12-14-2013

Diffusion Tensor Imaging in HIV and hepatitis C coinfection

Jodi Michelle Heaps
University of Missouri-St. Louis

Diffusion Tensor Imaging in Human Immunodeficiency Virus and Hepatitis C co-infection

by

JODI M. HEAPS
M.A. Psychology
B.A. Psychology

A DISSERTATION

Submitted to the Graduate School of the

UNIVERSITY OF MISSOURI- ST. LOUIS
In partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

in

PSYCHOLOGY
with an emphasis in Behavioral Neuroscience

December, 2012

Advisory Committee

Robert Paul, Ph.D. -Advisor
George Taylor, Ph.D.
Michael Griffin, Ph.D.
Beau Ances, M.D., Ph.D.

Abstract

Previous studies have demonstrated that infection with both human immune deficiency virus (HIV) and hepatitis C (HCV) is associated with impaired cognitive function. It is unclear whether co-infection is associated with neuroimaging markers of brain dysfunction. The purpose of the present study was to compare HIV+ individuals, HIV/HCV+ individuals, and seronegative controls using diffusion tensor imaging (DTI) to assess the microstructural integrity of white matter tissue.

Methods: Twenty-five HIV+ patients, 25 HIV/HCV+ patients, and 25 seronegative controls matched for age were included in the study. All participants completed an MRI session, neuropsychological testing, and an evaluation of clinical variables including liver health. White matter regions of interest (ROI) were determined using a semi-automatic method based on individual anatomy. DTI metrics including mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), and fractional anisotropy (FA) were compared across groups using ANOVA. A regression model including DTI metrics, an index of liver function, and self-reported physical and mental health was performed to determine the relationship between those variables and cognitive performance in the co-infected group.

Results: The co-infected group was similar to the mono-infected group in terms of HIV clinical variables. None of the participants met criteria for cirrhosis or fibrosis on the index of liver function. There were no differences between groups on DTI metrics in the frontal ROI. In the anterior corpus callosum there was a significant difference between the HIV+ groups compared to controls with both patient groups having lower FA values. Additionally, the HIV/HCV+ group had significantly higher RD compared to controls in the corpus callosum, particularly in the anterior sections. Increased RD in the corpus callosum was associated with performance on executive function/working memory measures only at a trend level ($p=0.07$) in the co-infected group.

Conclusions: Co-infection with HIV and HCV may result in significant alterations in white matter structural integrity as measured by DTI, especially in the anterior corpus callosum. The effect of

HIV/HCV co-infection was greater than the effect of HIV mono-infection compared to controls in all regions sampled. These results suggest that the combined effects of the viruses in the brain result in compromised white matter microstructural integrity.

Diffusion Tensor Imaging in Human Immunodeficiency Virus and Hepatitis C co-infection

Human immunodeficiency virus (HIV) and hepatitis C virus (HCV) share similar routes of transmission, most commonly through intravenous drug use or sexual contact. Approximately 1/3 of HIV patients are co-infected with HCV (Parsons et al., 2006) and it is believed that as many as 90% of HIV infected patients, who acquired the disease through intravenous drug use, may be co-infected with HCV. Liver disease has become the most common cause of death in HIV (Morgello et al., 2005) largely due to the number of co-infected individuals (Clifford, Evans, Yang, & Gulick, 2005).

The progression from fibrosis to cirrhosis of the liver is due to chronic inflammatory processes that are often magnified by factors such as HIV, other viruses, metabolic disorders and alcohol consumption. A recent meta-analysis by Thein and colleagues (Thein, Yi, Dore, & Krahn, 2008) reported that co-infection of HIV/HCV increased the risk to develop cirrhosis by a factor of two compared to an HCV mono-infected population. Higher levels of HCV-RNA have been found in co-infected individuals and increased inflammation of the liver and progression to end-stage liver disease is more common in co-infection than HCV mono-infection (Blackard & Sherman, 2008).

Both hepatitis C and HIV are each known to be associated with cognitive deficits (Forton et al, 2004; Hilsabeck et al, 2002; McAndrews et al, 2005). It is important to determine if the combined effects of HIV and HCV on the integrity of the brain are different from either infection alone, particularly given the high prevalence of co-infection and the potential additive/synergist impact on health outcomes. The primary purpose of this study is to determine the neuropsychological and neuroimaging impact of HIV/HCV co-infection using diffusion tensor imaging (DTI). I hypothesized that co-infected individuals would exhibit significantly abnormal DTI values in frontal white matter and the corpus callosum compared to HIV mono-infected individuals and controls. Additionally, I hypothesized that DTI variables would predict a larger percentage of the variance in cognitive performance than substance abuse, liver function, or self-reported physical and mental health.

Cognition in HIV

In the early years of HIV, AIDS dementia complex was a common diagnosis, affecting approximately 30% HIV patients (Heaton et al, 2011). Dementia due to HIV was considered an AIDS-defining illness and a cause of mortality in many patients. Since the advent of highly active antiretroviral therapy (HAART) the incidence of dementia has been reduced, but approximately 30-50% of patients continue to experience milder forms of cognitive impairment (Heaton et al, 2011). Practically, HAART has been very effective at restoring immune function, but have limited penetration into the central nervous system (CNS; Letendre et al 2005) and thus the brain may remain a reservoir for reproductive virus. In 2007 (Antinori et al., 2007), diagnostic criteria for identifying HIV-associated neurocognitive disorders (HAND) was developed, resulting in three primary categories of impairment: 1) HAD- HIV-associated dementia, 2) MND- mild neurocognitive disorder, or 3) ANI- asymptomatic neurocognitive impairment. In order to receive a diagnosis of HAND an individual must undergo neuropsychological testing in at least seven cognitive domains of function, score below local norms on at least 2 of the cognitive domains, and have an assessment of

activities of daily life. For a diagnosis of HAD an individual must score greater than two standard deviations (SD) below local norms in 2 cognitive domains and demonstrate marked impairment in daily activities. The diagnosis of MND requires an individual to score more than 1 SD below the mean on two domains, and have mild impairment in daily activities. An individual who receives the diagnosis of ANI would score 1 SD below normal in 2 of the cognitive domains, but demonstrate no impairment in daily activities. In all cases there cannot be any other explanation for the cognitive impairment, or a preexisting condition that may explain the cognitive deficit.

In general, HIV-associated neurocognitive disorders are characterized by a pattern of deficits commonly observed in other conditions with disruption of frontal-subcortical networks. These deficits include impairments in the domains of attention, learning, psychomotor speed, working memory, and executive functions. There has been evidence that HIV is present in higher concentrations in subcortical regions (Brew, 1995), and many neuroimaging studies report alterations in the basal ganglia and frontal white matter (For review see Tucker et al, 2004; see Paul, Cohen, Navia, Tashima, 2002;.. This is not to say that HIV only affects these brain regions. The alterations reported in white matter have a diffuse pattern and there have been a few studies to indicate alterations in parietal (Thompson, 2005),temporal regions (McArthur et al, 2005, Kuper et al., 2011), as well as cortical gray matter (Cohen et al 2010, Kallianpur et al 2012, Kuper, Togwood et al 2012;, Cohen 2010).

Viral factors

HIV does not directly infect neurons. Rather, immune cells such as T-lymphocytes (CD4 cells) and monocyte-derived macrophages (MDM) are the primary targets of the HIV virus (Nath, 2010). Once HIV enters the body it can attach to receptors on CD4 cells and through endocytosis may enter the cell. Once released into the cell, the viral envelope breaks apart, releasing viral particles, proteins, and HIV-RNA. This RNA is reverse-transcribed into DNA that is then inserted

into the cells of the host genome, referred to as proviral DNA. As the virus replicates, the host cell (usually a CD4 T lymphocyte) is weakened or destroyed. MDMs are less susceptible to the viral toxins and can provide a long-term sanctuary enabling continuous viral production over the life of these cells (Meltzer, et al., 1990). HIV infection may exist in a host for years without before an individual develops symptoms serious enough to see a physician... Over this time the number of CD4 cells in the body diminishes.

HIV is thought to enter the CNS in MDMs. Once inside the CNS these MDMs can produce substances that affect the integrity of the blood brain barrier (BBB). In the brain, there are few CD4 cells; however HIV infects and replicates in MDMs and microglia in the brain. Other support cells in the brain, such as astrocytes, respond to the infiltration by releasing neurotoxins, pro-inflammatory cytokines, and reactive oxygen species that can lead to disruption in neurotransmitter activity and ultimately, neuronal death (Lindl, Marks, Kolson, and Jordan-Sciutto, 2010). Astrocytes may be infected, though they are not centers of active HIV replication. However, these critical support cells are often affected by the free radicals and inflammatory environment in a manner that disrupts their normal physiological activity and interaction with neurons (Lindl et al 2010).

Viral proteins and particles are able to directly affect neuronal function and have been shown to induce neuronal apoptosis (Strazza, 2011). Viral proteins commonly associated with neuronal apoptosis are gp120, gp41, Tat, Nef, and Vpr (Grovit-Ferbas and Harris-White, 2010; Jayadev and Garden, 2009). The presence of these particles is associated with excitotoxicity that leads to neuronal death. Indirectly, these proteins may also lead to the production of other molecules that induce neuronal apoptosis. If the overall HIV burden can be minimized there should be fewer of these viral proteins and by-products in the CNS, resulting in improved neuronal function (Ellis, et al. 2000; Epstein and Gelbard, 1999).

Measurements of CD4, Viral load, and proviral DNA provide clinicians with information about an individual's HIV burden, and they can provide an indication about risk for HAND. Prior to

the introduction of HAART, CD4 count was commonly associated with cognitive status (Heaton et al., 2011); however, more recent studies have shown that current CD4 levels are not a reliable indicator of cognitive status (Ances, 2011; Cysique, 2010; Heaton et al., 2011; Robertson 2010). Nadir CD4, the lowest recorded CD4 level, is often associated with HAND with nadir CD4 count (<250cells/μL) relating to increased risk for cognitive impairment (Jernigan et al, 2011). Prior to HAART, plasma viral load correlated with poor cognitive status in many studies (Chang et al, 2002; McArthur, 1997; Robertson, 1998; Sactor et al 2003). Individuals who adhere to their medication regimen may not have a detectable plasma viral load, but still demonstrate cognitive impairment (Robertson et al., 2007), which is why current viral load is not the best predictor of cognitive impairment and does not provide information about latent virus in other areas of the body.

Antiretroviral therapy

Although HAART has greatly reduced the burden of HIV in the periphery, there may still be lingering virus in the CNS. The incidence of HAD in the HIV-infected population has dramatically decreased; however, the number of people with more minor forms of cognitive dysfunction have increased (Woods, Moore, Weber, and Grant, 2009). A number of anti-retroviral therapies do not easily cross the blood brain barrier. Letendre (2008) indexed the most common ART therapies to develop a rating scale of CNS penetration referred to as the central penetration effectiveness (CPE) score, to determine the impact of HAART penetration on cognitive status. Studies using the CPE have generally reported improvements in brain function with greater penetration, but some data also indicate that higher penetration may be toxic to neural integrity, possibly via drug-induced mitochondrial damage. (refs (Anthony, 2009). A drug holiday—a period of time during which no HIV meds are taken—could be beneficial, resulting in improvements in cognitive functioning (Robertson, 2010).

Because HAART has systemic effects that are unfavorable to a number of body systems over long periods of time the treatment is often not initiated until a person's CD4 count drops below 350

cells/μL. Recent studies have prompted a change however, indicating that treatment should be started when an individual's CD4 count reaches 500 cells/μL (JAMA-Beau I am not finding this source). The time from initial infection until the point where CD4 reaches that level could be several years and, as previously stated, it is known that the virus is present in the brain even at very early stages of infection. The lag time between infection and treatment may result in extended inflammatory effects that take place in the brain, leading to more severe impairments.

There is a well-documented reciprocal relationship between cognitive functioning and adherence to HIV medication. Individuals who do not strictly adhere to their regimen are more likely to have cognitive deficits (Catz 2000; Hinkin 2004, McPhail and Robertson, 2012). Further, prospective studies have demonstrated that individuals with poor cognitive function also exhibit poor adherence to the medication regimen, with deficits in executive function serving as the strongest predictor of poor adherence (Ettenhofer, Foley, Castellon, Hinkin, 2010). Inconsistent use of medications can also lead to drug resistance and mutations in HIV that are less responsive to the current therapies.

Substance use

It is common for individuals who are HIV+ to have a history of drug use, abuse, and dependence. A number of studies have attempted to determine the specific interaction of a given drug and HIV on cognition; however, given that most drug abusers are polysubstance users this has been a difficult task. Furthermore, it is important to assess the drug use history of current or past users to determine the individual or potentially additive effects of drug use on cognition in an HIV population. Below is a brief review of drugs commonly associated with HIV.

Cocaine-

Cocaine is associated with a number of factors that may lead to an increased risk of developing HAND. Cocaine has been implicated in affecting the permeability of the BBB, allowing the entry of virus and viral particles into the CNS that otherwise may not be able to pass. Studies

have shown that cocaine potentiates HIV replication in a number of cell types, and upregulates receptors to facilitate HIV infection in those cells (Buch, Yao, Guo, Mori, Su, and Wang, 2011). Additionally, cocaine potentiates HIV protein effects on glial cell activation and may increase the cytotoxic effects of gp120 and Tat on neuronal cells (Buch et al., 2011).

Methamphetamine-

The use of methamphetamine (meth) is associated with patterns of neurocognitive impairment similar to those seen in HIV+ patients, with seronegative addicts showing poor performance on measures of executive function, episodic memory, and psychomotor skills (Kalechstein et al., 2003, Scott et al., 2007). Neuroimaging and animal studies show an effect of meth in HIV- individuals on striatal and fronto-cortical dopamine transporters and activation in the striatum and parietal lobe, especially with NMDA receptors (Volkow, 2001). Use of meth is associated with depletion of striatal DA and diminished metabolism in striatal regions Chang, Alicata, Ernst, Volkow, 2004; Nordahl, 2005). Since HIV is associated with fronto-striatal alterations, reduced DA activity and similar cognitive deficits, it is possible that HIV and meth use would result in additive neuronal dysfunction in those regions. At least one study (Archibald et al., 2011) indicates that meth and HIV might have opposing effects on these regions, such that the co-morbidity does not have an additive effect, but rather mitigates the effect of HIV or meth in these brain areas. In the context of HIV, neuroimaging studies indicate that meth use is associated with reduced cerebral blood flow (Ances et al., 2011) and abnormalities indicating glial activation and neuronal distress Chang, Ernst, Speck, and Grob, 2005). Furthermore, studies indicate that meth may affect viral replication specifically in the CNS. For example, in a simian model of HIV, administration of methamphetamine resulted in an increase in viral load in the brain, without a congruent increase in plasma viral load (Marcondes 2010).

Heroin-

Heroin is associated with higher impulsivity (Pau, Lee, and Chan, 2002) that may persist despite prolonged abstinence (Fu et al., 2008). Studies in HIV+ heroin users indicate similar patterns of impaired impulse control (Martin et al, 2004). Many studies report that heroin users are less likely to adhere to their antiretroviral regimen (Cohen et al., 2004). Additionally there may be interactions between HAART and heroin, or methadone, resulting in altered levels of either HAART or methadone, depending on the types of antiretrovirals in the combination therapy (Faragon, 2003). It is common for these interactions to result in undesirable side effects that may further impact adherence to antiretroviral therapy (Cohen et al., 2004) and increase the risk of HAND.

