Neuro-imaging in paediatric HIV, a MRI/DTI study

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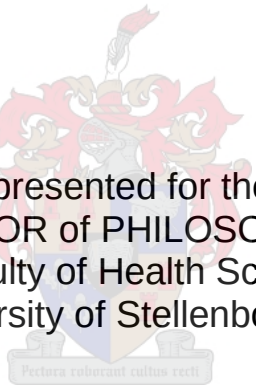

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Co-Supervisor: Prof Savvas Andronikou

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes 3 original papers published in peer reviewed journals and 1 unpublished publication (submitted awaiting result). The development and writing of the papers (published and unpublished) were the principal responsibility of myself, except where the acknowledgements indicate otherwise.

DEDICATION

To my parents, who faithfully supported me throughout my life and had complete faith that this project will be successful. I am profoundly sad that my mother is not here to share my joy.

To my husband for his positive influence in my life and with whom I can share both the good and bad knowing that he will always be there to support me.

To my wonderful children who are the light and laughter in my life.

Thank you.

ABSTRACT

The HIV epidemic has been largely controlled by antiretroviral treatment (ART) which improves neurodevelopmental outcomes. Nevertheless, many HIV-infected (HIV+) children on long-term treatment may have HIV-related brain injury, ongoing cognitive impairment and treatment-related neurological complications.

Magnetic resonance imaging (MRI) and in particular diffusion tensor imaging (DTI) are sensitive tools in assessing the integrity of white matter (WM) microstructure in HIV.

The pictorial review describes common causes of HIV-related cerebral WM disease as well as the role of neuro-imaging in managing these patients.

In the following chapters the characteristics of WM signal abnormalities on MRI and DTI (using DTI derived measures - fractional anisotropy (FA), mean (MD), axial (AD) and radial diffusion (RD)) in children with HIV, recruited as part of the Children with HIV early antiretroviral (CHER) trial and who started ART within the first year of life, are described. In the CHER trial, infants were randomized to early limited or deferred continuous ART.

Methods:

Structural MRI scans of children at mean age 39.1 months were reviewed and correlated with clinical and neurodevelopmental data, virological markers and time on ART.

DTI was acquired in a similar cohort (which included several children in the first study and control subjects) at mean age of 64.7 months. Voxel-based group comparisons were performed to determine regions where FA and MD differed between HIV+ and uninfected children. Associations of DTI parameters with timing of ART initiation and correlations of DTI parameters in abnormal WM with directed neurodevelopmental tests were examined.

Results:

MRI scans of 44 children were reviewed at mean age of 39.1 months: 10 on deferred and 34 on early CHER treatment arms, commencing ART at mean age of 18.5 and 8 weeks respectively. Multiple high signal intensity lesions on T2 /FLAIR were documented in 22 patients (50%), predominantly in frontal (91%) and parietal (82%) WM. There were no differences in neurodevelopmental scores in children with and without WM signal abnormalities. Neither lesion load nor distribution showed significant correlation with neurodevelopmental scores or neurological examination. There was a trend for association of WM signal abnormalities and longer time on ART (p=0.13) and nadir CD4% (p=0.08).

39 HIV+ children (15 male) and 13 controls (5 male) were imaged (using DTI) at mean age of 64.7 months. 2 Clusters with decreased FA and 7 clusters with increased MD were identified in the HIV+ group with symmetrical distribution predominantly due to increased RD, suggestive of decreased myelination. Children on early interrupted ART had lower FA compared to those receiving continuous treatment. The only neurodevelopmental domain with a trend of difference between the HIV+ children and controls (p=0.08), was personal social quotient which correlated to improved myelination of the forceps minor in the control group. As a combined group there was a negative correlation between visual perception and RD in the right superior longitudinal fasciculus and left inferior longitudinal fasciculus which may be related to these tracts, part of the visual perception pathway, are at a crucial state of development at age 5.

Conclusion:

Half of children at mean age of 39.1 months, referred with HIV-related brain disease had WM signal abnormalities on T2/FLAIR structural MRI. HIV+ children at 5 years have WM abnormalities measured by FA, despite early ART, confirming that early ART does not fully protect the WM either from peripartum or in utero infection. In contrast to adults, the corticospinal tracts are predominantly involved rather than the corpus callosum. Continuous early ART, however limits the extent of WM damage.

Even directed neurodevelopmental tests will underestimate the degree of microstructural WM damage detected by DTI. The visual perception deficit detected in the HIV study population should be further examined as it persists in longitudinal follow up of these patients at age 7.

ABSTRAK

Die MIV-epidemie is tans grootliks onder beheer deur effektiewe anti-retrovirale terapie (ART) en selfs wanneer dit onderbreek word, verbeter die neuro-ontwikkelingsuitkomste. Dit het daartoe gelei dat baie kinders met die siekte op langtermynbehandeling grootword, met gevolglike hoër risiko om MIV-verwante breinbesering, voortgesette kognitiewe inkorting en behandelingsverwante neurologiese komplikasies te ontwikkel.

Magnetiese resonansie beelding (MRI) en veral diffusie tensor beeldvorming (DTI) is effektiewe metodes om die integritiet van witstof-mikrostruktuur in MIV te assesseer.

Die eerste hoofstuk beskryf algemene oorsake van MIV-verwante serebrale witstof-siekte asook die rol van neuro-beelding in die behandeling van hierdie pasiënte.

In die volgende hoofstukke word die eienskappe van witstof sein abnormaliteite op MRI en DTI (met behulp van DTI afgeleide maatstawwe - fraksionele anisotropie (FA), gemiddelde (MD), aksiale (AD) en radiale diffusie (RD)) in kinders met MIV, en wat met ART in die eerste jaar van die lewe begin het, beskryf.

Metodes:

Strukturele MRI skanderings van kinders op gemiddelde ouderdom van 39,1 maande is hersien en gekorreleer met kliniese en neuro-ontwikkelingsdata, virologiese merkers en durasie van ART.

DTI is in 'n soortgelyke kohort (wat verskeie kinders in die eerste studie en kontroles insluit) op die gemiddelde ouderdom van 64,7 maande, verwerf. Voxel-gebaseerde groep vergelykings is uitgevoer om streke te bepaal waar FA en MD verskil tussen MIV + en onbesmette kinders. Assosiasies van DTI parameters met begin tydperk van ART en korrelasies van DTI parameters in abnormale witstof met direkte neuro-ontwikkelingsuitkomste was ondersoek.

Resultate:

MRI-skanderings van 44 kinders, gemiddelde ouderdom van 39,1 maande is geevalueer: 10 was op uitgestelde en 34 op vroeë CHER-behandelingsarms. ART is begin op gemiddelde ouderdomme van 18,5 en 8 weke onderskeidelik. Veelvuldige hoë sein intensiteit letsels op T2 / FLAIR is gedokumenteer in 22 pasiënte (50%), hoofsaaklik in frontale (91%) en parietale (82%) witstof. Geen verskille in neuro-ontwikkeling van kinders met en sonder witstof-seinafwykings is gevind nie. Geen letsellading of verspreiding het beduidende korrelasie met neuro-ontwikkelingstellings of neurologiese ondersoeke getoon nie. Daar was 'n tendens vir die assosiasie van witstof sein abnormaliteite en langer tyd op ART (p=0.13) en CD4% (p=0.08).

39 MIV+ kinders (15 manlik) en 13 kontroles (5 manlik) is op die gemiddelde ouderdom van 64,7 gebeeld met DTI. 2 gegroepeerde areas met verlaagde FA en 7 met verhoogde MD is in die MIV + -groep geïdentifiseer (oorwegende simmetriese verspreiding) as gevolg van verhoogde RD, wat dui op verminderde mielinisasie. Kinders met vroeë onderbreekte ART het laer FA vergeleke met diegene wat deurlopende behandeling ontvang het. Die enigste neuro-ontwikkelingsdomein met 'n tendens van verskil tussen die MIV + -kinders en kontroles (p = 0.08) was persoonlike sosiale kwotiënt wat verband hou met verbeterde mielinisasie van die forceps-minor in die kontrolegroep. As 'n gekombineerde groep was daar 'n negatiewe korrelasie tussen visuele persepsie en RD in die regter superior longitudinale fasciculus en linker inferior longitudinale fasciculus wat verband hou met die feit dat hierdie gebiede, wat deel vorm van die visuele waarnemingsbane, in 'n kritieke toestand van ontwikkeling is op die ouderdom van 5 jaar.

Gevolgtrekking:

Die helfte van kinders wat verwys is met MIV-verwante brein siekte, op 'n gemiddelde ouderdom van 39,1 maande, het witstof sein abnormaliteite op T2 / FLAIR strukturele MRI. MIV+ kinders op 5 jarige ouderdom het witstof-abnormaliteite, gemeet aan FA, ten spyte van vroeë ART, wat bevestig dat vroeë ART nie die witstof ten volle beskerm teen peripartum of in utero-infeksie met MIV nie. In teenstelling met volwassenes, is

die kortikospinale bane hoofsaaklik aangetas, eerder as die corpus callosum. Deurlopende vroeë ART beperk egter die omvang van witstof-skade.

Selfs gerigte neuro-ontwikkelingstoetse sal die mate van mikrostrukturele witstof-skade wat deur DTI bespeur kan word, onderskat. Die visuele persepsie tekort wat in die MIV-studiepopulasie waargeneem is, moet verder ondersoek word aangesien dit voortduur in longitudinale opvolg van hierdie pasiënte op die ouderdom van 7 jaar.

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ACRONYMS

AD	axial diffusivity
ADEM	acute disseminated encephalomyelitis
ANOVA	one-way analysis of variance
ART	combination antiretroviral therapy
Beery VMI	Beery visual motor integration test
CC	corpus callosum
CDC	centre for disease control
CHER trial	children with HIV early antiretroviral trial
CMV	cytomegalovirus
CNS	central nervous system
CST	corticospinal tract
DTI	diffusion tensor imaging
DWI	diffusion weighted imaging
FA	fractional anisotropy
fMRI	functional magnetic resonance imaging
GMDS	Griffiths mental development scales
HEU	HIV exposed uninfected
HIV	human immunodeficiency virus
HIV+	HIV-infected
HIVE	HIV encephalopathy
HSV	herpes simplex virus
HU	HIV unexposed
IFOF	inferior frontal occipital fasciculus
ILF	inferior longitudinal fasciculus
MD	mean diffusivity
MRS	magnetic resonance spectroscopy
PET	positron emission tomography
PML	progressive multifocal leucoencephalopathy
pMRI	perfusion magnetic resonance imaging
RD	radial diffusivity
ROI	region of interest

SAA	Sub-Saharan Africa
SLF	superior longitudinal fasciculus
SPECT	single photon emission computed tomography
TB	tuberculosis
TBM	TB meningitis
UF	uncinate fasciculus
WM	white matter
WMH	white matter hyperintensities
WMSA	white matter signal abnormality

Chapter 1: Introduction

8.1 Central nervous system HIV in children

Untreated childhood HIV infection causes high mortality and rapid disease progression. If left untreated more than a third of infected infants die during infancy and about half by 2 years of age. [1,2,3] The disease may have a variety of neurological complications and HIV infection should be suspected in children presenting with unexplained neurological manifestations and growth failure, posing a difficult clinical problem. [4]

With the recent improvements in HIV treatment, the disease has become chronic. The mean survival time of HIV-infected children is now 9-10 years, which is more than 4 times the mean age of such children who died in 1990. Yet, the prevalence of HIV encephalopathy has not decreased despite use of combination anti-retroviral therapy (ART). Rather, it is expected that as patients live longer, the prevalence of CNS manifestations will actually increase. [5] Since the experience of treatment of HIV-1 infections in adults cannot be easily translated to children, paediatric clinical trials are needed to answer questions specific to the unique characteristics of children. [6] Treatment options are complicated by long term toxicity of antiretroviral drugs, adherence issues as well as limited resources in our environment.

HIV- related encephalopathy is an important problem in vertically HIV-infected (HIV+) children. Infected infants may manifest early with catastrophic encephalopathy, loss of brain growth, motor abnormalities, and cognitive dysfunction. [7]

Neuropathology studies in children with AIDS typically reports atrophy with the most striking histopathological features that of inflammation, neuronal loss, demyelination and perivascular basal ganglia calcifications. [6] Initial entry of the virus into the central nervous system occurs very early in paediatric HIV infection, however the mechanisms are not yet entirely understood. The virus is primarily found in microglia and brain derived macrophages, not in neurons. The infected microglia may enhance the migration of immune-activated macrophages across the blood-brain-barrier and macrophage products such as cytokines, are highly likely to be neurotoxic. Circulating tumour necrosis factor has been found to be elevated in encephalopathic children suggesting a toxic effect on central myelin. The damage to neurons are there for

indirect, with several of the cytokines promoting apoptosis, the presumed mechanism of damage to neurons. Furthermore, components of the virus itself are thought to be neurotoxic. The susceptibility of astrocytes to infection may also be greater in children than in adults, and they harbour latent infection. [7]

Since the inception of this study, a number of trials have been completed in South Africa and abroad. [8–10] The *Children with HIV early antiretroviral* (CHER) trial [11] showed that early time limited ART in young infants is better than deferred ART over an extended period. HIVE was more common in the deferred treatment group, suggesting that early ART initiation could be neuroprotective.

The coverage of children with ARV stood at only 28% in 2011, which made it crucial to promote the early diagnosis and treatment in young infants, rather than only limiting interventions to prevent mother to child transmission.

Review of the literature

8.2 What is known from imaging the brain in adults with HIV

HIV is associated with central nervous system (CNS) changes that may affect cerebral blood flow, metabolism, structure, and diffusion. A variety of available neuroimaging techniques have been employed to gain a better understanding of the underlying neurological processes involved in disease progression, and while useful information has been gained, some of these techniques were not practical or effective to assess the latter. [12]

Each neuroimaging technique offers unique insight into the neural mechanisms underlying HIV, as well as a potential means of monitoring disease progression and treatment response. The results of published studies on MR Imaging suggest that neurological dysfunction and symptoms as well as neuroimaging findings can improve with ART. Treatment should be initiated before irreversible CNS damage occurs. [13]

SPECT (single-photon emission computed tomography) provides a measure of cerebral blood flow, and early studies in adult HIV patients found hypoperfusion in frontoparietal regions correlating with dementia, [14] which abated with treatment. [15]

PET (positron emission tomography) utilizes radioactive tracers to quantify neural changes related to cerebral glucose metabolism or blood flow. In a study by Pascal et al. an adult HIV patient group had asymmetry in glucose utilization compared to controls, most prominent in the pre-frontal and pre-motor regions. [16]

Volumetric analysis of MRI found a correlation between declining cognitive function and volume loss in specific brain structures including the basal ganglia and caudate nucleus in adult HIV patients compared to controls. [17,18] These studies however, are very labour intensive.

MRS (magnetic resonance spectroscopy) is a non-invasive way of analysing metabolite concentrations in targeted sites in the brain. Many studies have demonstrated abnormal metabolite concentrations and ratios in adult patients with HIV, but more importantly in patients without visible lesions on MRI. Abnormalities were most prominent in the white matter of the frontal lobes, basal ganglia and thalamus. [19,20]

fMRI (functional MRI) collects anatomical and functional scans reflecting alterations in blood oxygenation level dependant (BOLD) contrast, which can link a cognitive task performed in the magnet to brain activity over time. Chang et al. demonstrated that adult HIV patients had greater frontal and parietal activation during complex attention tasks. [21] Even before symptomatic cognitive decline patients with HIV may exert greater than normal effort to perform the same tasks. [22] A positive correlation was also found between activation during attention tasks and metabolites in the frontal lobes and basal ganglia. [23]

pMRI (perfusion MRI) measures the rate of blood flow through capillaries. During the scan, data are recorded at specific sites resulting in information about the relative cerebral blood flow (rCBF), cerebral blood volume (CBV) or the mean transit time from one point in the brain to another. Chang et al. found that patients with early HIV

cognitive motor complex had significant decline in rCBF in the lateral frontal lobes and the medial parietal lobes, with increases in rCBF in the posterior parietal white matter compared to controls. [24] In a study by Hall et al. there was an increase in CBV and CBF in the centrum semiovale of adult HIV+ patients with more advanced disease suggesting a relationship with changes in perfusion and atrophy/demyelination. [12]

DTI (diffusion tensor imaging) is derived from a set of diffusion gradients to measure the anisotropic diffusion of water molecules (see discussion later). DTI provides information about the integrity of white matter tracts and has been very helpful in the studying of diseases caused by demyelination. DTI is extensively used in brain imaging of HIV+ adults. The first studies already demonstrated decrease in anisotropic diffusion in the frontal subcortical white matter and genu of the corpus callosum despite normal appearing white matter on structural MRI compared to controls. [25] More specific findings of increased mean diffusivity and radial diffusion in HIV+ patients indicate that demyelination might be the main pathophysiological result of HIV associated white matter damage. [26]

The most common neuropathological feature of HIV-1 infection remains diffuse white matter pallor, especially in advanced HIV disease, [27] with MRI being the most sensitive in detecting early changes not yet evident on CT.

AIDS dementia complex is one of the most common causes of HIV-associated morbidity in adults, with early symptoms often subtle and overlooked, delaying appropriate treatment. Two histopathological patterns have been described in AIDS dementia complex: HIV encephalitis - representing active infection of the brain and meninges with neuropathological studies demonstrating accumulation of multinucleated giant cells, inflammatory reactions and often focal necrosis [28], and HIV leukoencephalopathy (more diffuse white matter involvement with defined clinical criteria), which can be distinguished radiologically.[13] HIV encephalitis present as patchy areas of T2 high signal, whereas HIV encephalopathy has a butterfly-like appearance, more diffuse increased T2 signal intensity of the white matter. Cerebral atrophy however remains the most common finding in AIDS dementia complex.

Focal brain lesions seen in adults prior to ART were predominantly toxoplasma encephalitis and primary CNS lymphoma which demonstrated a dramatic decline in the post ART era most likely as a direct result of immune reconstitution. [29]

8.3 Brain imaging in HIV+ children

MRI has become the preferred modality for neuroimaging in the HIV+ child, being able to detect subtle white matter signal abnormalities and vascular complications. The use of more sophisticated techniques is now the subject of further research.

