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Medication Treatment and Neuropsychological Functioning in Perinatal HIV

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MEDICATION TREATMENT AND NEUROPSYCHOLOGICAL FUNCTIONING IN PERINATAL HIV

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This study confirmed whether children on the current treatment of choice for HIV infection, Highly Active Antiretroviral Treatment (HAART), exhibit better immune functioning than children on earlier forms of treatment, including sole exposure to Monotherapy/Combination Therapy (Mono-Combo) and "upgrading" from Mono-Combo to HAART (Conversion). It explored whether HAART protects areas of neuropsychological functioning previously found to be compromised in children perinatally infected with HIV more effectively than these earlier treatments. This study includes a unique population (i.e., predominantly minority, low SES status, and largely bilingual), and controls for a number of treatment variables that have not been previously considered. Using the Neurodevelopmental Model and the literature, it was hypothesized that more global functions (i.e., IQ indices besides processing speed) and functions developing earlier in life (i.e., language) would be less affected than more specific functions developing later in life (i.e., visual-motor integration and processing speed). Treatment groups included Mono-Combo, Converters, and HAART. Participants (N=161, 3 to 20 years) were assessed in language, visual-motor integration, processing speed, and IQ using standardized measures and procedures. Three MANCOVAS and an ANCOVA compared groups on immune and neuropsychological measures using age antiretroviral medications were started and years on antiretroviral medications as

covariates. Results showed children on HAART have significantly better immune functioning than the Mono-Combo and Converter groups. Consistent with other studies that have controlled for demographic factors, language functioning was not affected by treatment type. Contrary to expectations, visual-motor integration was also not affected by treatment type. Interestingly, Converters were found to perform worse on processing speed than children only exposed to Mono-Combo or HAART. Consistent with expectations, the other IQ indices (i.e., VCI, POI, and FDI) were not affected by treatment type. Findings support the use of HAART globally to improve immune functioning. However, they also provide evidence that HAART does not more effectively protect areas of neuropsychological functioning previously found to be compromised than these former treatments, even when controlling for agents that cross the blood brain barrier, age medications were started, and years on medications. They also bring into question the possible effect of frequency and timing of regimen changes.

For all of my loved ones, who have supported me through every road I have chosen to take throughout my life. And for my mother and father in particular, who have shown me the value of unconditional love, hard work, and the fulfillment that comes with the pursuit of dreams.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

There are multiple complex factors associated with the functional deficits seen in children with chronic illnesses in which the disease process itself and/or its treatment adversely affects the central nervous system (CNS). Perinatal infection with the Human Immunodeficiency Virus (HIV) is one chronic illness in which the virus adversely affects multiple brain structures and pathways, and in which these adverse affects are evident in performance on neuropsychological tests. The aim of this study is to confirm prior research that children on the current treatment of choice for HIV infection, Highly Active Antiretroviral Treatment (HAART), exhibit better immune functioning than children on earlier forms of treatment, Mono and Combination Therapy. This study also expands on prior research by determining if HAART protects certain areas of neuropsychological functioning that have previously been found to be compromised in children perinatally infected with HIV more effectively than these earlier forms of treatment. A neurodevelopmental model is used along with the literature to guide the specific hypotheses of the study. Further, this study includes a population unique to those used in prior research, as well as controls for a number of treatment variables that have not been considered in the past.

Epidemiology

Since HIV was first identified in children in 1982 (CDC, 1982, n. 48 and 49), perinatal transmission has become the primary mode of infection among new cases of

HIV in young children in the United States (U.S.) (Pizzo et al., 1988). A number of interventions, including the routine screening of pregnant women, avoidance of breastfeeding, use of elective cesarean delivery, and use of HAART therapy during pregnancy, has reduced rates of vertical transmission to less than 2% from 25% to 30% with no interventions. The estimated number of perinatally acquired AIDS cases has also decreased by 95% since its peak in 1992 (CDC, 2006).

Despite dramatic improvements in screening and prevention in the U.S., the continued rise in new infections among women and a lack of prenatal care for a number of them (CDC, 2006) have kept perinatal HIV research relevant in the U.S. According to 2005 CDC estimates, about 6000 to 7000 HIV-infected women give birth each year, resulting in 280 to 370 new perinatal infections. In addition, the U.S.'s success in reducing vertical transmission contrasts with situations in poorer, developing nations, where perinatal HIV and subsequent neurocognitive consequences remain a significant global health problem (CDC, 2006). State-of-the-art treatment (i.e., HAART) currently available in the U.S. and developed countries are not readily available or studied in much of the developing world, making research findings prior to the introduction of combined therapies highly relevant to these parts of the world. Furthermore, many youngsters in the U.S. began treatment as infants or young children when treatment options were much more limited and resembled those now available in developing countries. These youngsters may continue to experience the neurocognitive consequences brought about by the early severe effects of the virus on the brain despite current virologic control (Willen, 2006).

Biology and Neurology

HIV, an RNA retrovirus, mainly targets T4 (CD4+/helper/inducer) cells, white blood cells that warn the immune system of invaders (AIDSmeds.com, 2000; Armstrong et al., 1993 and 2003). HIV infects the T4 cell and uses it to replicate and release new virus particles, which weakens and eventually kills the cell (AIDSinfo, 2004; AIDS InfoNet, 2005; AIDSmeds.com, 2000; Armstrong et al., 2003; Gandhi et al., 1999; Schweizer and Hollingsworth, 1998). Unless treatment interrupts the HIV life cycle, the depletion of T4 cells and increasing HIV viral load results in progressive and irreversible immunosuppression (Armstrong et al., 1993 and 2003).

Unlike adults who are developmentally stable and in whom changes in immune status are predominantly caused by HIV infection, children are continually growing and developing in their CNS and immune functioning, making it more difficult to make connections between the virus, immune functioning, and developmental outcome. What is known about HIV in children, however, is that it is a devastating and complex illness that often involves both direct and indirect disruption of the developing CNS. This disruption leaves HIV-infected children, especially those perinatally infected, at risk for developing significant neurocognitive deficits (i.e., learning disabilities) (Armstrong et al., 1999; Willen, 2006).

Indirectly, HIV-immunosuppression leads to opportunistic infections that attack the CNS and may impact the structure and function of the brain over time (e.g., cytomegalovirus, cryptococcus, toxoplasmosis, herpes simplex) (Mintz and Epstein, 1992). Directly, HIV appears to invade the CNS through the migration of infected macrophages across the blood-brain barrier (Persidsky et al., 2001 as cited in Mitchell,

2006), a process that occurs early in the disease process, especially in infants and children (Belman et al., 1988). The result of HIV's direct attack of CNS tissue includes (1) reduced brain mass (cortical atrophy or microencephaly) associated with damage to and a reduction in white matter, (2) calcifications in the basal ganglia (involved in general and visual motor control and cognitive functions), cerebellum, frontal lobe (involved in controlling attention & executive functions), and periventricular white matter, and (3) abnormal myelination of the brain (e.g., demyelination of nerve tracks) leading to a reduction in white matter (Armstrong et al., 1993 and 2003; Berk, 2005; Belman, et al., 1988; Epstein, et al., 1987; Harris, 1999; Mintz, 1999; Mintz and Epstein, 1992; Mitchell, 2001). Adequate myelination, in particular, is necessary for neuronal conduction and the complex integration of function (Berk, 2005). Therefore, an interruption in myelination is likely to result in developmental delay, especially in higher cortical functions thought to be mediated by the frontal and parietal regions (Gay et al., 1995), including visual-spatial functioning (e.g., visual-motor integration), processing speed, sensory-motor functioning, sensory integration skills, sequencing, attention, abstract reasoning, and verbal memory (Gay et al., 1995; Harris, 1990).

Natural Progression

In untreated or poorly managed pediatric HIV infection, one of the most devastating manifestations of HIV's direct effect on the brain or neuromuscular system is encephalopathy, or neuropsychological deterioration (e.g., loss of developmental milestones and intellectual function). Early in the epidemic, HIV encephalopathy was considered a cardinal feature of HIV and AIDS, as it affected a great proportion of

perinatally infected children (20% to 90% according to estimates) (Pizzo et al., 1988; Mitchell, 2006), particularly during the first year of life in the absence of treatment (Tardeiu, et al., 2000 as cited in Lindsey et al., 2007). However, HIV-related encephalopathy could also show up years later (Mintz and Epstein, 1992).

Two types of CNS encephalopathy are currently identified in children with HIV: static and progressive (Belman et al., 1988; Epstein et al., 1987; Willen, 2006). Static encephalopathy is characterized by severely delayed cognitive functioning and neuromotor deficits that vary in severity without progressive deterioration (Armstrong et al., 1993). New skills are acquired much slower than expected for the child's age (Willen, 2006). Children may have lower (but not deteriorating) IQs, often ranging from low average to markedly impaired (Belman et al., 1988; Epstein et al., 1987), or have selective impairments in certain areas while appearing to have relatively normal overall cognitive function (Mitchell, 2006).

Progressive encephalopathy has two courses, a subacute and plateau course (Armstrong et al, 1993; Willen, 2006). Subacute progressive encephalopathy results in a loss of function over time, with new developmental milestones not acquired and old ones lost as well (Armstrong et al., 1993; Belman et al., 1988; Gay et al, 1995). On standardized measures, both raw and standardized scores are likely to decline (Gay et al., 1995). Children may remain relatively stable during brief periods of neurological stability or plateau before a new loss is appreciated, leading to overall deterioration in function over time (Armstrong et al., 1993; Belman et al., 1988).

Plateau progressive encephalopathy involves a slowing or cessation of developmental progress, with no deterioration in function for relatively long periods of

time (Armstrong et al., 1993; Belman et al., 1988). While previously acquired developmental milestones are not lost, new ones are not attained (Belman et al., 1988; Gay et al., 1995; Willen, 2006). On standardized measures, raw scores are likely to remain stable, while standardized scores are likely to decline over time (Belman, 1990 as cited in Gay et al., 1995). While declines in cognitive function and brain growth are subtler than in the subacute course, these children do eventually deteriorate and lose previously acquired skills (Armstrong et al., 1993; Belman et al., 1988; Willen, 2006).

Despite plateau periods, progressive encephalopathy is associated with the deterioration of already acquired abilities resulting in more severe cognitive and motor delays than those seen in stable encephalopathy (Armstrong et al., 1993; Belman et al., 1988; Epstein et al., 1987; Gay et al., 1995). Before the advent of modern drug therapies, progressive encephalopathy was significantly associated with fatal outcome, with CNS abnormalities appearing as early as the first month of life and death occurring 1 to 28 months after the onset of neurologic deterioration (Belman, et al., 1988; Armstrong et al., 1993). While the most severe forms of encephalopathy are now seldom seen in the U.S., they are still a concern in developing countries that do not have access to the more advanced treatments (Bailey, et al., 1999 as cited in Armstrong et al., 2003).