Smoking/Nicotine-

Smoking is prevalent in the HIV population and has been shown to negate some of the benefits of antiretroviral therapy, particularly in women (Feldman et al 2006). At least one study has shown that smoking also is predictive of non-adherence, which may further reduce the benefits of ART (Webb, Vanable, Carey, Blair, 2009). A systemic review of the literature revealed that smoking is not associated with HIV disease progression (Furber 2006).

Cognition and HCV

HCV is a chronic disease in the majority of cases (Pawlotsky, 2004) and has been found to result in cognitive deficits. As noted above, the hepatitis virus attacks the liver, and in chronic infection, liver damage progresses to fibrosis and cirrhosis. Impaired liver function has been associated with a number of cognitive deficits among HCV-infected patients as well as other liver diseases (Collie, 2005). The most commonly reported cognitive deficits in HCV patients are in the areas of attention (Forton et al., 2002; Hilsabeck, Hassanein, Carlson, Ziegler, & Perry, 2003; Weissenborn, Bokemeyer, Krause, Ennen, & Ahl, 2005), working memory (Fontana et al., 2007; Forton et al., 2002), learning (Hilsabeck et al., 2003; McAndrews et al., 2005) and reaction time (Hilsabeck et al., 2003). Interestingly, while many of these patients exhibit significant liver disease, a current review of cognitive impairments in persons with HCV mono-infection revealed that

deficits on neuropsychological examinations have been found even in the absence of fibrosis or cirrhosis (Perry, Hilsabeck, & Hassanein, 2008).

In addition to liver disease, patients with HCV have a number of other common comorbid conditions. These are similar to HIV comorbidities and include substance abuse, depression, anxiety and fatigue. As noted above, these factors have also been associated with deficits in cognition independent of HCV, and could be considered confounds when examining the effect of the virus on cognitive function. However, several studies report no relationship between complaints of depression, anxiety and fatigue and cognitive dysfunction in this population (Fontana et al., 2007; Forton et al., 2002; Hilsabeck, Castellon, & Hinkin, 2005; Kramer et al., 2005; McAndrews et al., 2005; Weissenborn et al., 2005).

Studies of HCV mono-infection have reported cognitive deficits that were partially explained by history of substance use (Forton, Thomas, & Taylor-Robinson, 2004), while other studies have identified cognitive deficits in HCV patients without histories of intravenous drug use, or substance abuse (Perry et al., 2008). Further, Paul et al. (2007) reported no relationship between substance use based on frequency, duration, and chronicity of use and cognitive performance in a co-infected sample, suggesting that substance abuse may not fully explain the findings of greater cognitive impairment in this population

Cognition in HIV/HCV co-infection

A number of studies have revealed increased cognitive deficits among HIV/HCV co-infected individuals compared to HIV mono-infected individuals. A recent study using a comprehensive neuropsychological battery indicated that co-infection is related to poorer neuropsychological impairment across domains (Devlin et al., 2012). Co-infected subjects are more likely to exhibit global impairment than mono-infected subjects (Cherner et al., 2005; Letendre et al., 2005), slower reaction times (Martin et al., 2004; Perry et al., 2005; von Giesen et al., 2004), abstraction deficits

(Cherner et al., 2005), and attention and concentration difficulties (Perry et al., 2005). Murray et al. (2008) found a trend for lower scores in abstraction and executive function in a co-infected group compared to the mono-infected group. Likewise, Clifford et al. (2005) reported that HIV/HCV co-infected patients performed more poorly on tests of attention, motor skills, and visuomotor coordination compared to an HIV mono-infected group, even after controlling for possible confounds. One additional study revealed that both HCV mono-infected and HIV/HCV co-infected patients performed significantly poorer on tests of attention, visual scanning, and psychomotor speed, compared to normal controls; however, the difference in performance on the tasks between the HCV mono-infected group and the co-infected group was not significant (Perry et al., 2005).

Chang et al. (2008) reported impaired visual memory and fine motor skills among co-infected patients compared to HCV mono-infected patients. However, a recent study by Clifford et al. (2009) revealed no significant effect of HCV co-infection (in controlled HIV) on cognitive function when comparing co-infected individuals with a detectable plasma HCV viral load to mono and co-infected individuals with a non-detectable HCV viral load. However, HIV-positive individuals with a positive HCV titer performed more poorly than HIV-positive individuals without HCV antibodies on 2 of 3 neuropsychological tests administered (Trailmaking B and Digit Symbol Substitution test). These findings suggest that the effects of co-infection may not be due to active replication of HCV in the blood, but rather reflect chronic effects of HCV presence in the brain. It is also possible that among treated patients, there is minimal additional impact of co-infection on cognitive status. A recent study reviewing the effect of HIV/HCV coinfection did not report any increased neurocognitive impairment in a large cohort of women when controlling for other confounding factors (Crystal et al, 2012). It was unclear if the co-infected individuals in this study were undergoing treatment for HCV at the time of testing.

Neuroimaging

HIV has been associated with structural brain changes in both the pre- and post- HAART era. An early autopsy study examined the distribution of HIV infected cells in the brain (Brew, 1995) and reported that the areas most commonly infected were the basal ganglia, corpus callosum and frontal white matter. White matter was two to three times more likely to have infected cells than the cortex. This and other early autopsy studies (Hawkins, McLaughlin, Kendall, and McDonald, 1993; Kieburtz et al., 1990) verified MRI evidence of white matter alterations that are more pronounced in progressed disease.

DTI is a neuromaging method that is ideally suited to measure the structural integrity of white matter. Practically, DTI is a calculation of the rate of water diffusion along a gradient or neural tract (Correia et al., 2008). Axonal fibers restrict the random movement of water by restricting the flow of water along the fibers. Common metrics from DTI acquisitions include fractional anisotropy (FA) and mean diffusivity (MD). FA measures the amount of diffusion that is anisotropic, or directional, compared to the diffusion that is isotropic or random and MD is a measure of total diffusion occurring in a given voxel (Basser & Pierpaoli, 1996). More recently, measures of axial diffusivity (AD) and radial diffusivity (RD) have been measured as they are believed to provide specific information about the integrity of either the axon itself, or the myelin surrounding the axons in white matter respectively (Song et al. 2002). Specifically, AD is the sum of diffusion values along the longitudinal plane, i.e. along the primary eigenvector, while RD is the sum of the diffusion values along the shorter axis, running perpendicular to the primary eigenvector. Pathological factors such as disease, trauma and inflammation are thought to impact the integrity and organization of white matter fibers (WMF). When the microstructure of WMF is disrupted, the pattern of direct diffusion along their lengths is altered, which is expressed as a reduction in FA, and typically an increase in MD and potential changes to AD or RD.

DTI in HIV

DTI studies of individuals infected with HIV have reported significantly lower FA in the frontal lobe, genu of the corpus callosum, and putamen compared to healthy controls (Filippi, Ulug, Ryan, Ferrando, & van Gorp, 2001; Thurnher et al., 2005; Wu et al., 2006). In addition, studies have reported that DTI metrics correlate with cognitive performance among HIV patients. In 2004, Ragin et al. reported that decreased FA correlated with the degree of cognitive impairment in HIV patients (Ragin, Storey, Cohen, Epstein, & Edelman, 2004). The same group later reported that increased MD in the putamen correlated with verbal memory impairment while decreased FA in this structure was correlated with global cognitive impairment (Ragin et al., 2005). Decreased FA in both the caudate and putamen was related to visual and working memory impairments. Additionally, the researchers reported a significant negative correlation between FA measures in the centrum semiovale and impaired performance on a visuoconstruction task. Using the same cohort, Wu and colleagues (2006) reported decreased FA in the splenium and genu that correlated to motor deficits and visual memory impairments respectively. Collectively, these studies indicate that DTI is sensitive to white matter deficits among HIV patients.

Neuroimaging in HCV

There have been few studies using neuroimaging indices to study the impact of HCV mono-infection on cerebral integrity. Studies using magnetic resonance spectroscopy (MRS) have noted metabolite abnormalities in non-cirrhotic HCV patients (Forton et al., 2004). Weissenborn and colleagues (Weissenborn et al., 2004) reported decreased N-acetyl-aspartate (NAA), a marker of mature neurons, in a group of non-cirrhotic HCV patients that also exhibited deficits in attention. Further research has revealed decreased NAA in frontal white matter in a group of methamphetamine using HCV subjects, with a large percentage demonstrating global cognitive impairment. Other studies have reported increased choline, a vital component in cell membrane structure and function, and decreased NAA in central white matter, which did not show a relationship to fibrosis (McAndrews et al., 2005). Similar metabolite abnormalities have been

reported among patients with HIV mono-infection. Importantly, no prior studies have been designed to examine neuriomaging abnormalities among patients with HIV/HCV co-infection.

Neuroimaging Studies of HIV-HCV Co-Infection

A few studies have examined co-infection as a risk factor for neuroimaging abnormalities (e.g. Jernigan et al., 2011, Gongvantana et al, 2011). A recent study revelaed that co-infected individuals who have acute HCV infection performed worse than seronegative controls on neurocognitive measures, and had indications of altered brain metabolites compared to HIV mono-infected patients (Winston et al, 2011). A recent study by Stebbins et al. (2007) utilized DTI to assess white matter integrity in a group of HIV positive patients compared to an HIV negative group. Whole brain FA and MD did not differ between the groups though both indices differed in specific brain regions. More important for the proposed study, secondary analyses revealed significantly increased whole-brain MD and a strong trend for reduced whole-brain FA in a small group of HIV/HCV co-infected patients (n= 11) enrolled in the study. The study was not sufficiently powered to detect group differences based on co-infection status, but the outcomes suggest that DTI is a robust index of brain abnormalities associated with co-infection.

In the present study DTI was employed to investigate differences in white matter integrity between HIV mono-infected and co-infected patients. Additionally, a neuropsychological battery was used to compare cognitive performance between the mono- and co-infected groups and to determine associations between cognitive performance and DTI indices.

Summary

The primary purpose of this study was to determine the neuropsychological and neuroimaging impact of HIV/hepatitis C (HCV) co-infection using DTI. It is estimated that almost a third of HIV-positive patients are co-infected with HCV. Most studies examining cognitive function in co-infected groups report worse performance on neuropsychological examinations in a number of cognitive domains compared to groups with HIV or HCV alone (Cherner et al., 2005; Clifford et al.,

2005; Hilsabeck et al., 2005; Letendre et al., 2005; Martin et al., 2004; Parsons et al., 2006; Ryan, Morgello, Isaacs, Naseer, & Gerits, 2004; von Giesen et al., 2004). Additionally, it has been reported that co-infected persons are significantly more likely to develop AIDS dementia complex (Ryan et al., 2004). However, a recent study of patients with controlled HIV and active or inactive HCV viral replication indicates that patients who have active HCV viral replication exhibit no added impairment when examined using a brief battery of tests (Clifford et al., 2009). Despite the findings of decreased cognitive functioning in co-infection, to date, there are no published reports using neuroimaging specifically designed to study the impact of co-infection in the brain. There are a few published studies that utilized MRS in HCV that identified markers associated with the breakdown of white matter integrity (Weissenborn et al., 2004). These studies suggest the effect of HCV on neuronal integrity as seen by MRS is similar to the effects of HIV on white matter integrity. Currently there are a limited number of studies using DTI in HIV, with findings indicating white matter compromise, even in regions of "normal appearing" white matter (Stebbins et al., 2007). The neuropsychological deficits evident in co-infection and the similar findings in HCV using MRS prompt additional research specifically designed to examine the effects of co-infection on the brain. DTI is an excellent tool to examine co-infection in the brain, due to its specific sensitivity to detect changes in white matter structural integrity.

Methods

Participants

A total of 75 patients (25 HIV+, 25 HIV/HCV+, 25 HIV- controls) were included in the study. A total of 25 HIV/HCV+, and 25 HIV+ individuals were recruited for the purpose of examining cognitive and neuroimaging differences between HIV+ and HIV/HCV+ individuals with a previous history of recreational drug use, preferably intraveneous drug use (IDU). In the HIV+ group, data for 10 of the 25 HIV+ individuals included in the study were extracted from an archival dataset with similar inclusion/exclusion criteria although prior drug use information and similar

neuropsychological measures were not available for all of these individuals. Twenty-five controls were selected from archival data and matched as closely as possible for age and education to the HIV/HCV+ population. Detailed drug use histories were available for approximately half of all the controls chosen.

All participants were initially recruited by referral from coordinators involved in ongoing HIV studies at Washington University, or by clinic nurses at either the Washington University ACTG clinic or the liver clinic at Barnes Jewish Hospital in St. Louis. Coordinators and clinic nurses at both locations identified patients who met the inclusion criteria for the study by reviewing medical histories and performing an initial screening. Interested patients were provided an informed consent form to review and were asked if they could be contacted about participation in the study. Each participant was individually selected and approached. Participants were paid 30 dollars for completing the neuropsychological assessments, and an additional 70 dollars for the MRI.

Inclusion/Exclusion

Participants ranged from 21-61 years of age and the HIV+ individuals were required to be on stable antiretroviral therapy for HIV. HIV/HCV+ individuals had to be naïve to treatment for HCV or discontinued HCV treatment early due to negative side effects Three of the HIV/HCV+ individuals had attempted HCV therapy more than one year before the study, but discontinued due to negative side effects of the medication.

All infected participants recruited had a previous history of drug use, including injection drug use (IDU) but were not actively using drugs at the time of testing. History of alcohol abuse (defined by DSM-IV criteria) in the last 6 months was exclusionary; however, alcohol use not meeting criteria for abuse was allowed. Participants with a history of mental disorders such as schizophrenia or bipolar disorder, and learning disability were not considered for the study. Presence of other potentially confounding neurological disorders such as multiple sclerosis, demyelinating diseases, and CNS conditions, a history of opportunistic CNS infections, or any loss of

consciousness lasting longer than 30 minutes resulted in exclusion from the study. Other conditions that resulted in exclusion from the study were pregnancy, possibility of pregnancy (based on recent unprotected sexual activity), diabetes, history of ascites, encephalopathy, esophageal variceal bleeding, hepatorenal syndrome, or non-HCV liver disease. Individuals with a history of claustrophobia or metal exposure were excluded. Patients who have a confirmed HIV positive serostatus as determined using an HIV antibody test and confirmed with Western Blot analysis, are routinely referred to the ACTG clinic and are aware of their status before recruitment into the study. HCV status was confirmed using a HCV antibody test. As most of the patients are seen regularly at the clinic, HCV status was known for all participants. Markers of liver function were obtained through routine blood work within 30 days of their MRI and neuropsychological evaluation.

Study Visit:

Upon recruitment patients were briefly screened to ensure inclusion criteria were met. The study visit included informed consent, a rapid urine sample to screen for current drug use, and completed a brief demographic questionnaire. Participants were then escorted to their one-hour MRI appointment, followed by neuropsychological testing.

Neuropsychological evaluation- Co-infected group

Neuropsychological measures were administered uniformly in the co-infected group only. The majority of the HIV+ group and HIV- controls were administered minimal overlapping neuropsychological measures.