In the pre- and early ART era neuroimaging findings of vertically-infected HIV+ children were described as cerebral atrophy, symmetrical calcifications of the basal ganglia or periventricular white matter, as well as focal white matter lesions on CT and MRI, which in turn have an association with advanced immune and clinical staging. [30–33]

Over time, distinct patterns of CNS involvement, different from those in adults, are being recognized. [34] The atrophy pattern encountered in children with HIV is specific: a central atrophy, primarily affecting the subcortical white matter and basal ganglia regions. [7]

Opportunistic infections and brain tumours are rarely reported in children compared to adults. [5] HIV-1 involves almost exclusively the CNS in children, sparing the peripheral nervous system. [33] Vascular complications in children are associated with end-stage HIV disease with aneurysms and ischemic infarctions as the most common reported lesions. [35]

As with adults, **MRS** studies have shown increased myo-inositol (a glial cell marker) and decreased N-acetyl aspartate (a marker of neuron density and integrity) in the white matter of children with encephalopathy [36], and more specifically that HIV+ children do not demonstrate a normal age related increase in NAA in the frontal white matter and hippocampus. [37] These findings underline the significant developmental impact of early HIV infection.

DTI has now become a very popular imaging method to assess white matter damage in vertically infected HIV children, demonstrating clear differences between HIV-infected and control groups. Lower functional anisotropy (FA) and higher mean diffusion (MD) in specific white matter tracts, particularly in the corpus callosum have been reported. [38–41] These studies included wide age ranges over developmental phases during which there is significant physiologic increase in both white matter volume and FA .

8.4 What are diffusion weighted imaging and diffusion tensor imaging?

Diffusion is a physical process that involves the movement of molecules along random paths, colliding and moving past each other - so called Brownian motion. The distance and extent to which water molecules move per unit time in tissues is affected by physical properties such as viscosity and temperature as well as the presence of cellular structures (i.e. membranes or myelin sheaths) which provide barriers to free movement. Diffusion in such circumstances is said to be restricted. [42]

If diffusion is the same in all directions it is termed isotropic (free diffusion).

In tissues that have a highly organised structure, diffusion may be more restricted along one direction than another. For example, in myelinated white matter fibres diffusion across the fibre is much more restricted than along the fibre. In such circumstances diffusion it is called anisotropic.

A defining characteristic of neuronal tissue is the fibrillar structure, consisting of tightly packed and aligned axons, surrounded by glial cells. The result is increased movement of water in the direction of the fibres and hindrance perpendicular to them. The distribution of diffusion is further influenced by the fact that neuronal tissues and specifically white matter tracts run in various orientations. [43]

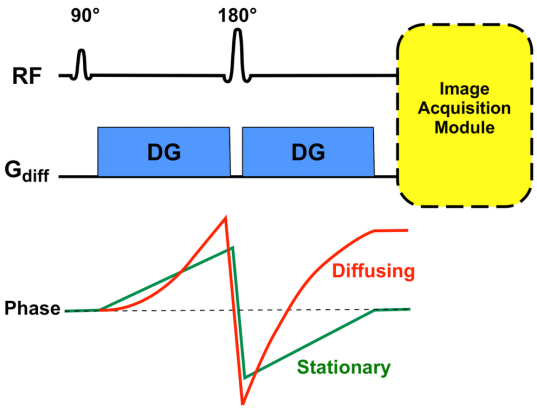
Experimental evidence suggests that the biggest contributor to anisotropic diffusion in white matter is not the myelin sheaths but rather the cell membrane. The degree of myelination further modulates anisotropy. [43]

MRI technique of DWI

DWI was introduced in the mid 1980's, and provided a novel contrast mechanism for MRI, and a non-invasive method of measuring the mobility of water molecules in various tissues. [42,44]

The typical diffusion time used in DWI is around 50ms, and the average distance of movement of water molecules in the brain is around 10µm. It is this movement that is measurable by MRI.

Diffusion weighting can be applied to almost any MRI pulse sequence by adding two gradient pulses. The first labels the initial position of the water molecules by introducing a phase shift that is dependent on the strength of the gradient. The second "reads" the final position of the molecules after they have had time to diffuse. Before the application of the second gradient, a RF (radio frequency) pulse of 180º is applied to reverse the phase shift induced by the first gradient. All spins remaining in the same location along the gradient axis during the 2 pulses will return to their initial state resulting in a measured MRI signal.

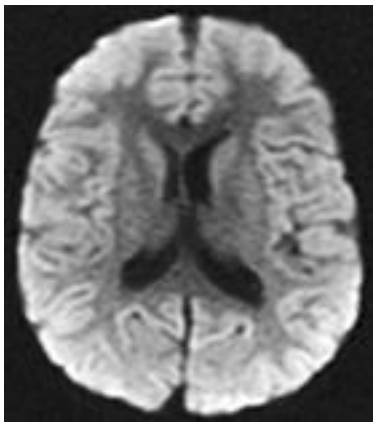


DG = diffusion sensitizing gradients. (MRIquestions.com)

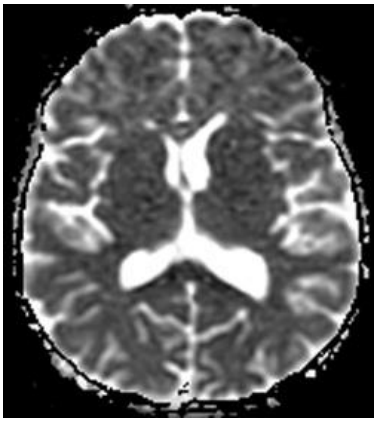
If the molecules changed position by diffusion the MRI signal is not refocused properly by the second gradient and image intensity is reduced. The resultant image shows low signal intensity in regions where diffusion along the applied diffusion gradient is high, for example in CSF. [42,43]

The degree of diffusion sensitivity or weighting is expressed as a *b*-value, where larger *b*-values are related to greater degree of diffusion sensitivity in a sequence. ($b \sim q^2 \times \Delta$, where q=gradient strength of the MRI and Δ=diffusion time interval). The direction of the diffusion gradients can be changed so that diffusion of water can be measured along different directions within the brain.

The interpretation of DWI is made easier by an image that reflects only diffusion, called the ADC map (apparent diffusion coefficient) derived from signal intensities of images acquired with different *b*-values (diffusion sensitivity). ADC imaging is based on a 3D isotropic diffusion model (spherical voxel; diffusion equal in all directions), not taking into account the orientation of axonal bundles and the anisotropic nature of diffusion encountered in white matter (cigar shaped voxel; diffusion dominant in one direction). [42,43]



DWI trace



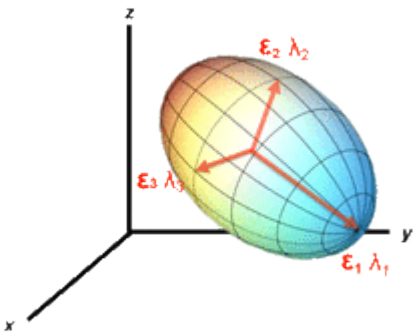
ADC map

To characterise diffusion within a white matter tract, at least 6 gradient directions must be applied. A mathematical calculation of the 6 diffusion direction coefficients results in a diffusion tensor rather than a single coefficient. This tensor is a 3x3 matrix which fully describes the sum of diffusion in 3D space, and is usually ellipsoid.

The mathematical nature of the data makes it possible to be analysed in 3 different ways, providing information on microstructure and architecture for each voxel imaged: the mean diffusivity, the main direction of diffusion and the degree of anisotropy.

Mean diffusivity (MD, also called the trace) describes the overall mean-squared displacement of molecules and the presence of obstacles to diffusion, in other words the degree of water diffusion within an imaging voxel. It has a similar appearance to an ADC map, derived from 3 diffusion gradients.

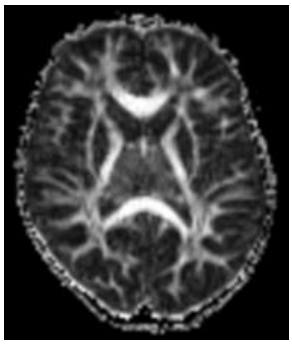
The main direction of diffusion is derived by computing eigenvectors and eigenvalues from the tensor. Eigenvectors are orthogonal to each other, each with a value describing the properties of the tensor. The eigenvector with the largest value is the main direction of diffusion. If the eigenvectors differ significantly, diffusion is called anisotropic. [43]



Representation of the diffusion as an ellipsoid with three unit eigenvectors (MRIquestions.com)

Fractional anisotropy (FA) is used to describe the shape of diffusion by using a scalar value derived by comparing each eigenvalue with the mean of all the eigenvalues within the voxel. The FA is a simple and robust method, it reflects the degree of anisotropic diffusion: so will be high (close to the value 1) in regions of highly organised tissues i.e. corpus callosum, and low in regions where the predominant diffusion is not specifically orientated and close to zero (value 0) in free fluids i.e. CSF. [45] It is viewed as a FA map, demonstrating high intensity in regions of the brain with

anisotropic diffusion and low intensity in those regions with isotropic diffusion, making it an excellent map delineating white matter tracts. [42–44]



Fractional anisotropy map, grayscale display of FA values across the image. Brighter areas are more anisotropic than darker areas. (MRIquestions.com)

The most practical way of viewing diffusion tensor data, is colour coding the data according to the principal direction of diffusion. The accepted coding system allocates red, to diffusion along inferior-superior (x) axis, blue, to diffusion along the transverse (y) axis, and green, to diffusion along the posterior-anterior (z) axis. The intensity of the colour is proportional to the FA, producing the well-known images of DTI. [43]

8.5 Previous work using DTI in adults with HIV

DWI has been used to great advantage in the adult population.

In HIV+ patients, apparent diffusion coefficient ratios obtained by diffusion-weighted imaging are significantly greater in lesions due to Toxoplasma encephalitis than in primary CNS lymphoma, providing a tool for distinguishing the two entities. [46]

Multiple studies in adults have demonstrated distinct differences in the HIV population compared to controls.

The diffusion constant (ADC) and anisotropy (FA) values in the subcortical white matter, corpus callosum and internal capsule in HIV+ adults are useful for detecting

abnormalities despite normal appearing white matter on conventional MR images and non-specific neurological examination. [27,47,48] Patients with the highest diffusion constant elevations and largest anisotropy decreases had the most advanced HIV disease. [25,27,49]

Significant increase in ADC in HIV patients, primarily in the frontal white matter has been correlated positively with a glial marker myo-inositol (MI) and negatively with performance, which suggests that increased diffusion may reflect glial activation or inflammation, in turn contributing to cognitive deficits in these patients. [50]

Furthermore, diffusion abnormalities have been identified in the splenium of the corpus callosum in HIV+ patients and these alterations were associated with dementia severity and motor speed losses. [51] Similar findings of loss of function within specific cognitive domains and DTI measures in subcortical regions of the brain have been reported, confirming that DTI is a sensitive tool for correlating neuroanatomic pathologic features with specific cognitive functions in patients with HIV infection. [47,52] Most of these studies used a 'priori' ROI (region of interest) analysis.

Subsequent studies, using a 'voxelwise', and in selected studies 'whole-brain', analysis have shown a more widespread white matter damage [26,52–55] and variable changes in DTI (conflicting FA values, both increased and decreased) [52], which may reflect both direct loss of axonal integrity (indicated by an increased MD and drop in FA) and a loss of complexity (indicated by a rise in FA) to the underlying axonal matrix (loss of crossing and other nonparallel fibres).

CNS injury is evident in patients with HIV despite effective antiretroviral treatment, [52,55] however a recent study suggested that initiating ART could lead to a reduction in neuroinflammation and therefore improvement in DTI measures [56], more specifically improvement in the mean diffusivity, in the corpus callosum and centrum semiovale.

It is very important to note that other factors may also influence the integrity and stability of white matter tracts in the adult population, most notably age and co-infection which both demonstrated an association with decreased FA and increased

diffusivity. [55] DTI measures have also shown significant correlation to duration of HIV infection [57,58], again an important factor in adults.

8.6 DTI in children

DWI has proven to be a sensitive supplemental sequence to routine cranial MR imaging in children, improving lesion detection and characterisation. [59] DWI has been demonstrated to be highly sensitive in identifying acute ischemic infarction when all other forms of neuroimaging are negative, with greatest lesion detection within a week of onset of symptoms. DWI has also been reported as being very effective in evaluating myelination. Water diffusion parallels the known course of brain maturation: as ADC decreases, FA increases. The importance of this is that apparent anisotropy precedes the signal on T1 or T2 weighted MR images routinely used to assess myelination. [59–61] In dysmyelinating and demyelinating conditions, DWI provides information that is not yet apparent on the T1 or T2 sequences, which may be used to prognosticate and study the evolution of these disorders as well as provide additional criteria to further classify undefined white matter disorders. [59,62]

DWI is also a highly sensitive tool for the evaluation of meningoencephalitic lesions with restricted diffusion, and is more sensitive than T2 and FLAIR sequences. [59,63,64]

DTI studies in children have demonstrated its utility to assess the microstructural development and myelination of white matter. [60,65,66] DTI has been used in a wide variety of other neurodevelopmental research in the paediatric population: childhood psychiatric disorders, traumatic brain injury, delineation of brain tumours pre-operatively and differentiation between low and high grade tumours, autism and metabolic diseases. [67–71]

Although DTI has also become an accepted and robust tool for assessing HIV-associated white matter disorders, there are only few studies of DTI in the paediatric HIV+ population, specifically looking at FA and correlation with clinical, laboratory and treatment parameters.

The **broad aim** of this study is to correlate neurodevelopmental scoring with the extent of white matter disease represented by FA values derived from DTI.

Specific objectives:

-To describe general pathological conditions manifesting as white matter abnormalities of the brain in perinatal HIV infection.

-To determine the incidence of white matter abnormalities in perinatal HIV infection.

-To compare DWI and ADC with T2 and FLAIR sequences in children with HIV with regards to presence and distribution of white matter changes.

-To determine geographical distribution of white matter signal abnormalities.

-To correlate white matter lesion load and distribution with clinical groups (encephalopathy, neurodevelopmental delay, focal neurology) and developmental profile.

- To determine the extent and nature of white matter abnormalities using the DTI-derived metrics (FA and MD) and to examine the ameliorating effects of ART.

Chapter 2: Spectrum of white matter diseases in HIV

Rationale for the inclusion of published work

The manuscript included in this chapter gives an overview of the MRI features of common white matter disease entities encountered in the HIV- infected paediatric population in the form of a pictorial review.

Baseline MRI imaging of the brain is currently not standard practice in our institution, for either perinatally infected or newly diagnosed HIV infection in children.

Children are referred for neuroimaging when they present with focal neurology as a result of opportunistic infections, tumours or for confirmation of clinically suspected HIV encephalopathy that does not fully comply with WHO criteria.

The baseline structural MRI studies are often normal; however non-specific white matter lesions are frequently encountered. This article discusses the differential diagnosis of white matter disease according to: clinical presentation, MRI imaging features, distinct characteristics and associated immunological parameters typically found in the separate disease entities.

It also sheds light on the differences in CNS involvement between HIV+ adults and children and discusses specific MRI parameters that can be utilized for monitoring patients.

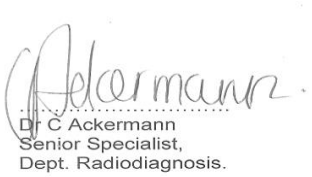
This chapter sets the scene for the remainder of the thesis and informs on the general difficulty that the diagnostic radiologist faces as part of the primary HIV care team.

Declaration by the candidate:

With regards to chapter 2, *Spectrum of white matter diseases in HIV,* the nature and scope of my contribution were as follows:
First author, preparation of images and artwork 80%
The following co-authors have contributed:

Prof Savvas Andronikou: development of the layout and suggestions for producing artwork, editing 15%

Prof Ronald van Toorn: editing 5%



Signature of candidate: Date: 20 February 2019

Declaration by co-authors:

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 2
2. No other authors contributed besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 2 of this dissertation.

Declaration with signature in possession of candidate and supervisor.

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PICTORIAL ESSAY

Human immunodeficiency virus-related cerebral white matter disease in children

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Abstract
The human immunodeficiency virus (HIV) epidemic seems largely controlled by anti-retroviral treatment with resultant large numbers of children growing up with the disease on long-term treatment, placing them at higher risk to develop HIV-related brain injury, ongoing cognitive impairment and treatment-related neurological complications. Cerebral white matter involvement is a common radiologic finding in HIV infection and the causes of this have overlapping appearances, ranging from diffuse widespread involvement to focal lesions. The varied pathophysiology is broadly grouped into primary effects of HIV, opportunistic infection, vascular disease and neoplasms. White matter changes in children can be different from those in adults. This review provides guidance to radiologists with the diagnostic dilemma of nonspecific cerebral white matter lesions in children with HIV. The authors discuss common causes of HIV-related cerebral white matter disease as well as the role of neuroimaging in the management of these children.

Keywords Central nervous system · Children · Computed tomography · Human immunodeficiency virus · Magnetic resonance imaging · White matter

Introduction

Cerebral white matter involvement is a common radiologic finding in HIV infection and its causes have overlapping appearances, ranging from diffuse widespread involvement to focal lesions. The varied pathophysiology is broadly grouped into primary effects of HIV, opportunistic infection, vascular disease and neoplasms. White matter changes in children can also exhibit specific differences in comparison to findings in adults with HIV.

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HIV-related white matter damage includes demyelination and axonal injury with dysfunction. Myelin injury is postulated to induce disruption of the brain–blood barrier, which is essential for HIV-1 entrance to the brain. HIV infection also adversely influences cerebral re-myelination, a process that requires proliferation, migration and survival of oligodendrocyte progenitor cells [1].

Previously, conventional neuroimaging played a vital role in the diagnosis of HIV-related cerebral atrophy, vasculopathy, opportunistic infections and tumors as well as monitoring the evolution of brain lesions and response to therapy [2–4]. Structural cerebral imaging alone in HIV-infected patients does not reveal the extent of HIV-related white matter abnormalities. In addition, it has limited value in asymptomatic patients [5] because of poor diagnostic yield; despite this, some clinicians continue to advocate that all people newly diagnosed with HIV infection undergo baseline imaging and that there should be a low threshold to image people with minimal neurological symptoms because they could have significant central nervous system disease [6, 7].

This review gives a concise summary of common causes of HIV-related cerebral white matter disease in children and provides guidance to radiologists with the diagnostic dilemma of nonspecific cerebral white matter lesions in children with HIV.

Published online: 29 November 2018

Introduction:

Cerebral white matter involvement is a common radiological finding in HIV infection and its causes have overlapping appearances, ranging from diffuse widespread involvement to focal lesions. Varied pathophysiology exists, broadly grouped into primary effects of HIV, opportunistic infection, vascular disease and neoplasms. White matter changes in children may also exhibit specific differences in comparison to HIV-infected adults.

HIV-related white matter damage includes demyelination and axonal injury with dysfunction. Myelin injury is postulated to induce disruption of the brain blood barrier which is essential for HIV-1 entrance to the brain. HIV-infection also adversely influences cerebral re-myelination, a process which requires proliferation, migration and survival of oligodendrocyte progenitor cells [72].