Fortunately, HIV encephalopathy is not universal (Belman, et al., 1988) and not all children infected with HIV develop early CNS disease and significant developmental delay (Gay et al., 1995). While some only experience relatively mild neurocognitive deficits (e.g., subtle deterioration in functioning over time, low-average to borderline IQ, or average IQ with specific learning problems) (Armstrong et al., 1993; Belman et al.,

1988; Belman, 1990), others never develop signs of neuropathology (Scott et al., 1989 as cited in Armstrong, et al., 1993).

Treatment and Neurodevelopment

Given the natural progression of the virus (rapid immunosuppression), HIV's early history in the U.S. (1980s) before the use of combination therapies created a morbid clinical picture for infected children. This included the early acquisition of serious AIDS-defining opportunistic infections (between 1.5 and 26 months), cardiac abnormalities, encephalopathy, microcephaly, myelopathy, developmental disabilities, peripheral neuropathies, and frequent (as many as 5) and prolonged (up to two months) hospitalizations between birth and 20 months of age, all leading to premature death between 5 months and 5 years of age in 20% to 25% of cases (Armstrong et al., 1993; Belman, et al., 1988; CDC, 1982, n. 49; Oleske et al., 1983; Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006). These morbid outcomes led early neurodevelopmental studies to mainly focus on untreated or minimally treated infants and young children (Armstrong et al., 2003; Willen, 2006).

Overall, these early studies found significant global delays in cognitive and motor functioning when compared to seroreverters or nonexposed children, with substantial numbers of infected infants and young children meeting criteria for developmental disability and mental retardation (Armstrong et al., 1993). HIV effects were found even when accounting for such confounding factors as prenatal drug exposure, ethnicity, socioeconomic status, maternal separation and death (as a result of maternal HIV positive status), prematurity, birthweight (Gay et al., 1995), and immune functioning (Lindsey et

al., 2000). Further, some studies showed infants with immune suppression to be more vulnerable than non-immune-suppressed HIV-infected or –affected infants with respect to neurodevelopmental functioning (Lindsey et al., 2007). Early encephalopathy with developmental delay was also shown to be associated with early mortality (Rigardetto et al., 1999 as cited in Armstrong et al., 2003).

However, over the past 20 years, children perinatally infected with HIV living in the U.S. have been placed on increasingly effective treatment regimens containing antiretroviral agents that inhibit some aspect of the HIV life cycle. These treatments have improved symptom management and immune functioning, prolonged lifespan, and improved quality of life (McConnell et al., 2005). Drug therapy guidelines have changed from suboptimal single medication treatment to combination therapy that includes up to four different classes of antiretroviral agents (McConnell et al., 2005; Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006).

Until 1995, the only drugs available for treating HIV infection were Nucleoside/ Nucleotide Reverse Transcriptase Inhibitors (NRTIs/NtRTIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), both of which act at an early stage of replication to inhibit reverse transcription of viral RNA into DNA and prevent proviral integration into the host DNA (AIDSmeds.com, 2000; Gandhi et al., 1999; Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006). Zidovudine (ZDV) was the first NRTI studied in adult and pediatric clinical trials, and the first antiretroviral agent FDA approved for the treatment of HIV infection in adults (1987) and children (1990), as well as prophylactically for the prevention of perinatal transmission (1994).

Even early studies of infants receiving monotherapy showed some improvements in neurocognitive functioning. Pizzo et al. (1988) examined the continuous intravenous infusion of ZDV alone in 21 symptomatic children (ages 14 months to 12 years) infected with HIV perinatally or through transfusions who were free of opportunistic infections and other medications. Sixty-two percent of these children showed clinical evidence of encephalopathy before therapy, including lower IQ scores than those without encephalopathy. After continuous infusion with ZDV, all children with and without neurodevelopmental abnormalities improved significantly ($M = 15.3 \pm 3.3$ overall IQ points) after 3 and 6 months of therapy, with improvement also in both verbal and performance IQ measures and no difference in the degree of improvement between the groups. Improvements were also seen in initially abnormal computed tomography (CT) and positron emission tomography (PET) scans, and increased appetite, weight gain, and CD4 levels. In some patients, improvement in encephalopathy occurred despite the absence of immunologic improvement. Thus, the continuous infusion ZDV schedule led to significant and, in a number of patients, sustained improvement of AIDS-related encephalopathy, even after ensuring improvements were not due to practice effects or other changes in patients' clinical condition. Additionally, the increase in IQ scores observed in the children without evidence of encephalopathy suggested for the first time that HIV infection may produce subtle, subclinical detrimental effects on cognitive function that may be among the earliest manifestations of AIDS encephalopathy (Pizzo et al., 1988).

Unfortunately, trials of ZDV administered orally or intermittently did not produce the same dramatic improvements in cognitive functioning. In Mintz's study (1992),

children treated with ZDV who had initially improved neurologically subsequently showed deterioration clinically 6 to 12 months later (Mintz and Epstein, 1992). These conflicting findings were likely due to the fact that NRTIs act prior to integration and thus have little to no effect on chronically infected cells that already have proviral DNA integrated into their DNA. Scientists quickly realized that, when monotherapy or antiretroviral treatment that does not maximally suppress replication is used, the magnitude and speed of HIV replication during all stages of infection lead to the development of viral strains that are resistant to the drugs (Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2005).

In 1995, combination therapy emerged as the preferred treatment modality for HIV infection when researchers discovered that treatment with combinations of AZT and other NRTIs was more effective than treatment with AZT alone. For example, Dideoxycytidine (ddC) and AZT administered in combination were shown to produce dramatic behavioral improvement among children with encephalopathy (Pizzo et al., 1990 as cited in Armstrong et al., 1993).

Also in 1995, the first licensed Protease Inhibitor (PI), Saquinavir (SQV), went to market and was approved for use in adults and adolescents older than age 16 in combination therapy. PIs act at a later stage of replication to block viral assembly after proviral DNA has been transcribed into viral RNA and RNA has been translated into viral proteins. Because PIs act after integration, they effectively inhibit replication in both newly and chronically infected cells (AIDSmeds.com, 2000; Gandhi et al., 1999; Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006). Due to its role in the HIV life cycle, the introduction of PIs into

combination therapies decreased HIV mortality in children by 70% (Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006).

In 1996, triple-drug combinations with a PI were shown to reduce the levels of HIV circulating in the blood so dramatically that the virus was often undetectable in the blood within 16 to 20 weeks with standard tests (AIDSinfo, 2002). These triple-drug therapies (a.k.a. HAART) aggressively suppress viral replication and slow the progression of HIV disease to AIDS, leading to reduced HIV-related hospitalizations and deaths (AIDSinfo, 2002; CDC, 2006). As a result of these findings, HAART became widespread during 1996 (CDC, 2003), but was not officially written in the treatment guidelines for use with children until 1998 (Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2005). Furthermore, the first and only Fusion Inhibitor (FI) was approved in 2003, further advancing HAART. FIs prevent viral entry and block the binding and fusion process (AIDSmeds.com, 2000; Gandhi et al., 1999; Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006).

Specifically among children less than 13 years of age, HAART has brought a significant decline in the proportion of AIDS cases, with a decrease from 1.4% (1981 to 1995) to 0.2% (2001 to 2004) (CDC, 2006). As a result of its consistent positive impact on survival (McConnell et al., 2005), immune functioning (Lindsey et al., 2007), growth (Buchacz et al., 2001 and Nachman et al., 2005 as cited in Lindsey et al, 2007), and overall quality of life, HAART is currently considered the "best practice" treatment regimen prescribed to maintain good immune functioning in individuals infected with HIV. By suppressing viral replication, HAART may also reduce the number of HIV-

infected cells entering the CNS, potentially reducing the incidence of severe CNS damage (Lindsey et al, 2007).

As children have lived longer due to the advent of new therapies, studies on neurocognitive functioning have moved from a focus on infants to preschoolers and school-age children, and recently to adolescents (Armstrong et al., 2003). As with infants, some studies have found children for whom treatment has been ineffective and who show a greater degree of encephalopathy and symptomatology to be at greater risk for impaired cognitive functioning (Armstrong et al., 2003; Willen, 2006). Several longitudinal investigations have also suggested that substantial neurocognitive effects lead to poorer morbidity and mortality, even with access to combined therapies (Pearson et al., 2000 and Shanbhag et al., 2005 as cited in Willen, 2006).

However, in contrast to research findings in infants, global neurocognitive impairment has not been typically found in school-age children (Fishkin et al., 2000), likely because the more severely affected infants either did not survive into childhood, or did survive as a result of improved treatment options (Havens et al., 1993; Willen, 2006). For the most part, clinically asymptomatic children and adolescents have instead tended to show more subtle and specific areas of deficit involving an interruption or slowing in the developmental acquisition of neuropsychological abilities (Mintz, 1992). The most common areas of deficit have been reported along the visual-spatial-motor continuum (Fishkin et al., 2000; Harris, et al., 1999). More specifically, children perinatally infected with HIV have been found to exhibit deficits in visually-guided behavior, including visual attention, visual-motor integration (i.e., the VMI), visual-spatial planning, manipulation, memory, and integration (i.e., Spatial Relations), and visuo-construction

abilities (Fishkin et al., 1998; Harris, 1999), as well as processing speed (Harris, 1999), fine motor coordination (i.e., the Grooved Pegboard) (Armstrong et al., 1993; Harris, 1999), and graphomotor speed (Harris, 1999), among other areas (Harris, 1999; Havens et al., 1993). In general, the literature has shown that the more complex the visual-spatial task, the more difficult it is for these children to master it (Fishkin et al., 1998; Harris, 1999).

In addition, perinatally infected children have rarely been found to exhibit deficits in receptive or expressive language (Havens et al., 1993) or verbal learning (Harris, 1999) when considering environmental factors, such as when compared to seroreverted and control children matched for age, sex, race, prenatal substance exposure, and foster care (Havens et al., 1993). Language and verbal delays have been found to be common in both at-risk and infected populations and are often not specific to HIV infection (Fishkin et al., 2000; Gay et al., 1995).

HIV and the Neurodevelopmental Model

During HIV's early history, most children exhibiting neurodevelopmental deficits were either too young or too ill to be involved in traditional educational programs. As a result, there was minimal concern for their progress or performance in school (Armstrong et al., 1993). Over the years, early identification and prevention of vertical transmission (McConnell et al., 2005), advances in treatment (McConnell et al., 2005; Mintz and Epstein, 1992), and the improved management of HIV-related symptoms and opportunistic infections with prophylactic antibiotics and antiretrovirals has increased the length of survival (McConnell et al., 2005). Thus, while HIV still has no identifiable cure

and remains a terminal illness, there is now less concern about acute, life-threatening complications. HIV is now managed as a chronic illness, with children living well into adulthood with proper care (McConnell et al., 2005).

