/neuroAIDS, as it reflects the severity of cognitive impairments, effectiveness of therapy, and risk of death.

2.1.10. Glial fibrillary acidic protein (GFAP)—GFAP is a type III intermediate filament protein that has a characteristic structure composed of a highly conserved central-helical rod domain flanked by nonhelical head and tail domains (Fuchs, 1996; Fuchs and Weber, 1994). It is expressed in all astroglial cells but predominantly in the fibrillary

astrocytes and has been widely used as an astrocyte marker (Eng *et al.*, 2000). GFAP expression is developmentally and pathophysiologically regulated. It begins to express as astrocytes mature (Chiu and Goldman, 1985), and its expression is always increased in reactive astrocytes, or astrocytosis, which is one of the main characteristics of the astrocytic reaction commonly observed in CNS injury, either as a result of physical, pathological, or chemical insults (Norenberg, 1998). GFAP is mainly located in cytoskeletal compartments, while little is found in the cytosolic (Bertelli *et al.*, 2000). In normal and pathological conditions GFAP leaks out from the central nervous system to the CSF (Rosengren *et al.*, 1995)

The serum and CSF GFAP levels have been shown to be a sensitive and specific indicator for a number of subacute or chronic CNS diseases including AD (Schmued and Hopkins, 2000), normal pressure hydrocephalus (Lord and Papoian, 2004; Ridet *et al.*, 1997), cerebral vasculitis (Lord and Papoian, 2004; Ridet *et al.*, 1997), multiple sclerosis (Kreutzberg, 1996), Lyme neuroborreliosis (de Olmos *et al.*, 1994) and trypanosomiasis (Norton *et al.*, 1992; O'Callaghan and Sriram, 2005). In HIV infection, one study shows only slight to moderate increase of the CSF GFAP level in some HIV-infected patients without detectable CNS complications (Andersson *et al.*, 2006), while the other two studies show no differences in the CSF GFAP levels and the frequency of increased GFAP levels between the three groups of HIV-infected patients with different degrees of neuroAIDS (Andersson *et al.*, 1998; Sporer *et al.*, 2000). The difference may be likely due to the small size of the samples in these later two studies. Thus, to further evaluate the exact sensitivity and specificity of the CSF GFAP as a HIV/neuroAIDS biomarker is clearly warranted.

2.2 Immune activation biomarkers

Infiltration of HIV-infected macrophages, monocytes and T cells into the CNS establishes a cascade of inflammation that leads to the activation of microglia, astrocytes, and perivascular macrophages. The combination of cytokines and chemokines produced by these infiltrated cells induces more inflammation and invites more immune cells from the periphery, contributing to neuronal injury. TNF-α, IL-1-β, and IFN-γ produced by infected monocytes and T cells activate microglia and astrocytes. These cells in turn produce IL-6, MCP-1, and macrophage inflammatory protein 1 (MIP-1), which bring in more immune cells into the CNS and contribute to neuroinflammation and injury (Hong and Banks, 2015). We have summarized the most commonly used immune activation biomarkers for HIV/neuroAIDS in the order of their significance along with their detection methods, strengths, and limitation (Table 2).

Several soluble factors in both plasma and CSF of HIV patients have been studied as biomarkers for immune activation before and after the advent of cART. Early studies show that CSF and serum IL-1β, IL-6, and TNF-α are more frequently detected in HIV patients presenting with HAD (Perrella *et al.*, 1992). Elevation of plasma and CSF HIV RNA, TNF-α, MCP-1, matrix metalloprotease-2 and macrophage colony stimulating factor have also been shown to be predictive of HAD (Sevigny *et al.*, 2004). Higher levels of chemokines such as MCP-1 and interferon gamma-induced protein 10 (CXCL-10) are also reported in the CSF of HIV patients with dementia (Mehla *et al.*, 2012). Astrocytes are the major

producers of MCP-1 following exposure to or expression of HIV-1 Tat protein (Conant *et al.*, 1998). CSF MCP-1 has been reported to be particularly higher in CSF of HIV patients with dementia, suggesting that monocyte infiltration of CNS is closely correlated with severe cognitive impairment. Other studies have confirmed the significant correlation of CSF MCP-1 with cognitive impairment and found that other immune activation biomarkers of CSF such as IL-6, interleukin-8 (IL-8), MIP-1α, CXCL10, and IFN-γ are elevated in HIV independent of cognitive status (Kamat *et al.*, 2012). A recent study has shown that HIV-positive individuals with a specific genotype of MCP-1 have significantly higher CSF levels of this chemokine and suffer more severe cognitive decline (Thames *et al.*, 2015). CSF MCP-1 has been shown to be correlated with neopterin (Price *et al.*, 2007), and both are elevated in HAD, dropping with cART regimen with more CNS penetration (Tiraboschi *et al.*, 2015). Elevated MCP-1 in CNS induces leukocytosis (Eugenin *et al.*, 2006) and is associated with increased CSF viral loads following the interruption of cART (de Almeida *et al.*, 2006). Transmigration of CD8 T cells into the CNS resulting in elevated CSF IFN-γ has specifically been correlated with increased risk of HIV-associated cognitive impairments (Schrier *et al.*, 2015). These findings have encouraged combinational analysis of cytokines and chemokines for the purpose of monitoring severity and progression of neurological impairments in HIV infection. In general, most of the immune activation markers with the exception of monocyte/macrophage markers have shown normalization within one year after the initiation of cART (Wada *et al.*, 2015). This observation maintains combinational monitoring of soluble immune activation biomarkers for HIV/neuroAIDS.