The Repeatable Battery for the assessment of neuropsychological status (RBANS) has been used previously to study cognitive impairment associated with liver disease (Mooney et al., 2007) and demonstrated sensitivity to detection of a subcortical pattern of impairment commonly seen in liver disease. Cognitive impairment in HIV and Hepatitis C (mono and co-infection) follows a

subcortical pattern of impairment similar to that seen in liver disease. As such, the neuropsychological battery consisted primarily of subtests from the RBANS

Simple Attention

Digit Span from the RBANS was administered to assess attention. The Digit Span task requires individuals to repeat a series of numbers (starting with 3 numbers and progressing to 9 numbers) in the manner it was recited to them. Number correct was the outcome measure. Additionally, Symbol Search from the WAIS III was also administered. Symbol search requires individuals to scan rows containing five different symbols and two target symbols. For each row an individual must determine whether or not one of two target symbols are included in the five symbols grouped in each row and presented adjacent to the targets. Each person has two minutes to scan as many rows as quickly as they can and indicate "yes" the target is included or "no" the target is not present. Number of correct responses is the outcome variable.

Psychomotor Speed

Digit Symbol from the WAIS III, Trail Making A, and Grooved Pegboard--dominant and non-dominant hand provided measures of psychomotor speed. Digit Symbol presents numbers associated with symbols that participants must then copy the symbol under the numbers presented in a series of rows. The test takes 90 seconds. Trails A is a pen and paper task where individuals are required to connect a series of numbers starting at 1 and ending at 25 around a page. Grooved Pegboard consists of a board with 25 holes that have a squared and a rounded side and pegs that are designed to fit in the holes. The holes rotate in rows and the participant must use only one hand at a time to manipulate the peg so that it fits properly into the hole. The time to completion was the outcome measure for Trail Making A and Grooved Pegboard. Number correct was used as the outcome measure for Digit Symbol.

Working memory/ Executive Function

Executive function was assessed using Trail Making B, and Letter Number Sequencing (LN). An additional subtest, color-word interference, from the Delis-Kaplan Executive Function System (D-KEFS) was administered to some of the subjects for secondary analyses. Trails B is a pen and paper measure that requires the participant to connect a series of numbers and letters in order around a page (e.g. 1 to A, 2 to B and so on). Letter-Number Sequencing presents participants with a series of letters and numbers and they must order the numbers first in numerical order, then order the letters presented after the numbers in alphabetical order. Color-Word Interference has three trials: 1) blocks of color are presented and participants must name the colors across the rows presented as fast as they can without error. 2) Color Words are presented in black ink and participants must read the words across the rows as fast as they can without errors, 3) Color words are presented in an ink color that is incongruent with the color word (e.g. the word green typed in red ink). Participants must say the color of the ink, not read the word. The time to completion will be the outcome measure for Color-Word Interference and Trails B. Number of appropriate words generated will be the outcome measure for Verbal Fluency and number correct for Letter-Number sequencing.

Memory

Both Immediate and Delayed Memory was assessed with the RBANS. Immediate memory consists of List Learning and Story Memory. Delayed memory consists of List Recall, List Recognition, Story Recall and Figure Recall. List learning presented participants with 10 words, on four separate trials, each with immediate recall. For story memory, a story was presented to the subjects twice with an immediate recall after the presentation, followed by a free recall of the story approximately 20 minutes later. List recall is the free recall of the words learned in list learning approximately 20 minutes after presentation. List Recognition presented the participant with words that were learned in List recognition as well as items that were not on the list. Figure recall wass preceded by figure copy, where participants were required to copy a geometric figure, then

approximately 20 minutes after the figure is copied they were asked to recall the figure and draw it again.

Semantic Fluency

The fluency subtest from the RBANS was used to assess semantic fluency. Each participant was asked to name as many fruits or vegetables as they could think of in a one minute time frame. Number of responses was recorded and used as the outcome measure. Items that were repeated (perseverations) or did not match the semantic category (set-loss error) were not included in the number of responses.

Quality of life/ psychiatric questionnaires

The Short Form-36 (SF-36;(Ware & Sherbourne, 1992)) questionnaire was administered to measure health-related quality of life, and the Fatigue Severity Scale (FSS;(Krupp, LaRocca, Muir-Nash, & Steinberg, 1989) was administered to assess physical fatigue. These scales were administered to determine if the mono and co-infected groups differ significantly in terms of self-reported feelings of health and fatigue.

The Center for Epidemiological Scale for Depression (CES-D) was used to assess current depression. This depression scale is commonly used in HIV research. Scores for each response were totaled (reverse scoring of items will be used where appropriate) and the total score provided the outcome measure. Patients also completed the Lawton and Brody Activities of Daily Living scale (ADL; (Lawton & Brody, 1969) to determine the ability to complete both basic and instrumental ADLs. This scale has been found to detect functional impairments in the HIV-mono and HIV/HCV-co-infected populations (Ryan et al., 2004).

Drug Use

A detailed alcohol/drug use and dependence questionnaire was administered to determine illicit drug use and to determine if participants met DSM-IV criteria for abuse or dependence. Additional information about the frequency, duration, and amount of each drug used was assessed

using the Kreek-McHugh-Schluger-Kellogg Scale (KMSK; (Kellogg et al., 2003). The KMSK allows for the quantification of drug use severity based on amount, duration, and frequency of use. The scale used for this study was modified to include marijuana use and has been used previously in NIH-funded studies of HAND. As indicated previously a rapid urine screen was administered to assess recent drug use. Positive results were further examined by asking if use was prescription, or non-prescription and time of most recent use. A positive result did not lead to exclusion because some drugs (e.g., marijuana) may be detected for weeks after use. Additionally, some HIV therapies result in a positive test for marijuana. Therefore, the toxicology results were used to cross-check the validity of self-reported classes of drug use and current use.

Blood draws and hepatic tests

Blood work was collected during routine clinic visits and took place within 30 days of MRI and neuropsychological testing. Liver function tests (LFT) were derived from a standard blood chemistry draw during a routine clinical visit. The main LFT variables used for this study was AST and platelets. CD4 count and viral load (for HIV and HCV) was also recorded.

Determination of cirrhosis or fibrosis

The ratio of AST to platelets (APRI) was calculated as the outcome variable for measuring hepatic function. This measure is a non-invasive method to assess possible liver damage, and although less sensitive than liver biopsy, APRI has been shown to reliably predict fibrosis and cirrhosis (Carvalho-Filho et al., 2008; Shaheen & Myers, 2007; Tural et al., 2009). In addition, another non-invasive measure of fibrosis, the FIB-4 was derived. The FIB-4 incorporates AST, ALT, age and platelet count to determine a degree of hepatic fibrosis (Trang, Petersen, & Snyder, 2008; Tural et al., 2009). These data were used only for secondary analysis, as DTI indices represented the primary variables of interest in this study.

DTI

Imaging was completed at Washington University Mallinckrodt Institute of Radiology. A Siemens 3T Tim Trio was used for scans completed by the HIV+ and HIV/HCV+ group. Approximately half of the controls and two of the HIV/HCV co-infected individuals were scanned using a Siemens 3T Allegra. A T1-weighted three-dimensional magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence (Time of repetition (TR) /inversion time (TI)/ echo time (TE) = 2400/1000/3.16 ms, flip angle = 8°, and voxel size = 1 × 1 × 1 mm^3) was obtained. A T2-weighted fast spin echo (T2W FSE) scan (TR = 4380 ms, TE = 94 ms, 1 × 1× 3 mm) was also acquired. Images were visually inspected at the time of scanning and an additional scan was performed if significant motion artifacts were observed. Two acquisitions of diffusion-weighted images were collected for the assessment of white matter microstructural integrity (2 x 2 x 2 mm voxels, TR=9,900 ms, TE=102 ms, flip angle =90^o, 23 directions with b-values between 0 and 1400 s/mm^2).

DTI Registration

A detailed review of the registration methods has been previously described (Shimony, Burton, Epstein, McLaren, Sun, Snyder, 2006). In brief, affine transforms computed by image registration were used to define the spatial relationships between all images. Multi-modal image registration between the anatomical images (T2W FSE and T1W MPRAGE) was accomplished using vector gradient measure (VGM) maximization (Rowland, Garbow, Laforest, Snyder, 2005). The first DTI volume (I_0, with b = 0) was registered to the T2W FSE image, which was then registered to the T1W MPRAGE image. Subsequently the image was registered to an atlas that conformed to the Tailairach system (Lancaster, Glass, Lankipalli, Downs, Mayberg, and Fox, 1995; Talairach and Tournoux, 1988).

DTI Motion Correction

The DTI dataset was motion-corrected by first aligning each DTI volume to the geometric mean volume of each group of images that shared similar degrees of diffusion sensitization. The geometric mean volume was then recomputed before aligning to the first acquired I_0 image. Next, transforms between the different images were algebraically composed. Repeating these steps three times yielded realignments with errors that had an estimated internal consistency < 0.1 mm. All transforms were 9 parameter affine (rigid body + scanner axis stretch) computed by VGM maximization (Rowland et al, 2005). Conventional intensity correlation maximization was used to align the I_0 volumes of each DTI dataset. The final motion-corrected results were obtained by algebraically composing all transforms and averaging datasets after application of the composed transforms using cubic spline interpolation.

DTI Computations

DTI post-processing was performed using previously described methods (Shimony et al., 2009). Briefly, the diffusion tensor and the 3 eigenvalues were calculated using log-linear regression in each voxel. Using standard methods (Basser, Mattiello, LeBihan, 1994), the three eigenvalues were used to calculate RD, AD, and MD. Anisotropy was expressed as FA, which is normalized to assume a range from 0 to 1. Diffusion parameters were measured and sampled for each individual by averaging particular regions of interest within the corpus callosum (CC) and frontal forceps.

Despite motion correction, in the HIV/HCV+ group DTI data were compromised due to registration errors, and eddy distortions in the frontal lobe that rendered some images unusable for ROI analysis. In addition, DTI metrics were highly abnormal (>4SD from the mean of the entire group) for two cases in the HIV+ group and one case in the HIV/HCV+ group and as such were not

included in analyses. As a result the final numbers for the neuroimaging analysis were HIV+ n=23, HIV/HCV+ n=21, and controls n=25.

Region of Interest Analysis

DTI parameters were obtained for the CC and frontal forceps using manually-drawn regions of interest (ROI) created in Analyze v. 11.0 (Mayo Clinic, Rochester, MN; Figure 1). ROIs for both regions were manually delineated using the threshold option (FA= 0.3) with adjustments where needed. The threshold of FA equal to or greater than 0.3 was set based on parameters used in previous studies of HIV and DTI (Gongvatana, 2010; Wright 2012). Additionally, the threshold of 0.3 provides confidence that the voxels selected are primarily white matter volumes rather than voxels consisting of partial gray/partial white matter. The genu, body, and splenium of the CC were drawn on the mid-sagittal slice of each subject's FA map as seen in Figure 1. Each region was sampled across five slices that were centered upon this slice. DTI parameters were calculated based on the mean of the five slices. Additionally, the CC was segmented into five sections based on anatomical connections to cortical areas as described in Rosas et al. (2010). These sections are as follows: CC1-prefrontal, CC2- premotor and supplementary motor, CC3/CC4-sensory motor, CC5-parietal, temporal, occipital. ROIs for the frontal forceps were also drawn using the threshold option in Analyze and manual adjustments when needed. ROIs were drawn in atlas space and were sampled from five axial slices as shown in Figure 2.

Statistical Analysis

Descriptive analyses were carried out prior to the analyses of the aims to characterize the groups. Age, education, and drug use were analyzed between groups using ANOVA.. An independent measures t-test was used to compare the HIV+ and HIV/HCV+ groups on clinical markers such as CD4 count, nadir CD4 count, plasma VL, time since diagnosis of HIV+ infection, and APRI.

Aim 1

The purpose of aim 1 was to identify microstructural alterations in the brain associated with co-infection using DTI. I hypothesized that co-infected individuals would exhibit significantly decreased FA and increased MD, AD and RD in white matter in two regions of interest. The frontal forceps and the corpus callosum compared to HIV mono-infected individuals and controls. Using separate ANOVAs for each of the dependent DTI measures, a main effect of group status on frontal white and corpus callosum DTI indices (FA, MD, AD, and RD) values were examined. A Hochberg G2 post-hoc test was used to determine where the group differences occur (Hochberg and Tamhane, 1987).

Aim 2

The second aim of the study was to identify neurological and neurobehavioral predictors of cognitive performance among co-infected with HIV/HCV. Data were analyzed using a regression model within the co-infected group to determine factors that are most predictive of cognitive deficits. Deficits were determined by group mean scores within cognitive domains such as memory, learning, psychomotor speed and executive function. A z-score for each measure was calculated based on current normative scores adjusted for age, education, and gender. These z-scores were then summed and averaged to create a sum z-score for each domain that were further summed across domains to determine a global measure of cognitive function. Independent factors included DTI indices, substance use (summed across different drugs of abuse), liver function, and self- reported physical/mental health (from SF-36). Neuropsychological measures were administered uniformly in the co-infected group only, therefore all analyses of cognitive functioning were for the co-infected group only.

Results

Analysis of demographic and drug use variables

Preliminary analyses of demographic and drug use information are presented in Table 1. There were no significant group differences in age or education between the three groups although

the control group did have a slightly higher level of education. Additionally, the control group scored significantly higher on the WRAT-3, a measure of reading ability compared to both the HIV+ and HIV/HCV+ groups.

The KMSK was administered to quantify drug use (see Table 2). For the individuals who provided responses on this measure there were no significant differences in terms of alcohol and tobacco use between the groups; however, for the most part the HIV+ and HIV/HCV+ groups reported current tobacco use, whereas many of the controls had a past history of drug use, but did not report current use of tobacco. Cocaine use was significantly higher in the infected groups compared to controls, but did not differ significantly between the HIV+ and HIV/HCV+ groups. Marijuana use was significantly different between all of the groups with the HIV+ mono-infected group having the highest scores on the KMSK and the controls having the lowest scores.

The HIV+ and HIV/HCV+ groups were similar in terms of current and nadir CD4 counts and HIV viral load. There was a significant difference between the groups in terms of time since HIV diagnosis with the HIV/HCV+ group having been infected on average 14 years with HIV, and the HIV+ group having been infected an average of 9 years with HIV ($p=0.032$, see Fig 8).

DTI- CC

There was a significant effect of group status on FA in the CC, with controls having significantly higher FA than both the HIV+ ($p=0.007$) and the HIV/HCV groups ($p=0.001$). The HIV+ group did have slightly higher FA values than the HIV/HCV group, however the difference was not significant. There was no difference between the groups in terms of AD, however there was a trend for lower MD in the control group compared to the infected groups. Additionally, there was a significant difference between controls and the HIV/HCV group on RD ($p=0.019$) but only a trend for increased RD between the HIV+ group and controls ($p=0.055$, see Fig. 1).

In order to determine a regional effect of the differences in the CC, this ROI was segmented into five sections, based on the anatomical connections into frontal, parietal, and occipital regions.

Since FA and RD were significantly different between the control and infected groups, a MANOVA was conducted for each of the 5 sections of the CC in order to identify group differences in specific regions of the CC. There was a significant difference between the co-infected groups and the controls in terms of RD in the anterior two-fifths and the central region of the CC with the co-infected group having higher RD than the controls (see Figs.2-6).

An additional analysis was conducted applying the same threshold of .3 to the CC. The control group had significantly more area in the CC compared to both the HIV+ ($p= 0.014$) and HIV/HCV groups ($p<0.001$), with the HIV/HCV having the smallest ROI sampled within the threshold limits.