There is a need to find ways to improve early diagnosis of HIV, especially related to the field of neuroimaging [73]. Previously, conventional neuroimaging played a vital role in the diagnosis of pediatric HIV patients by identification of HIV-related cerebral atrophy, vasculopathy, opportunistic infections and tumors. It also played an important role by excluding alternative causes of CNS symptomatology as well the monitoring of progression / evolution of brain lesions and response to therapy [12,73–76].

With the advent of advanced imaging techniques, it has become apparent that structural imaging of the brain in HIV-infected patients often underestimates the extent of the underlying pathology. Structural brain imaging has limited value in asymptomatic HIV+ patients [77,78] due to poor diagnostic yield, [78,79] yet others feel strongly that all newly diagnosed HIV+ patients should undergo baseline imaging and that there should be a low threshold to image patients with minimal neurological symptoms as they may have significant CNS disease [35,80].

The aim of this review is to give a concise summary of common causes of HIV-related cerebral white matter disease in children and to provide guidance to radiologists faced

with the diagnostic dilemma of nonspecific cerebral white matter lesions in HIV+ children.

Spectrum of HIV related white matter disease:

1. HIV Encephalopathy (HIVE):

HIVE is a broad clinical term and comprises deterioration of cognitive functions that are associated with white matter disease and cerebral atrophy. The WHO defines HIVE as at least one of the following, progressing over at least two months in the absence of another illness: failure to attain, or loss of, developmental milestones or loss of intellectual ability; OR progressive impaired brain growth demonstrated by stagnation of head circumference; OR acquired symmetric motor deficit accompanied by two or more of the following: paresis, pathological reflexes, ataxia and gait disturbances [81]. HIVE can furthermore be classified into: 1) progressive encephalopathy which is characterized by a step wise deterioration of mental status associated with severe immunodeficiency, pathologically characterized by diffuse loss of myelin in the deep WM, scattered multinucleated giant cells and microglia but scarce or absent inflammatory reaction and 2) static encephalopathy characterized by less severe cognitive dysfunction but inability to maintain age related developmental milestones [75].

HIV encephalitis (meningoencephalitis), as opposed to encephalopathy, represents active infection of the brain and meninges and is characterized by acute symptoms such as headache, neck stiffness, confusion and seizures. Neuropathological studies demonstrate accumulation of multinucleated giant cells, inflammatory reactions and often focal necrosis [28].

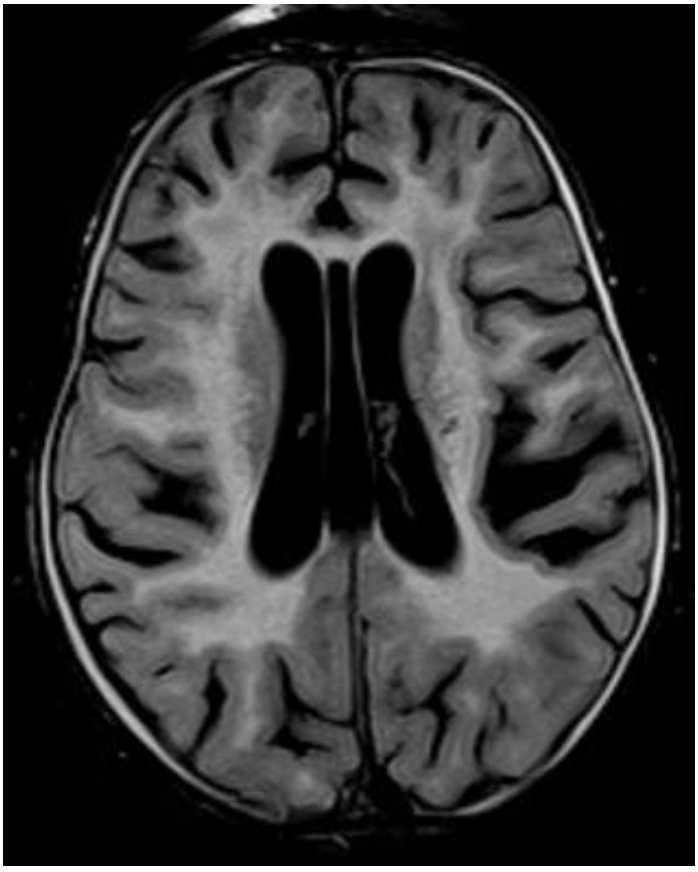
The hallmark of HIV infection in infants and children is early cerebral involvement; especially when the virus was acquired perinatally [7,82]. HIV can cross the blood brain barrier (BBB) either during primary infection or at a later stage. Haematogenous neuroinvasion via perivascular pathways is also described, [82] explaining why the largest concentration can be found in the central periventricular WM as well as in the

basal ganglia [83–85]. Viral latency may delay onset of CNS symptoms in adults. In contrast, the developing brain is more vulnerable to early CNS involvement [76,86,87].

Imaging: This will mirror the known pathologic findings, with bilateral symmetrical hyperintense T2 and FLAIR signal change of the periventricular WM. Typically there is no mass effect or contrast enhancement. If associated with cerebral atrophy, can indicate advanced disease.

Figure 2.1

A three-year-old HIV+ girl with unknown ART status, presented with right sided focal seizures. Axial FLAIR MRI demonstrates severe central atrophy with extensive symmetrical hyperintense signal change of the periventricular white matter, typical of HIVE

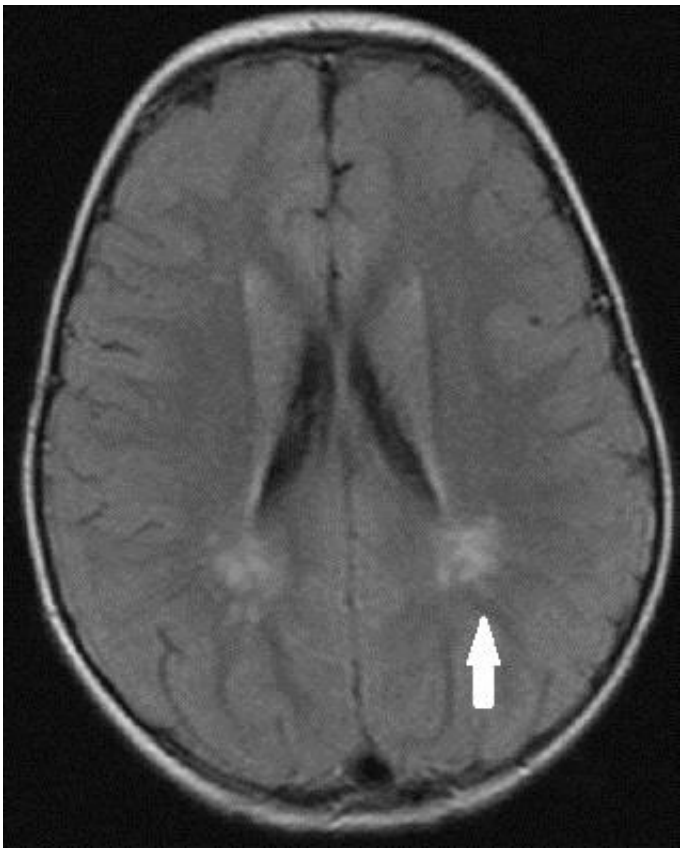


The diagnosis can be difficult to make in cases which are complicated by opportunistic infections. Caution is advised when interpreting WM changes in children under the age of 18 months as myelination of the centrum semi ovale and peritrigonal WM (which

may be delayed even beyond 2 years) is incomplete and may easily be confused with pathological WM changes [75,88,89].

Figure 2.2

A 2-year-old HIV+ girl on ART presented with neurodevelopmental delay. Axial FLAIR image (TR/TE 8000/109 IR 2340) demonstrates bilateral, symmetrical peritrigonal, linear hyperintensities (white arrow), in keeping with white matter high signal associated with normal perivascular spaces.



Alternative diagnoses to consider would be HIV related WM abnormalities, progressive multifocal leukoencephalopathy (PML) and lymphoma. These disease entities have a distinct clinical presentation, with the latter 2 usually only seen in advanced HIV disease with CD4 counts below 100 cells/mm^3.

2. White matter hyperintensities (WMH) in HIV-infected children:

WMH have been reported in healthy HIV-uninfected children and adolescents. In adults, there is an association with cerebrovascular disease and normal aging whilst

in children the pathogenesis is not well understood and may be multifactorial [90]. The significance of WMH in healthy children remains unclear. Some authors consider WMH in children above the age of 1.5 years as abnormal, whilst others report a prevalence of WMH as high as 31% in healthy children and adolescents [91,92,93]. The significance as an incidental finding in HIV+ children also requires further clarification.

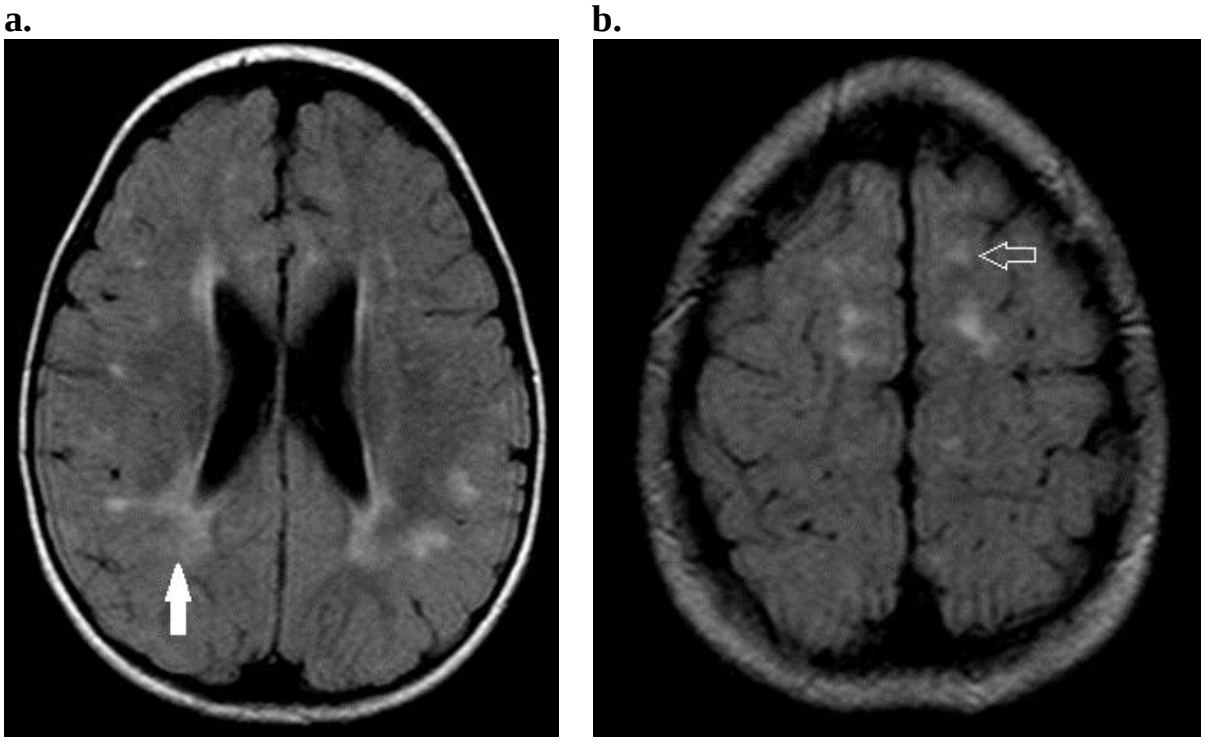
Imaging: WMH on MRI in HIV-infected children tend to be well described and most often located in the subcortical and deep WM [32,89,94]. Predilection for the frontoparietal lobes was found in one study [89] whilst more recent studies report no specific lobar predominance [95]. The lesions vary from pin point foci to larger confluent WM lesions.

Figure 2.3

A one-1-year-nine-month-old HIV+ boy on ART presented clinically with brisk reflexes and increased tone in the lower limbs but normal milestones.

(a) Axial FLAIR imaging at the level of the lateral ventricle bodies

(b) and over the convexities demonstrates bilateral, asymmetric focal and confluent hyperintensities of the periventricular and subcortical WM. Clinical criteria for HIVE were not met and thus the MRI findings were in keeping with HIV-associated WMH



In a study by Cohen et al. comparing cerebral injury in perinatally HIV infected children with controls, WMH were also demonstrated in 18% of controls. This should be considered when reporting WM lesions in the HIV+ paediatric population[95].

3. Acute disseminated encephalomyelitis (ADEM):

ADEM is a monophasic demyelinating disorder of the CNS associated with various viral infections such as HIV, influenza virus, Ebstein Barr virus (EBV), Herpes simplex virus (HSV) and cytomegalovirus [88,96–99]. It is extremely rare in perinatal HIV infection. In older children and adults, it typically presents with a monophasic, multifocal CNS disorder during seroconversion when the immune system is still competent. HIV-related immune dysfunction may also result in more aggressive and atypical presentations of ADEM such as tumefactive lesions, corpus callosum demyelination and recurrent and relapsing disease. Information regarding the patient's immune status is therefore important [100].

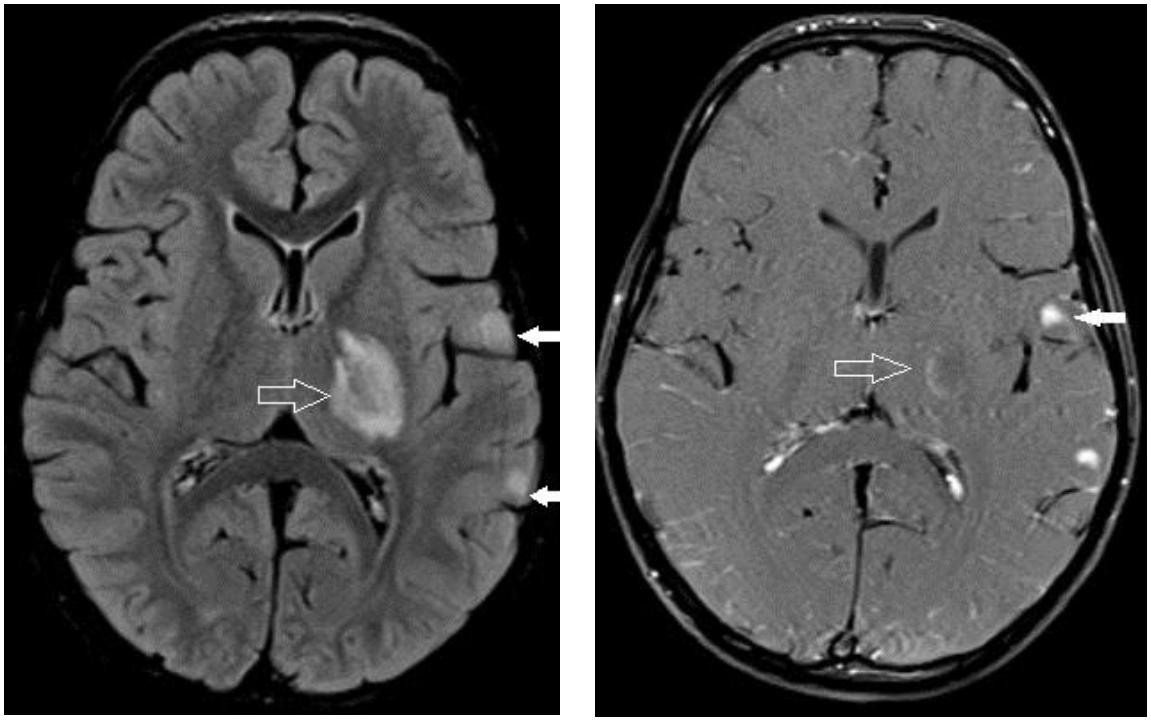
Imaging: WM lesions seen in ADEM are multifocal, asymmetric, ill-defined T2 and FLAIR hyperintensities. Subcortical WM is nearly always involved with lesions also seen in central WM, basal ganglia, brainstem and spinal cord. Nodular, diffuse or incomplete peripheral enhancement post contrast is common [88,97,100,101]. ADEM may also present as large tumefactive lesions with surrounding vasogenic oedema and mass effect. [99,102]

Figure 2.4

A six-year-old HIV+ boy, not on ART, presented with a new right CVA with the arm more affected than the leg

(a) Axial FLAIR MRI demonstrates a large hyperintense lesion in the left putamen and thalamus with bridging of the posterior limb internal capsule, as well as smaller subcortical lesions involving the left occipito-temporal region

(b) Axial Gadolinium enhanced T1W MRI demonstrates incomplete ring and nodular peripheral enhancement of the lesions, typical of ADEM. There was also involvement of the proximal cervical cord (not shown here)



a. **b.**

4. Progressive multifocal leukoencephalopathy (PML):

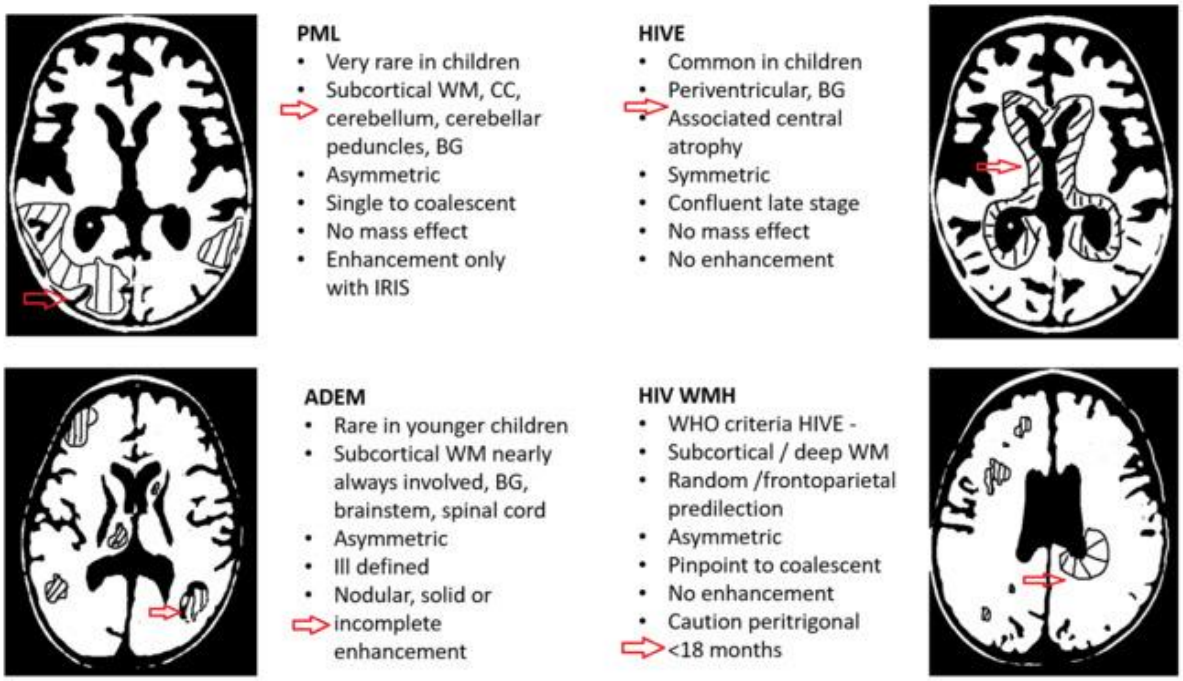
PML is a progressive nervous system disorder of demyelination almost exclusively seen in immunocompromised patients caused by the John Cunningham virus (JCV). In contrast to HIV which primarily infects astrocytes and microglia, JVC predominantly infects and damages oligodendrocytes, causing further demyelination [72].