2.3 Lipid biomarkers

Disruption in the lipid metabolism in the CNS is a common feature of CNS disorders with acute or chronic inflammation. As the second fattiest tissue in the body, the CNS, the high concentration of lipid components in the CNS, is susceptible to the insults of cytokines, chemokines, and reactive oxygen species. Other than HIV, the presence of activated microglia in the CNS capable of producing copious amounts of inflammatory factors has been observed in neurodegenerative disorders such as AD, Parkinson, Huntington, and MS (Takeuchi *et al.*, 2005). There is elevation of lipid metabolites in the brain and CSF for several above-mentioned disorders; for example, ceramide, a product of sphingomyelinase activation, is elevated in astroglia of frontal cortex and CSF of AD and ALS patients (Satoi *et al.*, 2005), contributing to neuronal apoptosis. Accumulation of ganglioside GD3 has been shown in the CSF of MS (Miyatani *et al.*, 1990) and prion disease (Ohtani *et al.*, 1996). Free fatty acids and their carrier, carnitine, have been documented to increase in meningitis, seizures, neuropathies (Shinawi *et al.*, 1998), traumatic brain injury, and stroke (Pilitsis *et al.*, 2003).

Cytokines and chemokines produced by glial cells are capable of inducing enzymes involved in lipid metabolism such as phospholipase A2 (PLA2), phospholipase C (PLC), and cyclooxygenase (Farooqui *et al.*, 2007). This induction of enzymes occurs through transcription factors that control cytokine and chemokine gene expression. For example, nuclear factor kappa light-chain enhancer of activated B cells directly stimulates PLA2, resulting in phospholipid membrane disintegration and release of arachidonic acid along with the generation of reactive oxygen species (Shmelzer *et al.*, 2003). Phospholipid

oxidation of neural membranes also occurs in the process of ongoing inflammation in the CNS, leading to the accumulation of oxidized lipid products, which can influence the inflammatory process, themselves. Peroxisome proliferator-activated receptors (PPAR) are a family of nuclear hormone receptors that exist in different isoforms in neural tissue (α, γ, δ). PPAR have anti-inflammatory properties once activated by their ligands most of which are composed of long-chain poly-unsaturated fatty acids, eicosanoid derivatives, and phospholipids. Deficiency in PPARs or modifications in their ligands, including oxidation of phospholipids, have been associated with delay in anti-inflammatory responses (Drew *et al.*, 2006).

Aside from their indirect effects on lipid metabolism, cytokine and chemokines produced in the CNS directly bind to receptors that are coupled with PLA2 and PLC and induce their enzymatic activity against lipids. TNF-α and IL-1β, for instance, have been shown to stimulate PLC and sphingomyelinase, leading to increased ceramide generation (Machleidt *et al.*, 1996). Ceramide is also produced as a result of oxidative assault on lipid membranes in the CNS; its accumulation has been reported in CSF of HIV/neuroAIDS patients. Inhibition of inducible nitric oxide synthase through minocycline treatment resulted in normalization of this metabolite in CSF (Sacktor *et al.*, 2014). We have summarized the lipid HIV/neuroAIDS biomarkers in the order of their significance along with their detection methods, strengths, and limitation (Table 3).

Initial investigations of lipid accumulation in CSF of HIV patients began with cell-specific lipid species. Ganglioside GD3, for example, is considered a microglial/macrophage- and astrocyte-enriched lipid component and has been found to be elevated in CSF of HIV patients. This elevation has been associated with the activation of microglia and astrocytes in HIV infection, resulting in a higher cell turnover (Andersson *et al.*, 1998). Then a global, mass spectrometric analysis of CNS lipid components was introduced and has revealed the accumulation of sphingolipids and ceramide in frontal, parietal, and temporal cortices of brain with HIV infection (Haughey *et al.*, 2004). It has also shown that the degree of sphingolipid and ceramide accumulation is correlates with severity of cognitive impairments of HIV (Haughey *et al.*, 2004). Using the same approach, additional elevated components such as triglyceride C52 and vitamin E have also been detected in the CSF and correlated with the stage of cognitive impairment progression (Bandaru *et al.*, 2007). Sphingolipid accumulation is correlated with moderate cognitive dysfunction in earlier stages of HAD when anti-oxidant defense barriers of the brain fails to protect CNS cells against acute oxidative and inflammatory storm. Ceramide is produced from sphingolipid in later stages and are associated with declining and more severe cognitive impairments. A mass-based metabolomics study shows elevation in phospholipids, and free fatty acids associated with the activation of phospholipases in SIV encephalitis (Wikoff *et al.*, 2008). Recent efforts in characterizing the relationship between lipidoses of the CNS and HIV infection indicate similarities of HIV/neuroAIDS to lysosomal storage disorders, suggesting disruptive effects of HIV proteins on the endolysosomal system (Bandaru *et al.*, 2013). Worsening of cognitive impairment is associated with a shift in lipid accumulation in the CNS from single lipid species to multiple species. These findings together support the utility of lipids as prognostic and staging biomarkers for HIV/neuroAIDS.