DTI- frontal horns

There were no significant differences between the groups on any of the DTI metrics in the frontal area; however, when assessing the area selected (based on the threshold of FA greater or equal to 0.3) the HIV/HCV group had significantly less white matter in the frontal forceps that fell within the threshold limits compared to controls resulting in smaller frontal ROIs for the HIV/HCV+ group compared to controls. The HIV+ group also had a smaller area of white matter voxels within the threshold limits; however, the difference in white matter area was not significantly lower between the HIV+ group and controls, or significantly higher than the HIV/HCV group. This is of note as the ROIs were drawn based on the individual anatomy of each participant using a marker (FA) of white matter integrity. As white matter integrity is compromised the value of FA can decrease to levels below the applied threshold.

Correlational analyses

Correlational analyses did not reveal any relationship between the individual scores on the KMSK and any of the DTI measures. There was a moderate relationship between depression symptomatology, MD and RD values in the corpus callosum for the co-infected group (Fig. 7). The measure of depression symptoms, the CES-D, was only collected from 15 of the 23 individuals in the

HIV+ mono-infected group, but there was no relationship between depression symptoms and DTI variables in the HIV+ mono-infected group.

Regression analysis-

A regression model including substance use scores (KMSK), liver status (APRI), self-reported physical and mental health scores (SF-36), and DTI indices was completed to determine theI ndividual and combined effects of these variables on neurocognitive function. This analysis was conducted for the co-infection group only. DTI indices included in the analysis were the variables that significantly differed from the control groups (FA and RD in frontal and CC white matter). The overall regression model did not identify significant associations between these variables and peformance performance for any of the cognitive domains, or global cognition. Additionally, none of the individual variables were significantly associated with cognitive performance in the co-infected group; however, in the domain of working memory/executive function there was a trend for CC RD to relate to performance on these tasks ($p=0.078$, see Fig. 9).

Discussion

This study identifies an additional impact of HIV/HCV co-infection on white matter structural integrity. In general, the co-infected group had the lowest FA, and the highest values for MD, AD and RD of the groups, however only FA and RD were significantly different in the co-infected group compared to the controls in the anterior regions of the CC. The reductions seen in FA and increases in RD primarily in the anterior areas of the brain indicate that the integrity of myelin is compromised in co-infection (Avram, Guidon, and Song, 2010), particularly in the anterior regions of the brain. This is effect is greater than the effect of HIV mono-infection, as demonstrated by the lack of significant differences between the HIV+ and control groups. Prior studies have associated co-infection with lower FA and MD in various brain regions (Gongvatana et al., 2011) and increased MD in a whole brain analysis (Stebbins et al., 2007) compared to HIV+ mono-infected individuals. These previous studies did not examine the indices of AD and RD, nor did they compare HIV/HCV+

groups to controls. On the contrary, this study focused on the CC and frontal forceps, and did not measure DTI metrics in other white matter fiber tracts. The primary reason for the selection of the CC is the high directionality of fibers, providing a good measure of overall white matter integrity (Wright et al., 2012). Additionally, the CC connects with cortical regions in an organized manner that are related to specific cognitive functions (Rosas et al., 2010). This provided an ideal means of assessing white matter integrity and cognitive function.

One potential explanation for the greater effect of co-infection that has been raised previously is liver dysfunction in the HIV/HCV groups. A proposed theoretical model defines liver dysfunction as the mechanism for the release of pro-inflammatory cytokines and astrocytic swelling that can progress to hepatic encephalopathy (Haussinger and Schliess, 2008). However, HCV has been found in brain tissue of autopsy patients (Vargas et al., 2002). This finding provides an alternate mechanism, whereby the presence of HCV in the brain leads to an upregulated immune response (Letendre et al., 2007). There are no studies reporting any direct infection of neurons by HCV. In this study the APRI score, an index of cirrhosis and fibrosis, was significantly higher in the co-infected group than the mono-infected group, though neither group had APRI or FIB-4 values suggestive of advanced liver disease (Shaheen and Myers, 2007). In fact, none of the participants in the study had scores indicative of cirrhosis or fibrosis.

The alterations in DTI metrics in the co-infected group further extends prior studies indicating that cognitive changes, and neuroimaging abnormalities exist in individuals who have not progressed to the later stages of liver disease (Hinkin, et al 2008; Ryan et al., 2004; Weissenborn et al, 2004). The results of this study do not provide information about the white matter structural integrity in those individuals who have developed cirrhosis or fibrosis. Future neuroimaging work should compare HIV/HCV individuals with normal liver function to individuals with compromised liver function.

Another potential explanation for the alterations in DTI metrics in the co-infected group that has been raised previously is recreational drug use (see Letendre and Paul publication). In the present study the co-infected group had significantly higher self-reported use of recreational drugs, especially cocaine and marijuana compared to the controls. However, there was no difference between the mono-infected group and the co-infected group in terms of drug use, and the correlational analysis did not reveal associations between the scores on the KMSK and any of the DTI metrics. Drug use histories were recorded from self-report measures and require participants to recall information about drug use that occurred primarily in the past. It is possible that the participants did not accurately recall their drug use, although this would be likely to occur in both patient groups. Of note, the KMSK did not account for all possible types of drug use and therefore drug use cannot be entirely ruled out as a potential mechanism of the alterations in white matter evident in the HIV/HCV+ group.

Both the co-infected and mono-infected groups were similar in terms of their current clinical profiles, with similar current CD4 levels and low or undetectable viral loads in the majority of individuals, with a couple of outliers in each group. Interestingly, neither age, education, nor time since diagnosis correlated with any of the imaging markers in any of the groups. Both groups also had similar nadir CD4 counts. Correlational analyses controlling for age revealed a moderate relationship between nadir CD4 and MD ($r=-.56$, $p=.01$) and RD ($r=-.47$, $p=.04$) values in the CC in the HIV+ group; however this relationship is not evident in the HIV/HCV+ group (See Fig.XX). Since both the HIV+ group and HIV/HCV+ group had similar nadir CD4 levels the lack of correlation between this variable and the imaging markers indicates that the alterations in white matter in the HIV/HCV+ group may be affected by different factors.

In the HIV/HCV+ group there was a moderate correlation between depression indices and both MD and RD in the CC. Although this correlation was not similarly observed in the HIV+ group only half of the HIV+ group provided information about their depressive symptomatology.

Depression has been previously linked to both volumetric and white matter alterations outside of HIV, and is a common comorbidity in HIV and HCV. A number of studies have linked inflammation to depression. For example, medical conditions associated with inflammation or immune dysfunction have a higher prevalence of depression (for review see: Hannestad, 2008), and when anti-inflammatory agents are initiated many patients report a decrease in depressive symptomatology. A number of studies have also shown an increase in pro-inflammatory cytokines in depression, although this is not a consistent finding across studies (Hannestad, 2008) Considering the mechanism for altered microstructural integrity in HIV and co-infection is believed to be the result of damage from pro-inflammatory cytokines it is possible the higher levels of depression symptomatology in this cohort may be due to an increase in inflammatory agents in the brain due to HIV and HCV that are affecting white matter integrity.

It is possible that the presence of HCV itself results in a greater change in the white matter of the co-infected subjects. Studies have shown that HCV is present in the CNS and may replicate in the CNS of HIV/HCV co-infected patients (Forton 2004, Letendre, 2007, Morgello, 2005), especially among individuals with detectible HIV viral loads in the CSF. At least one study has shown that microglia and astrocytes can be infected by HCV (Wilkinson, 2009). HCV is associated with an up-regulation of pro-inflammatory cytokines that have been shown to affect white matter (Letendre; others). This has been evidenced in a number of immune-mediated diseases such that higher levels of central and peripheral cytokines and chemokines result in white matter demylination. Using H-MRS, co-infection with HIV/HCV is associated with markers of decreased mI and increased choline, markers of microglial functioning and inflammation respectively. Using DTI, the alterations specific to FA and RD indicate that the integrity of myelin is affected in HIV/HCV co-infection. In other HIV neuroimaging studies, when HCV co-infection is entered as a variable of interest it has consistently been associated with an increased risk for white matter alterations (Gongvatana, 2010) and neuropsychological deficits (Wright, 2012). The result of this study show that although the

difference in white matter microstructural integrity between the mono- and co-infected groups is not significantly different, co-infected individuals are more likely to have alterations in the white matter when compared to seronegative individuals.

Although there was no significant relationship between the selected DTI metrics and neuropsychological domains there was a trend for RD in the anterior CC to predict performance in executive functioning/working memory and psychomotor speed measures. The sample was relatively small, which may have reduced power to detect a significant relationship between the DTI metrics and neuropsychological measures. Still this finding is important, as previous studies of co-infection and DTI have not specifically assessed the relationship between areas of altered DTI metrics and cognitive performance. The use of archival data limited the complete assessment of neuropsychological domains across the groups. Although all individuals included in the study received some neuropsychological testing the battery was not standardized across the three groups. However, the HIV/HCV group received a uniform set of measures allowing the assessment of cognition in this group and the ability to determine the relationship between specific cognitive domains and DTI metrics. The use of z-scores to determine the contribution of DTI, substance use, and liver function on neuropsychological domains provided a measure of how this group performed compared to a normative population. As seen in Table 3 the co-infected group performed below the normative mean (as indicated by –z scores) on almost all of the measures.

All of the HIV/HCV+ participants in the study were treated for HIV, but untreated for HCV. Use of HCV therapies is commonly associated with fatigue, flu-like symptoms, depression, anxiety, and sleep disturbances. These symptomscan affect the validity of neuropsycological testing. Recently there have been advances in the treatment of HCV that limit the negative side effects of commonly used therapies like interferon and ribavirin. As some studies of HIV have shown a 'normalization' of DTI variables with HAART (Wright et al., 2012, Becker, 2012) it would be beneficial to investigate changes in DTI parameters in these patients who currently are not on HCV treatment, or had a poor

response to treatment to determine if DTI metrics become normalized after HCV treatment is initiated. If so, this may provide more information about the mechanisms associated with white matter abnormalities in co-infection.

The course of HAND has been defined as fluctuating, such that individuals may show deficits and improvements in cognitive function that change over the course of the disease. Recent longitudinal studies using neuroimaging have shown what appears to be a reconstitution of integrity in HIV+ individuals with the commencement of HAART (Ances 2012, Becker 2012). It is possible that levels of inflammatory markers mediate changes in neuroimaging indices and neuropsychological performance throughout the course of disease. Additional longitudinal studies exploring the relationship between inflammation, neuroimaging, and neuropsychological performance are needed to further elucidate the relationship. It is also imperative to find anti-inflammatory agents to minimize the impact on neural tissue in HIV and HCV, as an early effort to reduce inflammation using minocycline was not effective in improving cognitive function (Sacktor et al., 2011)

In summary, this study identifies a significant impact of HIV/HCV+ co-infection on white matter microstructural integrity. Changes to DTI indices of FA and RD indicate an impact of co-infection on the myelin, resulting in increased diffusion perpendicular to axon fibers primarily in the anterior regions of the CC. These changes had a trend level association with cognitive measures that rely on prefrontal and motor cortices, areas connected with the sections of the CC that were significantly altered. Although there was an effect of HIV mono-infection on FA in the CC, the effect of co-infection was stronger. Future studies should assess the impact of HCV mono-infection as well as the impact of new HCV therapies on DTI metrics and neuropsychological function.

Table 1.

Group demographics and descriptive statistics				
	Control	HIV+	HIV+/HCV+	Significance
AGE				
Mean	46.32	44.65	49.48	p =0.134
SD	10.18	7.28	5.06	
n	25	23	21	
Education				
Mean	13.44	13.22	12.45	p=0.334
SD	2.40	2.75	1.83	
n	25	23	21	
CD4abs				
Mean		462.48	488.4	p=.78
SD		338.99	249.65	
n		23	21	
HIVRNAViralLoad				
Mean		19980.	1005	p=0.26
IQR		50-72	20-121.5	
n		23	21	
NadirCD4				
Mean		197.48	150.76	p=0.35
SD		186.13	132.30	
n				
Years since HIV diagnosis				
Mean		8.78	13.81	p=0.022
SD		6.08	7.73	
n		23	21	
APRI				
Mean	.	0.2889	0.7252	p=0.001
SD		0.12605	0.61205	
n		23	21	

Table 2

Self-report drug use (KMSK)				
	Control	HIV+	HIV/HCV+	
Alcohol				
Mean	8.6	10.6111	9.96	p=0.19
SD	3.47051	2.03322	2.93655	
n	10	18	25	
Tobacco				
Mean	9.6364	8.5625	8.64	p=0.71
SD	2.11058	2.94321	4.41475	
n	11	16	25	
Cocaine				
Mean	2.4	10.2105	10.64	p<0.001
SD	4.37671	5.12704	5.31413	
n	10	19	25	
Heroine/Opiates				
Mean	1.4444	0.3125	1.68	p=.39
SD	4.33333	0.87321	3.60231	
n	9	16	25	
Marijuana				
Mean	2.6154	9.7222	6.75	p<0.001
SD	3.40437	3.25044	3.23365	
n	13	18	24	

Table 3

Working Memory and Psychomotor speed measures

	N	Minimum	Maximum	Mean	Std. Deviation
DSzscore	19	-1.75	1.33	-.9037	.87988
pegszscore	21	-1.90	2.00	.2857	1.28308
pegsndomzscore	21	-1.50	3.00	.4381	1.13907
TrailsAzscore	21	-1.90	2.90	.2429	1.03033
TrailsBzscore	21	-2.70	2.70	-.1762	1.38272
LNSzscore	21	-2.75	1.00	-.7224	1.08012
Valid N (listwise)	19				

Mean scores for the coinfected group on neuropsychological measures in the domains of working memory and psychomotor speed.