A wide ranging clinical presentation is seen, ranging from cognitive dysfunction, visual loss, gait and speech disorders to limb weakness and cranial nerve palsies, mostly described in adults**.** PML is rare in the pediatric population as the seroprevalence of JC virus rises according to age from 16% in children to 34 % in adults by ages 21-50. In a study by Schwenk et al. in 2014 there were only 19 published reports of PML in HIV+ children. The rarity of PML in children was thought to be due to most patients demising before manifestation of the disease. the incidence was expected to rise with HIV becoming a chronic disease – but this has not materialized [103,104].

Imaging: As opposed to HIVE, PML affects the subcortical white matter (subcortical u-fiber involvement can cause sharp contrast with overlying cortex) in an asymmetrical distribution with predominate involvement of the occipital, parietal and frontal W/M [105]. Lesions are single, multifocal or become confluent as disease progresses. They are hyperintense on T2 and FLAIR and usually do not cause mass effect or show enhancement. PML may involve the corpus callosum, basal ganglia, cerebellar peduncles and cerebellum. In the immune reconstitution inflammatory syndrome, usually seen within weeks of starting ART, the lesions may have a more aggressive appearance with irregular peripheral enhancement and mass effect [103–105].

Figure 2.5

Schematic summary and comparison of the predominant white matter lesions seen in pediatric HIV



5. HIV-associated cerebral vasculopathy and infarction:

HIV-associated cerebral vasculopathy predominantly affects the medium sized cerebral vessels causing disease manifestations that include aneurysmal dilatation (in particular fusiform aneurysms reported to be much more common in the pediatric than adult HIV population), [106] arterial stenosis complicated by ischemic infarction and secondary moya-moya syndrome [107,108].

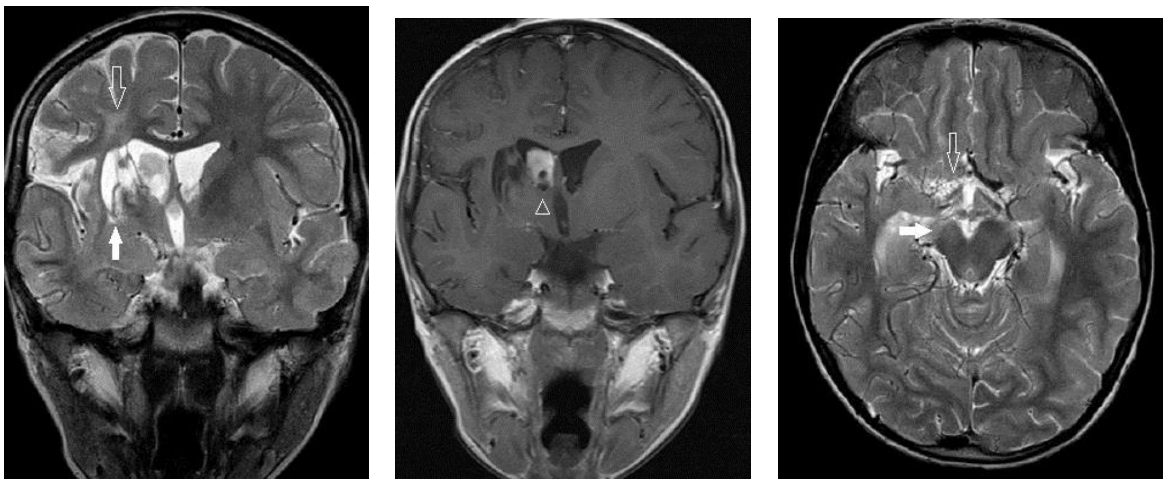
Figure 2.6

An HIV+ nine-year-old boy on ART presented with severe headache and neck stiffness

(a) T2 and

(b) T1W coronal MRI demonstrate signal change in the right frontal WM, large chronic infarcts involving the right basal ganglia with associated atrophy and an acute intraventricular hemorrhage

(c) Axial T2W MRI demonstrates the HIV vasculopathy with complete occlusion of the right internal carotid and proximal MCA with multiple small collaterals consistent with moya-moya disease.



a. **b**. **c.**

Secondary infarctions due to opportunistic infections such as TB, VZV and Herpes virus represent the other end of the spectrum. The incidence of cerebrovascular disease increases with disease severity and predominately occurs in children with perinatally acquired HIV [35,106]. Most children are asymptomatic in the early stages of disease, which justifies the importance of vascular imaging with MRI [35,80].

6. Infective lesions and edema:

WM changes related to viral infections can be either due to direct viral infection of the CNS with resultant encephalitis or secondary inflammatory or autoimmune response to the virus such as ADEM and vasculitis (discussed above) [88]. Focal infective lesions such as TB and toxoplasmosis also manifest as T2 and FLAIR WM hyperintensity due to surrounding vasogenic oedema.

HSV:

MRI reveals asymmetric T2 and FLAIR hyperintensity of the cortex and WM with frontoparietal lobe extension distinct from the typical medial temporal lobe involvement seen in adults. Leptomeningeal and gyral enhancement as well as petechial and confluent hemorrhage may be observed [88].

CMV:

CMV is common in patients with very low CD4 counts due to reactivation of a latent infection. Imaging findings of central nervous system (CNS) involvement with CMV are often non-specific and may even be normal. [109,110]

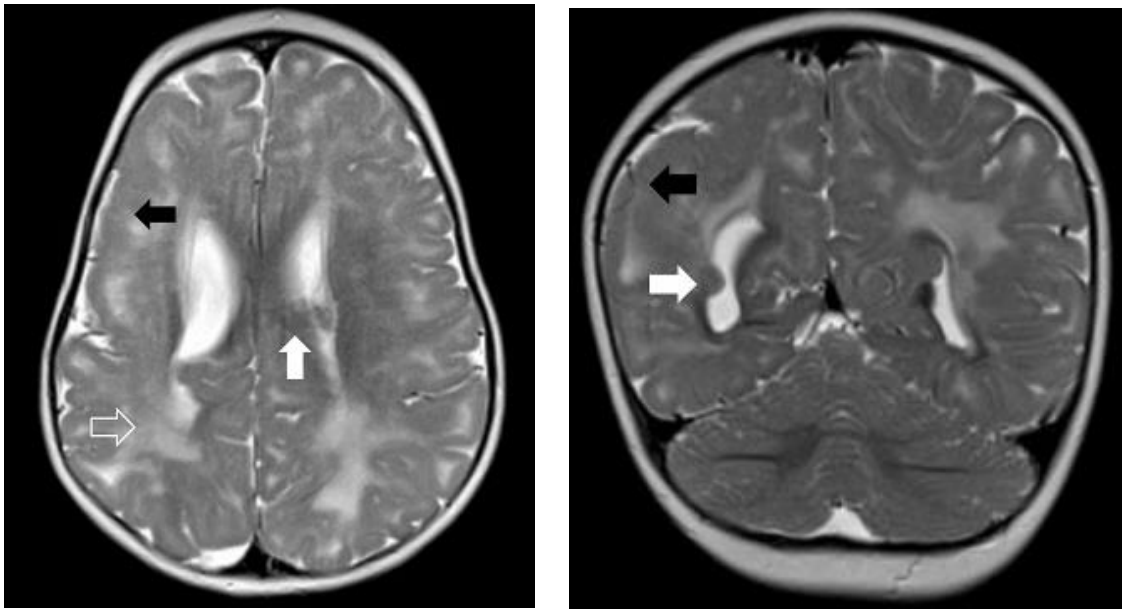
T2 and FLAIR periventricular WMH are seen with periventricular enhancement indicating acute ependymitis and ventriculitis [75,88,105,111].

Figure 2.7

An 11-month-old HIV+ girl, on ART with undetectable HIV viral load, presents with left arm and leg weakness. She had a very high cytomegalovirus viral load at birth. Axial and coronal T2-weighted images (TR/TE 5720/80) demonstrate

(a) bilateral, asymmetrical white matter hyperintensity, slightly more prominent posterior parietal (open arrow).

(b) Associated subependymal heterotopic grey matter (solid white arrow) bilateral at the lateral ventricles as well as right temporal-parietal and perisylvian polymycrogyria (solid black arrow) in (a) and (b) are typical findings in congenital CMV. Other typical imaging findings include coarse, peri-ventricular and basal ganglia calcifications, peri-ventricular cysts and atrophy (not shown here). White matter abnormalities are asymmetric, may be focal, patchy or confluent and can have a predominant frontal, parietal or posterior involvement, however this child was not myelinated enough for this to be assessed.



a. **b.**

TB:

The incidence of TB has reached epidemic proportions in Sub Saharan Africa due to the heavy HIV burden. Brain injury in TB meningitis (TBM) is a consequence of an immune-mediated vasculopathy causing infarctions. HIV-related immune dysfunction may prevent the production of thick basal meningeal exudates that result in cerebral parenchymal infarction and non-communicating hydrocephalus.

On **imaging** this manifests as fewer infarctions in the basal ganglia, decreased and more focal, asymmetric patterns of meningeal enhancement with more pronounced atrophy rather than hydrocephalus. (Figure 2.8) WMH is seen as a result of parenchymal oedema due to focal meningoencephalitis, granulomas or infarction [112,113]. (Figures 2.9 and 2.10)

Figure 2.8

Schematic representation of CNS TB in HIV

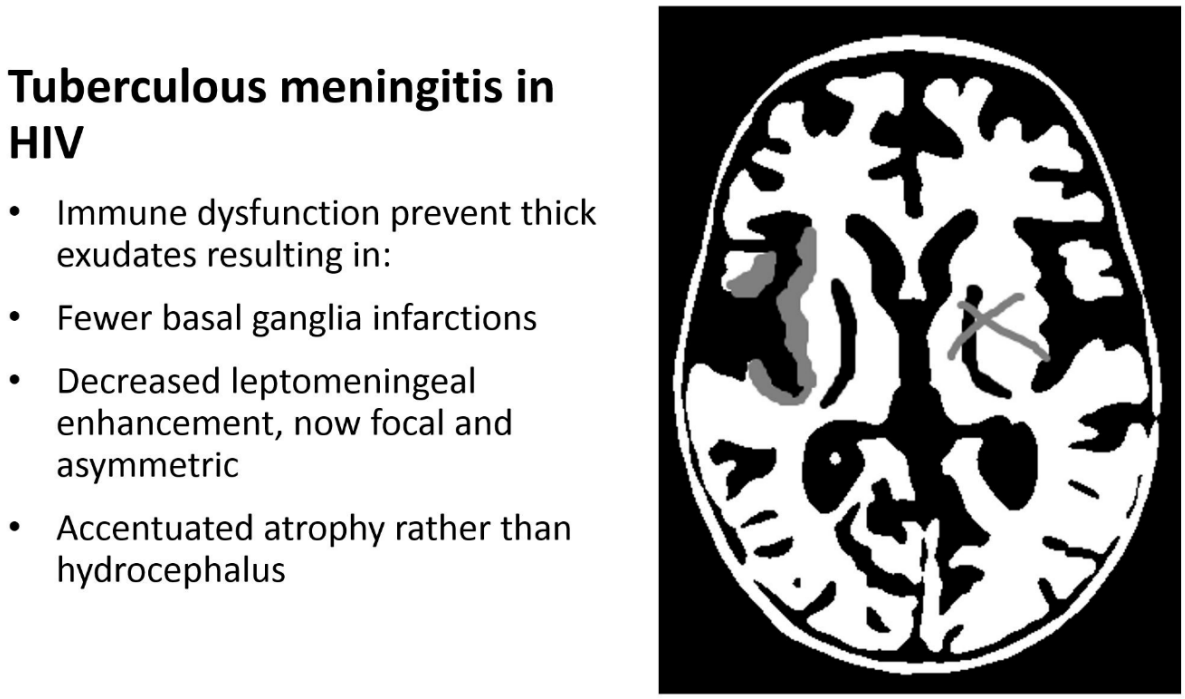


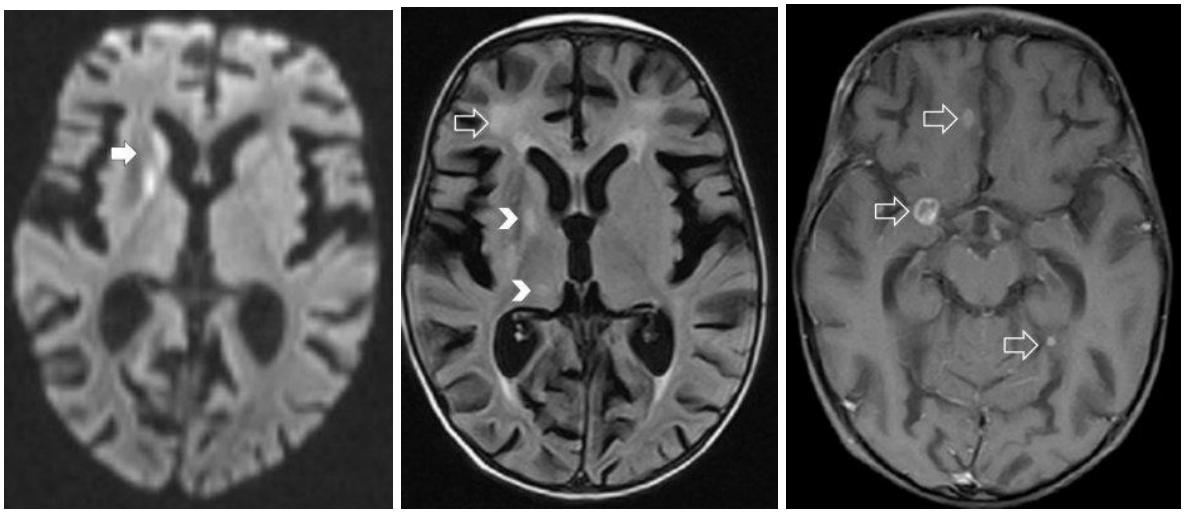
Figure 2.9

A twenty-two-month-old HIV+ girl, known with TB, presented with acute onset left hemiplegia. CT (not shown here) demonstrated acute hemorrhage in the right putamen

(a) Axial DWI demonstrates restricted diffusion in the head of the left caudate nucleus and globus pallidus (solid arrow) indicating an acute haemorrhagic infarction.

(b) FLAIR MRI demonstrate periventricular WM hyperintense signal change and central atrophy in keeping with HIVE. Hypointense foci in the right putamen correspond to hemorrhage, and in addition the hyperintense foci in the head of the right caudate nucleus, right globus pallidus and right thalamus (arrow heads), are in keeping with infarcts, secondary to inflammatory vasculitis as a result of TB meningitis.

(c) Axial post gadolinium T1W MRI demonstrates multiple enhancing lesions (open arrows) representing TB granulomas on a background of marked cerebral atrophy.



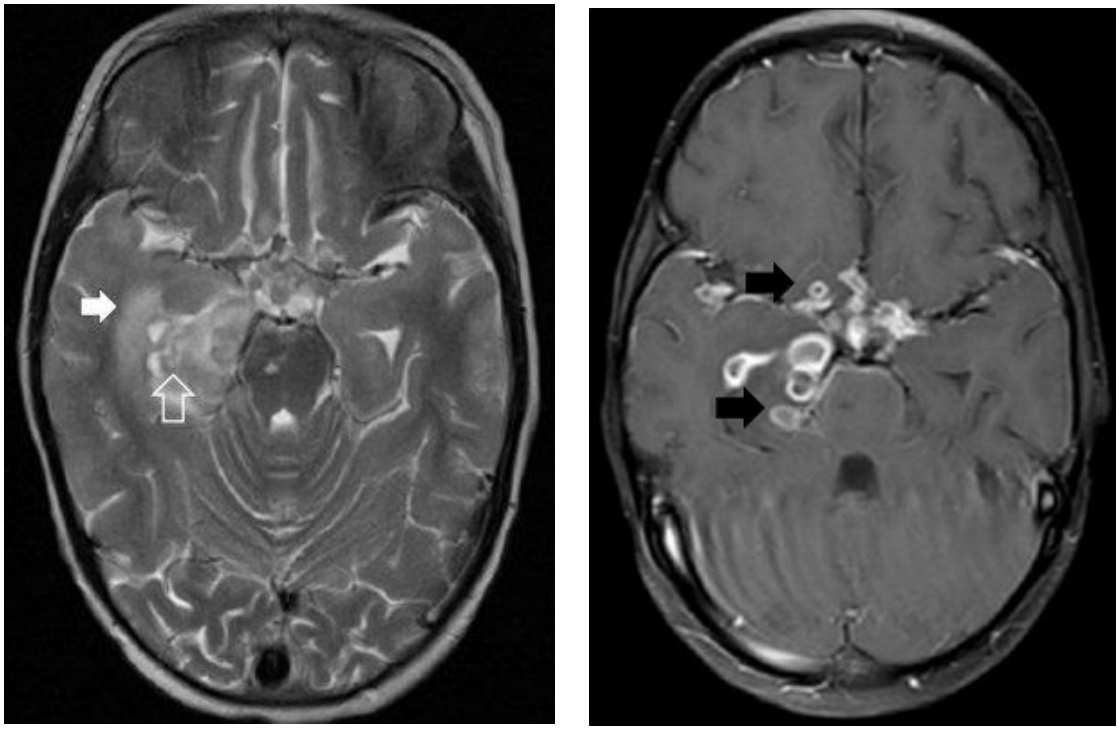
a. **b.** **c.**

Figure 2.10

A six-year-old HIV+ boy on ART with TBM.

(a) Axial T2-weighted image (TR/TE 4280/104) demonstrates multiple lesions in the right medial temporal lobe with T2 hypointense signal (open arrow), peripheral oedema (solid arrow).