2.4 microRNA (miRNA) biomarkers

Circulating, small, non-coding RNA have become attractive surrogates for evaluating disease progression, pathogenesis, therapeutic effect, and prognosis. Potential use of miRNA as biomarkers is supported by the accumulating evidence from numerous studies, which show that conditions of disease change the physiological expression patterns of these transcription-regulating molecules. Moreover, the resistance to degradation, the tissue specificity, the fast response to cellular environment, and the predictable effects on biological pathways also make miRNA ideal candidates for monitoring processes and aberrations in biological systems (Rao *et al.*, 2013). Extracellular miRNA can be detected in all biofluids in association with lipoproteins, proteins of the argonaute family, or exosomes, which adds to their value as resistant molecules capable of tolerating common problems of sample handling without degradation.

Studies on tractable miRNA signatures in the context of CNS disorders are particularly attractive because these brain-specific small RNA can be identified in the CSF or plasma and provide a rare snapshot of the status of the inaccessible brain tissues. Although several studies have successfully demonstrated differential expression patterns of brain-specific miRNA in plasma, CSF remains the biofluid of choice. The main reasons are the less heterogeneity of sources of miRNA in CSF compared to blood, the small volume of CSF imposing less diluting effects on miRNA concentrations, and the proximity to the source tissue. All of those unique properties allow use of miRNA to precisely predict the pathological process. miRNA profiling has been performed in the context of various CNS disorders including AD, Parkinson, Schizophrenia, ALS, and MS and have led to identification of specific miRNA expression patterns associated with each of the diseases (Jin *et al.*, 2013).

Investigating the global expression pattern of miRNA in response to HIV infection has also revealed disease-specific miRNA signatures that could potentially be used as biomarkers of staging, progression, and therapeutic monitoring. The initial study compared miRNA expression in peripheral blood mononuclear cells (PBMC) between HIV patients and healthy controls (Houzet *et al.*, 2008). Expression of 63 miRNA is altered in PBMC of HIV infected patients. Several mostly down-regulated miRNA are T- cell specific and have the anti-HIV property. In a follow-up study, HIV patients are divided into four groups: asymptomatic, cART naïve, cART treated, and cART resistant, significant correlation is found in miRNA expression between plasma and PBMC. In addition, two differentially expressed miRNA in both plasma and PBMC could potentially predict progression of disease and response to therapy (Munshi *et al.*, 2014; Witwer *et al.*, 2012). miRNA profile in PBMC of viremic subjects and elite controllers show the same pattern of down-regulation of anti-HIV miRNA in both groups, but also differentially expressed miRNA specific to patients with HIV viremia (Witwer *et al.*, 2012). Down-regulation of the let-7 miRNA family has been noted in HIV-infected T cells and correlated with increase in IL-10 expression and inhibition of T cell responses against HIV infection (Swaminathan *et al.*, 2012). Besides those studies, the other study also shows a general trend in down-regulation of anti-viral defenses in T cells and PBMC following infection with HIV (Duskova *et al.*, 2013). Overall down-regulation in miRNA expression in HIV infection has also been

associated with RNA silencing suppressor (RSS) activity of HIV Tat (Bennasser and Jeang, 2006; Hayes *et al.*, 2011) and Vpr (Coley *et al.*, 2010).

To date, only four comprehensive miRNA profiling studies have been conducted to identify suitable biomarkers for HIV/neuroAIDS. Two of these studies have been performed on archived and post-mortem brain tissue, one on plasma, and one on CSF. Parallel analysis of mRNA and miRNA expression profiles in the frontal cortex of HIV patients with or without HAD have shown 68 differentially expressed miRNA in HAD, 49 of which are down-regulated and 19 are up-regulated (Zhou *et al.*, 2012). Furthermore, gene ontology and clustering analysis reveal involvement of significantly altered miRNA in axon guidance and down-stream signaling. Five miRNA are identified as potential biomarkers of neurodegeneration based on the fact that they are all enriched in neurons and have previously been shown to be involved in processes related to pathology in AD, Huntington, MS, and Schizophrenia. miRNA expression profiling in the brains of four HIV patients with encephalitis shows that the majority of up-regulated miRNA are involved in the transcriptional control of gene products important in immune response and inflammation, nucleotide metabolism, and cell cycle control (Noorbakhsh *et al.*, 2010). These findings are consistent with infiltration and proliferation of immune cells in the CNS. Down-regulated miRNA are shown to be involved in cell death and resting state of glial cells (Noorbakhsh *et al.*, 2010). Paired miRNA profiling of the plasma in HIV patients with or without cognitive impairment shows that 30 pairs of miRNA are differentially expressed but only 10 of them can be used to distinguish cognitively impaired HIV patients from unimpaired (Kadri *et al.*, 2015). Interestingly, three of these identified miRNA targeted brain-derived neurotrophic factor (Kadri *et al.*, 2015).