As with other chronic illnesses, researchers have over the years become more aware of how the natural progression of HIV and its treatment can affect brain development and how cognitive abilities emerge over time. Awareness has led to increased concern about HIV's chronic and long-term functional impact for these children and adolescents, including the need for special education services and long-term medical management (Armstrong et al., 1993; Armstrong, 2006; Harris, 1999). The recognition of poor long-term neurocognitive outcomes in a number of chronic illnesses (e.g., cognitive late effects in childhood cancer) allowed researchers to begin to consider the possible disease- and treatment-related mechanisms that may be affecting these outcomes. Armstrong (2006) developed the Neurodevelopmental Model to help understand the cognitive late effects seen in chronic illnesses, including perinatal HIV infection.

Armstrong's model assumes that most chronically ill children follow a typical pattern of development and meet milestones within normal limits as long as they do not have other concurrent risk factors that have been shown to impact development. This pattern of development includes (1) improvement in gross motor abilities, verbal memory, expressive and receptive language, and oral language processing from 0 to 3 years of age, (2) the development and refinement of fine motor coordination, visual processing (i.e., visual memory, visual-spatial skills, and visual-motor coordination), the ability to process complex information quickly and accurately, and attention and

emotional regulation from 3 to 7 years of age, and (3) improvement in organization and planning skills, the ability to rapidly process complex visual and auditory information, and the capacity to store and retrieve complex content from 7 to 30 years of age.

In addition, the order in which cortical regions develop corresponds to the order in which different capacities or functional abilities emerge (Berk, 2005; Armstrong, 2006). Thus, diseases or treatments that affect any of the systems that directly or indirectly contribute to normal brain development may in turn affect the emergence of functional abilities. These effects may be either immediate and noticeable after the onset of the disease and/or treatment, or delayed and only noticeable when the functional ability associated with the disruption is detectable in typically developing children (Armstrong, 2006). These delayed effects are much more subtle than those seen acutely, as they indicate a failure of the brain to support specific functional abilities at the time they should typically emerge (Armstrong, 2006).

According to the model, whether a disease or treatment that affects the CNS also affects neurodevelopment depends on (1) the type of disease, (2) the type, intensity, and duration of the treatment, (3) whether the disease is successfully managed, (4) the timing of the disease or treatment in relation to brain development, or age of the child at the onset of the disease or treatment (Armstrong, 2006), (5) the time since the diagnosis or treatment, and (6) the age at assessment. Brain structures, processes, and functions that have developed prior to the onset of the disease or treatment generally appear to be intact and continue on a relatively normal developmental course (Armstrong, 2006). Those that typically develop afterward appear to be most at risk for impairment. Therefore, the younger the child at the onset of the disease or treatment, the more global and severe the

late effects will be, while the older the child, the fewer and more specific the functional impairments. The current study integrates this model into what is already known about the neurocognitive effects of HIV and its treatment.

The Current Treatment Study

As discussed in this review, advancements in antiretroviral therapies have improved immune functioning and prolonged lifespan into adulthood. Improvements in health maintenance have changed the management of HIV to that of a chronic illness. Longer lifespan has led to the emergence of more specific and subtle HIV effects on neurodevelopment, rather than the global effects found during the early years of the epidemic when children lacking appropriate treatments exhibited morbid outcomes, including progressive encephalopathy. These subtle effects and longer lifespan have led to greater concern about the educational prognosis of perinatally infected children now living through the high school years. While HAART is currently the treatment of choice for HIV due to its dramatic positive effects on immune functioning, little is known about its direct impact on neurodevelopment compared to former, less optimal treatments, which are still being used in poorer nations. Through the development of the Neurodevelopmental Model, Armstrong aims to provide a framework for studying and understanding the effects of HIV (among other chronic illnesses) and its treatment on the brain.

Language deficits are not typically seen in children with HIV who receive combination therapies and have lived longer than early childhood (when controlling for environmental factors). However, deficits in the visual-motor-spatial realm as well as

processing speed have been found. The Neurodevelopmental Model also assumes that functions associated with brain structures developing earlier in life (language in the first 3 years) will tend to remain intact and continue to progress compared to other abilities associated with structures that develop late (i.e., visual-motor integration and processing speed).

This study provides two unique contributions to the perinatal HIV and HAART literature. First, it includes a population consisting of an overwhelmingly underprivileged minority, unique when compared with populations included in prior research. It also includes a wide age range that permits comparisons in treatments among older children. Second, it controls for a number of treatment variables that have not been considered in the past.

There is currently a debate about the usefulness of agents that penetrate the blood brain barrier. Since HAART became available, there has been a proportional increase in the AIDS dementia complex compared with other AIDS-defining illnesses in infected adults (Melton et al., 1997 as cited in Armstrong et al., 2003; Dore et al., 1999 as cited in Lindsey et al, 2007). Recent studies have also raised concern that a more subtle form of encephalopathy, resembling that seen in adults, may be occurring among older perinatally infected children as a result of inadequate penetration of HAART drugs into the cerebrospinal fluid (CSF) (Mitchell, 2006). Furthermore, while several studies have suggested improved neurocognitive abilities as a result of combined therapies despite the absence of CNS penetrating medications (Ferrando et al., 2003 and Robertson et al., 2004 as cited in Willen, 2006), findings by Tamula et al (2003, as cited in Willen, 2006) suggest that a CNS penetrating agent is critical.

While there are 22 antiretroviral agents approved for use in HIV-infected adults and adolescents in the U.S. within the four drug classes of FDA-approved HAART drugs (13 of these approved for use with children) (Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006), not all cross the blood-brain barrier, or do so at the same rate. For example, ZDV has the best penetration, while PIs have very poor penetration (Mitchell, 2006). This lack of or variability in CNS penetration of HAART agents may allow the CNS to serve as a reservoir for latently infectious virus (Sonza, 2001 as cited in Lindsey, 2007). Given the debate on the usefulness of agents that penetrate the blood brain barrier, this study will aim to control for this possible confounding factor by ensuring all participants have always been on regimens including at least one agent that crosses the blood-brain barrier.

In addition to controlling for agents that cross the blood brain barrier, this study also aimed to control for non-psychosocial factors that, in their reviews of the pediatric HIV literature, Armstrong (2003) and Willen (2006) suggested researchers consider when examining the neurocognitive effects of HIV in children. These factors include (1) specific disease factors, such as viral load and CD4 count (collected for this study), (2) whether CNS damage was an isolated (acute) or prolonged (chronic) event (perinatal HIV is considered chronic), (3) the age at which the child experienced CNS infection (assumed to be at birth for perinatal HIV) (4) the age of the child at the time of the assessment (which also serves as the interval between CNS damage and assessment of neurocognitive abilities for vertically infected children), (5) the age at which HAART therapy was started, given its substantial and positive impact on morbidity and mortality (collected for this study, along with age at which the other regimens were started), and

(6) the neurocognitive abilities that the child should have developmentally acquired at the time of assessment (considered in this study by using the Neurodevelopmental Model as a guide).

Specific Aims and Hypotheses. This study had two main goals consisting of four hypotheses. The first aim of this study was to confirm prior research that children on HAART exhibit better immune functioning (i.e., viral load and CD4 count) than children on earlier forms of treatment, namely Mono and Combination Therapy.

Hypothesis 1.1. Children on HAART will have better immune functioning than children who converted to HAART after starting one of these earlier treatments (Converters).

The second aim of this study was to expand on prior research by determining if HAART protects certain areas of neuropsychological functioning that have previously been found to be compromised in children perinatally infected with HIV more effectively than these earlier forms of treatment. This consisted of three specific hypotheses:

Hypothesis 2.1. There will be no differences between the treatment groups in language (expressive and receptive),

Hypothesis 2.2. Children on HAART will exhibit better visual-motor integration functioning and processing speed than children on Mono or Combination Therapy and Converters, and

Hypothesis 2.3. There will be no differences on less sensitive measures of IQ, including verbal comprehension, perceptual organization, and freedom from distractibility. These hypotheses were based on previous findings as well as the Neurodevelopmental Model.

CHAPTER 2

Methods

Study Design and Procedure

This study used a subset of data from a larger study integrating neurodevelopmental assessment and intervention conducted at the University of Miami (UM), Mailman Center for Child Development and supported by the Ryan White Title I program. Participants for this Neurodevelopmental Study included children infected with HIV followed by physicians in the Division of Infectious Disease and Special Immunology. Specialty care services included, but were not limited to, regular blood work, prescription of antiretroviral and/or combination therapies, infection prophylaxis, and monitoring of toxicities and disease progression. Within this specialty care system, children also received primary care services.

As part of their pediatric care, these children received yearly neurodevelopmental evaluations, which were completed during one of their routine clinic visits. These assessments included a brief clinical interview with the child's caregiver (parent or legal guardian) to acquire demographic, prenatal, physical, cognitive, and developmental history. The assessment also included a full neuropsychological evaluation of the child consisting of a core battery of standardized, developmentally-normed instruments. Graduate students, interns, and post-doctoral fellows in pediatric psychology conducted all testing on an individual basis using standardized instructions and procedures, and under the supervision of a licensed pediatric psychologist. While examiners were aware of HIV status, they were blind to treatment group membership. Caregivers who agreed to

the clinical evaluation were subsequently asked to participate in the Neurodevelopmental Study, and were informed that a detailed review of their child's medical chart would be added to the clinical protocol for research purposes. Written informed consent was obtained from caregivers who agreed to participate in the study, and assent was acquired from each child whose caregiver had provided informed consent.

Participants

The Neurodevelopmental Study. A total of 424 children were tested for their yearly neurodevelopmental assessments from July 1993 to March 2005. Of these, 204 participants provided assent and caregiver consent for research. Of the remaining 220, 2 children completed their first evaluation after November 2004, when research consent for the study expired and IRB issues interfered with recruitment. As a result, all evaluations after this date, including those for these two children, were unable to be included. The remaining 218 children had at least one other evaluation conducted prior to this date. However, they were not consented as a result of either (1) caregiver refusal to participate in research, (2) an early determination by the child's medical team that he or she was infected after birth, or (3) a child's current placement in the foster care system.

