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Biomarkers for neuroAIDS: Recent Progress in the Field

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In June, 2012, we held our second annual colloquium at the University of Nebraska Medical Center on current research and clinical issues concerning the effects of HIV on the brain. This day-long event focused on “Biomarkers for NeuroAIDS: Recent Progress in the Field.” The talks, questions, and subsequent discussions were quite illuminating not only for progress, but to point out gaps in our knowledge as well as existing controversies. To help further advance efforts in disseminating information and spurring further work addressing issues raised, in conjunction with the Journal of Neuroimmune Pharmacology (and special thanks to the Managing Editor Robin Taylor and Editor-in-Chief Dr. Howard Gendelman), we are proud to publish a series of papers in this issue centering on biomarkers for neuroAIDS.

As the readers of this journal know, current treatments for HIV infection (combination antiretroviral treatment, cART) have dramatically decreased most of the complications due to HIV including HIV-associated dementia, the most severe form of neuroAIDS. Unfortunately, for those whom treatments are unavailable, brain disease caused by HIV itself as well as caused by secondary central nervous system opportunistic infections still afflict infected people. As HIV infection is more common in less developed countries, neuroAIDS still remains a significant global health issue, and, although less severe, impacts those with in developed countries as well.

HIV dementia and a wider spectrum of less severe neurocognitive states have now been classified under the umbrella designation HIV-associated neurocognitive disorder (HAND) (Antinori et al., 2007). Although the most severe form of HAND, HIV-associated dementia, has declined, the milder forms of HAND predominate in treated individuals. Given the chronicity of disease and the fact that infected individuals living longer, the overall prevalence of HAND is substantial (Heaton et al., 2011). Whereas in the pre-cART era many pathogenic factors contributing to HAND have been characterized (predominately inflammatory factors in HIV-associated dementia), it is unclear if the etiopathogenesis of HAND will be similar in those individuals in which cART is used, and the deficits more subtle. Both the virus and inflammation dominated the findings in the prior studies; now, with treatment, both appear reduced. Neuropathologically, while HIV encephalitis could be found in a high proportion of patients with HIV-associated dementia in the era before treatment, currently no distinct neuropathology of HAND can be identified (Everall et al., 2009).

Could the link between prior-to and cART eras be something as simple as “area under the curve,” *i.e.* early in the HIV pandemic, before treatment was available, those with CNS disorders had high levels of virus and inflammation in the brain for a relatively short period of time, whereas treatment has transformed this to low levels of virus and persistent low levels of inflammation over a now prolonged period of time? Or are factors driving HAND distinct, potentially including negative effects of the life-saving drugs used during treatment regimens? Control subjects with long-term use of cART simply do not exist; similarly many

of the comorbid factors often associated with HIV infection, such as substance abuse and infections with other agents, can be similarly difficult to control. Fortunately well-planned studies and generous participation of the community of HIV infected people are enabling researchers to make headway in this effort.

Biomarkers can be extremely helpful not only in correlation to disease, and thus aid in clinical diagnosis and serve as a surrogate endpoint in clinical studies. They may also perhaps point to key mechanistic pathways, spurring efforts on prevention and treatment. In this issue we present ten original studies, reviews and perspectives exemplifying the current active work in the biomarker field.

One difficulty in both clinical and research aspects of the effects of HIV on the brain concerns the ability to identify those with the now milder forms of HAND in a typical clinical setting, which at least here in the US is increasing limiting as far as clinicians' time, restricting the amount of specific testing that can be performed. In an original cross-sectional study, Cross *et al.* (this issue) successfully utilized the International HIV Dementia Scale in an outpatient, urban setting, finding a high prevalence of HAND (>40%). Multivariate analysis identified a number of risk factors for HAND including socio-demographic factors. As pointed out by the authors such studies need to be performed in matched HIV negative subjects as well as larger overall cohorts to assess the utility of this potentially useful instrument.

Objective laboratory values are thought of by many as ideal biomarkers. Given the limitations in sampling the central nervous system, utilizing measures from the blood would be a distinct advantage. Cassol *et al.* (this issue) present an informative perspective concerning inflammatory biomarkers in plasma for studies on HAND. A thorough review of findings, critical caveats, and an exploratory study are described, with a wealth of information on study design, data analysis, and potential for integration of additional information in carrying out biomarker studies. In this light, a unique approach was taken in an original research study by Marcotte *et al.* (this issue) to identify plasma biomarkers, examining four groups of infected patients, classified by both the presence of neurocognitive impairment and whether there was a change in classification at a subsequent visit. Using recursive partitioning, finding patterns of inflammatory biomarkers for diagnostic (predicting impairment at the first visit) purposes was difficult. However, utilizing biomarkers for prognostic (change in impairment state) required only two or three markers for many of the groups. Marcotte and colleagues' work now provides a limited set of analytes that can be tested and used in subsequent studies.