The first and only CSF miRNA profiling study compared frontal cortex tissue and CSF of HIV patients with or without neurocognitive impairment to healthy controls (Pacifici *et al.*, 2013). The study found 66 differentially expressed miRNA in CSF samples and 35 of those miRNA were also detected in frontal cortex. There were 4 miRNA with identical expression patterns between both sample sources and one miRNA with more than 20 fold higher expression in CSF compared to brain tissue. Computer-assisted target prediction of significantly altered miRNA indicated involvement in cytoskeletal remodeling, cell adhesion, chemokine expression, neurogenesis, axonal guidance, notch signaling, synaptogenesis, and nerve impulses.

Comparing the reported miRNA signatures of these four studies, we have found 6 brain-enriched miRNA with shared differential expression patterns. Interestingly, each of the identified miRNA have been reported as significant in various processes in the brain; miR-495 and miR-744 in BDNF regulation (Mellios *et al.*, 2008; Wu *et al.*, 2010), miR-19 in the pathogenesis of neurodegenerative disorders (Lee *et al.*, 2008), miR-16 in neurotransmitter transport regulation (Baudry *et al.*, 2010), miR-92 in neuronal differentiation (Bian *et al.*, 2013), and miR-139 in regulation of neuronal apoptosis (Qu *et al.*, 2014). We have summarized the miRNA studies on brain tissue and CSF of HIV patients along with shared signatures of expression with potential use as HIV/neuroAIDS biomarkers (Table 4).

More studies of miRNA profiling in both brain tissue and CSF of HIV patients need to be performed in order to define HIV/neuroAIDS-specific miRNA signatures. Overall, relevant disease-specific changes in miRNA have been noted in the above-mentioned studies, those miRNA could potentially enlighten the path to accurate biomarker discovery. This is particularly important with the changing dynamics of HIV/neuroAIDS and potential effects of the aging co-morbidity. Considering the close relationship of circulating miRNA with exosomes and the fact that these extracellular vesicles serve as significant intercellular communication vehicles between cells in the CNS, profiling of the exosome-associated miRNA in the CSF in the context of HIV infection is necessary for identification of potentially more specific miRNA for HIV/neuroAIDS.

3. Blood biomarkers for HIV/neuroAIDS

Compared to CSF, blood has the advantage of being easily sampled and is a desirable source for identification of biomarkers for diagnosis and management. However, this source has proved less useful than CSF in most of the CNS diseases including HIV/neuroAIDS. A number of studies have examined blood concentrations of the same type of biomarkers as discussed for CSF, without suggesting any clinical utility. One exception is that circulating proviral HIV DNA within peripheral blood CD16+ monocytes has recently been suggested as a prognostic marker for HIV/neuroAIDS (Shiramizu *et al.*, 2005). In the pre-cART era, monocytes from individuals with HAD exhibit increased expression of CD69, CD16, and TNF-α (Pulliam *et al.*, 1997). Subsequently, in cART-treated patients, circulating CD69 and CD16 monocytes are decreased compared to earlier observations, suggesting either inactive disease or that treatment might down-regulate monocyte activation even in patients with HAD/HIV encephalitis (HIVE) (Kusdra *et al.*, 2002). Proteomics has been used to analyze CSF and monocyte/macrophage products derived from HIV-infected individuals with varying cognitive impairments (Luo *et al.*, 2003). This methodology holds the great promise in discovering truly novel molecules associated with HIV/neuroAIDS.

4. Neuroimaging biomarkers for HIV/neuroAIDS

The rationale for applying neuroimaging modalities to HAD/HIVE parallels those for CSF biomarkers. In fact, neuroimaging has contributed significantly to defining the neurologic manifestations of HIV-1 infection since the onset of the AIDS pandemic; neuroimaging remains invaluable in distinguishing lesions due to opportunistic CNS disease. Computerized tomography (CT) and magnetic resonance imaging (MRI) were utilized early in the HIV epidemic to detect encephalitis and damage to white matter (WM) (Thompson and Jahanshad, 2015). These earlier studies found that WM and caudate loss was accompanied with a larger CNS space in HIV patients (Stout *et al.*, 1998). We have summarized the most commonly utilized neuroimaging methodologies in HIV/neuroAIDS (Table 5).

In the context of HIV/neuroAIDS, neuroimaging methods have the obvious advantage of anatomic definition that can be combined with various functional assessments. Ideally, a neuroimaging marker should be (1) specific for the diagnosis of CNS injury that underlies HIV associated neurocognitive impairment; (2) sensitive in detecting these processes during

the presymptomatic stages; and (3) able to define disease stage and track the effects of treatment. Morphometric MRI studies focus on quantitative volumetric changes of the brain or its subanatomic regions and have shown that the reduced size of various brain regions including basal ganglia, caudate nucleus, corpus callosum, cortex, hippocampus, and lateral ventricles in HIV/neuroAIDS patients is correlated with impaired neuropsychological test performance of these individuals (Cysique *et al.*, 2004; Hall *et al.*, 1996; Jakobsen *et al.*, 1989; Paul *et al.*, 2002; Thompson *et al.*, 2006; Thompson *et al.*, 2005). Smaller thalamic volume and larger frontal sulcal volume has been reported in MRI studies of HIV compared to controls (Pfefferbaum *et al.*, 2012). Cortical atrophy has also been reported from MRI studies of HIV-affected brain and is interpreted as a sign of neuronal and myelin loss (Archibald *et al.*, 2004).