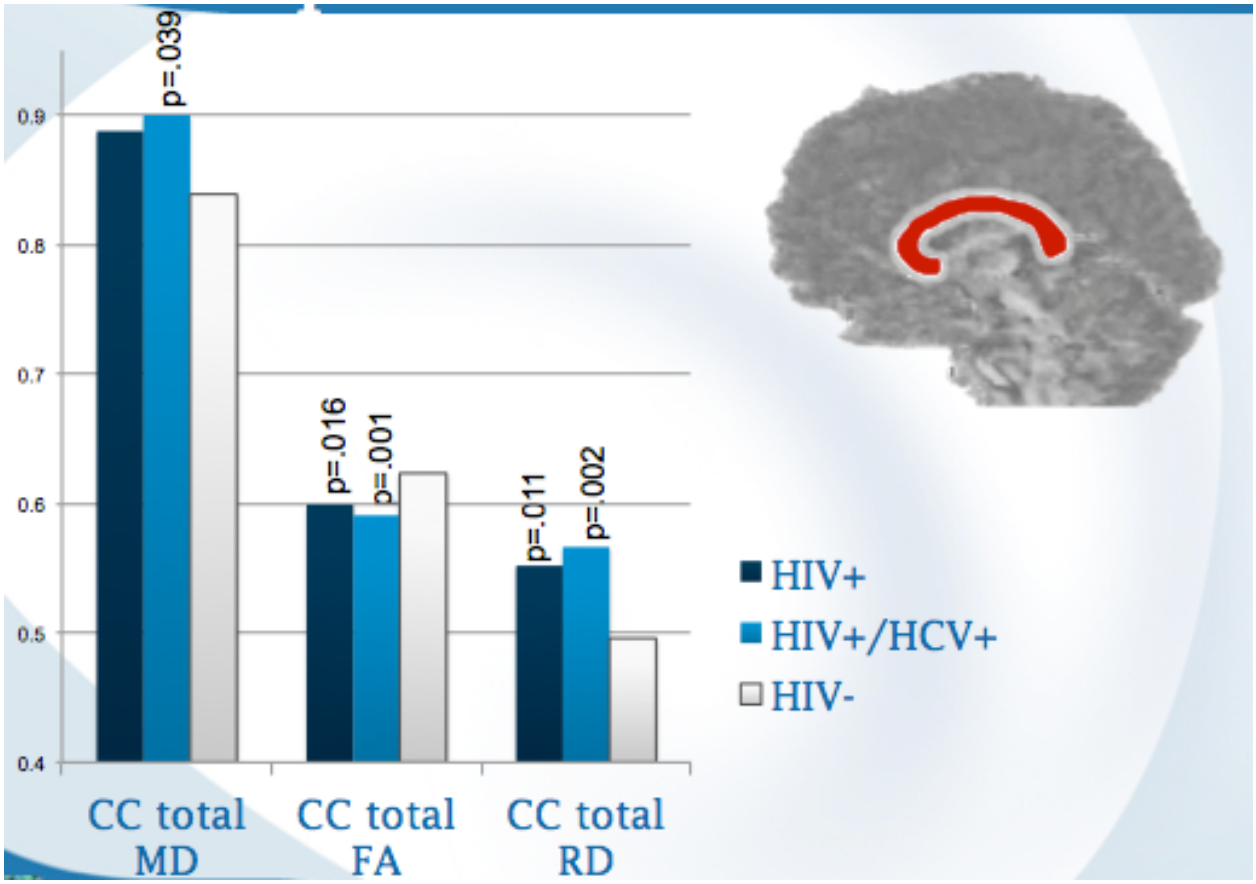


Figure 1 Shows the significant differences across the groups for MD, FA, and RD in the corpus callosum

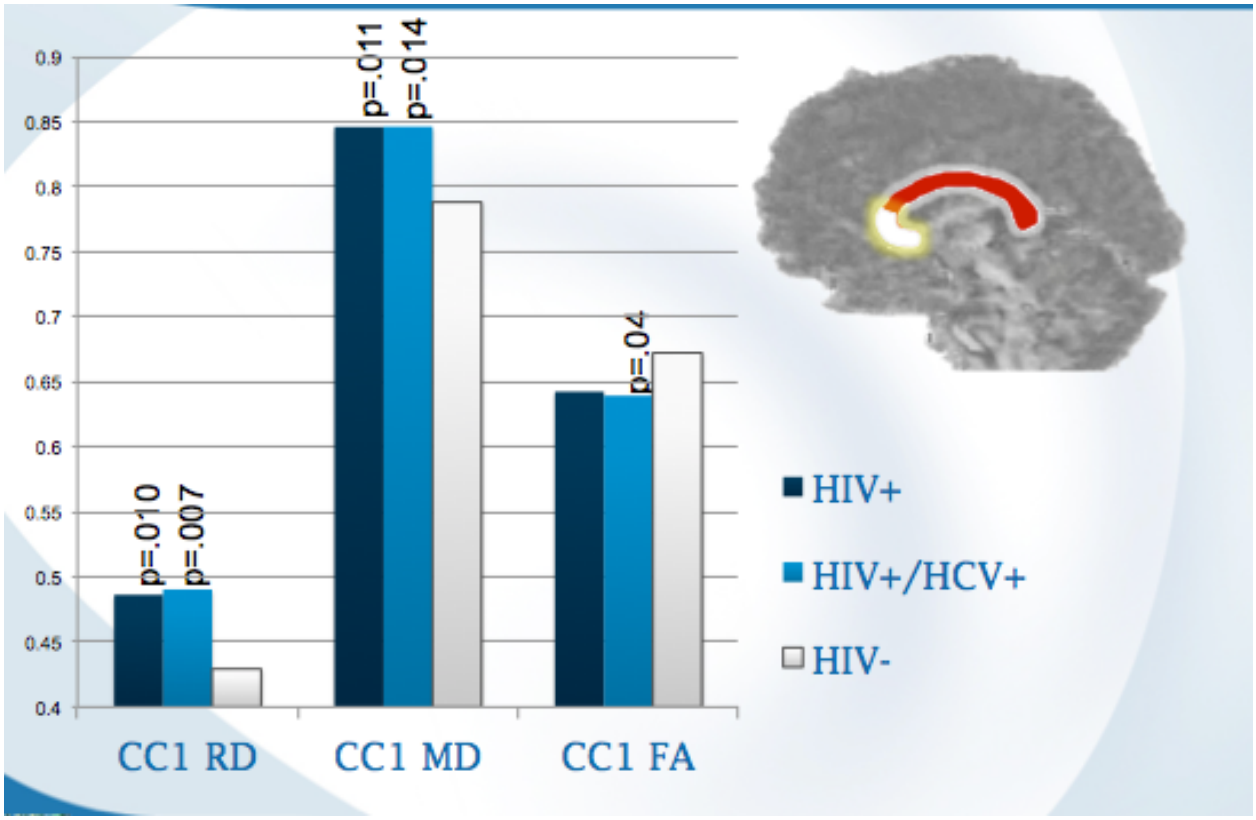


Figure 2 Shows the significant differences between groups in the Corpus Callosum (CC1 as highlighted on brain image) for RD, MD, and FA

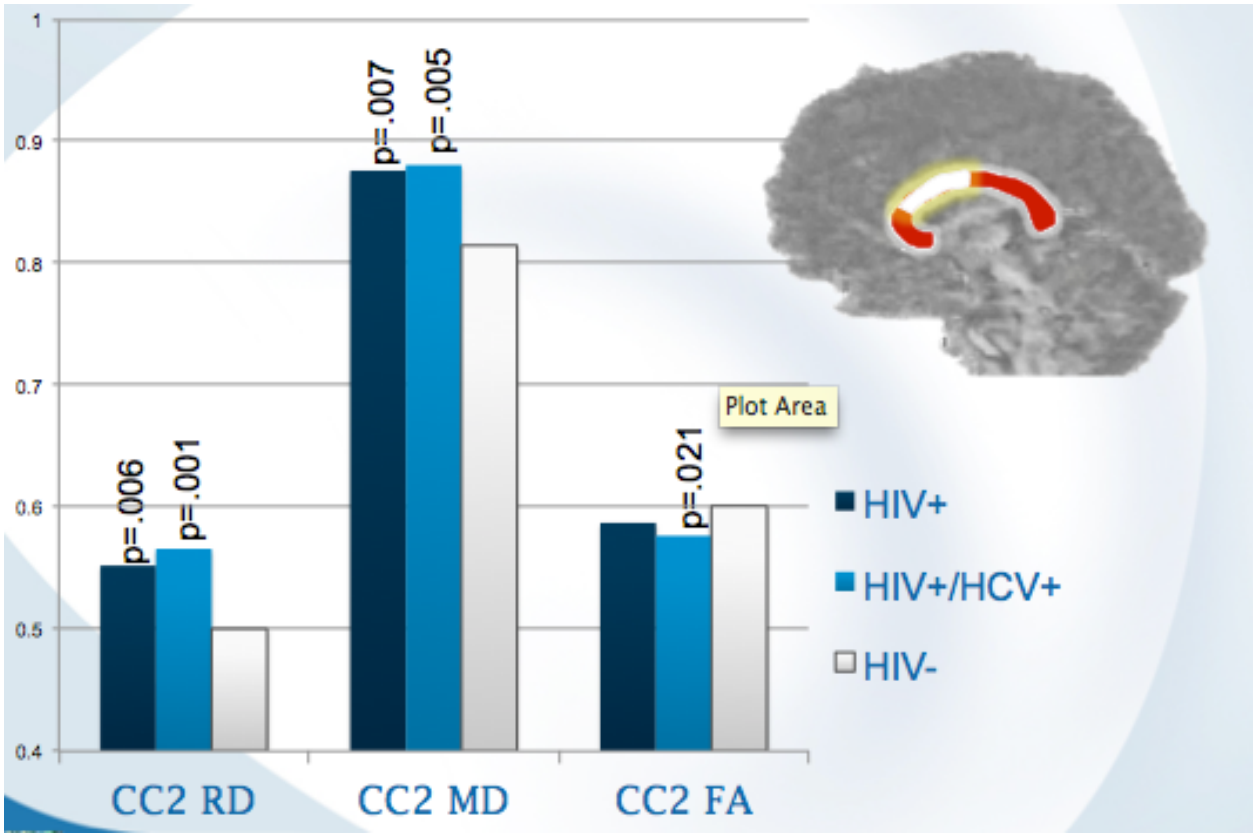


Figure 3 Shows the significant differences between groups in the Corpus Callosum (CC2 as highlighted on brain image) for RD, MD, and FA

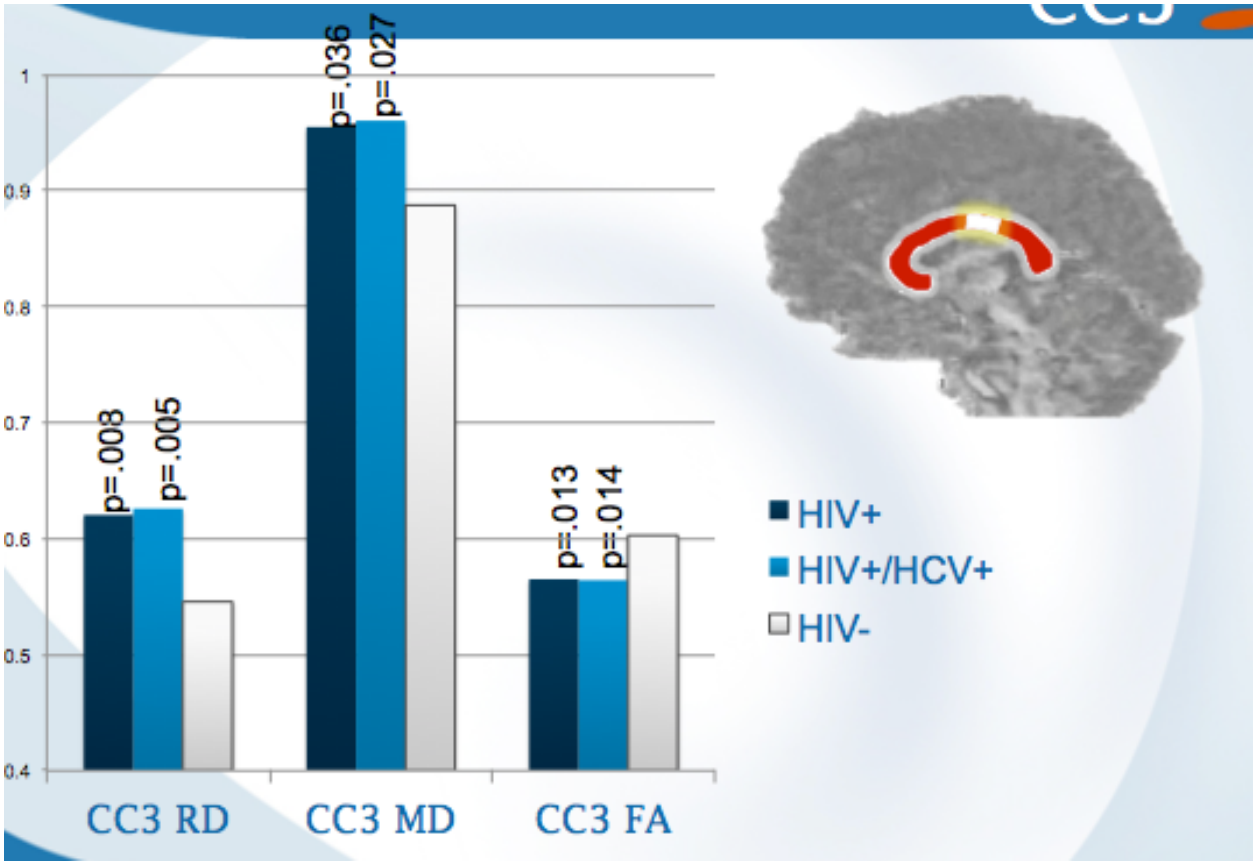


Figure 4 Shows the significant differences between groups in the Corpus Callosum (CC3 as highlighted on brain image) for RD, MD, and FA

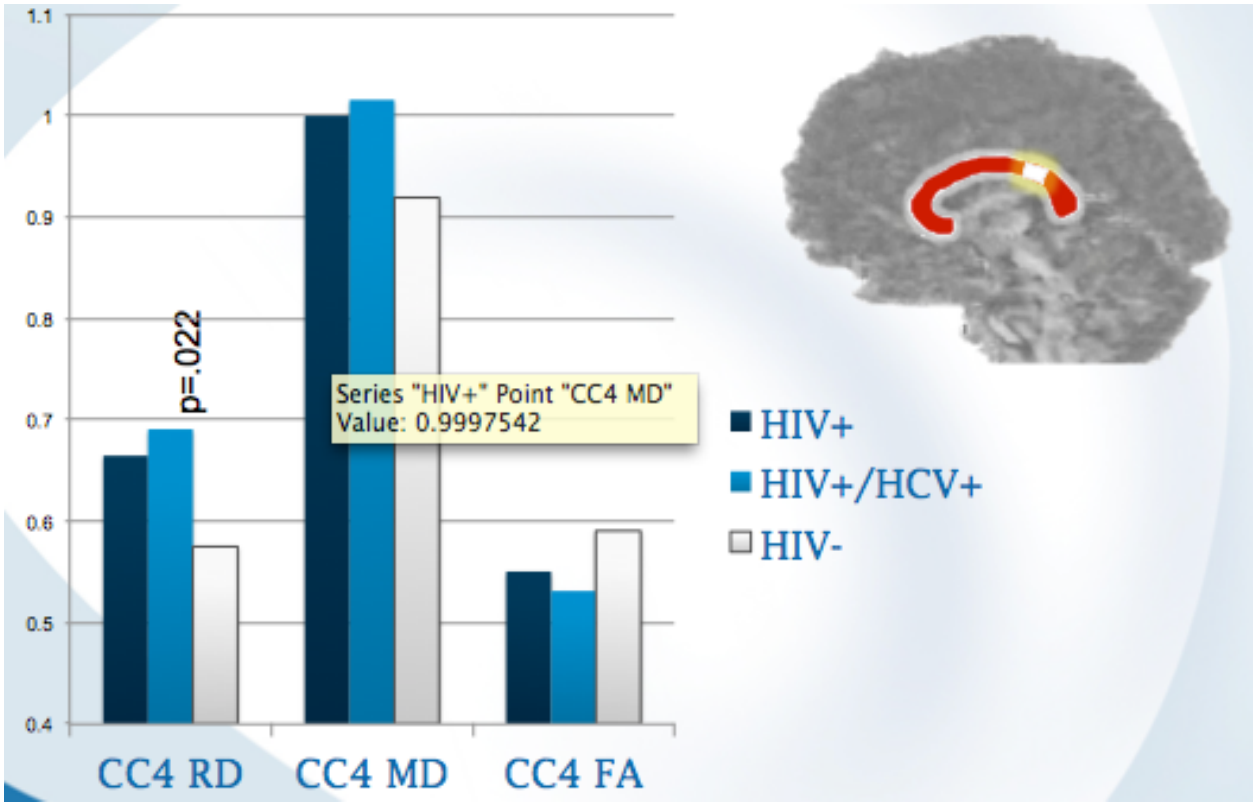


Figure 5 Shows the significant differences between groups in the Corpus Callosum (CC4 as highlighted on brain image) for RD, MD, and FA