(b) T1-weighted post-gadolinium image (TR/TE 739/14) demonstrates intense rim enhancement of these lesions (solid black arrows), representing TB granulomas.



a. **b.**

Toxoplasmosis:

There has been a dramatic decline in the incidence of toxoplasmosis in the post-ART era [114]. Infection in infants and young children is considered to be congenital in most cases, and in older children as the result of reactivation of latent infection, usually with CD4 counts below *50 cells/mm^3* [105,115].

Imaging: Disease is most commonly located in the basal ganglia, thalamus and at cortex/peripheral WM junction. On MRI focal lesions which are usually hyper to mixed intensity on T2 surrounded by hyperintense vasogenic oedema with nodular or ring-enhancement and occasionally peripheral hemorrhage are observed. The target sign consisting of a small eccentric nodule adjacent to an enhancing ring, has been described as highly suggestive of Toxoplasmosis but is insensitive and seen in less than 30% of cases [105]. Differential diagnosis includes lymphoma and TB, and repeat imaging after 2 weeks of toxoplasmosis treatment can be a useful method of confirming the diagnosis [75,105]. A positive response to therapy is judged by the regression in size of all lesions.

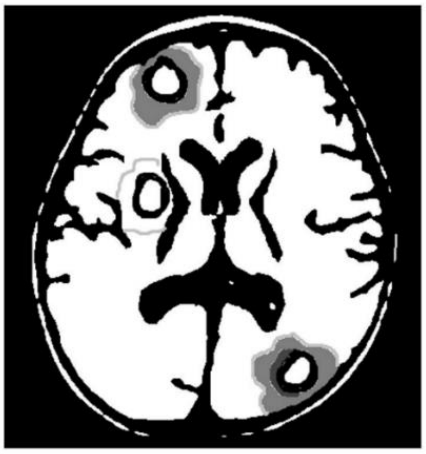
7. Tumors:

Primary CNS tumors occur less commonly in children compared to adults and when observed are usually associated with low CD4 counts and advanced disease. High grade B-cell lymphoma is the most common CNS malignancy related to HIV and is often associated with Epstein-Barr virus infection [75,116]. In adults, toxoplasmosis is one of the main differential diagnoses to consider.

Figure 2.11

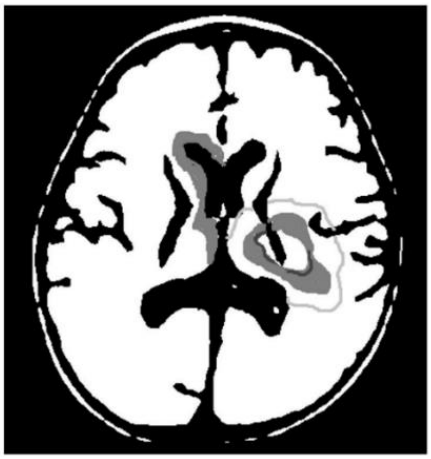
Schematic representation of the differences in imaging features of toxoplasmosis vs. lymphoma. Toxoplasmosis typically affects the basal ganglia and peripheral subcortical white matter (shaded area representing ring enhancement with surrounding oedema). Lymphoma is commonly seen subependymal and in the periventricular white matter (shaded area). Spectroscopy may confirm a raised choline in lymphoma (not shown here).

Toxoplasmosis



- Dramatic decline in presentation
- Basal ganglia, peripheral subcortical white matter
- Usually multifocal
- Ring enhancement
- Can have peripheral haemorrhage
- Spectroscopy: decreased choline

Lymphoma



- Most common malignancy
- Subependymal, periventricular, corpus callosum
- Diffuse or focal
- Ring enhancing in HIV
- Usually no haemorrhage prior to treatment
- Spectroscopy: increased choline

Imaging: MRI shows diffuse or focal ring enhancing mass lesions, predominantly periventricular (as opposed to more peripheral location of toxoplasmosis) [117] but also involves the basal ganglia and corpus callosum (CC). It can be very difficult to differentiate from toxoplasmosis, however lymphoma is much more common in the pediatric population and is more likely when lesions involve the corpus callosum [75,116].

Additional imaging findings related to HIV:

Atrophy

Central atrophy is predominant as the result of initial concentration of the HIV antigen within the basal ganglia, manifesting as enlarged lateral ventricles. The degree of atrophy is directly related to severity of disease and usually correlates with poorer neurocognitive performance. Cortical atrophy is seen later in the disease [7,33,73].

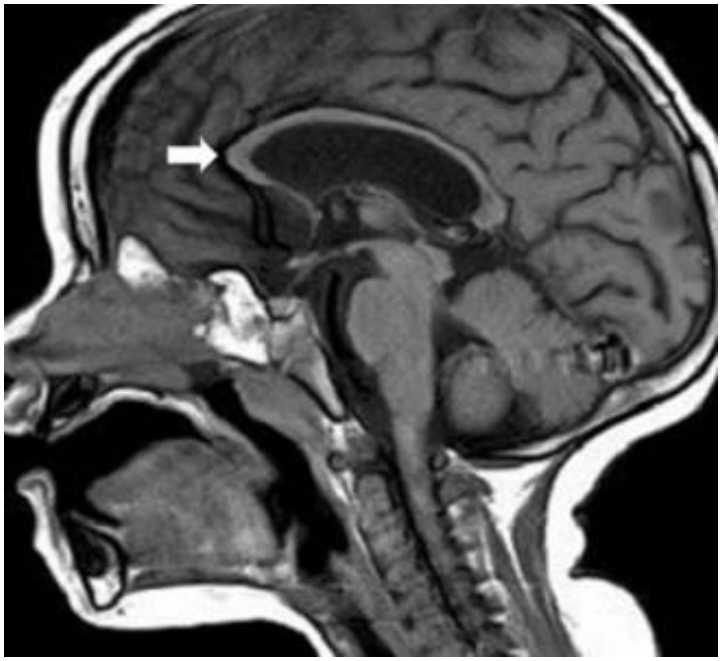
Cerebral atrophy has become an infrequent finding in virally suppressed children, which complicates early detection of white matter volume loss on conventional MR imaging [73,76,78].

Corpus Callosum thinning

In adults corpus callosum (CC) volume is affected by peripheral WM loss with significant thinning of predominantly the anterior portion [118–120]. Atrophy of the CC correlates with decreased CD4 levels. In a study by Andronikou in children, the length and motor segment of the CC emerged as possible surrogate biomarkers of HIV related CNS atrophy/disease [76].

Figure 2.12

A 3-year-old HIV+ girl on ART, presents with HIV-encephalopathy. Sagittal T1-weighted image (TR/TE 700/8.3) demonstrates atrophy of the corpus callosum. The genu of the corpus callosum (white arrow) in this patient measured 6.3 mm. The degree of thinning of the corpus callosum corresponds with the degree of cerebral volume loss and can act as a surrogate marker of cerebral atrophy.

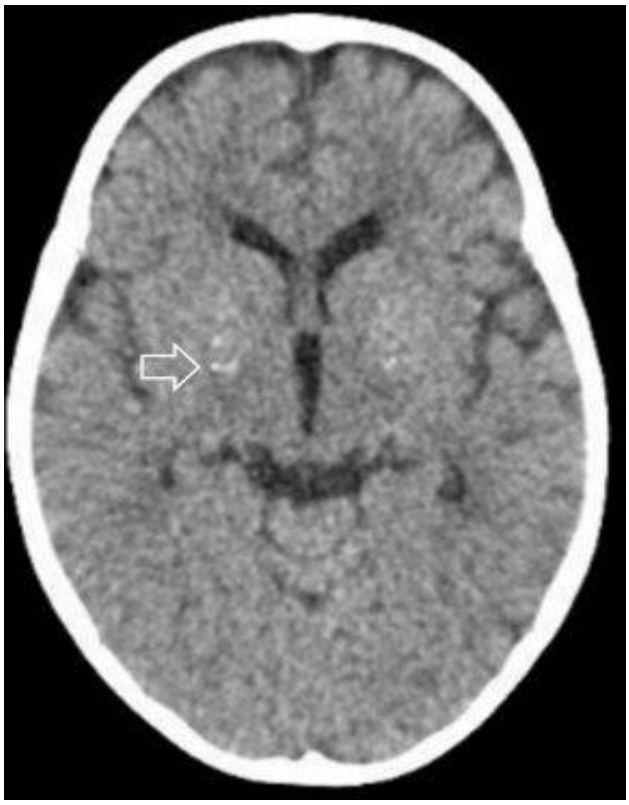


Calcification

Bilateral, symmetric basal ganglia calcification, traditionally deemed an indicator of congenital HIV, is now thought to rather represent a calcific vasculopathy based on neuropathology findings and the occurrence of progression on serial imaging. These calcifications are not commonly seen before 10 months of age [33,83,85]. BG calcifications and generalized atrophy are less frequently encountered in the post ART era [78].

Figure 2.13

A 2-year-old HIV+ girl on ART presented with meningitis. Axial uncontrasted CT scan of the brain demonstrates bilateral punctate calcifications (open arrow) in the basal ganglia due to HIV-associated calcific vasculopathy. Calcifications are usually bilateral symmetrical.



Imaging advances and objective imaging measures of disease:

CC thickness

Thickness of the CC correlates well with WM cerebral volume in pediatric HIV infected patients [121] and a simple caliper measurement of different segments of the CC is easy to perform in clinical practice. The use of the prefrontal CC thickness (genu) is advised due to the early development and stability of the genu over age. A median prefrontal CC segment thickness measurement of 9 mm (interquartile range 7.4 -10.3 mm) is considered normal for African patients and for European children the thickness is similar at 9.1 mm [121].

The length and the motor segment of the CC can also be used as surrogate biomarkers of CNS disease severity preceding HIVE [76]. In a study of 33 children with HIV related brain disease, the length of the CC correlated with microcephaly and the motor segment with neurodevelopmental score (general quotient on the Griffiths mental Development scales) [76]. These linear measurements are easy to perform and may assist with early diagnosis and monitoring of HIV related CNS progression.

Volumetric analysis of MRI

High resolution T1-weighted sequences such as MPRAGE have been found useful in quantitative volumetric analysis of specific structures or brain regions (white and grey matter). In HIV-infected adults such studies showed correlation of grey matter volume with neurocognitive and clinical outcome measures. Similar findings were reported in perinatally HIV infected adolescents in studies which measured regional and total grey matter volume [122,123].

Semi and fully automated techniques have been developed for, segmenting the brain, based on voxel signal-intensity properties of tissues, but these remain labour intensive and a level of expertise is needed for post processing [122]. A study comparing automated volumetric output software packages indicated that visual inspection of the segmented output with manual correction remains critical to ensure validity of results, regardless of the software used. Freesurfer (http://surfer.nmr.mgh.harvard.edu/) and Individual Brain Atlases using Statistical Parametric Mapping (IBASPM) (http://www.mathworld.com/) are examples of widely used and published software packages that are freely available and run on a wide variety of hard and software platforms [120,124].

Imaging in practice:

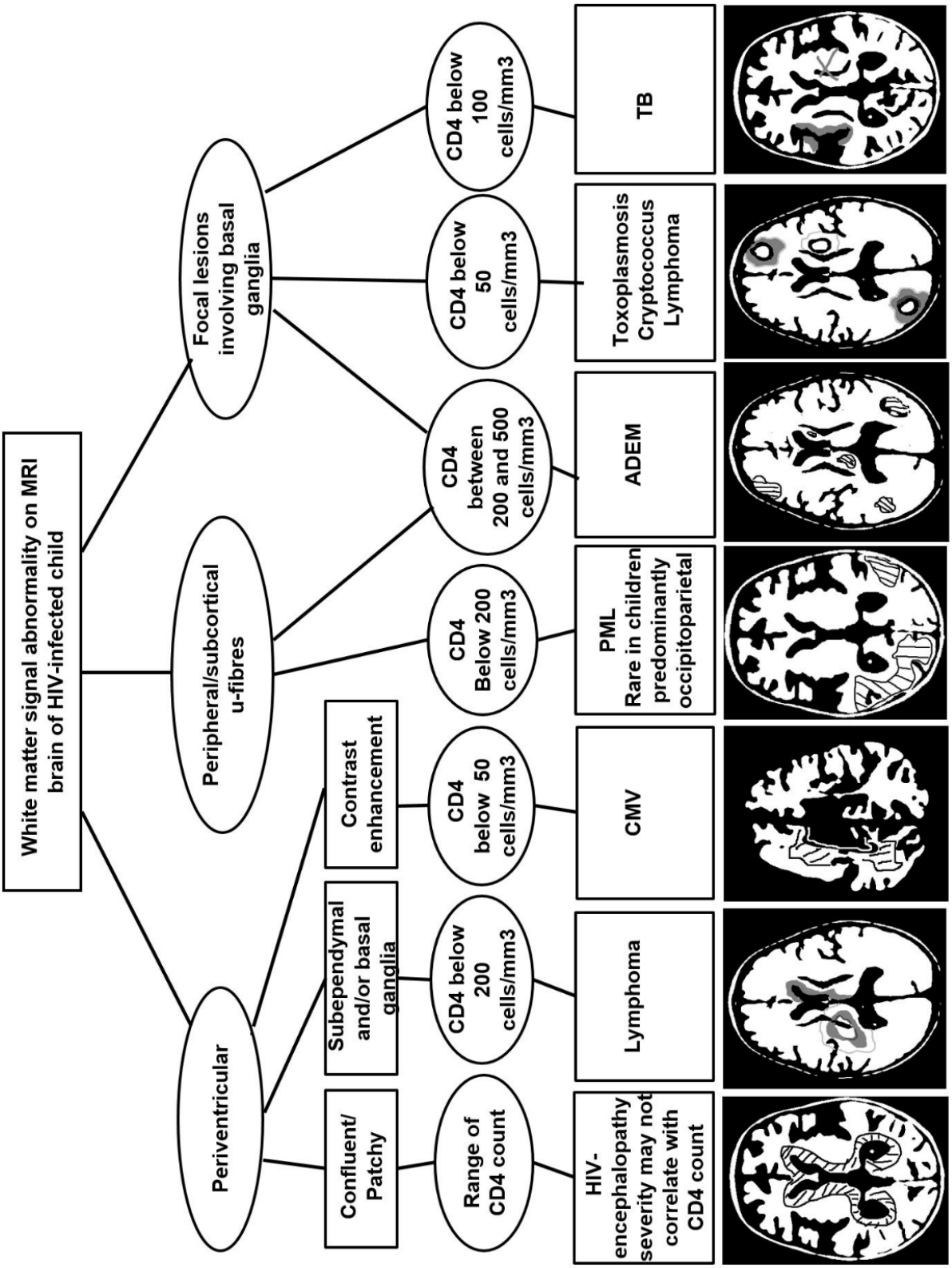
Computed tomography (CT) of the brain is more accessible than MRI but it is not sensitive enough to pick up early changes in HIV-related disease. CT contributes to the radiation burden in children and should be reserved for the assessment of acute neurological events such as CNS infection and vascular/ ischemic pathology. MRI is therefore, the principle modality used in imaging the brain in HIV. Standard structural scans may be normal in the presence of early WM pathology but remain valuable in evaluating brain volume and excluding alternative pathologies. The role of the radiologist in diagnosis and management of pediatric patients with HIV-related brain disease are:

- To diagnose HIVE and distinguish from other causes of WMH.
- To diagnose opportunistic infections and tumors associated with HIV.
- To establish baseline brain volume and white matter macro structural integrity.
- To alert the clinician of children at risk for progressive neurocognitive decline based on specialized MRI techniques.
- To monitor disease progression and the effects of ART as HIV has now become a chronic illness.

Figure 2.14

Flow diagram: guidance to diagnosis of commonly encountered white matter disease on MRI in HIV-infected children.

CMV (cytomegalo virus), PML (progressive multifocal leukoencephalopathy), ADEM (acute disseminating encephalomyelitis), TB (tuberculosis)



At the outset detailed clinical history including neurodevelopment, immunological parameters such as CD4 count and viral load should be taken into context when reporting as imaging findings usually relate to stage and severity of disease.

A CD4 count of < 350 mm^3 is immunological grounds for diagnosing advanced HIV disease with severe disease seen at CD4 count < 200 mm^3 in children 5 years and older.

Standard practice should include the assessment of volume using validated simple methods such as corpus callosum thickness and advising on the implication of finding atrophy.

Conclusion:

Cerebral white matter abnormality is a common radiologic finding in HIV infection, the cause of which can range from diffuse widespread involvement to focal lesions. The etiology is varied, with specific differences in pathology when compared to HIV infected adults. Radiologists are an integral part of the team in the diagnosis of HIV-related brain disease and it is therefore essential to have a working knowledge of relevant disease entities that might be encountered and the imaging features that can distinguish the multiple causes of white matter abnormalities on MRI.

Chapter 3: HIV related white matter disease on structural MRI

Rationale for inclusion of published work

This article was based on the pilot study data for the project which had several baseline objectives.

The children in the cohort of this study were aged 2 years and were followed longitudinally to age 5 years.

Objectives:

- Determine the frequency of white matter abnormalities that was seen in children with a spectrum of HIV related brain disease.
- To compare DWI and ADC with T2 and FLAIR sequences in children with HIV with regards to presence and distribution of white matter changes.
- To determine geographical distribution of white matter signal abnormalities: frontal, deep, peritrigonal, brainstem, corpus callosum and cerebellum on T2 and FLAIR.
- To correlate white matter lesion load and distribution with brain atrophy.
- To correlate white matter lesion load and distribution with neurodevelopmental status, and laboratory finings such as CD4 count and viral load.

Declaration by the candidate:

With regards to chapter 3, *HIV related white matter disease on structural MRI*, the nature and scope of my contribution were as follows:

First author, interpretation and production 43%

The following co-authors have contributed:

Prof Savvas Andronikou: co-supervisor and editing 42%

Barbara Laughton: clinical input and neurodevelopmental data as well as editing 5%

Martin Kidd: statistical analysis 5%

Els Doebels: clinical data 1%

Steve Innes: clinical data 1%

Ronald van Toorn: editing 1%

Prof Mark Cotton: supervisor and editing: 2%

Declaration by co-authors:

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 3
2. No other authors contributed besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 3 of this dissertation.

Declaration with signature in possession of candidate and supervisor.

White Matter Signal Abnormalities in Children With Suspected HIV-related Neurologic Disease on Early Combination Antiretroviral Therapy

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Background: The natural history and manifestation of HIV-related neurologic disease have been ameliorated by combination antiretroviral therapy (ART). We describe the characteristics of white matter signal abnormalities (WMSA) on magnetic resonance imaging in children with HIV-related neurologic disease.