HIV infection of the CNS is associated with accelerated and/or accentuated aging. Particularly in the post-cART era, an increasing number of HIV patients are presenting with more severe symptoms and co-morbidities of aging compared to their seronegative counterparts. Signs of accelerated aging are identifiable even through the MRI screening in the pre-cART era (Holt *et al.*, 2012). Later, voxel-based morphometry has been used to characterize age-related changes in the HIV-affected brain. Based on this method, change in the gray matter (GM) due to ageing becomes identifiable from HIV-related atrophy in the GM (Towgood *et al.*, 2012). HIV is still causing volume loss in WM and GM in the post-cART era and these reductions in volume are independent of ageing (Becker *et al.*, 2011). Contribution of HIV and aging to volume reduction in several brain regions are shown to be independent and distinct. Aging has been shown to be associated with atrophy in amygdala, while in HIV, a reduction in corpus callosum is observed (Ances *et al.*, 2012b). Also, HIV-associated atrophy in frontal, temporal, parietal, and cerebellar regions, as well as basal ganglia are found to be greater than age-related atrophy (Chang *et al.*, 2011). These studies confirm the advantage of imaging studies in differentiating respective assaults of HIV and aging on the HIV population that is experiencing dramatically improved life expectancy.

Both diffusion tensor imaging (DTI) and magnetization transfer imaging (MTR) are quantitative MRI modalities that provide information on brain microstructures. DTI is to measure diffusion parameters of water in the brain, while MTR is based on the study of interactions between protons in free and restricted (macromolecular) environments. DTI is a useful method in studying WM changes since neuroinflammation is associated with increase in fluid volume in the brain. Two main values are measured by DTI techniques: fractional anisotropy (FA), which reflects integrity and organization of WM fibers, and mean diffusion (MD), which indicates the average of diffusion along the axons (axial diffusion) and diffusion perpendicular to axon fibers (radial diffusion). This technique has enabled the detection of WM abnormalities in seemingly normal MRI screenings of the HIV brain (Pomara *et al.*, 2001b). Studies have shown using both strategies that structural changes in white matter, subcortex, and corpus callosum correlate with cognitive status measures, including the severity of dementia and the degree of impairment in specific cognitive domains (Cloak *et al.*, 2004; Filippi *et al.*, 2001; Pomara *et al.*, 2001a; Ragin *et al.*, 2004a). Also, higher diffusion and lower FA in several brain regions have been documented in HIV. In a one-year follow up study of neuroasymptomatic HIV patients, MD values are recorded as highly significant with lower FA in parietal WM at baseline. Follow-up evaluation

however shows increased MD in frontal and parietal WM, putamen and genu regions (Chang *et al.*, 2008). DTI has enabled the differentiation of age-related changes in WM, as well as changes associated with co-infections, from HIV-related abnormalities in brain regions. Reductions in FA of frontal regions has been shown to be an age-specific change in HIV, while a lower WM FA with higher diffusion in parietal and occipital regions indicates co-infection with the hepatitis C virus (Gongvatana *et al.*, 2011). These findings confirm the utility of DTI in differentiating the assaults of aging and other co-morbidities from HIV specific effects in an aging HIV affected population.

Magnetic resonance spectroscopy (MRS) is a widely available MRI modality that measures regional changes of the metabolites in the brain. These neurometabolites include *N*-acetylaspartate (NAA) which is a marker for neuronal and axonal damage, choline (CHO) and myoinositol (MI) which are indicators of membrane turnover and glial activation, and creatine (Cr) which is a marker of high energy metabolism associated with glial activation. MRS studies have shown that a reduced level of *N*-acetylaspartate and an increased level of myoinositol in frontal white matter are markers for neuronal or axonal loss or damage in basal ganglia and glial activation, respectively and have been correlated with the severity of cognitive impairment and the permeability of the blood-brain barrier (Dousset *et al.*, 1997; Ge *et al.*, 2003; Lee *et al.*, 2003; Ragin *et al.*, 2004b; Ragin *et al.*, 2005; Tarasow *et al.*, 2003; Wu *et al.*, 2006). Reduced NAA along with increased CHO has been noted in the frontal WM and lenticular nuclei of cognitively unimpaired HIV patients (Chong *et al.*, 1993; McCONNELL *et al.*, 1994). A recent proton MRS study on SIV model of rhesus macaques has shown that in acute infection, MRS glial markers MI, Cr, and CHO increase by 28%, 15%, and 10% respectively while NAA remains unchanged. These results indicate that activation of glia occurs prior to neuronal injury (Wu *et al.*, 2015). Another study on the basis for creatine alterations in a rapid SIV progression model in macaques shows that reduction of NAA and elevation in creatine in this model have been correlated with reduced synaptophysin and MAP-2 levels, indicating neuronal injury, while GFAP and Iba-I are significantly upregulated. These findings establish a relationship between astrocytosis and enhanced high-energy phosphate turnover as indicated by creatine levels (Ratai *et al.*, 2011).