The Current Drug Study. Each child's medical record was subsequently used to retrospectively confirm perinatal infection, as well as determine treatment group membership and immune functioning. As a result, all research subjects were required to have accessible medical charts. Medical record review occurred at regular intervals over a one and a half year span to maximize the odds of locating a child's medical chart. However, 23 children had inaccessible medical charts, which resulted in their exclusion

from the study. Once medical charts were located, children were included in the current study only if they were perinatally infected with HIV. That is, a child's medical record had to show he or she had been infected at some point during the pregnancy or birth process. This criterion resulted in the exclusion of two participants due to unknown modes of transmission. Six additional children were excluded as a result of being treatment naïve, and therefore not falling into one of the three treatment categories of interest for this study. Another five children were excluded due to having incomplete treatment information, making it impossible to accurately determine their treatment group membership. An additional two children were excluded as a result of having been exposed to HAART therapy, but having switched back to Mono or Combination Therapy at the time of testing. Finally, five children were excluded due to not being administered at least one of the neurodevelopmental measures of interest for this study. All exclusion criteria resulted in a final sample size of 161 for this study. Figure 1 illustrates the determination of the final sample size.

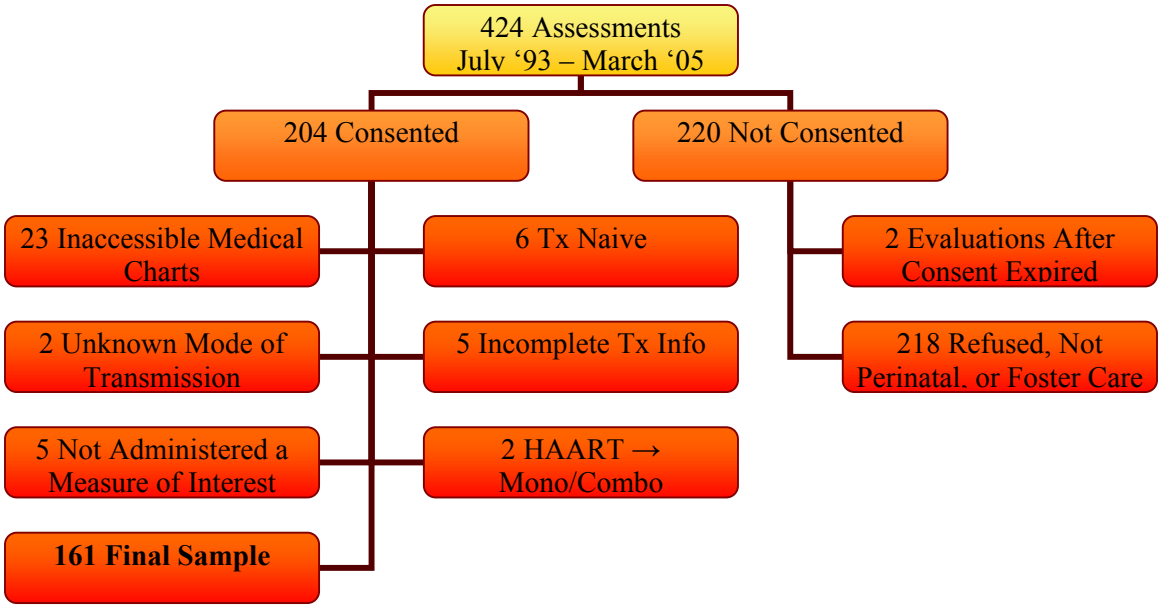

Figure 1. Determination of Final Sample Size.

Treatment Groups. Each child was assigned to one of three treatment groups based on their current and past medical treatment regimens. The first group included children who, at the time of the evaluation, had only been exposed to Mono Therapy or Combination Therapy, which were defined as treatment with one or two antiretroviral medications, respectively. The second group included children who were started on Mono or Combination Therapy, but were converted to HAART Therapy (Converters). HAART Therapy was defined as treatment with one of the following combinations of antiretrovirals:

1. One NRTI, one NNRTI, and one Protease Inhibitor or Fusion Inhibitor,
2. One NRTI or NNRTI, one Protease Inhibitor, and one Fusion Inhibitor,
3. Two NRTIs and one NNRTI, protease inhibitor, or fusion inhibitor, or
4. Two protease inhibitors and one NRTI, NNRTI, or fusion inhibitor.

The third group included children who had only been exposed to HAART Therapy at the time of evaluation.

Of note, only certain antiretroviral medications cross the blood brain barrier. Given that we were looking at treatment effects on brain functioning and we hypothesized that the more advanced treatment regimen (HAART) works to protect both immune and neuropsychological functioning, it was important to determine which of these medications had the ability to cross the blood brain barrier, and if each subject had taken such medications. Each of the children in our sample had been consistently exposed to at least one antiretroviral medication that crosses the blood brain barrier prior to and during the evaluation. Table 1 provides a list of all possible antiretroviral medications (generic name, brand name, and abbreviation), as well as their CSF-to-plasma concentration ratios (Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2005).

Table 1.
Antiretroviral Medications and Their CSF-to-Plasma Concentration Ratios.

Drug Class	Generic Name	Brand Name (Abbreviation)	CSF-to-Plasma Ratio
NRTIs	Abacavir*	Ziagen (ABC)	[B] 0.36
	Atripla	Emtriva + Sustiva + Viread	
	Combivir	Lamivudine + Zidovudine	[B]
	Didanosine*	Videx, Videx EC (ddI)	[B] 0.05
	Emtricitabine*	Emtriva, Coviracil (FTC)	
	Epzicom	Abacavir + Lamivudine	[B]
	Lamivudine*	Epivir (3TC)	[B] 0.11
	Stavudine*	Zerit (d4T)	[B] 0.16-0.97
	Tenofovir DF	Viread (TDF)	
	Trizivir	Abacavir + Lamivudine + Zidovudine	[B]
	Truvada	Emtricitabine + Tenofovir F	
	Zalcitabine	Hivid (ddC)	
	Zidovudine*	Retrovir (AZT, ZDV)	[B] 0.68
NNRTIs	Delavirdine	Rescriptor (DLV)	
	Efavirenz*	Sustiva (DMP-266, EFV)	
	Nevirapine*	Viramune (NVP)	[B] 0.45
PIs	Amprenavir *	Agenerase (APV, VX478)	
	Atazanavir	Reyataz (ATV)	[B] 0.0021-0.0026
	Darunavir	Prezista (DRV)	
	Fosamprenavir	Lexiva (f-APV, FPV)	
	Indinavir	Crixivan (IDV)	
	Kaletra*	Lopinavir + Ritonavir (ABT-378/r, LPV/r, RTV)	
	Nelfinavir*	Viracept (NFV)	
	Ritonavir*	Norvir (RTV)	
	Saquinavir	Fortovase, Invirase (SQV)	
	Tipranavir	Aptivus (TPV)	
FIs	Enfuviritide*	Fuzeon (T-20)	

Generic Names in ***Bold Italics*** are true 2- or 3-drug therapies (Kaletra is considered 1 drug).
* Approved for use in pediatric populations.
[B] Indicates the drug crosses the blood-brain barrier.

Descriptors. Three categories of variables were collected to describe the groups. These included sociodemographic, treatment, and immune descriptors. Individual ANOVAS for continuous variables and Chi-Squares for categorical, dummy coded variables were used to determine if there were any significant differences between the treatment groups on any of these descriptors. Any significant differences between the groups on these descriptors guided the choice of covariates for the main analyses.

Sociodemographic Descriptors. Sociodemographic variables were collected from parent interview as well as medical records. These included age at evaluation, gender, race/ethnicity, bilingual status, and socioeconomic status (SES) based on Federal Poverty Guidelines for the year the child was seen. Bilingual status was an essential demographic variable to collect given that our population of children is predominantly African American, Haitian, or Hispanic, with a large number bilingual in Spanish or Creole, and that bilingual children often demonstrate delays in verbal facility (McLaughlin, 1977 and Sattler, 1988 as cited in Harris, 1999). Participant characteristics for each of the three treatment groups are presented in Table 2. There were no differences between groups on any of the sociodemographic variables. Across treatment groups, the average age at assessment was 10 years, ranging from age 3 to 20, and 52% were male. The sample was 60% African American, followed by 23% Caribbean Black (including Haitian), and 16% Hispanic. Only 0.6% of the population (n = 1) was Caucasian American. While most were monolingual English speakers (65%), 35% were bilingual (including one trilingual child). Over 40% of the sample fell below the federal poverty guidelines.

Treatment Descriptors. Once treatment groups were determined, additional variables were collected to describe the groups. These included age at which antiretroviral medications were started, years on antiretroviral medications, years on Mono or Combination Therapy, age at which HAART therapy was started, and years on HAART Therapy. Table 2 shows Converters started antiretroviral medications at a significantly younger age (2.4 years) than children exposed only to Mono-Combo Therapy (4.7 years) or HAART alone (5.7 years). At the time of evaluation, Converters had also been taking antiretroviral medications for a significantly longer period of time

(8.2 years) than children only exposed to Mono-Combo Therapy (5.8 years), who in turn had been taking them or a significantly longer period of time than children who had only been exposed to HAART (3.6 years). However, children who had only been exposed to Mono-Combo Therapy had been on either Mono or Combination Therapy for a significantly longer period of time (5.8 years) than Converters at the time of evaluation (3.5 years). Finally, Converters had been on HAART for a significantly longer period of time (4.7 years) than children only exposed to HAART (3.6 years). There were no differences between groups on age at which HAART was started.

Immune Descriptors. Medical records collected included immune outcome variables that are described in the following section. The laboratory dates used for these immune variables were those closest to the date of evaluation for each child, either before or after the evaluation. Table 2 shows no significant difference between treatment groups as to when laboratory data was collected (mean of half a month before or after the assessment).

It is important to note that CDC Classification is one variable commonly used to describe immune functioning in populations with HIV/AIDS. However, reliable classifications can only be made by medical physicians based on a person's lifetime medical history, including CD4 T-Cell count (used to determine the immunologic category) and symptomatology (used to determine the clinical category) (CDC, 1994; CDC, 1992). Unfortunately, CDC classification was not always clearly documented at each clinic visit during the earlier years, limiting our ability to choose a classification close to the time of evaluation with any consistency. In addition, given a transition from handwritten medical files to electronic files and the loss of some data in transcription, it is

highly unlikely that our physicians were able to classify patients based on their lifetime medical history. As a result, our best alternative was to use laboratory data, specifically CD4 count, which has always been electronically recorded, to create the immunologic category of the CDC Classification for descriptive purposes. Table 2 shows the immunologic categories for the three treatment groups. While the majority of children on Mono-Combo Therapy had severe suppression (53%), the majority of Converters (60%) and children on HAART (49%) had no suppression. Significant differences between the groups on this measure were not examined, as CD4 count was used as an immune outcome variable (described below).

Table 2.
Participant Characteristics.