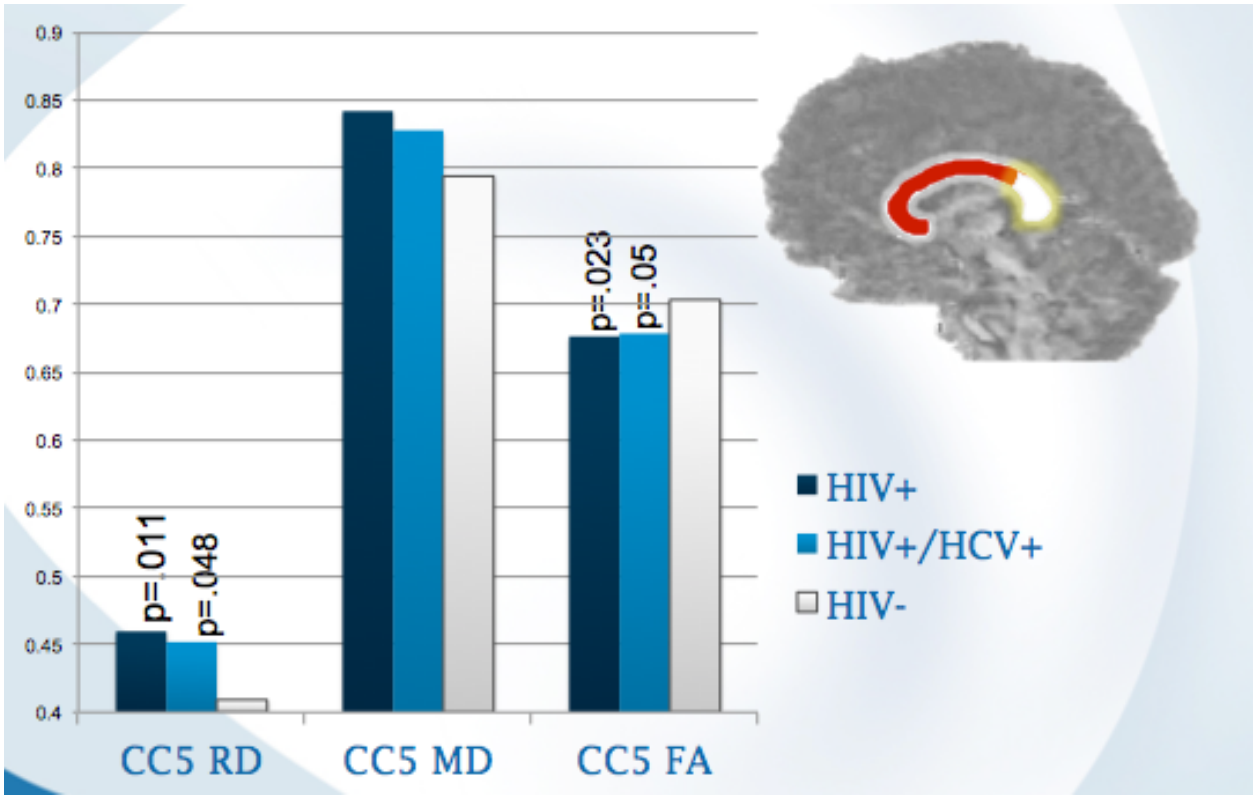
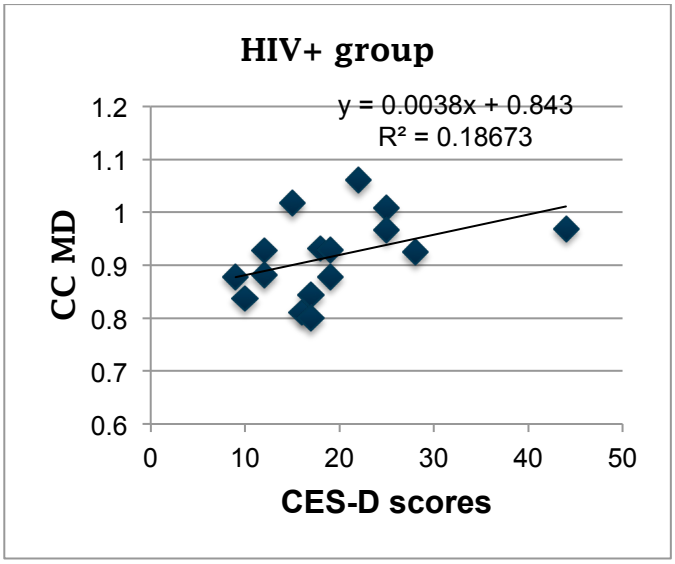


Figure 6 Shows the significant differences between groups in the Corpus Callosum (CC5 as highlighted on brain image) for RD, MD, and FA



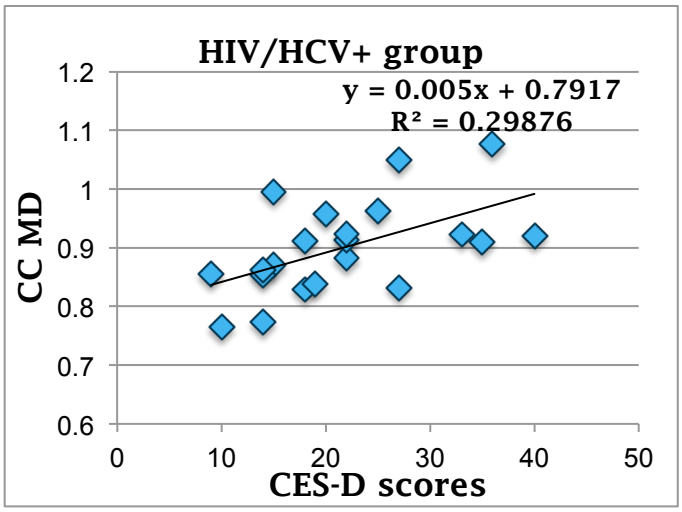


Figure 7 shows the significant relationship between MD in the corpus callosum and depression scores in the HIV+ and HIV/HCV+ group. The relationship is significantly stronger in the HIV/HCV+ group

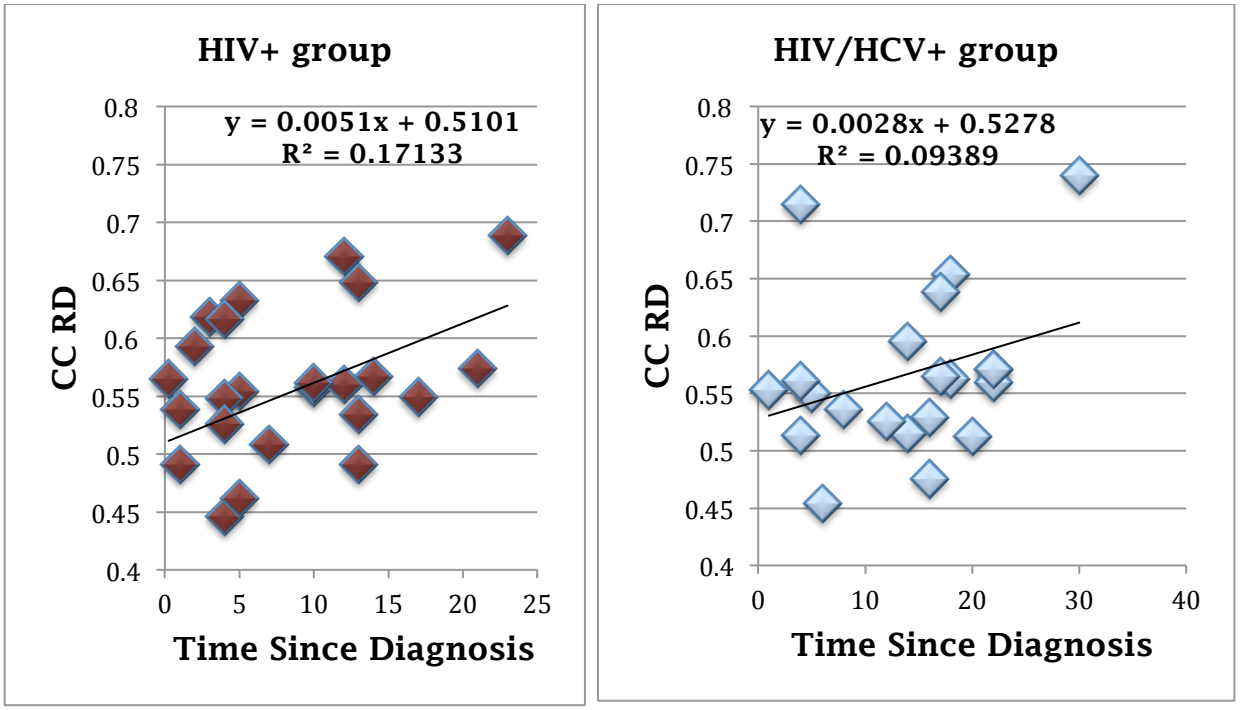


Figure 8 Shows the relationship between RD in the corpus callosum and time since diagnosis in the HIV+ and HIV/HCV+ group. The relationship was significant in the HIV+ group only.

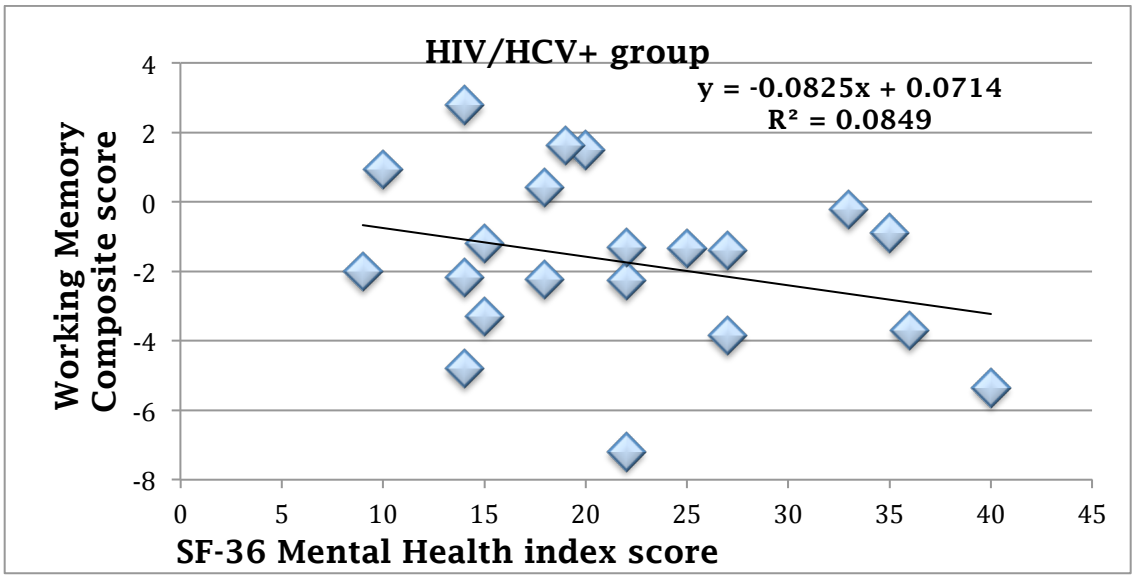


Fig. 9 Shows the significant relationship between scores of working memory and overall mental health as measured by the SF-36 in the HIV/HCV+ group only.

References

Ances, B.M., Ortega, M., Vaida, F., Heaps, J., Paul, R. (2012) Independent effects of HIV, Aging and HAART on brain volumetric measures. Journal of Acquired immune deficiency syndrome, 59 (5), 469-477. Doi: 10.1097/QAI.0b013e318249db17.

Ances, B. M., Vaida, F., Cherner, M., Yeh, M. J., Liang, C. L., Gardner, C., ... & Buxton, R. B. (2011). HIV and chronic methamphetamine dependence affect cerebral blood flow. *Journal of Neuroimmune Pharmacology*, *6*(3), 409-419.

Anthony, I.A., Norrby, K.E., Dingwall, T., Carnie, F.W., Millar, T., Arango, J.C., Robertson, R., Bell, J.E. (2010). Predisposition to accelerated Alzheimer-related changes in the brains of human immunodeficiency virus negative opiate abusers. Brain, 133, 3685-3698. Doi: 10.1093/brain/awq263

Antinori, A., Arendt, G., Becker, J.T., Brew, B.J., Byrd, D.A., Cherner, M., Clifford, D.B.,…Wojna, V.E. (2007). Updated research nosology for HIV-associated neurocognitive disorders. Neurology, 69 (18), 1789-1799. Doi: 10.1212/01.WNL.0000287431.88658.8b.

Archibald, S. L., Jacobson, M. W., Fennema-Notestine, C., Ogasawara, M., Woods, S. P., Letendre, S., ... & Jernigan, T. L. (2012). Functional interactions of HIV-infection and methamphetamine dependence during motor programming. *Psychiatry Research: Neuroimaging*.

Avram, A. V., Guidon, A., & Song, A. W. (2010). Myelin water weighted diffusion tensor imaging. *Neuroimage*, *53*(1), 132-138.

Basser, P. J., Mattiello, J., & LeBihan, D. (1994). MR diffusion tensor spectroscopy and imaging. *Biophysical journal*, *66*(1), 259-267.

Basser, P. J., & Pierpaoli, C. (1996). Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B, 111*(3), 209-219.

Blackard, J. T., & Sherman, K. E. (2008). HCV/ HIV co-infection: time to re-evaluate the role of HIV in the liver? *J Viral Hepat, 15*(5), 323-330. doi: JVH970 [pii]10.1111/j.1365-2893.2008.00970.x

Brew, B.J. (1995) AIDS dementia complex and HIV-1 brain infection: Clinical-Virological correlations. Annals of Neurology, 38, 563-570

Buch, S., Yao, H., Guo, M., Mori, T., Su, T. P., & Wang, J. (2011). Cocaine and HIV-1 interplay: molecular mechanisms of action and addiction. *Journal of Neuroimmune Pharmacology*, *6*(4), 503-515.

Catz, S. L., Kelly, J. A., Bogart, L. M., Benotsch, E. G., & McAuliffe, T. L. (2000). Patterns, correlates, and barriers to medication adherence among persons prescribed new treatments for HIV disease. *Health Psychology; Health Psychology*, *19*(2), 124.

Carvalho-Filho, R. J., Schiavon, L. L., Narciso-Schiavon, J. L., Sampaio, J. P., Lanzoni, V. P., Ferraz, M. L., & Silva, A. E. (2008). Optimized cutoffs improve performance of the aspartate aminotransferase to platelet ratio index for predicting significant liver fibrosis in human immunodeficiency virus/hepatitis C virus co-infection. *Liver Int, 28*(4), 486-493. doi: LIV1675 [pii]10.1111/j.1478-3231.2008.01675.x

Chang, L., Andres, M., Sadino, J., Jiang, C.S., Nakama, H., Miller, E., Ernst, T. (2011) Impact of apolipoprotein E ε4 and HIV on cognition and brain atrophy: Antagonistic

pleiotropy and premature brain aging. NeuroImage, 58, 1017-1027. DOI: 10.1016/j.neuroimage.2011.07.010.