MRS studies have proven to be useful in monitoring cART treatment. Initiation of cART has been shown to normalize NAA/Cr and Cho/Cr ratios (Chang *et al.*, 2004b; Stankoff *et al.*, 2001; Tarasów *et al.*, 1999), while lactate/Cr has been reported to be significantly higher in HIV patients receiving cART who have moderate to severe cognitive impairment (Roc *et al.*, 2007). Increase in lactate is an indicator of anaerobic glycolysis in macrophages and glia due to the ongoing inflammation despite HAART implementation. Decrease in NAA has been associated with more severe dementia in HIV (Holt *et al.*, 2012). MRS studies of cART-naïve HIV patients have shown that elevation of MI in frontal WM is linked to poor performance on executive function test while increase in CHO in the same region of the brain is correlated with slower performance in working memory tasks (Chang *et al.*, 2002).

Several groups have shown significant correlations between magnetic resonance data and other CSF biomarkers of HIV/neuroAIDS. A cross-sectional study of HIV patients investigating cognitive status, CSF biomarkers of HIV/neuroAIDS, and MRI structural evaluation has found that the severity of brain atrophy, signal changes in basal ganglia, and

degree of cognitive impairment are positively correlated with the amount of Tau protein in CSF (Steinbrink *et al.*, 2013). This study demonstrates that HIV/neuroAIDS can be differentiated from AD using this combinational approach to biomarker detection since p-Tau is unchanged by HIV infection. The recent study has looked at global biomarkers of chronic inflammation in plasma and CSF of HIV patients along with proton MRS of the brain (Anderson *et al.*, 2015). The study shows an association between increased MCP-1 in plasma and CSF with reductions in NAA/Cr of midfrontal cortex and basal ganglia. The study further shows that sCD14 in plasma and CSF is exclusively correlated with Glucose/Cr ratio. The inverse relationship between MCP-1 levels and NAA/Cr has been described previously as the link between transmigration of monocytes and neuronal injury in HIV/neuroAIDS (Chang *et al.*, 2004a). These results emphasize again the presence of persistent immune activation in chronic HIV and its role in neuronal injury.

MRS can be used to reliably differentiate age-related changes in neurometabolites in HAND from other cognitive impairments. Applying this technique in aging research has shown that in the process of normal aging, metabolites such as creatine, myoinositol, and choline increase in a linear fashion (Chang *et al.*, 1996). Discrimination of healthy aging individuals from subjects with mild cognitive impairment has also been reported by comparing MI/Cr ratios through MRS (Catani *et al.*, 2001). In HIV, changes in MRS metabolites due to accelerated aging of the brain have been documented in both cART-naïve and cART-treated subjects. A study on young, untreated HIV patients shows the usual reduction in NAA along with substantial increases in MI and CHO which are significantly higher than age-associated changes in these markers (Ernst and Chang, 2004). In cART-treated HIV patients, NAA/Cr ratios are lower even in young individuals compared to seronegatives; this ratio decreases with age. These data indicate that abnormalities in MRS metabolites resulting from pre-mature aging can be detected in cART-treated HIV patients. Evaluation of glutamate levels using MRS has shown that the levels of this metabolite are decreased in parietal region of HIV/neuroAIDS patients while patients without cognitive impairment have higher glutamate in basal ganglia (Ernst *et al.*, 2010). In addition, glutamate levels have been found to be lower in young HIV patients, which are comparable to the levels normally seen in seronegatives who are 10 years older. These MRS studies of aging HIV population suggest the great utility of this technique in the aged HIV population.

Functional MRI (fMRI) measures cerebral blood flow by detecting changes in the concentration of paramagnetic deoxyhemoglobin. This technology has allowed documentation of compensatory brain hyperactivation in mildly impaired patients, prior to the appearance of abnormalities on neuropsychological testing (Ernst *et al.*, 2002). The blood oxygen level-dependent contrast imaging (BOLD) method of fMRI can be used as an indirect measure of neuronal function and activity (Arthurs and Boniface, 2002). In HIV infection, fMRI studies have consistently shown reduced activity of normal attention networks along with increased usage of reserve networks of the brain (Chang *et al.*, 2004c). This increased involvement of reserve networks that acts as a compensatory mechanism to maintain normal cognitive performance is also observed in neuroasymptomatic HIV subjects. Greater need for the activation of brain reserve networks has also been shown in studies that employ both MRS and fMRI. Elevated glial metabolites such as MI, CHO, and Cr lead to stronger BOLD signals, associating neuroinflammation with greater need for