Methods: We reviewed magnetic resonance imaging scans of children with suspected HIV-related neurologic disease despite early ART and correlated with clinical, neurodevelopmental data, virologic markers and time on ART. These children were also on the Children with HIV Early Antiretroviral (CHER) trial.

Results: Magnetic resonance imaging scans were performed at a mean age 31.9 months (range 8–54) on 44 children: 10 on deferred and 34 on early treatment arms, commencing ART at mean age of 18.5 and 8 weeks, respectively. Multiple high signal intensity lesions on T2/fluid attenuated inversion recovery were documented in 22 patients (50%), predominantly in frontal (91%) and parietal (82%) white matter. No differences in neurodevelopmental scores comparing children with and without WMSA were found. Neither lesion load nor distribution showed significant correlation with neurodevelopmental scores or neurologic examination. Normal head growth was more common in the WMSA group ($P = 0.01$). There was a trend for association of WMSA and longer time on ART ($P = 0.13$) and nadir CD4% ($P = 0.08$).

Conclusions: Half of children referred with HIV-related brain disease had WMSA on T2/fluid attenuated inversion recovery. Our findings of the association with normal head growth and duration of ART require further study. We suspect that WMSA can occur early and that initiating ART by 8 weeks of life may be too late to prevent HIV from entering the central nervous system.

Key Words: HIV-encephalopathy, HIV-related brain disease, developmental score, white matter signal abnormality, MRI, early antiretroviral therapy

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HIV-encephalopathy is an AIDS defining event,[1] which in its most severe form, presents with developmental delay and motor dysfunction.[2] However, with combination antiretroviral therapy (ART), manifestations are likely to be subtle and have not yet been well-described in children receiving early ART. It is critical to identify a good marker of HIV-related manifestations in the central nervous system (CNS)[3] as early treatment can slow deterioration and partially improve manifestations.[4,5] HIV-encephalopathy demonstrates white matter signal abnormality (WMSA), mainly hyperintensity on magnetic resonance imaging (MRI).[6] In adults treated with ART, resolution of WMSA mirrors clinical improvement.[7,8] The imaging findings in children with HIV-encephalopathy show basal ganglia calcification and atrophy. One limited study (21 children) antedating the availability of ART, demonstrated deep white matter hyperintensity sparing the subcortical U-fibers in a third of children.[9] Our aim was to determine the prevalence, distribution and characteristics of WMSA on T2/ fluid attenuated inversion recovery (FLAIR) sequences in a larger number of children initiating ART from an early age, but with suspected HIV-related neurologic disease. We also sought to correlate WMSA with developmental scores, clinical presentation and laboratory studies.

MATERIALS AND METHODS

We conducted a prospective study over 2.5 years (August 2007 to April 2010) at Tygerberg Children's Hospital, Cape Town, South Africa on HIV-infected children referred for neuroimaging by their infectious diseases clinicians because of suspicion of HIV-related neurologic disease (either poor head growth, long tract signs or neurodevelopmental delay).

The Children With HIV Early Antiretroviral (CHER) trial[10,11] took place in the same hospital and contributed all of the children referred for MRI. The CHER trial was a randomized 2-center study in which HIV-infected infants between 6 and 12 weeks of age and CD4 ≥ 25% were randomized to 1 of 3 strategies: ART deferred until indicated, early limited ART for 40 weeks or early limited ART for 96 weeks. Continuous ART was initiated in the deferred arm or in the early limited ART arms after interruption if the CD4% declined below 20% (25% for ART deferred in the first year of life). Other criteria for continuous ART were Centers for Disease Control (CDC) stage C or protocol-defined severe CDC stage B disease. The latter included bronchiectasis and severe lymphoid interstitial pneumonitis, nephropathy and cardiology. Additional criteria were failure to thrive not meeting CDC stage C, severe oral candidiasis, recurrent pneumonia and any condition considered severe enough for ART (with approval of the study team). A small group with CD4 ≤ 25% at baseline were recruited in parallel and also received early continuous ART on the recommendation of the study's data safety monitoring board. First-line ART was lopinavir-ritonavir, lamivudine and zidovudine. Many mothers had participated in the prevention of mother-to-child transmission program, which included zidovudine antenatally from 32 weeks and single dose nevirapine

INTRODUCTION

HIV-encephalopathy is an AIDS defining event, [5] which in its most severe form, presents with developmental delay and motor dysfunction [125]. However, with ART, manifestations are likely to be subtle and have not yet been well described in children receiving early ART. It is critical to identify a good marker of HIV-related manifestations in the central nervous system (CNS) [126] as early treatment can slow deterioration and partially improve manifestations [7]. HIV-encephalopathy demonstrates white matter signal abnormality (WMSA), mainly hyperintensity on magnetic resonance imaging (MRI) [111]. In adults treated with ART, resolution of WMSA mirrors clinical improvement [13,127]. The imaging findings in children with HIV-encephalopathy show basal ganglia calcification and atrophy. One limited study (21 children) antedating the availability of ART, demonstrated deep white matter hyperintensity sparing the subcortical U-fibers in a third of children [32]. Our aim was to determine the prevalence, distribution and characteristics of WMSA on T2 /FLAIR (fluid attenuated inversion recovery) sequences in a larger number of children initiating ART from an early age, but with suspected HIV-related neurological disease. We also sought to correlate WMSA with developmental scores, clinical presentation and laboratory studies.

METHODS

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the first year of life). Other criteria for continuous ART were Centers for Disease Control (CDC) stage C or protocol-defined severe CDC stage B disease. The latter included bronchiectasis and severe lymphoid interstitial pneumonitis, nephropathy and cardiomegaly. Additional criteria were failure to thrive not meeting CDC stage C, severe oral candidiasis, recurrent pneumonia and any condition considered severe enough for ART (with approval of the study team). A small group with CD4 ≤ 25% at baseline were recruited in parallel and also received early continuous ART on the recommendation of the study's data safety monitoring board. First-line ART was lopinavir-ritonavir, lamivudine and zidovudine. The majority of mothers had participated in the prevention of mother to child transmission program, which included zidovudine antenatally from 32 weeks and single dose nevirapine at delivery. Mothers with CD4 count below 250 cells per mm^3 received ART antenatally. New-born infant received a single dose of nevirapine at birth and zidovudine for 7 days.

Children were in regular follow-up [2,11] with clinical assessments monthly, including neurological examination and head growth monitoring using CDC growth charts (< 3 years) and World Health Organization (WHO) percentile charts (> 3 years). Poor head growth was defined as downward crossing of at least 2 major centiles on CDC charts and 1 centile line on WHO charts. Neurodevelopmental screening assessment was performed every 6 months, and as part of a site-specific neurodevelopmental sub-study, the Griffiths mental development scales (GMDS) [128] were performed at 12, 18, 30 and 42 months. GMDS quotients were obtained from raw scores or age equivalents, using the United Kingdom Norms with a mean of 100 and standard deviation of 15 [128,129,130]. Significant developmental delay was defined as more than 2 standard deviations below the mean (developmental quotient below 70).

Patients were excluded if evidence of current or previous opportunistic CNS infection; CNS neoplasm; neurological disease caused by factors other than HIV; previous anoxic insults; persistent metabolic derangement or inadequate MRI scans (omitted sequences) were present. Post-gadolinium-enhancing lesions such as focal, ring or solid enhancement, enhancement of the dura, meninges or cranial nerves, were excluded from the study. Baseline and clinical data were obtained from participants' medical records. Viral Loads (VL) > 750 000 copies/mL were assigned as 750 001 and

those < 400 copies/mL as 399. Mean VL at baseline from the CHER trial was calculated, and VL closest to scan was categorized as < or > 400 copies/mL.

Scans were performed under general anaesthesia (standard practice at our institution for paediatric patients) on a Siemens Magnetom Symphony 1.5T. Sequences: Axial T2 spin echo (SE), Axial FLAIR, Sagittal T1 SE, Sagittal T2 turbo spin echo (TSE), diffusion weighted imaging (DWI), post gadolinium Axial T1 with a slice thickness of 5mm. A paediatric neuroradiologist, blinded to clinical findings at time of referral, performed the MRI readings. Any non-enhancing WMSA was recorded according to anatomical regions of the brain and topographic white matter fibre involvement. Predefined criteria were used to describe the WMSA as pinpoint lesions, measurable lesions < 1cm, measurable lesions > 1cm or 'larger' confluent lesions difficult to measure. The maximum diameter of the largest measurable lesion in each patient was determined on axial FLAIR sequences, using visual inspection to determine the slice. DWI and apparent diffusion co-efficient (ADC) map was used to determine any diffusion abnormality relating to the lesions. 'Lesion load' was arbitrarily determined by calculating the number of regions involved (divided into 17 zones: frontal, temporal, occipital, parietal left and right, corpus callosum, midbrain, pons, medulla, cerebellar hemispheres, cerebellar vermis, caudate, lentiform nucleus, thalamus irrespective of the size and number of the lesions.

Quantitative cytomegalovirus (CMV) polymerase chain reaction (PCR) was performed on stored plasma samples of all CHER subjects at screening in a separate sub-study using the RocheCOBAS AmpliPrep/COBAS TaqMan CMV Test (Roche Molecular Diagnostics, Branchburg, New Jersey). CMV values ≥150 copies/ml were considered positive for exploring a potential relationship with WMSA.

For statistical analysis, one-way analysis of variance (ANOVA) was conducted to compare continuous measurements between groups with and without WMSA. The Chi-square test was used to compare categorical variables (e.g. gender) between the two groups. Spearman correlations were used to test for relationships between developmental scores and 'lesion load' and also WMSA distribution. Two-way ANOVA was conducted to produce results corrected for gender.

Ethics approval, N07/09/208, for the study was obtained from Stellenbosch University.

RESULTS

Forty-four HIV-infected children (22 boys) were studied. Age ranged from 8 to 54 months (mean 31.9 and SD 9.9 months). Average time between referral request and MRI scan was 2.2 months (SD 1.6 months). There were no exclusions due to poor image quality (motion artefact). MRI demonstrated WMSA on T2/FLAIR in 22 children, 50% of the sample. Demographic, immunologic and virological data are shown in **Table 3.1**, which also shows comparisons in those with and without WMSA.

Prevalence and distribution of WMSA:

Sixteen children out of 22 (73%) with WMSA had a combination of lesions (representative scan in **Figure 3.1a**). Three children had only pinpoint lesions (**Figure 3.1b**), one had only measureable lesions <1cm and two children had confluent lesions. Lesion size ranged from 5 to 12mm, with an average of 7.2mm. In twelve children, the lesions were T1 iso-intense, three had T1 hypo-intense lesions and seven had combinations of lesion intensity. None of the lesions demonstrated enhancement. Predominantly frontal (20; 91%) (**Figure 3.1c**) and parietal (17; 77%) distribution was noted in subcortical and deep white matter (**Figure 3.1d**). **Table 3.2** summarizes WMSA distribution. Other areas of involvement included the peritrigonal regions in seven children (32 %), involving both right and left sides (unilateral in one child), left cerebellar hemisphere in one child (5%) and left lentiform nucleus in one child (5%). Twelve of 22 (54%) had WSMA in 4 or more zones. The maximum number of zones in a single child was 7.

Developmental score:

Assessments were performed at a mean age of 30 (range 11 - 48) months. Mean scores fell into the low average category with a mean general quotient (GQ) of 81.7 (range 67 – 101). The mean locomotor sub-quotient was 83 (range 50 – 116). The mean language sub-quotient was 79.7 (range from 57 – 118). These mean scores are around 1 standard deviation lower than previously described in HIV-uninfected

children at 21 months of age, from this community who had means of 95, 99.7 and 93.2 respectively for GQ, locomotor and language [131]. The time between GMDS and MRI ranged from 3.7 months before to 3.4 months after the scan. There was no difference between the groups (with and without WMSA) for time between scan and GMDS (p=0.87) or mean age at GMDS assessment (p=0.46).

*Correlating developmental scores with lesion load and distribution of WMSA***:**

There were no differences in developmental scores in those with and without WMSA (see **Table 3.1**). Lesion load also showed no correlation with developmental quotients (lesion load vs. GQ, p=0.99; lesion load vs. locomotor, p=0.80 and lesion load vs. language, p=0.50). However, the child with the most sites involved (n=7) also had the lowest overall GQ (67) and locomotor sub-quotient (50), both significantly delayed, with a language sub-quotient of 82, which is below average. This child, with a baseline CD4 of 13.4% and viral load above 750 000 copies/mL at 9.4 weeks of age received continuous ART. There were more boys in the group with WMSA (14 vs. 8), but no differences were found between the groups when controlling for gender on GMDS outcomes (2-way ANOVA results not shown).

Clinical indications, laboratory tests and WMSA:

For those with WMSA, significantly fewer (36%) had declining head growth as an indication for neuroimaging referral versus 64% without WMSA (p=0.01). There was no difference in the frequency of developmental delay, increased muscle tone or pathological tendon reflexes as a reason for referral between the children with or without WMSA.

There was no difference in baseline CD4 and viral load pre-ART, or CD4 and viral load closest to MRI scan between the groups. Those with the lowest CD4% nadir showed a trend to more WMSA (p=0.08), which diminished when controlling for gender (p=0.28 from 2-way ANOVA). We also noted a trend for CMV positivity and WMSA (p=0.055).

Antiretroviral Therapy:

Ten children in the deferred ART arm were referred for MRI. They had initiated ART at a median age 18.5 weeks due to immunological and/or clinical decline, and received ART for a median 114 weeks prior to the MRI. Three in this arm had WMSA. [2,11] Thirty-four children were in the early limited ART arms. They commenced early ART at a median age of 8 weeks and received ART for a median of 98 weeks. Of these, 2 children had not yet interrupted, 5 were in the ART interruption phase at time of neuroimaging and 17 had already restarted ART after a period of interruption. Ten children received early continuous ART. Seven with baseline CD4 ≥ 25% had already developed significant HIV-related disease (3 with failure to thrive and four with site-determined HIV-related brain disease). The remaining 3 had a CD4% below 25% at baseline.

One child had changed to second line ART (didanosine, abacavir and nevirapine) for 6 months at 17 months of age (2 years before neuroimaging) and then changed back to first line therapy; abacavir was added at 38 months of age (1 year before neuroimaging), due to virological failure.

For those with a baseline CD4% ≥ 25% and initially randomized to early limited ART, there was a trend to more WMSA in those not interrupting ART compared to those who interrupted ART (p=0.129, Fisher's Exact 2 tail test).

There was also a trend for more WMSA with longer time on ART (in weeks) with mean (standard deviation) 115.6 (43.7) (p=0.13 or p=0.20 after controlling for gender). There was no statistical difference when controlling for age and time on ART between the two groups. Of the 26 children randomized to early limited ART, 12 had WMSA and 14 did not.

DISCUSSION

Imaging findings in children with HIV in the pre- and post-ART eras include atrophy [5,29], calcification [30] and more recently, WMSA [31]. We have described, for the first time, the distribution and characteristics of WMSA in children who received early ART in infancy. Fifty percent of those referred for MRI because of concern of HIV-related brain disease, had WMSA on T2/FLAIR MRI. Lesions occurred most commonly in

superficial and deep white matter and predominantly in the frontal and parietal lobes. Most importantly, WMSA was present in children with early limited, early continuous and deferred ART. There was no correlation between the distribution of WMSA or the lesion load with immunological or developmental scores.

There was a surprising association between WMSA and normal head growth (p=0.01), rather than acquired microcephaly. However, there was no association with either developmental delay, or increased tone and tendon reflexes. These observations require further investigation in a larger cohort. A possible explanation may be that early ART is neuroprotective and that imaging findings in these children represent arrested brain disease but with ongoing inflammation or low-level viral replication. Unfortunately, head circumference-for- age Z-scores, which may have increased our ability to interpret this finding, were not documented over time.

The lack of correlation of WMSA and GMDS scores is possibly due to early identification of suspected HIV-related brain disease by performing the GMDS regularly in all children. Some GMDS scores were in the normal range, as other criteria for neurological compromise were met (poor head growth and acquired symmetric motor deficits). It may also be that the GMDS has insufficient sensitivity to assess the subtler effects corresponding to WMSA based on T2/FLAIR and the children were too young for more detailed neuropsychological assessments to assess specific domains of functioning. Stability of the GMDS over time is also not clear in South African children, since it was standardized in the United Kingdom [128,129] with varying reports of performance on the GMDS in South Africa [131,132]. We did not collect data on socioeconomic status, but these children are all from similar background low-socioeconomic communities.

There was a trend towards presence of WMSA and time on ART, with longer time on treatment associated with increased WMSA (p=0.13). The association is of unclear significance (power= 34%), and requires further study in larger groups, as WMSA could either represent more severe disease or cumulative ART toxicity, specifically as all guidelines recommend early continuous ART in all HIV infected infants [133]. Also, a trend towards more WMSA in those who, although randomized to early limited ART remained on continuous ART, suggests more severe HIV disease. No children were

on efavirenz, which may be neurotoxic in adults [134]. There was also no correlation between WMSA and different treatment arms on the CHER trial; however, this is a relatively small descriptive study of clinical referrals, and conclusions cannot be drawn from these small numbers.

There was no correlation between WMSA and viral loads, CD4 counts or CD4% closest to the time of scan. However, the time between the scans and these parameters ranged from 0 – 12 months, because it was not part of the research design to obtain these at the time of scan. There was a trend showing a negative association with nadir CD4%. In adults, nadir CD4 levels correspond to episodes of severely impaired immune function, which place the brain at greatest risk of HIV involvement [135]. The WMSA in our patients may represent damage from HIV during exposure to high viral loads prior to ART, or other pathogens may have accessed the brain. Focal white matter lesions without enhancement or mass effect have increased in HIV-infected children between 1991 and 1998, aetiologies including viral encephalitis, focal HIV-encephalopathy and progressive multifocal leukoencephalopathy (PML) [29]. The latter was a problem in the pre-ART era resulting in WMSA, possibly due to delayed initiation of ART and using medications with lower CNS penetration. Differential diagnoses to consider with WMSA include maternal recreational drug exposure, intrauterine CNS infections such as toxoplasmosis, CMV and cerebral malformations with cortical dysplasia [7]. Infants co-infected with CMV and HIV, have a higher rate of progression to symptomatic stages of AIDS as well as higher incidence of encephalopathy [7,111]. Although we showed a possible link between CMV at 6 weeks of age, data were missing for 6 children with and 3 without WMSA. Also, we could not distinguish congenital from acquired CMV infection. The lack of control subjects is a limitation of our study; however WMSA in children above 1.5 years of age is abnormal [92].