Chang, L., Wong, V., Nakama, H., Watters, M., Ramones, D., Miller, E.N., Cloak, C., Ernst, T. (2008). Greater than age-related changes in brain diffusion of HIV patients after 1 year. Journal of Neuroimmune Pharmacology. 3, 265-274. DOI: 10.1007/s11481-008-9120-8.

Chang, L., Ernst, T., Speck, O., & Grob, C. S. (2005). Additive effects of HIV and chronic methamphetamine use on brain metabolite abnormalities. *American Journal of Psychiatry*, *162*(2), 361-369.

Chang, L., Ernst, T., Witt, M.D., Ames, N., Gaiefsky, M., Miller, E. (2002) Relationships among brain metabolites, cognitive function, and viral loads in antiretroviral-naïve HIV patients. Neuroimage, 17 (3), 1638-1648. doi: 10.1006/nimg.2002.1254.

Chang, L., Smith, L. M., LoPresti, C., Yonekura, M. L., Kuo, J., Walot, I., & Ernst, T. (2004). Smaller subcortical volumes and cognitive deficits in children with prenatal methamphetamine exposure. *Psychiatry Research: Neuroimaging*, *132*(2), 95-106.

Chen, Y., An, H., Zhu, H., Stone, T., Smith, J.K., Hall, C., Bullitt, E., Shen, D., Lin, W. (2009) White Matter abnormalities revealed by diffusion tensor imaging in non-demented and demented HIV+ patients. NeuroImage, 47, 1154-1162. DOI: 10.1016/j.neuroimage.2009.04.080.

Cherner, M., Letendre, S., Heaton, R. K., Durelle, J., Marquie-Beck, J., Gragg, B., & Grant, I. (2005). Hepatitis C augments cognitive deficits associated with HIV infection and methamphetamine. *Neurology, 64*(8), 1343-1347. doi: 64/8/1343 [pii]10.1212/01.WNL.0000158328.26897.0D

Clifford, D. B., Evans, S. R., Yang, Y., & Gulick, R. M. (2005). The neuropsychological and neurological impact of hepatitis C virus co-infection in HIV-infected subjects. *AIDS, 19 Suppl 3*, S64-71. doi: 00002030-200510003-00011 [pii]

Clifford, D. B., Smurzynski, M., Park, L. S., Yeh, T. M., Zhao, Y., Blair, L., . . . Evans, S. R. (2009). Effects of active HCV replication on neurologic status in HIV RNA virally suppressed patients. *Neurology, 73*(4), 309-314. doi: 73/4/309 [pii]10.1212/WNL.0b013e3181af7a10

Cohen, M. H., Cook, J. A., Grey, D., Young, M., Hanau, L. H., Tien, P., ... & Wilson, T. E. (2004). Medically eligible women who do not use HAART: the importance of abuse, drug use, and race. *Journal Information*, *94*(7).

Cohen, R.A., de la Monte, S., Gongvatana, A., Ombao, H., Gonzalez, B., Devlin, K., Navia, B., Tashima, K. (2010). Plasm cytokine concentrations associated with HIV/hepatitis C coinfection are related to attention, executive and psychomotor functioning. Journal of neuroimmunology 233, 204-210. Doi:10.1016/j.jneuroim.2010.11.006

Cohen, R.A., Harezlak, J., Gongvatana, A., Buchthal, S., Schiffitto, G., Clark, U., Paul, R., …Navia, B. (2010) Cerebral metabolite abnormalities in human immunodeficiency virus are associated with cortical and subcortical volumes. Journal of Neurovirology, 16 (6), 435-444

Collie, A. (2005). Cognition in liver disease. *Liver Int, 25*(1), 1-8. doi: LIV1012 [pii]10.1111/j.1478-3231.2005.01012.x

Correia, S., Lee, S. Y., Voorn, T., Tate, D. F., Paul, R. H., Zhang, S., . . . Laidlaw, D. H. (2008). Quantitative tractography metrics of white matter integrity in diffusion-tensor MRI.

Neuroimage, 42(2), 568-581. doi: S1053-8119(08)00643-5 [pii]10.1016/j.neuroimage.2008.05.022

Crystal, H., Kleyman, I., Anastos, K., Lazar, J., Cohen, M., Liu, C., Pearce, L., …Manly, J. (2012) Effects of Hepatitis C and HIV on Cognition in women: Data from the women's interagency HIV study. Journal of Acquired Immune Deficiency Syndrome, 59 (2), 149-154

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nature Reviews Neuroscience*, *9*(1), 46-56.

Devlin, K. N., Gongvatana, A., Clark, U. S., Chasman, J. D., Westbrook, M. L., Tashima, K. T., ... & Cohen, R. A. (2011). Neurocognitive Effects of HIV, Hepatitis C, and Substance Use History. *Journal of the International Neuropsychological Society*, *18*(1), 68.

Ellis, R. J.; Gamst, A. C.; Capparelli, E.; Spector, S. A.; Hsia, K.; Wolfson, T.; Abramson, I.; Grant, I. ; McCutchan, J.A.(2000) Cerebrospinal fluid HIV RNA originates from both local CNS and systemic sources. Neurology, 54 (4), 927.

Epstein, L.G. and Gelbard, H.A., (1999). HIV-1-induced neuronal injury in the developing brain. Journal of Leukocyte Biology, 65, 453-457.

Ettenhofer, M. L., Hinkin, C. H., Castellon, S. A., Durvasula, R., Ullman, J., Lam, M., ... & Foley, J. (2009). Aging, neurocognition, and medication adherence in HIV infection. *The American journal of geriatric psychiatry: official journal of the American Association for Geriatric Psychiatry*, *17*(4), 281.

Faragon, J. J., & Piliero, P. J. (2003). Drug interactions associated with HAART: focus on treatments for addiction and recreational drugs. *AIDS READER-NEW YORK-*, *13*(9), 433-450.

Filippi, C. G., Ulug, A. M., Ryan, E., Ferrando, S. J., & van Gorp, W. (2001). Diffusion tensor imaging of patients with HIV and normal-appearing white matter on MR images of the brain. *AJNR Am J Neuroradiol, 22*(2), 277-283.

Fontana, R. J., Bieliauskas, L. A., Lindsay, K. L., Back-Madruga, C., Wright, E. C., Snow, K. K., . . . Padmanabhan, L. (2007). Cognitive function does not worsen during pegylated interferon and ribavirin retreatment of chronic hepatitis C. *Hepatology, 45*(5), 1154-1163. doi: 10.1002/hep.21633

Forton, D. M., Thomas, H. C., Murphy, C. A., Allsop, J. M., Foster, G. R., Main, J., . . . Taylor-Robinson, S. D. (2002). Hepatitis C and cognitive impairment in a cohort of patients with mild liver disease. *Hepatology, 35*(2), 433-439. doi: S0270913902540994 [pii]10.1053/jhep.2002.30688

Forton, D. M., Thomas, H. C., & Taylor-Robinson, S. D. (2004). Central nervous system involvement in hepatitis C virus infection. *Metab Brain Dis, 19*(3-4), 383-391.

Forton DM, Hamilton G, Allsop JM, Grover VP, Wesnes K, O'Sullivan C, Thomas HC, Taylor-Robinson SD. (2008). Cerebral immune activation in chronic hepatitis C infection: a magnetic resonance spectroscopy study. *Journal of Hepatology. 49*(3), 316-22.

Fu, L. P., Bi, G. H., Zou, Z. T., Wang, Y., Ye, E. M., Ma, L., & Yang, Z. (2008). Impaired response inhibition function in abstinent heroin dependents: an fMRI study. *Neuroscience letters*, *438*(3), 322-326.

Furber, A. S., Maheswaran, R., Newell, J. N., & Carroll, C. (2007). Is smoking tobacco an independent risk factor for HIV infection and progression to AIDS? A systemic review. *Sexually transmitted infections*, *83*(1), 41-46.

Gannon, P., Khan, M.Z., Kolson, D.L. (2011) Current understanding of HIV-associated neurocognitive disorders pathogenesis. Current Oninion in Neurology, 24, 275-283. DOI: 10.1097/WCO.0b013e32834695fb

Gongvatana, A., Cohen, R. A., Correia, S., Devlin, K. N., Miles, J., Kang, H., ... & Tashima, K. T. (2011). Clinical contributors to cerebral white matter integrity in HIV-infected individuals. *Journal of neurovirology*, *17*(5), 477-486.

Grovit-Ferbas, K., & Harris-White, M. E. (2010). Thinking about HIV: The intersection of virus,neuroinflammation and cognitive dysfunction. Immunologic Research, 48(1-3), 40-58.

Hannestad, J., DellaGioia, N., & Bloch, M. (2011). The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology*, *36*(12), 2452-2459.

Haussinger, D. and Schliess, F. (2008) Pathogenic mechanisms of hepatic encephalopathy. Gut, 57, 1156-1165. DOI: 10.1136/gut.2007.122176.

Hawkins, C.P., McLaughlin, J.E., Kendall, B. E., McDonald, W.I., (1993) Pathological findings correlated with MRI in HIV infection. Diagnostic Neuroradiology, 35 (4), 264-268. DOI: 10.1007/BF00602609

Heaton, R.K. et al., (2011). HIV-associated neurocognitive disorders before and during the era of combination anti-retroviral therapy: differences in rates, nature and predictors. Journal of Neurovirology, 17, 3-16 DOI: 10.1007/s13365-010-0006-1.

Hilsabeck, R. C., Castellon, S. A., & Hinkin, C. H. (2005). Neuropsychological aspects of coinfection with HIV and hepatitis C virus. *Clin Infect Dis, 41 Suppl 1*, S38-44. doi: CID35341 [pii]10.1086/429494

Hilsabeck, R. C., Hassanein, T. I., Carlson, M. D., Ziegler, E. A., & Perry, W. (2003). Cognitive functioning and psychiatric symptomatology in patients with chronic hepatitis C. *J Int Neuropsychol Soc, 9*(6), 847-854. doi: 10.1017/S1355617703960048S1355617703960048 [pii]

Hinkin, C., Castellon, S., Levine, A, Barclay, T., Singer, E. (2008) Neurocognition in Individuals Co-infected with HIV and Hepatitis C. Journal of Addictive Diseases. 27(2), 11-17.

Hinkin, C. H., Hardy, D. J., Mason, K. I., Castellon, S. A., Durvasula, R. S., Lam, M. N., & Stefaniak, M. (2004). Medication adherence in HIV-infected adults: effect of patient age, cognitive status, and substance abuse. *AIDS (London, England)*, *18*(Suppl 1), S19.

Kellogg, S. H., McHugh, P. F., Bell, K., Schluger, J. H., Schluger, R. P., LaForge, K. S., . . . Kreek, M. J. (2003). The Kreek-McHugh-Schluger-Kellogg scale: a new, rapid method for quantifying substance abuse and its possible applications. *Drug Alcohol Depend, 69*(2), 137-150. doi: S0376871602003083 [pii]

Jayadev, S. and Garden, G. (2009). Host and viral factors influencing the pathogenesis of HIV-associated neurocognitive disorders. Journal of Neuroimmune Pharmacology, 4, 175-189. DOI: 10.1007/s11481-009-9154-6.

Jernigan, T.L., Archibald, S.L., Fennema-Notestine, C., Taylor, M.J., Theilmann, R.J., Julaton, M.D., …Grant, I. (2011) Clinical factors related to brain structure in HIV: the

CHARTER study. Journal of Neurovirology. 17, 248-257. doi: 10.1007/s13365-011-0032-7.

Kalechstein, A.D., Newton, T.F., Green, M., 2003. Methamphetamine dependence is associated with neurocognitive impairment in the initial phases of abstinence. J. *Neuropsychiatry Clin. Neurosci. 15*, 215–220.

Kallianpur, K.J., Kirk, G.R., Sailasuta, N., Valcour, V., Shiramizu, B., Nakamoto, B.K., Shikuma, C., (2012) Regional Cortical Thinning associated with decectable levels of HIV DNA. Cerebral Cortex, 22, 2065-2075. doi: 10.1093/cercor/bhr285.

Kieburtz KD, Ketonen L, Zettelmaier AE, Kido D, Caine ED, Simon JH. Magnetic Resonance Imaging Findings in HIV Cognitive Impairment. *Arch Neurol.* 1990;47(6):643-645. doi:10.1001/archneur.1990.00530060051016.

Kramer, L., Hofer, H., Bauer, E., Funk, G., Formann, E., Steindl-Munda, P., & Ferenci, P. (2005). Relative impact of fatigue and subclinical cognitive brain dysfunction on health-related quality of life in chronic hepatitis C infection. *AIDS, 19 Suppl 3*, S85-92. doi: 00002030-200510003-00014 [pii]

Krupp, L. B., LaRocca, N. G., Muir-Nash, J., & Steinberg, A. D. (1989). The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol, 46*(10), 1121-1123.

Kuper, M, Rabe, K., Esser, K., Gizewski, E.R., Husstedt, I.W., Maschke, M., Obermann, M. (2011) Structural gray and white matter changes in patients with HIV. Journal of Neurology, 258 (6), 1066-1075. doi: 10.1007/s00415-010-5883-y

Lancaster, J. L., Glass, T. G., Lankipalli, B. R., Downs, H., Mayberg, H., & Fox, P. T. (2004). A modality-independent approach to spatial normalization of tomographic images of the human brain. *Human Brain Mapping*, *3*(3), 209-223.

Lawton, M. P., & Brody, E. M. (1969). Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist, 9*(3), 179-186.

Letendre, S. L., Cherner, M., Ellis, R. J., Marquie-Beck, J., Gragg, B., Marcotte, T., . . . Grant, I. (2005). The effects of hepatitis C, HIV, and methamphetamine dependence on neuropsychological performance: biological correlates of disease. *AIDS, 19 Suppl 3*, S72-78. doi: 00002030-200510003-00012 [pii]

Letendre, S., Paulino, A. D., Rockenstein, E., Adame, A., Crews, L., Cherner, M., ... & Masliah, E. (2007). Pathogenesis of hepatitis C virus coinfection in the brains of patients infected with HIV. *Journal of Infectious Diseases*, *196*(3), 361-370.

Letendre, S., Marquie-Beck, J., Capparelli, E., Best, B., Clifford, D., Collier, A.C., (…), Ellis, R. (2008) Validation of the CNS penetration-effectiveness rank for quantifying antiretroviral penetration into the central nervous system. Archives of neurology, 65 (1), 65-70. doi: 10.1001/archneurol.2007.31.

Lindl, K. A., Marks, D. R., Kolson, D. L., & Jordan-Sciutto, K. L. (2010). HIV-associated neurocognitive disorder: Pathogenesis and therapeutic opportunities. Journal of *Neuroimmune Pharmacology, 5*(3), 294-309.

Marcondes, M. C. G., Flynn, C., Watry, D. D., Zandonatti, M., & Fox, H. S. (2010). Methamphetamine increases brain viral load and activates natural killer cells in simian immunodeficiency virus-infected monkeys. *The American journal of pathology*, *177*(1), 355-361.

Martin, E. M., Novak, R. M., Fendrich, M., Vassileva, J., Gonzalez, R., Grbesic, S., . . . Sworowski, L. (2004). Stroop performance in drug users classified by HIV and hepatitis C virus serostatus. *J Int Neuropsychol Soc, 10*(2), 298-300. doi: 10.1017/S135561770410218XS135561770410218X [pii]

Martin, E. M., Pitrak, D. L., Weddington, W., Rains, N. A., Nunnally, G., Nixon, H., ... & Bechara, A. (2004). Cognitive impulsivity and HIV serostatus in substance dependent males. *Journal of the International Neuropsychological Society*, *10*(7), 931-938.