reserve network activation (Ernst *et al.*, 2003). A longitudinal fMRI study of cognitively unimpaired HIV patients and seronegative controls has shown greater baseline BOLD signals in occipital, cerebellar, and right prefrontal regions of HIV brain during the completion of visual attention tasks. Followup measurements one year after the baseline reading have shown significantly increased BOLD activity in prefrontal and parietal regions of HIV brain and decreased BOLD activity in seronegatives (Ernst *et al.*, 2009). These results are consistent with signs of HIV-associated premature aging, as increased activation of reserve networks have also been observed in normal aging brains following visual attention tasks. Evaluating resting cerebral blood flow (CBF) and its alterations in response to visual stimulation has shown that HIV patients have reduced CBF when compared to seronegatives who are 15 years older. Assessing activation of brain regions during visual tasks, HIV patients show a reduced activity that is equivalent to the brain activity of seronegatives who are 21 years older (Ances *et al.*, 2010). The data from fMRI studies of HIV brain suggest that although this technique can identify changes in the activity of brain networks and cerebral blood flow in the context of HIV infection, it cannot discriminate the similar effects from the aging brain. However, this technique along with MRS is useful in demonstrating increased need for brain reserves in HIV infection. Moreover, despite the technical complexity of fMRI that limits its general utility in clinical settings, advances in areas associated with language lateralization (e.g., prior to temporal lobe surgery for intractable epilepsy) suggest that this technique should soon be more readily available in clinical settings.

5. Conclusion

Clinical symptoms, neuropsychological testing and post-mortem pathology have been the main approaches for diagnosis of HIV/neuroAIDS. A great deal of studies have been attempted to identify more objective laboratory-based quantitative CSF/blood biomarkers for HIV/neuroAIDS. Neuroimaging for HIV/neuroAIDS still remains an attractive option, despite the nature of being expensive and not readily accessible. It is conceivable that proteomics and metabolomics will expedite the HIV/neuroAIDS biomarker discovery and validation and will surely facilitate development of anti-HIV/neuroAIDS therapeutics and clinical management of HIV/neuroAIDS. Considering the variable specificity of each biomarker, a combinational approach in clinical implementation of multiple HIV/neuroAIDS biomarkers is expected to improve the detection, staging, and therapeutic monitoring of HIV/neuroAIDS.

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List of Abbreviations

Aβ42	β-amyloid of 42 kiloDaltons
AD	Alzheimer’s disease

APP	amyloid precursor protein
ALS	(amyotrophic lateral sclerosis
BOLD	blood oxygen level-dependent contrast imaging
cART	combination anti-retroviral therapy
CBF	cerebral blood flow
CHO	choline
CNS	central nervous system
Cr	creatine
CSF	cerebrospinal fluid
CT	computerized tomography
CXCL-10	interferon gamma-induced protein 10
DTI	diffusion tensor imaging
FA	fractional anisotropy
fMRI	functional magnetic resonance imaging
GFAP	glial fibrillary acidic protein
GM	grey matter
HAD	HIV-associated dementia
HAND	HIV-associated neurocognitive disorders
HIV	human immune deficiency virus
HIVE	HIV encephalitis
IF	intermediate filament
IFN-γ	interferon γ
IL-1β	interleukin-1β
IL-6	interleukin-6
IL-8	interleukin-8
IL-10	interleukin-10
LPS	lipopolysaccharide
MCMD	mild cognitive and motor disorder
MCP-1	monocyte chemo-attractant protein-1

MD	mean diffusion
MI	myoinositol
MIP-1	macrophage inflammatory protein
miRNA	microRNA
MRI	magnetic resonance imaging
MS	multiple sclerosis
MRS	magnetic resonance spectroscopy
MTR	magnetization transfer imaging
NAA	N-acetylaspartate
NF	neurofilament protein
PLA2	phospholipase A2
PLC	phospholipase C
PMBC	peripheral blood mononuclear cells
PPAR	peroxisome proliferator-activated receptors
PrPC	protease resistant protein, cellular isoform
p-Tau	hyperphosphorylated Tau protein
S100B	calcium binding protein B
sAPP	soluble amyloid precursor protein
sCD14	soluble CD14
sCD163	soluble CD163
SIV	simian immune deficiency virus
sPrPC	soluble protease resistant protein, cellular isoform
TNF-α	Tumor necrosis factor α
t-Tau	total Tau protein
WM	white matter
YKL-40	human cartilage glycoprotein 39

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Table 1

Cellular biomarkers for HIV/neuroAIDS

Biomarker	Detection Method	Strengths	Limitations	References
NFL	ELISA	-Levels are reliable reflection of disease progression or therapy -Differentiates primary, chronic, and neuroasymptomatic HIV -Correlates positively with plasma viral load and negatively with CD4 count	-Also elevated in other CNS disorders -Detectable only in mid-late phase of disease	Abdulle et al. 2007 Jessen Krut et al. 2014 McGuire et al. 2015
Neopterin	HPLC,ELISA	-Levels are reliable reflection of disease progression or therapy -Correlates positively with NFL levels -Reliable biomarker of macrophage and microglial activation	-Also elevated in other CNS Disorders -Detectable only in mid-late phase of disease	Griffin et al. 1991 Abdulle et al. 2007 Fuchs et al. 1988
SB100	ELISA	-Levels are reliable indicator of early damage to BBB and astrocyte activation -Correlates with severity of neurocognitive Impairment	-Also elevated in other CNS disorders	Rohlwink et al. 2014 Pemberton et al. 2001
sCD14	ELISA	-Correlates with severity of neurocognitive impairment -Correlates with neuroimaging biomarkers -Levels predict risk of death	-Also elevated in other CNS disorders	Lyons et al. 2011 Ryan et al. 2001 Sandler et al. 2011
YKL-40	ELISA	-Correlates with productive HIV infection of microglia and with acute neuroinflammation	- Also elevated in other CNS Disorders -Few human studies conducted	Kolson et al. 2008 Bonneh-Barkay et al. 2012
sCD163	ELISA	-Correlates with viral load in plasma and with monocyte expansion -Levels are reliable reflection of disease progression or therapy	-Detectable only in plasma - Also elevated in other CNS disorders	Burdo et al. 2011 Burdo et al. 2013
PrPC	ELISA	-Levels correlate with CNS compromise and with monocyte influx into CNS	- Also elevated in other CNS -Does not correlate with viral load or CD4 count	Roberts et al. 2010 Megra et al. 2013
Tau	ELISA	-Correlates with severity of neurocognitive impairment	-Also elevated in other CNS disorders -Results not consistent between groups	Peterson et al. 2014 Gisslen et al. 2009
GFAP	ELISA	-Correlates with astrocytosis	-Also elevated in other CNS disorders -Few studies conducted	Norenberg 1998 Andersson et al. 2006