Changes occurring in the CNS in the earlier stages of HIV-1 infection remain poorly understood and the evidence is conflicting. Progressive encephalopathy can be associated with normal imaging studies, [5] and there may even be abnormalities on CT scan in asymptomatic children.

HIV leukoencephalopathy, visualized as WMSA on MRI, is a triad of diffuse myelin loss, astroglial proliferation, and infiltration by mono and multinucleated macrophages [13,136]. The myelin pallor is diffuse, involves deep white matter and spares superficial (subcortical) white matter and corpus callosum [136], in contrast to PML which tends to involve subcortical and periventricular white matter, corpus callosum, internal and external capsules and the myelinated fibres of the deep grey nuclei [104,137]. Our imaging findings suggest that superficial white matter is not spared as WMSA involving the subcortical white matter was noted in many children in our series. It may be difficult to distinguish from other causes of WMSA such as prenatal/perinatal injury [7], especially in the peritrigonal area, which is susceptible to global hypoxic insults and is also a terminal zone of maturation [138]. However, only 7 (16%) of our patients showed pathological asymmetrical WMSA in the peritrigonal region, with birth asphyxia having been excluded in our study.

Documenting WMSA is important in the clinical management of HIV-infected adults as it provides supporting evidence for HIV-1 associated cognitive motor complex [127], correlates with clinical improvement following ART and suggests that disease regression in patients with AIDS dementia complex on ART can be characterized and monitored by MRI [13]. Currently, neurodevelopmental testing is performed routinely in HIV-infected children to assess cognitive function and effectiveness of ART in the CNS. By the time cognitive deficits are detected however, significant brain injury may already have occurred [139] as shown in the Paediatric Randomized Early versus Deferred Initiation in Cambodia and Thailand (PREDICT) trial. Here children between 1 and 12 years of age were randomized to early or deferred ART, performed worse than HIV-uninfected control children on intelligence quotient, Beery Visual Motor Integration, Binet memory and Child Behavioural Checklist [10].

The utility of detecting WMSA in children requires prospective study. Qualitative MRI analysis of early white matter changes in HIV-encephalopathy can be difficult [127]. Alternative quantitative imaging (e.g. MRI spectroscopy and relaxometry) can detect encephalopathy, disease progression or improvement on treatment [79]. DTI (Diffusion Tensor Imaging) provides quantitative information on the integrity of white matter tracts and correlates with cognitive impairment in adults [53]. In children, this offers new challenges relating to immaturity of myelination, which may affect measurable

fractional anisotropy and because these studies are time consuming. Our limited MRI resources at the time curtailed our capacity to perform these advanced sequences but they are included in our continuing work.

CONCLUSION

Our results demonstrate that half of the children referred for suspected HIV-related neurological problems have WMSA on T2/FLAIR, involving mainly the frontal and parietal lobes, superficially and in the deep white matter. The lesion load and distribution did not correlate with the developmental scores or viral load. We suspect that WMSA can occur early and that initiating ART by 7 to 8 weeks of life may already be too late to prevent HIV from entering the CNS.

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TABLE 3.1: Comparison between children with and without WMSA

	WMSA present (n=22)	**WMSA absent** (n=22)	**p-value**	**Effect size*****
Male gender (%)	14 (64%)	8 (36%)	0.07*	Cramer's V=0.27
Mean age at MRI (months)	32.8(9.4)	30.9(10.4)	0.53^	0.20
Baseline mean(SD)				
CD4 absolute count (cells/mm^3)	1756 (778.2)	1859 (984)	0.70^	0.12
CD4%	32.0 (11.9)	35.6 (9.6)	0.27^	0.34
Viral load (copies/mL)	591931(261119)	571449(234191)	0.79^	0.08
Time on ART before MRI (wks) mean(SD)	115.6 (43.7)	94.4 (46.4)	0.13^*	0.48
Mean age of ART initiation (weeks)	10	13.6		
Arms on CHER trial:				
Baseline CD4 ≥ 25%:				
ART-Def	3	7	0.4	
Early ART until W40	7	7		
Early ART until W96	4	6		
Early continuous ART ≈	5	2		
Baseline CD4 <25%:				
Continuous ART	3	0	NS	
Viral load closest to MRI (%)				Cramer's
<400 HIV RNA copies/mL	48%	52%	0.86	V=0.05
>400 HIV RNA copies/mL	55%	45%		
Time between Viral Load and MRI (days) Mean (median)	88.5 (56.5)	83.3 (50)	0.59**	0.04
CD4 count closest to MRI scan				
Absolute count (cells/mm^3)	1490 (819.6)	1441 (432.2)	0.81	0.08
CD4%	34.2 (8.1)	33.8 (9.2)	0.88	0.05
Time between CD4 and MRI (wks) Mean (median)	35.1 (18.5)	25.5(17.5)	0.53**	0.18

Nadir CD4 closest to MRI				
CD4 count (cells/mm^3)	1184.3 (680.5)	1048.6 (487.5)	0.45	0.23
CD4%	18.9 (6.5)	22.4 (6.5)	0.08	0.55
Mean time between scan and nadir CD4 (wks)	89.0 (54.4)	68.6 (43.2)	0.18	0.43
CMV DNA positive at baseline§	6 (75%)	2 (25%)	0.055	
CMV DNA negative at baseline	10 (37%)	17 (63%)		
Reason for MRI request: n (%)				Cramer's
Developmental delay	19 (51%)	18 (49%)	0.68	V=0.06
Poor head growth	10 (36%)	18 (64%)	0.01*	0.38
Increased muscle tone	5 (42%)	7 (58%)	0.50	0.10
Pathological reflexes	14 (48%)	15 (52%)	0.75	0.05
Griffiths Mental Development Scales:				
Age at assessment mean (SD) in months	31.0 (8.5)	29.0 (9.3)	0.46	0.23
Time between scan and GMDS in months	3.4 (3.0)	3.2 (4.2)	0.87	0.05
General Quotient	83.2 (7.4)	80.3 (15.4)	0.44	0.25
Locomotor sub-quotient	81.5 (11.9)	84.4 (15.1)	0.49	0.22
Language sub-quotient	79.8 (8.9)	79.5 (13.1)	0.94	0.03

WMSA: white matter signal abnormalities, MRI: magnetic resonance imaging

CHER: children with HIV early antiretroviral therapy

ART: combination antiretroviral therapy

ART-Def: ART deferred therapy

CMV: cytomegalovirus

^ F-test degrees of freedom 1,42

* Significant p-value

** Mann Whitney U-test

*** Cohen's D unless otherwise specified

§ CMV ≥150 copies/ml; no CMV data for 6 with WMSA and 3 without WMSA

≈ Received continuous ART although randomized to early limited ART.

TABLE 3.2: Distribution of WMSA in children with HIV related brain disease (N=22) by number of patients with at least one lesion in the listed location (note that some patients had more than one site involved)

Location	Cumulative number of patients	Right		Left	
		Superficial	Deep	Superficial	Deep
Frontal	20 (91%)	18 (82%)	5 (23%)	17 (77%)	7 (32%)
Parietal	17 (77%)	11 (50%)	9 (41%)	12 (55%)	8 (36%)
Temporal	1 (5%)	1 (5%)	0	1 (5%)	0
Occipital	3 (14 %)	3 (14 %)	0	1 (5%)	0

WMSA: white matter signal abnormalities

Figure 3.1a:

Axial FLAIR MRI: pin-point WMSA (black arrows) in addition to larger measurable lesions (white arrow) of various shapes and sizes all less than 1cm in both of the superior frontal lobes.

Figure 3.1b:

Axial FLAIR MRI: multiple bilateral pin point WMSA, involving predominantly subcortical (white arrows) and to a lesser degree deep white matter in the superior frontal lobes.

Figure 3.1c:

Axial FLAIR MRI: Two right frontal sub-centimetre focal lesions.

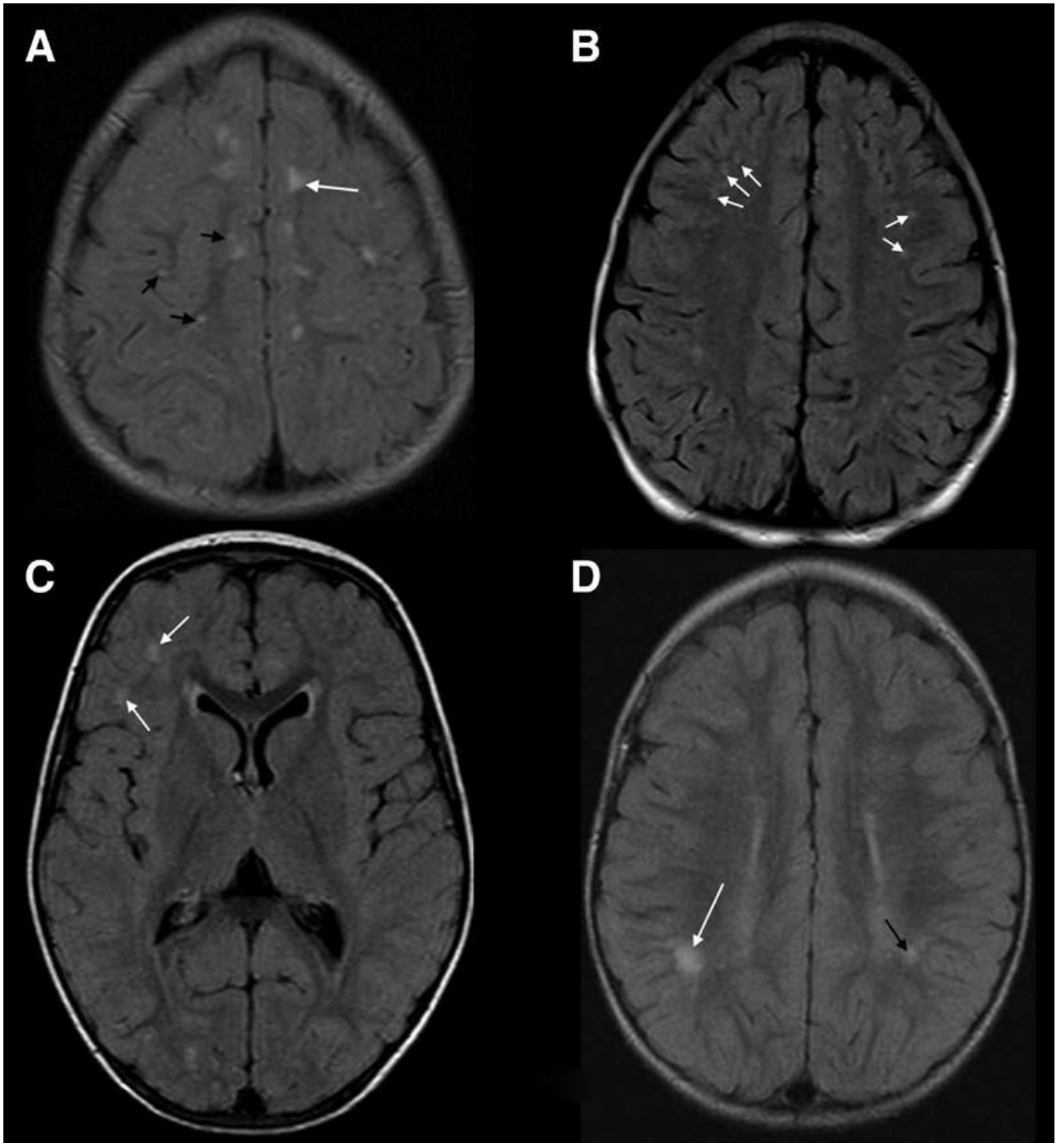
Figure 3.1d:

FLAIR MRI: Bilateral parietal WMSA, on the right larger than 1cm (white arrow) and on the left less than 1cm (black arrow) extending from the subcortical to the deep white matter.

FLAIR: fluid attenuation inversion recovery

MRI: magnetic resonance imaging

WMSA: white matter signal abnormalities



Chapter 4: White matter disease in HIV defined by DTI

Rationale for inclusion of published work

This article forms the basis of the second part of my study with the following hypothesis and aim:

Hypothesis

DTI (diffusion tensor imaging) in children with HIV will demonstrate altered diffusion (MD) and decreased Fractional Anisotropy (FA) values in white matter as well as poorer white matter integrity when starting ART after 12 weeks of age.

Aim

To determine the spatial distribution and nature of white matter abnormalities at age 5 years in a cohort of HIV+ children beginning ART well within the first year of life. An additional aim was to explore associations of timing of ART initiation and DTI-derived parameters (FA, AD, RD), to interrogate potential protection of early ART on WM microstructure.

This article is one of few studying a cohort of patients with early HIV diagnosis and initiation of ART well before 1 year of age. The ART treatment regimens were well documented and varied little. The children were in regular follow up with comprehensive clinical and laboratory data available**.**

Declaration by the candidate:

With regards to chapter 4, *White matter disease in HIV defined by DTI*, the nature and scope of my contribution were as follows:

First author, interpretation and production 50%

The following co-authors have contributed:

Prof Savvas Andronikou: co-supervisor and editing 5%

Barbara Laughton: clinical input as well as editing 5%

Martin Kidd: statistical analysis 10%

Muhammad Saleh: technical support and data processing 15%

Ernesta Meintjes: data post processing and editing 8%

Ali Alhamud: data post processing 1%

Andre van der Kouwe: data post processing 1%

Prof Mark Cotton: supervisor and editing: 5%

Declaration by co-authors:

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 4
2. No other authors contributed besides those specified above, and
3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 4 of this dissertation.

Declaration with signature in possession of candidate and supervisor.

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ORIGINAL RESEARCH
PEDIATRICS

Early Antiretroviral Therapy in HIV-Infected Children Is Associated with Diffuse White Matter Structural Abnormality and Corpus Callosum Sparing

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ABSTRACT

BACKGROUND AND PURPOSE: Fractional anisotropy in the frontal white matter, corpus callosum, and internal capsule is abnormal in human immunodeficiency virus–positive (HIV+) adults. We describe the distribution and nature of white matter abnormalities in a cohort of children who started antiretroviral therapy within the first year of life and the benefit of early treatment by using DTI measures (fractional anisotropy and mean, axial, and radial diffusion).

MATERIALS AND METHODS: DTI was performed on children in a neurodevelopmental substudy from the Children with HIV Early Antiretroviral trial. Voxel-based group comparisons were obtained to determine regions where fractional anisotropy and mean diffusion differed between HIV+ and uninfected children. Associations of DTI parameters with the timing of antiretroviral therapy initiation were examined.

RESULTS: Thirty-nine HIV+ children (15 boys; mean age, 5.4 years) and 13 controls (5 boys; mean age, 5.7 years) were scanned. Two clusters with lower fractional anisotropy and 7 clusters with increased mean diffusion were identified in the HIV+ group, with symmetric distribution predominantly due to increased radial diffusion, suggestive of decreased myelination. Corticospinal tracts rather than the corpus callosum were predominantly involved. Children on early-interrupted antiretroviral therapy had lower fractional anisotropy compared with those receiving continuous treatment.

CONCLUSIONS: HIV+ children at 5 years of age have white matter abnormalities measured by fractional anisotropy, despite early antiretroviral therapy, suggesting that early antiretroviral therapy does not fully protect the white matter from either peripartum or in utero infection. In contrast to adults, the corticospinal tracts are predominantly involved rather than the corpus callosum, possibly due to early antiretroviral therapy. Continuous early antiretroviral therapy can limit white matter damage.

ABBREVIATIONS: AD = axial diffusivity; ART = antiretroviral therapy; CC = corpus callosum; CHER = Children with HIV Early Antiretroviral trial; CST = corticospinal tract; FA = fractional anisotropy; HIV = human immunodeficiency virus; HIV+ = human immunodeficiency virus–positive; MD = mean diffusivity; RD = radial diffusivity

White matter structural abnormalities can be assessed by using quantitative parameters determined from DTI.[1] Fractional anisotropy (FA) provides information about the microstructural integrity of highly oriented microstructures but is not specific to the type of injury. Mean diffusivity (MD) is a measure of average molecular motion independent of any tissue directionality.

Loss of axonal integrity decreases FA and increases MD; however, increased FA may also indicate loss of complexity in the underlying axonal matrix due to loss of crossing and other nonparallel fibers. Increased radial diffusivity (RD), a marker of excessive axonal packing attenuation and/or poor myelination,[2] and

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Introduction:

White matter (WM) structural abnormalities can be assessed using quantitative parameters determined from Diffusion tensor MRI [52]. Fractional anisotropy (FA) provides information about the microstructural integrity of highly oriented microstructures, but is not specific to the type of injury. Mean diffusivity (MD) is a measure of average molecular motion independent of any tissue directionality.

Loss of axonal integrity decreases FA and increases MD, however increased FA may also indicate loss of complexity in the underlying axonal matrix due to loss of crossing and other nonparallel fibres. Increased radial diffusivity (RD), a marker of excessive axonal packing density and/or poor myelination [42], and decreased axial diffusivity (AD), an index of axonal damage, occur in HIV-associated WM injury. [26,74,140] FA in the frontal subcortical WM, corpus callosum (CC) and internal capsule are abnormal in HIV-infected (HIV+) adults. [25,27,53] Those with the most advanced HIV disease have the highest diffusion constant elevations and largest anisotropy reductions, specifically in the CC and frontal WM. [25] Most early studies used *a priori* ROI analyses. Subsequently, more widespread WM damage has been shown using voxelwise and whole-brain analyses.[26,52–55] Animal neuro-AIDS models also show WM damage - macaques show reduced FA in the CC genu [141] and mice have reduced FA (mainly due to increased RD) and increased MD in the CC. [142]

Few studies have used DTI to examine HIV-associated alterations in WM in children. Lower FA, higher MD and RD in the CC and higher MD in the superior longitudinal fasciculus have been demonstrated in ART-naïve children (8-12 yrs.) compared to age-matched controls [38], while ART failure was associated with decreased FA in the left superior and right posterior corona radiata and decreased AD in the left inferior cerebellar peduncle in 50 children on first line ART (6-15 yrs.). [39] Regional and whole brain decreases in FA, and increased MD and RD, compared to controls, have been reported in HIV+ children and adolescents (6-20 years) [41,143] irrespective of treatment status. Regional alterations were related to past disease severity, measured by nadir CD4% and peak viral loads. [41] ART-naïve children (6-11 yrs.) showed reduced myelin compared to children on ART (6-16 yrs.), but were also younger. These studies did not document ART history.

Adolescents stable on ART (n=15, 13-17 yrs., mean age at ART initiation 9.5 yrs.) had lower FA in the CC, superior and posterior corona radiata, frontal and parietal WM, pre-and post-central gyrus and superior longitudinal fasciculus (mainly due to increased RD) than controls (n=26). [144]

Despite consistent evidence of HIV-related WM alterations, studies have included wide age ranges over developmental phases when both WM volume and FA increase significantly. [65,145,146] Few studies have controlled adequately for age or ART regimens. To date, no DTI studies have been performed in younger children, and none in children receiving standardised early ART (within the first year of life).