Many studies have examined cerebrospinal fluid (CSF) for biomarkers for HAND as well as a myriad of other neurodegenerative diseases due to its proximity to the site of pathology. The study by Fields *et al.* (this issue) presents original research findings on a set of vesicular transport proteins (chosen since they are important in HIV replication) measured in the CSF as well as within the brain in HAND. In addition an inflammatory (interferon-stimulated) marker, ISG-15, and HIV viral load were studied for correlation to HAND. Indeed correlations and trends were identified, indicating that combining such makers may aid in the development of HAND biomarkers in the CSF. As a nice correlate, brain tissue itself was examined, revealing increased expression of some of these markers in neurons as well as astrocytes. Also examining brain tissue was the original study of Tatro *et al.* (this issue) in which microRNA profiling was performed in a distinct class of HIV infected individuals with a comorbid condition, abuse of methamphetamine. A neuron-specific microRNA, miR-9, was found to be elevated in the frontal cortex of the HIV infected methamphetamine abusers. In vitro models found evidence consistent with miR-9 induction by HIV and methamphetamine, and identified a specific splice variant of a component of the potassium

channel as a target of miR-9, which was then verified in brain tissue as being reduced. This study provides a mechanism by which the combination of methamphetamine and HIV could affect neuronal physiology. While not examined, it would be interesting to assess the level of miR-9 in the CSF of such individuals compared to controls.

Three reviews then examine the progress, challenges, and future perspectives for biomarker studies on CSF. Haughey *et al.* (this issue) discuss an important aspect of the “omics:” lipidomics, an oft-ignored subject as it departs from the typical triad of DNA to RNA to protein. Given their abundance and importance in the brain, structurally, metabolically, and in signaling, lipids represent a rich source of potential biomarkers. Studies on sphingolipids and ceramide, and importantly their links to crucial neurobiological pathways and mechanisms are linked to intriguing hypotheses on therapeutics for HAND in this article. With the rise of the “omics” continued debate of hypothesis-driven versus exploratory scientific investigation intensified. However, one must remember that in the formulation of the scientific method one of the key methods in using hypotheses is to create them to explain observations. What better way to observe than to perform an “omics” experiment? Regardless, Price *et al.* (this issue) describe both targeted, hypothesis-driven, and proteomic untargeted exploration of the CSF in HAND. A nice framework is presented upon which studies can be based, including the choice of biofluid for studies, with the advantages and disadvantages of CSF, and issues on interpretation. Furthermore, as also pointed out by other studies in this issue, expansion of the molecules examined from just the typical inflammatory cytokine markers can be useful, as the phenotype of immune cells themselves, and other molecules such as neopterin (a non-protein inflammatory marker) and even proteins derived from injured neurons can lead to important information in targeted studies. Approaches to untargeted studies are then illustrated through a proteomics experiment on CSF. The third paper on CSF is from Megra *et al.* (this issue) and describes studies on a quite intriguing molecule, PrP^c, the cellular (non-pathogenic isoform) of the prion protein. These investigators have found, in both the SIV model and in humans with HAND, increased PrP^c in the brain as well as the CSF. The cellular biology and physiological functions of PrP^c are discussed relative to how this relates to neuronal damage in HAND, and in particular its dual potential damaging and protective roles. In conjunction with others’ studies, PrP^c may link parts of the etiopathogenesis of HAND to other neurodegenerative disorders, in addition to being one of the potential CSF biomarkers in study panels for HAND.

Another approach to studying specific biomarker molecules is to examine analytes that result from mechanisms linked to the disease process itself. Uzasci *et al.* (this issue) discuss strategies to examine modified proteins, specifically by oxidation and nitrosylation, two reactive species found in HIV infected brain. Oxidative stress has long been implicated in a number of neurodegenerative conditions as well as HAND. The mechanisms by which reactive species are produced, how they modify proteins, and importantly how such modifications are detected by mass spectrometry are described. This paper explains the flow from discovery to validation of these disease-linked modifications as biomarkers in HAND. Related to this approach Epstein *et al.* (this issue) present a multi-faceted original study focusing on metabolites, the end products of normal and abnormal cellular physiology. As such, metabolites can represent excellent biomarkers, and in fact are well utilized in clinical medicine. However measurement of metabolites in the brain, and linking these to specific regions and neuropathological processes, presents significant challenges. Epstein and colleagues address this through the presentation of a series of experiments in mice. Combining a distinctive tissue preservation technique, proton magnetic resonance spectroscopy, mass spectroscopy, and immunohistochemistry their studies reveal methods to enable the accurate measurements of key metabolites in the brain. Further they expand these studies to two models for HAND, including one utilizing treatment with a nanomedicine

approach to antiretrovirals, enabling long-term treatment. Correlation of neuropathology and metabolites with the viral responses revealed the strength of these methods and their ability to be utilized to identify metabolic biomarkers for HAND.

These papers will serve as an excellent source of knowledge and data to those examining biomarkers for HAND, as well as other neurodegenerative disorders. We look forward to exciting studies following up on these as we move forward to try to understand, prevent, and treat the neurological complications of HIV.

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