Table 2

Immune activation biomarkers for HIV/neuroAIDS

Biomarker	Detection Method	Strengths	Limitations	References
MCP-1	ELISA	-Correlates with Monocyte infiltration and with severity of cognitive impairment -Correlates with Neopterin levels and CSF viral load -Can be used for cART monitoring	-Also elevated in other CNS disorders	Thames et al. 2015 Price et al. 2007 Tiraboschi et al. 2015
IL-1β, IL-6, TNF-α CXCL10	ELISA	-Can be used to monitor infiltration of HIV infected cells into the CNS -Correlate with early stage of disease prior to astrocyte activation	-Also elevated in other CNS disorders -Do not correlate with cognitive status	Hong et al. 2015 Kamat et al. 2012
IFN-γ	ELISA	-Correlates with CD8 T cell transmigration into CNS -Associated with increased risk of cognitive impairment	-Also elevated in other CNS disorders	Schrier et al. 2015

Table 3

Lipid biomarkers for HIV/neuroAIDS

Biomarker	Detection Method	Strengths	Limitations	References
Ceramide, GD3 2004	Immunoblotting Immunostaining	-Correlate with more severe cognitive impairment in late stages of HAND -Associated with activation of microglia and astrocytes -Accumulation of multiple species indicator of worsening cognitive status	-Also elevated in other CNS disorders -Only detectable in late stages	Haughey et al. Andersson et al. 1998 Bandaru et al. 2013
C52, Vitamin E 2007 Sphingolipid	Mass Spectrometry	-Correlate with stage of cognitive impairment -Correlates with earlier stage of disease and with moderate cognitive dysfunction	-Also elevated in other CNS disorders	Bandaru et al.

Table 4

MicroRNA biomarkers for HIV/neuroAIDS

Group	Sample Source	Subjects	Findings	Identical expression signatures
Zhou et al. 2012	Frontal cortex tissue	HIV patients with or without HAD	68 differentially expressed miRNA in HAD with potential involvement in axon guidance and neurodegeneration process	None
Noorbakhsh et al. 2010	Brain tissue	HIV patients with encephalitis	34 differentially expressed miRNA in HIV encephalitis with potential involvement in inflammation, nucleotide metabolism, cell cycle control, cell death, and resting state of glial cells	
Kadri et al. 2015 Plasma		HIV patients with or without cognitive impairment	30 differentially expressed miRNA in HIV encephalitis, 10 miRNA confirmed to distinguish cognititve impairment in HIV were involved in BDNF regulation, neurogenesis synaptic regulation, and maitenance of white matter	miR-495: involved in BDNF regulation miR-744: Involved in BDNF regulation
Pacifici et al. 2013	Frontal cortex tissue and CSF	HIV patients with or without neurocognitive impairment	66 differentially expressed miRNA in HIV encephalitis, 11 miRNA were identified as statistically significant	miR-16: involved in neuronal differentiation miR-92: Involved in neuronal differentiation miR-139: Involved in neuronal apoptosis miR-19: involved in neurodegeneration
			significant in encephalitis with involvement in cytoskeletal remodeling, cell adhesion, chemokine regulation and neurogenesis	

Table 5

Neuroimaging biomarkers for HIV/neuroAIDS

Biomarker	Detection Method	Strengths	Limitations	References
NAA,CHO,MI,Cr	MRS	-Detects glial activation (early phase of neuroAIDS)		Wu et al. 2015
		and neuronal damage (late phase of neuroAIDS)		Holt et al. 2012
		-Detects severity of neurocognitive impairment		Chang et al. 2004
		and BBB permeability		Steinbrink et al. 2013
		-Can be used for cART monitoring		
		-Can differentiate neuroAIDS from other CNS disorders and normal assaults of aging	Cost Lack of availability in clinical settings	
FA,MD	DTI	-Detects brain structure changes in neuroasymptomatic HIV patients -Can differentiate neuroAIDS from normal assaults of aging		Pomara et al. 2001 Gongvatana et al. 2011
HHb	fMRI(BOLD)	-Detects brain structure changes in neuroasymptomatic HIV patients -Can differentiate neuroAIDS from normal assaults of aging		Ernst et al. 2002 Ances et al. 2010