Demographic or Treatment Variable	Mono-Combo Therapy	Converters	HAART Therapy	Overall
Age (yr)				
Mean (SD)	10.5 (2.7)	10.7 (2.8)	9.3 (3.9)	10.3 (3.1)
Range	4.2 – 19.5	4.4 – 20.8	3.4 – 17.4	3.4 – 20.8
Gender (%)				
Male	52.0	55.7	46.3	52.2
Female	48.0	44.3	53.7	47.8
Race/Ethnicity (%)				
African American	64.0	61.4	53.7	60.2
Caribbean Black	18.0	18.6	36.6	23.0
Hispanic/Latino	18.0	20.0	7.3	16.1
Caucasian American	0.0	0.0	2.4	0.6
Socioeconomic Status (%)				
≤ 100% of Poverty	28.6	47.8	42.5	40.5
101 – 200% Above Poverty	53.1	36.2	45.0	43.7
> 200% Above Poverty	18.4	15.9	12.5	15.8
Bilingual (%)				
Monolingual (English)	62.0	65.7	68.3	65.2
Bilingual/Trilingual	38.0	34.3	31.7	34.8
Age Medications Started (yr)***				
Mean (SD)	4.7 (3.0)	2.4 (2.5)	5.7 (3.9)	4.0 (3.3)
Range	0.7 – 14.5	0.0 – 12.5	0.2 – 14.0	0.0 – 14.5
Time on Medications (yr)***				
Mean (SD)	5.8 (3.1)	8.2 (2.2)	3.6 (1.5)	6.2 (3.0)
Range	0.3 – 12.6	4.1 – 14.0	0.1 – 7.5	0.1 – 14.0
Time on Mono-Combo (yr)***				
Mean (SD)	5.8 (3.1)	3.5 (2.4)	----------	----------
Range	0.3 – 12.6	0.0 – 9.4		
Age HAART Started (yr)				
Mean (SD)	----------	6.0 (3.2)	5.7 (3.9)	----------
Range		0.2 – 15.3	0.2 – 14.0	
Time on HAART (yr)**				
Mean (SD)	----------	4.7 (1.5)	3.6 (1.5)	----------
Range		0.5 – 7.6	0.1 - 7.5	
Lab Time (months)				
Mean (SD)	0.6 (1.2)	0.4 (0.7)	0.6 (0.9)	0.5 (0.9)
Range	0.0 – 5.9	0.0 – 3.0	0.0 – 5.2	0.0 – 5.9
CDC Immunologic Category (%)				
No Suppression	44.7	60.3	48.7	52.6
Moderate Suppression	2.1	5.9	20.5	8.4
Severe Suppression	53.2	33.8	30.8	39.0

** p<.01. *** p<.001.

Selection of Evaluations When Participant Was Able to Contribute to Two Groups. It is important to note that 43 children from the final sample were evaluated more than once from 1993 to 2005 and could have provided an evaluation to either the Mono-Combo group (when younger) or Converter group (when older). The evaluation that was chosen for each subject provided the most data, as well as contributed to balancing the treatment groups. Given this method of selection, individual ANOVAS for continuous variables and Chi-Squares for categorical, dummy coded variables were used to determine if there were any significant differences on any of the sociodemographic, treatment, and immune descriptors between the 43 participants that could contribute to more than one treatment group, and those that could only contribute to a single group. Participant characteristics for each of the two groups are presented in Table 3.

There were no significant differences on sociodemographic descriptors, years on antiretroviral medications, and years on Mono-Combo Therapy between children in these two groups. Children able to contribute evaluations to only one group began using antiretroviral medications at a significantly younger age (3.6 years) than children able to contribute evaluations to both the Mono-Combo and Converter groups (4.9 years). Children able to contribute evaluations to only one group also had blood tests completed closer to the time of evaluation (0.4 months) than children able to contribute evaluations to both the Mono-Combo and Converter groups (0.8 months). Despite this significant difference, both groups had blood tests completed less than 4 weeks before or after the date of assessment, on average. There was also a striking difference between the groups on the CDC Immunologic Category. The majority of children able to contribute evaluations to only one group (62.3%) exhibited no suppression, while the majority of

children able to contribute evaluations to both the Mono-Combo and Converter groups (72.5%) exhibited severe suppression. This is possibly the result of clinical judgment, as children doing less well immunologically tended to receive more frequent evaluations to closely monitor their progress. Again, significant differences between the groups on this measure were not examined, as CD4 count was used as an immune outcome variable.

Table 3.
Comparing Participants Able to Contribute to One Versus Two Treatment Groups.

Demographic or Treatment Variable	Able to Contribute to 1 Group	Able to Contribute to 2 Groups
Age (yr)		
Mean (SD)	10.1 (3.4)	10.7 (2.4)
Range	3.4 – 20.8	4.2 – 15.3
Gender (%)		
Male	50.8	55.8
Female	49.2	44.2
Race/Ethnicity (%)		
African American	60.2	60.5
Caribbean Black	23.7	20.9
Hispanic/Latino	15.3	18.6
Caucasian American	0.8	0.0
Socioeconomic Status (%)		
≤ 100% of Poverty	45.2	27.9
101 – 200% Above Poverty	38.3	58.1
> 200% Above Poverty	16.5	14.0
Bilingual (%)		
Monolingual (English)	66.9	60.5
Bilingual/Trilingual	33.1	39.5
Age Medications Started (yr)*		
Mean (SD)	3.6 (3.4)	4.9 (3.1)
Range	0.0 – 14.0	0.7 – 14.5
Time on Medications (yr)		
Mean (SD)	6.4 (3.0)	5.8 (3.2)
Range	0.1 – 14.0	0.3 – 12.8
Time on Mono-Combo (yr)		
Mean (SD)	4.5 (3.3)	4.5 (2.4)
Range	0.0 – 12.6	0.3 – 9.0
Lab Time (months)*		
Mean (SD)	0.4 (0.7)	0.8 (1.3)
Range	0.0 – 5.2	0.0 – 5.9
CDC Immunologic Category (%)		
No Suppression	62.3	25.0
Moderate Suppression	10.5	2.5
Severe Suppression	27.2	72.5

* $p<.05$.

Final Outcome Variables

A number of outcome variables were chosen from the already existing data acquired for the Neuropsychological Study. It is important to note that, given the clinical nature of the research, the standardized tests used for evaluation purposes somewhat changed over the years in order to remain relatively up to date with the more recent versions of the tests (e.g., changing the PPVT-R to the PPVT-III). This resulted in inconsistency in testing over the years, thereby limiting the number of subjects having the same measures that were administered. Table 4 lists each of the outcome variables, including immune outcome measures derived from medical chart review, and neuropsychological outcome measures derived from standardized testing.

Table 4.
Outcome Variables.

Outcome Type	Main Analysis Group	Variables Included	Variable Score or Conversion
Immune	Immune	Viral Load (VL)	Log 10[1]
		CD4 Count (CD4)	Square Root
Neuropsychological	Language	Receptive (PPVT-3)	Standard Score
		Expressive (EVT)	Standard Score
	Visual-Motor Integration	Beery VMI-4	Standard Score
	IQ	WISC-III Indices	
		Verbal Comprehension (VCI)	Standard Score
		Perceptual Organization (POI)	Standard Score
		Freedom from Distractibility (FDI)	Standard Score
		Processing Speed (PSI)	Standard Score

Immune Outcome. Immune outcome variables included Viral Load (using a log 10 conversion) and CD4 Count (using a square root conversion). CD4 cell counts and Viral Load have consistently been used in the literature, particularly by the CDC to

[1] The $\log_{10}$ conversion of Viral Load has been commonly used in the adult and pediatric HIV literature (Lindsey et al., 2000; Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children).

monitor the changing spectrum of HIV disease (CDC, 2006). In the adult literature, Viral Load and CD4 cell counts are valued as prognostic markers for progression to AIDS or death (Mellors et al., 1997 and Riddler and Mellors, 1997 as cited in Lindsey et al., 2000). The pediatric HIV literature has also reported viral load, CD4 cell counts, and CD4 percentages to have a predictive value on disease progression and mortality (Lindsey et al., 2000; Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006). While we initially also collected CD4 percent and Historic Low values for CD4 count and percent (the lowest CD4 count or percent for the child at or prior to the evaluation), these were excluded from analyses for two reasons. First, correlations between dependent variables revealed high correlations (greater than .70) between CD4 count and percent, CD4 count and Historic Low CD4 count, and Historic Low CD4 count and percent. Second, the literature pointed to the greater utility of CD4 count as an immunologic marker when evaluating treatment efficacy (Lindsey et al, 2000).

Neuropsychological Outcome. Depending on the severity and pattern of CNS involvement, functional impairment in perinatal HIV can be either global and pervasive across multiple functional areas, or can be specific in nature (Belman, 1990 and Hittleman, 1990 as cited in Armstrong, 1993). As a result, the assessment of functioning in perinatal HIV requires consideration of both global and specific areas of functioning (Brouwers et al., 1991 and Cohen and Diamond, 1992 as cited in Armstrong et al., 1993). To look at both global and specific areas of functioning, neuropsychological outcome variables were grouped into language, visual-motor integration, and IQ outcome categories. All scoring was checked for accuracy by the author.

The standard scores of the Expressive Vocabulary Test (EVT) (Williams, 1997) and Peabody Picture Vocabulary Test – Third Edition (PPVT-3) (Dunn and Dunn, 1997) were used as the measures of language. The EVT is an individually administered, norm-referenced measure for individuals 2.5 to 90 years of age that assesses expressive vocabulary knowledge using labeling and synonyms. The EVT was co-normed with the PPVT-3, an individually administered, norm-referenced measure that serves as an achievement test of receptive (hearing) vocabulary attainment for standard English, as well as a screening test of verbal ability.

The standard score for The Beery-Buktenica Developmental Test of Visual-Motor Integration – Fourth Edition (VMI-4) (1997) was used as an overall measure of visual-motor integration. The VMI-4 is an individually-administered or group-administered, norm-referenced measure for individuals 3 to 17 years of age that assesses the ability to integrate visual and motor abilities (eye-hand coordination) by copying a developmental sequence of geometric forms. We initially considered also including the Woodcock-Johnson Tests of Cognitive Abilities – Revised (WJC-R) Spatial Relations subtest, as it assesses visual-spatial ability alone. However, only a small percentage of participants were administered this subtest, thereby limiting the power of the main analyses. As a result, this subtest was not included. We also initially considered including the Dominant and Non-Dominant Hand standard scores for the Grooved Pegboard test, as these assess fine-motor coordination alone. However, it has been our clinical experience with our population that these scores are relatively inconsistent. For example, during one evaluation, a child may obtain higher Dominant than Non-Dominant scores, but in

contrast obtain the reverse pattern on a following evaluation. As a result of this instability in scores, we decided to not include this test as well.