McAndrews, M. P., Farcnik, K., Carlen, P., Damyanovich, A., Mrkonjic, M., Jones, S., & Heathcote, E. J. (2005). Prevalence and significance of neurocognitive dysfunction in hepatitis C in the absence of correlated risk factors. *Hepatology, 41*(4), 801-808. doi: 10.1002/hep.20635

McArthur, J.C., Brew, B.J., Nath, A. (2005) Neurological complications of HIV infection. Lancet Neurology, 4, 543-555

McArthur, J.C., McClernon, D.R., Cronin, M.F, Nance-Sproson, T.E., Saah, A.J., St. Clair, M., Lanier, R. (1997) Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and berain. Annals of Neurology, 42 (5), 689-698. doi:10.1002/ana.410420504.

McPhail, M. E., & Robertson, K. R. (2011). Neurocognitive impact of antiretroviral treatment: thinking long-term. *Current HIV/AIDS Reports*, *8*(4), 249-256.

Meltzer, M.S, Nakamura, M., Hansen, B.D., Turpin, J.A., Kalter, D.C, Gendelman, E.(1990) Macrophages as susceptible targets for HIV infection, persisten viral reservoirs in tissue and key immunoregulatory cells that control levels of virus replication and extent of disease.. AIDS Research and Human Retroviruses, 6(8),967-971. doi:10.1089/aid.1990.6.967.

Mooney, S., Hasssanein, T. I., Hilsabeck, R. C., Ziegler, E. A., Carlson, M., Maron, L. M., & Perry, W. (2007). Utility of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) in patients with end-stage liver disease awaiting liver transplant. *Arch Clin Neuropsychol, 22*(2), 175-186. doi: S0887-6177(07)00002-9 [pii]10.1016/j.acn.2006.12.005

Morgello, S., Estanislao, L., Ryan, E., Gerits, P., Simpson, D., Verma, S., . . . Sharp, V. (2005). Effects of hepatic function and hepatitis C virus on the nervous system assessment of advanced-stage HIV-infected individuals. *AIDS, 19 Suppl 3*, S116-122. doi: 00002030-200510003-00018 [pii]

Nath, A. (2010) Human immunodeficiency virus-associated neurocognitive disorder. Pathophysiology in relation to drug addiction. Annals of the New York Academy of Sciences: Addiction Reviews 2, 1187, 122-128. doi: 10.1111/j.1749-6632.2009.05277.

Nordahl, T. E., Salo, R., Natsuaki, Y., Galloway, G. P., Waters, C., Moore, C. D., ... & Buonocore, M. H. (2005). Methamphetamine users in sustained abstinence: a proton magnetic resonance spectroscopy study. *Archives of general psychiatry*, *62*(4), 444.

Parsons, T. D., Tucker, K. A., Hall, C. D., Robertson, W. T., Eron, J. J., Fried, M. W., & Robertson, K. R. (2006). Neurocognitive functioning and HAART in HIV and hepatitis C virus co-infection. *AIDS, 20*(12), 1591-1595. doi: 10.1097/01.aids.0000238404.16121.4700002030-200608010-00003 [pii]

Pau, C. W., Lee, T., & Chan, S. F. F. (2002). The impact of heroin on frontal executive functions. *Archives of clinical neuropsychology*, *17*(7), 663-670.

Paul, R., Cohen, R., Navia, B., Tashima, K. (2002) Relationships between cognition and structrual neuroimaging findings in adults with human immunodeficiency virus type-1. Neuroscience and Biobehavioral Reviews, 26 (3), 353-359. doi: 10.1016/S0149-7634(02)00006-4.

Paul, R.H., Letendre, S., Dearborn J. (2007). Cognitive function in patients co-infected with hepatitis C and human immunodeficiency virus. Current Hepatitis Reports, 6, 145-152.

Pawlotsky, J. M. (2004). Pathophysiology of hepatitis C virus infection and related liver disease. *Trends Microbiol, 12*(2), 96-102. doi: 10.1016/j.tim.2003.12.005S0966842X03003329 [pii]

Perry, W., Carlson, M. D., Barakat, F., Hilsabeck, R. C., Schiehser, D. M., Mathews, C., & Hassanein, T. I. (2005). Neuropsychological test performance in patients co-infected with hepatitis C virus and HIV. *AIDS, 19 Suppl 3*, S79-84. doi: 00002030-200510003-00013 [pii]

Perry, W., Hilsabeck, R. C., & Hassanein, T. I. (2008). Cognitive dysfunction in chronic hepatitis C: a review. *Dig Dis Sci, 53*(2), 307-321. doi: 10.1007/s10620-007-9896-z

Pulliam, L., Rempel, H., Sun, B., Agadjian, L., Calosing, C. and Meyerhoff, D.J.. (2011) A peripheral monocyte interferon phenotype in HIV infection correlates with a decrease in magnetic resonance spectroscopy metabolite concentrations. AIDS 25, 1721-1726 DOI: 10.1097/QAD.0b013e328349f022

Ragin, A. B., Storey, P., Cohen, B. A., Epstein, L. G., & Edelman, R. R. (2004). Whole brain diffusion tensor imaging in HIV-associated cognitive impairment. *AJNR Am J Neuroradiol, 25*(2), 195-200.

Ragin, A. B., Wu, Y., Storey, P., Cohen, B. A., Edelman, R. R., & Epstein, L. G. (2005). Diffusion tensor imaging of subcortical brain injury in patients infected with human immunodeficiency virus. *J Neurovirol, 11*(3), 292-298. doi: UX5L5173X04U0312 [pii]10.1080/13550280590953799

Richardson JL, Nowicki M, Danley K, Martin EM, Cohen MH, Gonzalez R, Vassileva J, Levine AM.(2005). Neuropsychological functioning in a cohort of HIV- and hepatitis C virus-infected women. AIDS, 19(15), 1659-67.

Robertson, K., Fiscus, S., Kappor, C., Robertson, W., Schneider, G., Shepard, R., …Hall, C. (1998). CSF, Plasma viral load and HIV associated dementia. Journal of Neurovirology, 4 (1), 90-94.

Robertson, K., Smurzynski, M., Parsons, T.D., Wu, K., Bosch, R.J., Wu, J., … Ellis, R.J., (2007) The prevalence and incidence of neurocognitive impairment in the HAART era. AIDS, 21(14), 1915-1921. doi: 10.1097.QAD.0b013e32828e4e27

Robertson, K, Su, Z., Margolis, D., Krambrink, A., Havlir, D., Evans, S., Skiest, D. (2010) Neurocognitive effects of treatment interruption in stable HIV-positie paitents in an observational cohort. Neurology, 74 (16), 1260-1266. doi: 10.1212/WNL.0b013e3181d9ed09.

Rosas, H. D., Lee, S. Y., Bender, A. C., Zaleta, A. K., Vangel, M., Yu, P., ... & Hersch, S. M. (2010). Altered white matter microstructure in the corpus callosum in Huntington's disease: implications for cortical “disconnection”. *Neuroimage*, *49*(4), 2995-3004.

Rowland, D. J., Garbow, J. R., Laforest, R., & Snyder, A. Z. (2005). Registration of [18F] FDG microPET and small-animal MRI. *Nuclear medicine and biology*, *32*(6), 567.

Ryan, E. L., Morgello, S., Isaacs, K., Naseer, M., & Gerits, P. (2004). Neuropsychiatric impact of hepatitis C on advanced HIV. *Neurology, 62*(6), 957-962.

Sacktor, N., Miyahara, S., Deng, L., Evans, S., Schifitto, G., Cohen, B. A., ... & Clifford, D. B. (2011). Minocycline treatment for HIV-associated cognitive impairment Results from a randomized trial. *Neurology*, *77*(12), 1135-1142.

Scott, J.C., Woods, S.P., Matt, G.E., Meyer, R.A., Heaton, R.K., Atkinson, J.H., Grant, I.,(2007). Neurocognitive effects of methamphetamine: a critical review and metaanalysis.*Neuropsychol. Rev. 17*, 275–297.

Shaheen, A. A., & Myers, R. P. (2007). Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis C-related fibrosis: a systematic review. *Hepatology, 46*(3), 912-921. doi: 10.1002/hep.21835

Shimony JS, Sheline YI, D'Angelo G, Epstein AA, Benzinger TL, Mintun MA*, et al.* Diffuse microstructural abnormalities of normal-appearing white matter in late life depression: a diffusion tensor imaging study. *Biol Psychiatry* 2009,**66**:245-252.

Shimony, J. S., Burton, H., Epstein, A. A., McLaren, D. G., Sun, S. W., & Snyder, A. Z. (2006). Diffusion tensor imaging reveals white matter reorganization in early blind humans. *Cerebral Cortex*, *16*(11), 1653-1661.

Song, S. K., Sun, S. W., Ramsbottom, M. J., Chang, C., Russell, J., & Cross, A. H. (2002). Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. *Neuroimage*, *17*(3), 1429-1436.

Stebbins, G. T., Smith, C. A., Bartt, R. E., Kessler, H. A., Adeyemi, O. M., Martin, E., . . . Moseley, M. E. (2007). HIV-associated alterations in normal-appearing white matter: a voxel-wise diffusion tensor imaging study. *J Acquir Immune Defic Syndr, 46*(5), 564-573.

Strazza, M., Pirrone, V., Wigdahl, B., & Nonnemacher, M. R. (2011). Breaking down the barrier: The effects of HIV-1 on the blood-brain barrier. Brain Research, 1399, 96-115.

Talairach, J., & Tournoux, P. (1988). *Co-planar stereotaxic atlas of the human brain* (Vol. 147). New York:: Thieme.

Thein, H. H., Yi, Q., Dore, G. J., & Krahn, M. D. (2008). Natural history of hepatitis C virus infection in HIV-infected individuals and the impact of HIV in the era of highly active antiretroviral therapy: a meta-analysis. *AIDS, 22*(15), 1979-1991. doi: 10.1097/QAD.0b013e32830e6d5100002030-200810010-00010 [pii]

Thompson, M., Aberg, J., Hoy, J., Telenti, A., Benson, C., Cahn, P. Eron, J., Gunthard, H, Hammer, S., Reiss, P., Richman, D., Rizzardini, G., Thomas, D., Jacobsen, D., Volberding, P. (2012) Antiretroviral treatment of adult HIV infection:2012 recommentations of the International Antiviral Society-USA panel. Journal of the American Medical Association, 308 (4), 387-402. DOI: 10.1001/jama.2012.7961.

Thompson, P.M., Dutton, R.A., Hayashi, K.M., Toga, A.W., Lopez, O.L., Alzenstein, H.J., Becker, J.T. (2005) Thinning of the cerebral cortex visualized in HIV/AIDS reflects CD4+ T lymphoccyte decline. PNAS, 102 (43) 15647-15652. doi: 10.1073/pnas.0502548102.

Thurnher, M. M., Castillo, M., Stadler, A., Rieger, A., Schmid, B., & Sundgren, P. C. (2005). Diffusion-tensor MR imaging of the brain in human immunodeficiency virus-positive patients. *AJNR Am J Neuroradiol, 26*(9), 2275-2281. doi: 26/9/2275 [pii]

Togwood, K. Pitkanen, M, Kulasegaram, R., Fradera, A., Kumar, A., Soni, S… Kopelman, M. (2012) Mapping the brain in younger and older asymptomatic HIV-1 men: Frontal volume changes in the absence of other cortical or diffusion tensor abnormalities. Cortex, 48, 230-241. doi 10.1016/j.cortex.2011.03.006.

Trang, T., Petersen, J. R., & Snyder, N. (2008). Non-invasive markers of hepatic fibrosis in patients co-infected with HCV and HIV: comparison of the APRI and FIB-4 index. *Clin Chim Acta, 397*(1-2), 51-54. doi: S0009-8981(08)00358-6 [pii]10.1016/j.cca.2008.07.009

Tucker, K.A., Robertoson, K.R., Lin, W., Smith, J.K., An, H., Chen, Y., Aylward, S.R., Hall, C.D. (2004) Neuroimaging in human immunodeficiency virus infection. Journal of Neuroimmunology, 157, 153-162.

Tural, C., Tor, J., Sanvisens, A., Perez-Alvarez, N., Martinez, E., Ojanguren, I., . . . Clotet, B. (2009). Accuracy of simple biochemical tests in identifying liver fibrosis in patients co-infected with human immunodeficiency virus and hepatitis C virus. *Clin Gastroenterol Hepatol, 7*(3), 339-345. doi: S1542-3565(08)01168-3 [pii]10.1016/j.cgh.2008.11.019

Volkow et al., (2001) Higher cortical and lower subcortical metabolism in detoxified methamphetamine abusers. American Journal of Psychiatry, 158 (3), 383-389 DOI: 10.1176/appi.ajp.158.3.383.

von Giesen, H. J., Heintges, T., Abbasi-Boroudjeni, N., Kucukkoylu, S., Koller, H., Haslinger, B. A., . . . Arendt, G. (2004). Psychomotor slowing in hepatitis C and HIV infection. *J Acquir Immune Defic Syndr, 35*(2), 131-137.

Ware, J. E., Jr., & Sherbourne, C. D. (1992). The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care, 30*(6), 473-483.

Webb, M. S., Vanable, P. A., Carey, M. P., & Blair, D. C. (2009). Medication adherence in HIV-infected smokers: the mediating role of depressive symptoms. *AIDS Education and Prevention*, *21*(3_supplement), 94-105.

Weissenborn, K., Bokemeyer, M., Krause, J., Ennen, J., & Ahl, B. (2005). Neurological and neuropsychiatric syndromes associated with liver disease. *AIDS, 19 Suppl 3*, S93-98. doi: 00002030-200510003-00015 [pii]

Weissenborn, K., Krause, J., Bokemeyer, M., Hecker, H., Schuler, A., Ennen, J. C., . . . Boker, K. W. (2004). Hepatitis C virus infection affects the brain-evidence from psychometric studies and magnetic resonance spectroscopy. *J Hepatol, 41*(5), 845-851. doi: S0168-8278(04)00358-7 [pii]10.1016/j.jhep.2004.07.022

Winston, A., Garvey, L., Scotney, E., Yerrakalva, D., Allsop, J. M., Thomson, E. C., ... & Taylor-Robinson, S. D. (2009). Does acute hepatitis C infection affect the central nervous system in HIV-1 infected individuals?. *Journal of viral hepatitis*, *17*(6), 419-426.

Woods, SP., Moore, D.J., Weber, E., Grant, I. (2009) Cognitive neuropsychology of HIV-associated neurocognitive disorders. Neuropsychology review, 19 (2), 152-168.

Wright, P. W., Heaps, J. M., Shimony, J. S., Thomas, J. B., & Ances, B. M. (2012). The Effects of HIV and combination antiretroviral therapy on white matter integrity. *AIDS*, *26*(12), 1501-1508.

Wu, Y., Storey, P., Cohen, B. A., Epstein, L. G., Edelman, R. R., & Ragin, A. B. (2006). Diffusion alterations in corpus callosum of patients with HIV. *AJNR Am J Neuroradiol, 27*(3), 656-660. doi: 27/3/656 [pii]