The Wechsler Intelligence Scale for Children – Third Edition (WISC-III) (1991) was administered to assess each child's overall level of cognitive functioning. The WISC-III is an individually administered norm-referenced instrument for children ages 6 through 16 years. The standard scores for the four indices were used, including the Verbal Comprehension Index (VCI), Perceptual Organization Index (POI), Freedom from Distractibility Index (FDI), and Processing Speed Index (PSI).

CHAPTER 3

Results

Statistical Analyses

All analyses for this study included a final sample size of 161 participants. For the preliminary analyses, individual ANOVAS for continuous variables and Chi-Squares for categorical, dummy coded variables were used to determine which demographic, immune, or treatment descriptors would be included as covariates in the main analyses. Covariates were candidates for inclusion in the main analyses if they were significantly different between all three groups (Mono-Combo Therapy, Converters, HAART Therapy). As described in the Methods section, only two variables were found to be significantly different between the groups: (1) age at which antiretroviral medications were started and (2) years on antiretroviral medications. These two variables were thus included as covariates in the main analyses. As stated previously, age at which antiretroviral medications were started was also significantly different between children capable of contributing to one versus two treatment groups. Using this descriptor as a covariate in the main analyses also controlled for this difference as well.

Pearson Correlations were run between all outcome variables and covariates to detect multicollinearity. A Pearson r-value above .70 between any two variables was chosen to indicate a high level of collinearity between the two. If this occurred, it was determined that one variable or the other would be chosen for the main analyses unless these high correlations were expected due to the nature of the measures. Table 5 shows all correlations between the outcome variables fell below .70 with two exceptions. First,

the WISC-III VCI was highly correlated with the EVT ($r = .780$, $p<.001$), but this was expected given they both involve verbal ability. Second, the VCI and POI were highly correlated ($r = .712$, $p<.001$). These were still kept in analyses given their consistent use on the literature as different measures of IQ. Once the outcome variables were finalized, individual boxplots were used to determine that they were all normally distributed.

For the remaining outcome variables and covariates, Table 5 shows that Viral Load was correlated with CD4 Count ($r = -.441$, $p<.001$), age at which medications were started ($r = .211$, $p<.01$), and years on medication ($r = -.230$, $p<.01$). CD4 count was also correlated with age at which medications were started ($r = -.408$, $p<.01$). Expressive language was correlated with receptive language ($p = .645$, $r<.001$), visual-motor integration ($r = .518$, $p<.001$), perceptual organization ($r = .655$, $p<.001$), freedom from distractibility ($r = .661$, $p<.001$), and processing speed ($r = .481$, $p<.001$). Receptive language was associated with visual-motor integration ($r = .417$, $p<.001$), verbal comprehension ($r = .676$, $p<.001$), perceptual organization ($r = .665$, $p<.001$), freedom from distractibility ($r = .504$, $p<.001$), processing speed ($r = .268$, $p<.01$), and age at which medications were started ($r = .197$, $p<.05$). Visual-motor integration was correlated with verbal comprehension ($r = .490$, $p<.001$), perceptual organization ($r = .579$, $p<.001$), freedom from distractibility ($r = .457$, $p<.001$), and processing speed ($r = .370$, $p<.001$). Verbal comprehension was associated with freedom from distractibility ($r = .656$, $p<.001$) and processing speed ($r = .471$, $p<.001$). Perceptual organization was correlated with freedom from distractibility ($r = .601$, $p<.001$) and processing speed ($r = .512$, $p<.001$). Freedom from distractibility was associated with processing speed ($r = .476$,

$p<.001$). Finally, age at which medications were started was correlated with years on medications ($r = -.518$, $p<.001$).

Overall, consistent with expectations, disease factors and treatment descriptors were significantly correlated with each other. However, they were not generally correlated with neuropsychological functioning. More specifically, lower detectability of the virus (Viral Load) was associated with better immune functioning (CD4 Counts). In addition, the younger the age that medications were started, the longer the time spent on these medications, the lower the detectability of the virus, and the higher the immune functioning. Contrary to expectations, the older the age that medications were started, the better the receptive language. It is possible that children who were started on medications at an older age exhibited better immune functioning, thereby allowing the delay in prescribing these medications. If this is the case, good immune functioning in the early years would have allowed children time to develop their receptive language skills, which develops earlier in children than any of the other neuropsychological functions measured.

Also consistent with the literature, measures of neuropsychological functioning were correlated with each other, such that higher functioning in one area was associated with higher functioning in another. More specifically, better expressive language abilities were associated with better receptive language abilities, and better performance on both language measures were associated with better visual-motor integration and better performance on all four IQ indices. Higher visual-motor integration scores were associated with higher scores on all four IQ indices. Finally, better performance on each IQ index was associated with better performance on all other IQ indices.

Table 5.
Correlations Between Outcome Variables.

	CD4	EVT	PPVT-3	VMI-4	VCI	POI	FDI	PSI	Age Meds Started	Time on Meds
VL	-.441***								.211**	-.230**
CD4	-----								-.408**	
EVT		-----	.645***	.518***	.780***	.655***	.661***	.481***		
PPVT-3			-----	.417***	.676***	.665***	.504***	.268**	.197*	
VMI-4				-----	.490***	.579***	.457***	.370***		
VCI					-----	.712***	.656***	.471***		
POI						-----	.601***	.512***		
FDI							-----	.476***		
PSI								-----		
Age Meds Started									-----	-.518***
Time on Meds										-----

* p<.05. ** p<.01. *** p<.001.

Main analyses aimed to confirm prior research that children on the current and more advanced HAART regimen exhibit better immune functioning than children on former treatments. In the current study, former treatments included Mono-Combo Therapy and conversion from Mono-Combo to HAART (Converters). Analyses also aimed to expand on previous research by determining if HAART serves to protect certain areas of neuropsychological functioning above and beyond these former treatments. Three MANCOVAS were used to compare the three treatment groups on immune, language, and IQ outcome. A single ANCOVA was used to compare the three treatment groups on visual-motor integration. All four analyses used age at which antiretroviral medications were started and years on antiretroviral medications as the two covariates, as these were found to be significantly different between the three treatment groups in preliminary analyses.

Immune Outcome

The first goal of this study was to examine whether children on HAART exhibit better immune functioning than children on Mono-Combo Therapy and Converters. This was assessed using Viral Load and CD4 Count. It was hypothesized that children who have only been exposed to HAART would show better immune functioning than children who had ever been treated with Mono-Combo Therapy, even if they had converted to HAART at a later point prior to testing.

A multivariate analysis of covariance (MANCOVA) was conducted to determine the effect of the three types of HIV treatments (Mono-Combo Therapy, Converters, HAART Therapy) on the two dependent variables, Viral Load and CD4 Count, using age at which antiretroviral medications were started and years on antiretroviral medications as the two covariates. Due to unequal group sizes (i.e., the largest *n* was greater than 1.5 times the smallest *n*), Pillai's Trace was used as the multivariate significance test. As expected, significant differences were found among the three treatment groups on the immune measures, Pillai's Trace = .08, $F\ (4, 274) = 2.77$, $p < .05$. The multivariate η^2 based on Pillai's Trace was small, .04.

Analyses of Variances (ANOVA) on each dependent variable were conducted as follow-up tests to the MANCOVA. Each ANOVA was tested at the .05 level. The ANOVA for Viral Load was significant, $F\ (2, 137) = 3.61$, $p < .05$, $\eta^2 = .05$, as was the ANOVA for CD4 Count, $F\ (2, 137) = 3.56$, $p < .05$, $\eta^2 = .05$.

Post hoc analyses to the univariate ANOVA for Viral Load and CD4 Count consisted of conducting pairwise comparisons to find which treatment group affected immune outcome most strongly. Each pairwise comparison was tested at the .05 level.

Examination of the pairwise comparisons for Viral Load showed that children only exposed to HAART had significantly lower viral loads than children only exposed to Mono-Combo Therapy ($p < .05$). There was also a trend for children only exposed to HAART to exhibit lower viral loads than Converters ($p = .066$). The Mono-Combo Therapy group and Converters were not significantly different from each other. Examination of the pairwise comparisons for CD4 Count showed that children only exposed to HAART had significantly higher CD4 Counts than children only exposed to Mono-Combo Therapy ($p < .05$). There were no significant differences between the Converters and either the Mono-Combo Therapy group or HAART Group. Table 6 contains the unadjusted and adjusted means, standard deviations, standard errors, and sample sizes for the immune outcome measures within each of the three groups. Figures 2 and 3 illustrate the adjusted mean Viral Load and CD4 Count, respectively, for each group, as well as the standard errors for each group. Overall, findings suggest that children on HAART do exhibit better immune functioning than children on Mono-Combo Therapy, and possibly also Converters.

Table 6.
Unadjusted and Adjusted Means, Standard Deviations, Standard Errors, and N's for the Immune Outcome Measures by Treatment Group.

	Mono-Combo Therapy (N = 43)		Converters (N =61)		HAART Therapy (N = 36)	
Immune Measure	*Unadjusted M(SD)*	*Adjusted M(SE)*	*Unadjusted M(SD)*	*Adjusted M(SE)*	*Unadjusted M(SD)*	*Adjusted M(SE)*
VL*	3.80 (0.80)	3.74 (0.14)	3.53 (0.92)	3.76 (0.13)	3.50 (1.11)	3.20 (0.17)
CD4*	20.46 (8.79)	21.28 (1.26)	24.96 (8.86)	23.89 (1.21)	25.59 (10.06)	26.39 (1.58)

* $p < .05$.

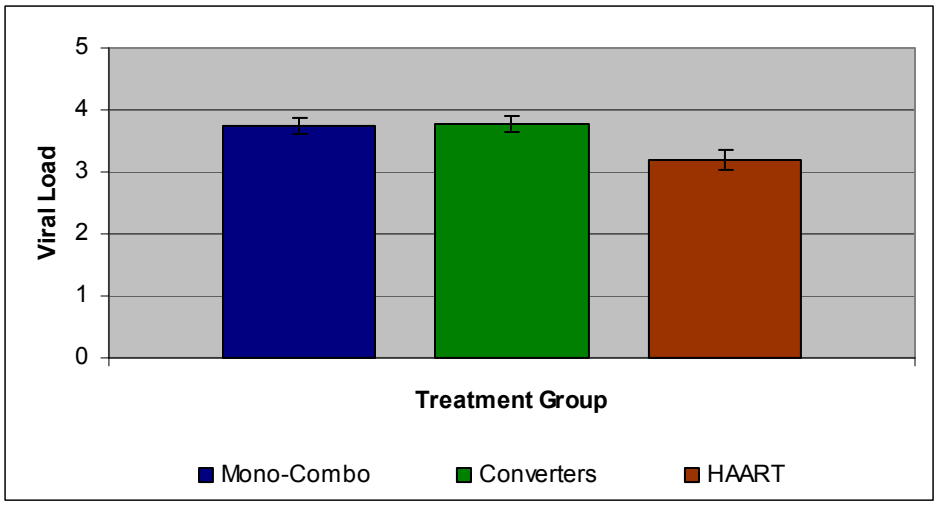

Figure 2. Viral Load. Adjusted mean *Viral Load* (log conversion) for Mono-Combo Therapy (n = 43), Converters (n = 61), and HAART Therapy (n = 36).

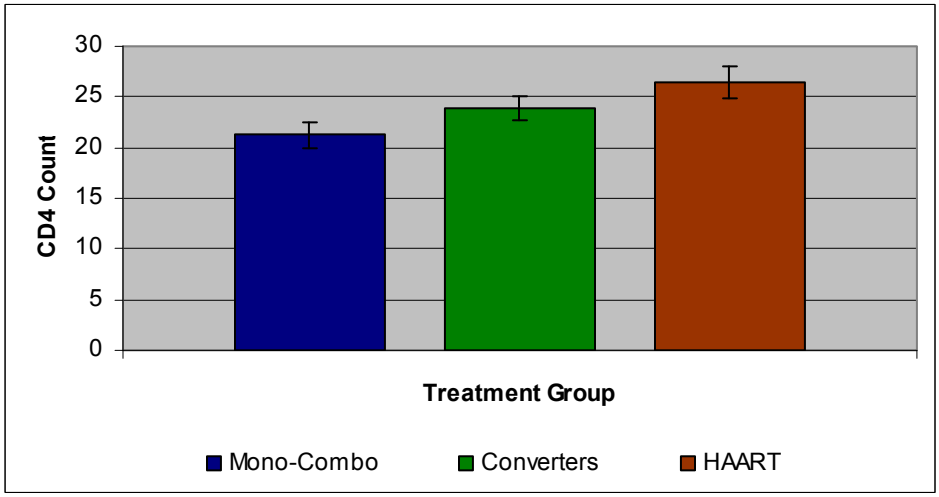

Figure 3. CD4 Count. Adjusted mean *CD4 Count* (square root conversion) for Mono-Combo Therapy (n = 43), Converters (n = 61), and HAART Therapy (n = 36).

Language Outcome

The second goal of this study was to examine whether HAART serves to protect language functioning more effectively than Mono-Combo Therapy and conversion from Mono-Combo to HAART. This was assessed using standard scores from the EVT and

PPVT-3. Given that (1) the left hemisphere has a smaller proportion of white matter than grey matter compared to the right hemisphere, (2) the left hemisphere needs white matter integrity only to develop functions (rather than develop and maintain them), and (3) children with later onset of or slower disease progression have more time for functional reorganization of the brain and may even experience normal brain development in the early years, during which language rapidly develops (Neurodevelopmental Model), it was hypothesized that the treatment groups would not differ in their language functioning, as HAART would not serve to protect this area more effectively than previous treatments.

A multivariate analysis of covariance (MANCOVA) was conducted to determine the effect of the three types of HIV treatments (Mono-Combo Therapy, Converters, HAART Therapy) on the two dependent variables, expressive language and receptive language, using age at which antiretroviral medications were started and years on antiretroviral medications as the two covariates. Due to unequal group sizes (i.e., the largest *n* was greater than 1.5 times the smallest *n*), Pillai's Trace was used as the multivariate significance test. As expected, results showed no significant effect for this analysis, indicating no significant differences in language functioning between the treatment groups, Pillai's Trace = .05, $\underline{F}$ (4, 196) = 1.23, $\underline{p}$ = .300, η^2 = .02. Table 7 contains the means, standard deviations, and sample sizes for the language outcome measures within each of the three groups.

Table 7.
Means, Standard Deviations, and N's for the Language Outcome Measures by Treatment Group.

	Mono-Combo Therapy (N = 20)		Converters (N =53)		HAART Therapy (N = 30)	
Language Measure	*M*	*SD*	*M*	*SD*	*M*	*SD*
EVT	84.00	13.58	77.64	14.98	83.67	12.76
PPVT-3	83.60	11.42	80.34	12.19	82.10	12.32

Visual-Motor Integration Outcome

The third goal of this study was to examine whether HAART serves to protect visual-motor integration skills more effectively than Mono-Combo Therapy and conversion from Mono-Combo to HAART. This was assessed using the standard score of the VMI-4. Given previous findings in the literature of deficits in the visual-motor-spatial continuum in children with HIV, it was hypothesized that children who have only been exposed to HAART would show better visual-motor integration skills than children who had ever been treated with Mono-Combo Therapy, even if they had converted to HAART at a later point prior to testing.

An analysis of covariance (ANCOVA) was conducted to evaluate the relationship between the independent variable, treatment group (Mono-Combo Therapy, Converters, HAART Therapy), and the dependent variable, visual-motor integration, using age at which antiretroviral medications were started and years on antiretroviral medications as the two covariates. Contrary to our prediction, there were no significant differences found among the three treatment groups on visual-motor integration. However, there was a trend with an associated small effect size, F (2, 141) = 2.87, p = .060, η^2 = .04. Table 8 contains the unadjusted and adjusted means, standard deviations, standard errors, and

sample sizes for the visual-motor integration outcome measures within each of the three groups.

Table 8.
Means, Standard Deviations, and N's for the Visual-Motor Integration Outcome Measure by Treatment Group.

	Mono-Combo Therapy (N = 47)		Converters (N = 64)		HAART Therapy (N = 35)	
VMI Measure	*M*	*SD*	*M*	*SD*	*M*	*SD*
VMI-4	84.55	12.53	79.97	12.76	83.14	13.28

IQ Outcome

The fourth and final goal of this study was to examine whether HAART serves to protect IQ functioning more effectively than Mono-Combo Therapy and conversion from Mono-Combo to HAART. This was assessed using the IQ indices of the WISC-III (VCI, POI, FDI, and PSI). Given previous findings in the literature of deficits in processing speed in children with HIV and associated neurological findings, it was hypothesized that children who have only been exposed to HAART would show better functioning in processing speed than children who had ever been treated with Mono-Combo Therapy, even if they had converted to HAART at a later point prior to testing. However, no other differences were expected, as children have usually not been shown to have deficits in global intelligence, and the VCI, POI, and FDI are less sensitive measures.

A multivariate analysis of covariance (MANCOVA) was conducted to determine the effect of the three types of HIV treatments (Mono-Combo Therapy, Converters, HAART Therapy) on the four dependent IQ Index variables, Verbal Comprehension, Perceptual Organization, Freedom from Distractibility, and Processing Speed, using age at which antiretroviral medications were started and years on antiretroviral medications

as the two covariates. Due to unequal group sizes (i.e., the largest *n* was greater than 1.5 times the smallest *n*), Pillai's Trace was used as the multivariate significance test. As expected, results showed significant differences with a small effect size among the three treatment groups on the IQ measures, Pillai's Trace = .15, $\underline{F}$ (8, 210) = 2.08, $\underline{p}$ <.05. The multivariate η^2 based on Pillai's Trace was small, .07.

Analyses of Variances (ANOVA) on each dependent variable were conducted as follow-up tests to the MANCOVA. Each ANOVA was tested at the .05 level. As expected, the ANOVA for Processing Speed showed a significant and small to medium effect, $\underline{F}$ (2, 107) = 4.82, $\underline{p}$ <.01, η^2 = .08. Also as expected, the ANOVAs for the other three IQ Index measures were not significant: Verbal Comprehension, $\underline{F}$ (2, 107) = 2.35, $\underline{p}$ = 0.100, η^2 = .04; Perceptual Organization, $\underline{F}$ (2, 107) = 1.10, $\underline{p}$ = 0.336, η^2 = .02; and Freedom from Distractibility, $\underline{F}$ (2, 107) = 0.36, $\underline{p}$ = 0.702, η^2 = .00.

Post hoc analyses to the univariate ANOVA for Processing Speed consisted of conducting pairwise comparisons to find which treatment group affected processing speed outcome most strongly. Each pairwise comparison was tested at the .05 level. Examination of the pairwise comparisons for Processing Speed showed Converters had significantly lower scores than children only exposed to either Mono-Combo Therapy (p <.05) or HAART alone (p <.05). The Mono-Combo Therapy and HAART groups were not significantly different from each other. Table 9 contains the unadjusted and adjusted means, standard deviations, standard errors, and sample sizes for the IQ Index outcome measures within each of the three groups. Figure 4 illustrates the adjusted mean PSI and standard error for each group.

Table 9.
Unadjusted and Adjusted Means, Standard Deviations, Standard Errors, and N's for the IQ Index Outcome Measures by Treatment Group.

	Mono-Combo Therapy (N = 38)		Converters (N =50)		HAART Therapy (N = 24)	
IQ Measure	*Unadjusted M(SD)*	*Adjusted M(SE)*	*Unadjusted M(SD)*	*Adjusted M(SE)*	*Unadjusted M(SD)*	*Adjusted M(SE)*
VCI	78.18 (14.33)	78.30 (2.45)	76.70 (14.96)	76.33 (2.49)	86.08 (15.04)	86.68 (3.72)
POI	82.82 (16.89)	83.08 (2.78)	83.02 (16.36)	82.17 (2.83)	88.50 (18.10)	89.86 (4.22)
FDI	87.47 (14.40)	87.47 (2.47)	84.76 (14.99)	84.77 (2.51)	88.67 (15.40)	88.65 (3.75)
PSI**	94.87 (16.65)	95.33 (2.92)	84.70 (19.16)	83.13 (2.97)	96.75 (16.27)	99.29 (4.43)

** p <.01.

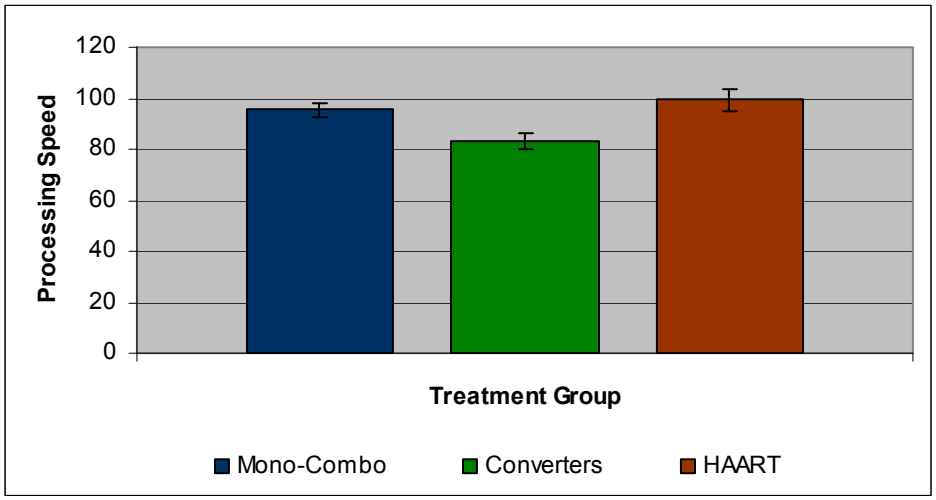

Figure 4. Processing Speed. Adjusted mean *Processing Speed* for Mono-Combo Therapy (n = 38), Converters (n = 50), and HAART Therapy (n = 24).

CHAPTER 4

Discussion

The children participating in this study present with risk factors that have been shown to impact development and neuropsychological functioning in addition to their chronic illness. An overwhelming majority were of minority status (99%) from impoverished households (84%). A large proportion also lived in bilingual homes (35%). In addition, across all areas of neuropsychological functioning assessed, performance scores fell within the borderline to low average range across groups, with the exception of processing speed, which fell in the low average to average range. This indicates that these children may exhibit some difficulty in school without meeting criteria for special education services.

This study provided two unique contributions to the literature, the first being its unique population with a wide age range, making comparisons among treatment groups possible in older children and adolescents. In addition, it controlled for a number of treatment variables not previously considered in the perinatal HIV treatment literature. Overall, results of this study confirm previous findings that children on HAART therapy exhibit significantly better immune functioning than children on earlier, less optimal forms of treatment. It will thus be important to move toward more global use of HAART as resources within each nation permit.

However, despite better immune functioning, this study provides evidence that HAART does not serve to more effectively protect those areas of neuropsychological functioning that have previously been found to be compromised in children perinatally

infected with HIV than these earlier forms of treatment. This is true even when controlling for antiretroviral agents that cross the blood brain barrier, as well as age at which medications were started and years on medications. It seems then, what may be happening in the brain cannot be assumed from what is seen in blood test results (viral load and CD4 count). Therefore, while HAART serves to improve immune functioning, which previous research has shown thereby prolongs lifespan, it does not serve to protect neuropsychological functioning, leaving perinatally infected children at risk for developing neuropsychological deficits and needing special education services later in life. Nevertheless, it is a partial sigh of relief for healthcare providers in poorer, developing countries, as the less sophisticated forms of treatment still prevalent in these countries protect the CNS as well as HAART does.

There are some possible explanations for both of the unexpected findings in this study, including the lack of visual-motor integration findings and poor Converter performance in processing speed abilities. The lack of findings on visual-motor integration could be the result of when HAART was typically started in this population. For both the Converters and HAART groups, HAART was started around the age of 6 on average. This age is a crucial time for fine-tuning visual-motor integration skills as children are learning to perfect writing in school. It may be possible that a switch or start in therapies in combination with the time it takes to reach an optimal level of dosing for each individual child (which may take months), may have both negatively affected performance for Converters (least optimal performance with an associated trend), who had previously been on a regimen that was likely not working, as well as washed out

effects of treatment for children starting antiretrovirals for the first time on HAART, as therapy may not have had enough time to take effect in such as subtle area of functioning.

It seems possible for the poor performance in processing speed abilities seen in Converters to be attributed to frequency of changes in regimen. McConnell and her colleagues (2005) reported a significant increase in the proportion of patients using newer antiretroviral drugs and proportion receiving their third or greater sequential triple-therapy regimen from 1997 (4%) to 2001 (17%), as well as a significant decrease in the duration of sequential triple therapy regimens (from 13 to 7 months for her cohort from the CDC's Pediatric Spectrum of HIV Disease Study). Doctors may change regimens often as a result of patient adherence, response to therapy, previous regimens, and the presence of viral resistance (McConnell et al., 2005). However, multiple changes in antiretroviral drug regimens can quickly exhaust treatment options and should be avoided unless required as a result of severe toxicity, intolerance, or significant clinical, immunologic, or virologic progression (Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006). Looking at both adjusted and unadjusted means, it is evident that there are only minimal differences in processing speed scores between children who had only been exposed to Mono-Combo and those who had only been exposed to HAART. It is possible that these two treatment groups experienced less changes in regimen type (and only in the use of specific drugs within regimen type) than Converters, who may have experienced changes from Mono Therapy to Combination Therapy and finally to HAART. However, we did not look at the number of changes in regimen prior to assessment, and this has not been studied as of yet in terms of neuropsychological functioning. It would be interesting for future studies to look at

frequency in regimen changes as a possible barrier to improved neuropsychological functioning, even within the HAART regimen.

Furthermore, it is possible that Converters were simply sicker children at the time they were converted to the more potent HAART regimen. As a result, their poorer performance could be attributed to a more significant effect of the virus on the CNS that later viral suppression on the HAART regimen could not reverse. In addition, it is important to consider both the impact of non-adherence and other psychosocial factors not controlled for in this study, which may have impacted results and are discussed below.

The significantly poorer performance of Converters in processing speed, as well as their poor performance in visual-motor integration (trend) may have some implications for prescribing. For one, providers may now hesitate to convert a child from Mono Therapy to HAART, for example, given the possibility of added negative neuropsychological effects due to frequent regimen changes. There is already an emerging concern for clinicians with regard to the use of HAART, as drug resistance has been clinically observed for adolescents who have been exposed to HAART agents that cross the blood brain barrier. In an attempt to prevent the emergence of resistant viral strains, a number of providers currently choose to keep a relatively healthy, immune-suppressed child on a less optimal treatment as long as the child is doing well and the treatment is working to suppress the virus. These providers only choose to convert the child to a more advanced treatment when the less optimal treatment ceases to work. Given the findings from the current study, this approach may also help to maintain a child's neuropsychological functioning. However, withholding HAART in order to

prevent poorer neuropsychological functioning or risk the emergence of resistant strains would not be prudent for a child who needs a regimen change as a result of failing treatment and subsequent poor immune functioning.

Limitations and Future Directions in Research

The interpretation of research findings related to the neurodevelopmental functioning of children perinatally infected with HIV is complicated by factors other than HIV that effect a child's development (Armstrong et al., 1993). Lindsey and her colleagues (2007) found that both HIV infected and HIV affected infants and young children in the era before HAART became available displayed a negative developmental trajectory, with indices on the Bayley Scales of Infant Development declining at similar rates from birth to age 2. They posited these results might reflect the relative detrimental impact of genetic susceptibility and psychosocial environment often characterized by poverty and other risk factors often observed in HIV-affected families and known to influence cognitive development (Lindsey et al., 2007). Similarly, Fishkin and his colleagues (2000) found mean IQ scores for both HIV infected and a comparison group of non-infected preschoolers to be more than one standard deviation below WPPSI-R standardized sample norms and not significantly different from each other.

As a result of such findings, it is important to consider all possible biological and environmental factors that may impact neurodevelopment in this population. This would include greater consideration of birth complications (e.g., prematurity), prenatal exposure, hospitalizations and frequency of medical visits limiting access to education (Armstrong, et al., 1999), malnutrition, family history, extreme poverty and

unemployment leading to lack of opportunity for stimulation during sensitive periods of development, family stress associated with death of the primary caregiver, family support, developmental disabilities unrelated to HIV (difficult to tease apart often), and use of early intervention services. All of these variables were not carefully considered in this study due to lack of availability of such specific information. It would be helpful for future studies to assess treatment effects while also controlling for some of those demographic and psychosocial factors that are less studied.

Given the incomplete nature of converting to electronic charting, and the complexity of HIV-related diseases, it was difficult to systematically get a clear picture of what other infectious processes a child had experienced, let alone when they had experienced them. This served as a limitation to the study, as certain infections affect the brain, thereby negatively impacting neuropsychological performance. Future studies should aim at using only electronic charts, as these are more readily available and more accurately reviewed. It will not only be important to document infectious processes experienced by the child, particularly those that affect the train (e.g., toxoplasmosis and cytomegalovirus), but side effects also experienced by the child as a result of medication. For example, exposure to anti-retroviral medication (e.g., AZT) often results in chronic anemia (Hermans, 1995, Kalichman, 1995, Miles, 1995, Moore et al., 1998, Scadden, 1997, Sullivan et al., 1997, and Watson et al., 1998 as cited in Harris, 1999), which can also have negative consequences on cognitive functioning.

Laboratory tests conducted in order to collect desired disease factors were not always collected on the day of testing. While blood tests were completed on the same day for a large proportion of our participants, the time interval from laboratory date to

assessment date ranged out to almost 6 months. According to Melna et al. (1986) and Epstein et al. (1986; as cited in Armstrong et al., 1999), significant changes in health status and cognitive functioning may occur in an interval as small as 2 to 3 weeks. As a result, future studies should aim to limit collection of immune markers to the same day plus or minus two to three weeks.

Another limitation of this study was not controlling for adherence, which may have affected the results. Research suggests that adherence rates of 95% or better are necessary for adequate reduction in viral replication and to prevent the development of drug resistance (Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006). However, actual adherence rates are typically much lower (Pontali et al., 2001 and Reikert and Drotar, 2002 as cited in Willen, 2006). Barriers to adherence in the pediatric HIV population include drug palatability, the number of medications and frequency of their administration, the complexity of medication schedules and their impact on quality of life, ability of the child and/or their caregiver to administer these complex regimens, side effects, and stigma associated with the disease, leading parents to changing or hiding labels of medications (Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children, 2006). There is no easy way to assess adherence, and researchers in various fields have stressed the importance of assessing adherence through multiple methods, such as self-report, parent report, pharmacy refill checks, and pill counts. While the current study was limited in its ability to assess adherence and control for it, future studies can aim to do so in order to account for the effect of adherence on a treatment's efficacy in improving a particular area of functioning.

Other limitations of this study include not controlling for practice effects as a result of repeated testing using the same instruments, as well as multiple revisions of standardized tests throughout the years that thereby limits the sample size and power. Maintaining consistency in testing measures is difficult in clinical research, as it is important to remain up to date on tests in order to assist children in receiving the best services possible.

Overall, the study required more power in order to examine more of the above factors effectively. In addition, larger cohorts could have allowed for the examination of treatment effect according to age group to determine if more subtle differences emerged over time for these same abilities. Future analyses within the Neurodevelopmental Study can, however, examine developmental trajectories over time for each treatment cohort, which would add to the current cross-sectional findings.

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VITA

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