The aim of the present study was to determine the spatial distribution and nature of WM abnormalities at age 5 years in a cohort of HIV+ children beginning ART well within the first year of life. An additional aim was to explore associations of timing of ART initiation and DTI-derived parameters (FA, AD, RD), to interrogate potential protection of early ART on WM microstructure.

We hypothesized poorer WM integrity when starting ART after 12 weeks of age.

Methods:

Subjects

We present data for fifty-two of 62 children enrolled in a neurodevelopmental sub study of the Children with HIV Early Antiretroviral (CHER) trial [2,11] in Cape Town, South Africa. The group comprised HIV+ children on ART and age-matched controls from a parallel vaccine study, with informed consent from parents or caregivers. [147]

Exclusions were: six with mixed ancestry, one HIV+ child whose structural dataset was motion corrupted, one control child with incidental periventricular leukoencephalopathy and two HIV+ children with data inter-slice instabilities.

The CHER trial was a two-centre study in which HIV+ infants between 6 and 12 weeks of age and CD4 ≥25% were randomized to one of three treatment strategies: ART-

Deferred (ART-Def) until indicated; early limited ART for 40 weeks (ART-40W); or early limited ART for 96 weeks (ART-96W). Infants with a CD4% < 25% were enrolled into a separate group (part B), initially to be randomised into ART-40W and ART-96W, but then retained on early continuous ART. The entire cohort comprised 451 HIV-infected infants below 12 weeks of age. Four hundred and eleven infants had baseline CD4 ≥ 25%, of whom 377 were reported in the main trial. [11]

Continuous ART was initiated in ART-Def when the CD4 declined below 25% in the first year of life and 20% thereafter or for Centres for Disease Control severe stage B or C disease. These criteria also applied to restarting ART in ART-40W and ART-96W. Since some children in ART-Def began ART early, we stratified children into those starting ART after (Late ART) or before 12 weeks (Early ART), irrespective of treatment arm. Also, as some in ART-40W and ART-96W arms met endpoint during primary therapy, the early ART group was sub-divided into those with or without treatment interruption.

First-line ART was lopinavir-ritonavir, lamivudine and zidovudine. Most mothers participated in the prevention of mother to child transmission program, which included zidovudine antenatally from 32 weeks and single dose nevirapine at delivery. Mothers with CD4 count below 250 cells per mm^3 received ART antenatally. New-born infants received a single dose of nevirapine at birth and zidovudine for 7 days.

Children were in regular follow-up with three-monthly clinical assessments.

Baseline laboratory and clinical data at enrolment and within 6 months of MRI scan, including CD4, CD8 parameters and viral load (VL) were obtained from participant medical records and the CHER database. VL >750 000 copies/mL were assigned as 750 001 and those <400 copies/mL as 399 (viral suppression).
Ethics approval for the study was obtained from ethics boards of all institutions involved.

MRI Data Acquisition

The children enrolled in the neurodevelopmental sub study were imaged on a 3T MRI using structural T1 imaging followed by 2 DTI acquisitions with opposite phase encoding directions using a twice-refocused spin echo sequence. [148] The 3D echo EPI-navigated [149] multiecho MPRAGE [150] (MEMPR) sequence was acquired in a sagittal orientation with the following parameters: FOV 224×224 mm, 144 slices, TR 2530 ms, TE 1.53/3.19/4.86/6.53 ms, TI 1160 ms, flip angle 7°, voxel size 1.3×1.0×1.0 mm^3. DWI was performed in 30 directions with b-value 1000 s/mm^2, voxel size 2×2×2 mm^3, TR/TE 9500/86 ms, and 4 volumes with b = 0 s/mm^2.

Data analysis:

Pre-processing

Diffusion weighted volumes with signal dropout or motion corrupted slices were removed,[151] and diffusion encoding scheme adjusted, with a constraint that the same volumes be removed in both DTI acquisitions. Co-registration and susceptibility correction were performed.[152,153] Briefly, co-registration of individual volumes to the first unweighted image was performed using linear affine (12 degrees of freedom) transformation (FLIRT) in FSL (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, Oxford, UK). Subsequently, these images were imported to MATLAB (Mathworks, Natick, MA) for susceptibility correction and outlier rejection. [153] Outliers of each acquisition were examined by first calculating z-scores based on 25 and 75 percentile limits; data points above 3 standard deviations beyond the mean were discarded. The two acquisitions were combined into a single corrected image; FA, MD and eigenvalue (e_1, e_2, and e_3) images were generated. The first eigenvalue (e_1) was AD; the remaining two were used to compute RD ($e_{23} = [e_2 + e_3]/2$).

Co-registration

The FA images were first co-registered to corresponding structural images to achieve intra-subject alignment. Structural images of all subjects were then co-registered to a 'most representative' control image, then subsequently co-registered to the National

Institutes of Health paediatric MRI Data repository T1-template image for children aged 4.5 – 8.5 years with isotropic resolution 1.0 × 1.0 × 1.0 mm^3 using linear (FLIRT) and non-linear (FNIRT) co-registration algorithms in FSL. [154] FA images were warped using the same transforms for inter-subject alignment. The same transforms were applied to MD, AD and RD images. A WM binary mask was generated for each subject by applying a FA threshold of 0.2. Individual masks were multiplied to generate a final binary image representing WM regions where FA ≥ 0.2 in all subjects. The binary image was multiplied with the co-registered FA and MD images of each subject to localise statistical analyses, explained below, to the same WM regions.

Statistical analysis

Voxel-based group comparisons were performed in FSL to determine regions where FA and MD differed significantly between HIV+ and control children, and between HIV+ children starting ART late or early, and those with and without interruption. To account for multiple comparisons when determining significant clusters, AFNI's AlphaSim command was used with overall significance level α = 0.05 and individual voxel-wise significance level $p = 0.01$. FWHM values ranged between 3.8-5.2 mm across the masked thresholded WM masks and we performed 5000 Monte Carlo simulations. [155] Clusters of at least 258mm^3 were significant at these levels.

Locations of clusters showing group differences were identified using the Harvard-Oxford cortical and subcortical and John Hopkins University WM tractography atlases provided in FSL and an MRI atlas of human WM anatomy. [156,157] For each cluster, average FA and MD, and corresponding AD and RD values, were extracted.

Categorical variables were summarised using frequency and percentage frequency distributions overall and by group. Continuous measurements were summarised using means and standard deviation. Variables were compared between the groups using ANOVA and Chi-square tests.

Results:

After exclusions, we present data for 13 healthy controls (mean age 5.7 ± 0.5 yrs., 5 male) and 39 HIV+ children (5.4 ± 0.3 yrs., 15 male). Demographic and clinical data of HIV+ children are presented in **table 4.1**.

Ten children receiving early ART fulfilled criteria for continuous ART. Sixteen children interrupted after primary therapy, and 3 had not re-started ART by the time of MRI scan. Parents of one child randomized to ART-96W initially withheld ART without knowledge of the investigators. This child was included in the late treatment group.

Four children started ART under part B, 2 were interrupted and 2 were on continuous ART.

The cumulative period on ART was longest for those receiving early continuous ART.

Eighty-seven percent (n=34) had VL suppression (< 400 copies/ml). Of the 13% (n=5) unsuppressed at MRI, four were in the late ART group (with VL = 3 590, 5 980, 8870 and >750 000 HIV RNA copies/ml) and one in the early ART interrupted group (VL = 204 000 HIV RNA copies/ml).

Imaging:

On the T1W MR sequences structural abnormalities were identified in three HIV+ children: mild cerebellar atrophy, mild generalised atrophy, pineal multilobed cyst and none in controls.

Two clusters were identified in the right corticospinal tract (CST) where FA was lower in HIV+ children than controls (mean FA ± standard deviation: 0.42 ± 0.03 versus 0.46 ± 0.03; and 0.43 ± 0.04 versus 0.49 ± 0.04). Differences in FA were attributable to increased RD (p<0.01, **table 4.2**. Left-sided similar clusters were also seen, but did not survive cluster size correction.

Seven clusters showed higher MD at $p<0.01$, in infected children than controls, the largest being 7503 mm^3 which included several tracts. Both AD and RD contributed to the increased MD **(table 4.3)**.

Comparison of FA between children starting ART before and after 12 weeks of age:

Children starting ART later did not demonstrate poorer white matter integrity as measured by FA. Rather, one cluster was identified in the brainstem in the left CST where FA was lower in early compared to late ART initiation. When comparing early continuous and early interrupted ART individually against late ART, we found lower FA *only* in the early interrupted ART group, suggesting that interruption is harmful to WM. No regions showed FA differences between early continuous and late ART. The reduced FA in the children on early interrupted ART was attributable to increased RD and AD.

The FA cluster values (FA and RD) for the child with VL > 750 000 HIV RNA copies/ml at scan were below the average values for the remaining group but not the lowest overall.

No significant difference in FA values was noted between the other 4 unsuppressed children and the remaining group.

Discussion:

We demonstrated WM areas with significantly reduced FA in HIV+ children initiating ART at a median age of 4 months compared to uninfected controls.

No frontal or parietal white matter predilection for abnormal findings:

Our findings confirm the presence of FA abnormalities found in HIV+ adults and adolescents but differ in volume and distribution. Young children on early ART had very few regions with abnormal FA. The predilection for frontal lobe involvement described in adults [47,49,51] was not seen. We previously reported multi-focal WM signal abnormalities on standard T2W MRI sequences in frontal (91%) and parietal WM

(82%) of HIV+ children at mean age 31.9 months. [89] Twenty of these children are also included in the present study. Ten had WM signal abnormality on FLAIR. Unfortunately, a limitation in the present study was an absence of FLAIR, thus an inability to assess interval WM signal change. However, absence of frontal and parietal involvement on FA does suggest interval improvement on ART.

Although clusters showing left-sided FA differences did not survive cluster size correction, FA reductions were bilateral in the CST. The MD differences were more widely distributed and included the inferior longitudinal fasciculus (bilateral), CST, inferior fronto-occipital fasciculus, forceps minor and uncinate fasciculus.

As frontal WM myelination continues into adulthood, children demonstrate inherently lower frontal FA values than adults. [66] To exclude frontal predominance of WM abnormality being maturational [60] we determined areas of significant FA difference between HIV+ and age-matched controls from the same ethnic group, using voxelwise group comparisons, noting the small sample size of the control group as a limitation. The predominant contribution to decreased FA was RD, while the increased MD was due to both RD and AD, indicating both reduced myelin and loss of axonal integrity.[158] Age difference between the HIV+ and control group was only a few months, not considered clinically significant. Our study has a much narrower age range than previous studies, facilitating improved comparison to controls representing the age-related normal developing brain.

Children's age and ART relevance:

The higher FA values in those beginning ART after 12 weeks was surprising as we expected this in those beginning ART before 12 weeks. However, the difference was attributable to ART interruption, possibly negating the benefits of early ART, rather than neurotoxicity due to longer ART exposure. [159]

The timing of interruption may be important with reference to WM maturation. There are 3 phases of maturation observed by FA: rapid change in first 3-6 months, slower change until 24 months and relative stability thereafter. Most WM tracts are formed at birth then increase in size together with FA over 24 months. [160] Deep WM structures

such as the CC and internal capsule have high FA at birth which rapidly rises. In contrast, frontal WM has low FA, increasing to intermediate values around 24 months. In neonates, the CST is present within the brainstem but size and signal intensity on MRI are much lower than in the older brain. [62] Autopsy studies however, show that the CST and the superior cerebellar peduncles mature early. [161] ART was interrupted at 40 weeks (around 10 months of age) which may have coincided with a critical stage of CST maturation.

Notably, we found no CC involvement in HIV+ children. HIV-associated FA abnormalities in the CC are reported in adults and in children. [25,27,38,53] In contrast, our children started ART early compared to other studies. Of interest, CC volume and thickness were similar to controls in a study by Andronikou et al. which included the 20 HIV+ children previously reported.[89,121] The CC genu demonstrates a variable growth spurt at 2 months of age, followed by similar growth in the splenium by 4-6 months, with myelination being visible on T1W MRI from 4-6 months. [162] Our data support early ART being neuroprotective for the CC.

That no FA differences were noted between the late and early continuous groups may have been a 'survivor effect'. Eight children in ART-Def died in the first year of life and were not studied. Also, those on early continuous rather than early limited ART, were more severely affected by HIV, having already reached a trial endpoint during primary ART, thus ineligible for interruption. All participants on continuous therapy had suppressed VL at the time of scan. Also, those from Part B had baseline CD4 below 25% and therefore had more advanced HIV. Nevertheless, the early interrupted treatment group children had the most WM damage, suggesting that WM is more vulnerable at this time.

This cohort of children is enrolled in a longitudinal neuroimaging study that includes DTI at age 7 and 9, which will provide vital information on the continuous effect of ART. Our data strongly suggests that WM damage, although not prevented by early ART, can be ameliorated or reversed, possibly through reduced neuroinflammation. [56]

Conclusions:

HIV+ children at 5 years of age have WM fibre abnormalities measured by FA despite early ART, suggesting that early ART does not fully protect the WM either from peripartum or in utero infection. In contrast to adults, the CSTs rather than the CC, are predominantly, possibly due to the timing of myelination/development and the relationship with timing of institution of early ART. Continuous ART can limit WM damage.

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Table 4.1: Sample characteristics of HIV infected children

	Late ART (>12 weeks)	**Early ART (<12 weeks) interrupted**	**Early ART (<12 weeks) not interrupted**	**p**
N	13	16	10	
Gender	4M/9F	5M/11F	6M/4F	0.30
Age at scan (yrs.)	5.3 (0.30)	5.4 (0.24)	5.6 (0.43)	0.20
Age ART started (weeks)	36 (17)	8 (2)	8 (2)	**<0.01**
Time on ART (weeks)	241 (22)	203 (59)	285 (22)	**<0.01**
Time interrupted (weeks)*	n/a	85 (90)	n/a	
Clinical measures at baseline				
CD4 count	2064 (711)	1969 (1118)	1720 (978)	0.57
CD4%	37 (7)	35 (10)	30 (13)	0.21
CD8	1751 (1109)	1460 (675)	1978 (945)	0.34
VL>750 000 %, (n)	69 (9)	56 (9)	40 (4)	0.37
400<VL<750 000 %, (n)	31 (4)	44 (7)	60 (6)	
Clinical measures within 6 months of scan				
CD4 count	1027 (392)	1110 (460)	1289 (592)	0.58
CD4%	37 (8)	34 (7)	36 (10)	0.49
CD8 count	902 (450)	1083 (544)	1087 (625)	0.57
VL>750 000 %, (n)	8 (1)	0	0	0.14
400<VL< 750 000 %, (n)	23 (3)	6 (1)	0	
Suppressed VL %, (n)	69 (9)	94 (15)	100 (10)	

NA = applicable, VL = viral load

Values: mean (SD)

*calculated up to time of scan in 3 children who had not restarted ART.

Table 4.2: Clusters where FA was lower in HIV+ children compared to controls

Cluster Location	Size mm^3	Co-ordinates	AD			RD		
			HIV+	Control	*p*	HIV+	Control	*p*
CST Right internal capsule	365	27, -23, -1	1.18 (0.03)	1.20 (0.03)	0.10	0.60 (0.10)	0.56 (0.02)	<0.001
CST Right parietal lobe	294	19, -24, 42	1.18 (0.06)	1.22 (0.07)	0.08	0.60 (0.04)	0.56 (0.03)	<0.001

Values: mean (SD)

Table 4.3: Clusters where HIV+ children had significantly greater MD compared to controls

Cluster Location	Size mm^3	Co-ordinates	AD			RD		
			Control	HIV+	*p*	Control	HIV+	*p*
ILF/SLF								
Right temporal	7503	32,0, -24	1.19 (0.03)	1.23 (0.03)	0.001	0.62 (0.02)	0.65 (0.03)	<0.001
Left putamen	6916	-29,-26,-2	1.20 (0.03)	1.25 (0.03)	0.001	0.59 (0.02)	0.62 (0.03)	<0.001
CST								
Right brainstem	916	21,-15,-9	1.25 (0.04)	1.29 (0.03)	0.01	0.58 (0.02)	0.63 (0.02)	<0.001
IFOF								
Left temporal	555	-37,-10,-17	1.19 (0.05)	1.25 (0.04)	0.003	0.66 (0.02)	0.70 (0.04)	<0.001
Forceps minor								
Left frontal	336	-19,43,14	1.20 (0.06)	1.24 (0.06)	0.04	0.63 (0.03)	0.67 (0.05)	0.0030
Left frontal	266	-17,42,-1	1.22 (0.06)	1.26 (0.06)	0.05	0.62 (0.03)	0.65 (0.05)	0.0040
UF								
Right frontal	330	15,38,-12	1.19 (0.05)	1.25 (0.06)	0.003	0.64 (0.03)	0.67 (0.04)	0.0046

Values: mean (Standard Deviation).

ILF /SLF (inferior/superior longitudinal fasciculus), IFOF (inferior fronto-occipital fasciculus), UF (uncinate fasciculus)

Chapter 5: DTI and Neurodevelopmental changes in HIV

Rationale for inclusion of unpublished work

This article brings into context the relationship of white matter damage with developmental changes encountered in the HIV population.

The observation continues in the same group of children, which was reported on in the preceding article and now concentrates on the developmental impact of HIV.

In this unique population, the children were on ART from a very early age and were followed up regularly with intervention when needed. This may account for the differences in outcomes in this study when compared to similar studies reported in literature.

This article has been submitted for publication, currently awaiting review, Journal: AIDS research and therapy, BIOMED central.

Declaration by the candidate:

Concerning chapter 5, *DTI and Neurodevelopmental changes in HIV*, the nature and scope of my contribution were as follows:

First author, interpretation and production 60%

The following co-authors have contributed:

Prof Savvas Andronikou: co-supervisor and editing 5%

Muhammad Saleh: technical support and data processing 5%

Martin Kidd: statistical analysis 8%

Prof Mark Cotton: supervisor and editing: 2%

Barbara Laughton: clinical input as well as editing 10%

Ernesta Meintjes: data post processing and editing 10%

Declaration by co-authors:

The undersigned hereby confirm that

1. The declaration above accurately reflects the nature and extent of the contributions of the candidate and the co-authors to Chapter 5
2. No other authors contributed besides those specified above, and

3. Potential conflicts of interest have been revealed to all interested parties and that the necessary arrangements have been made to use the material in Chapter 5 of this dissertation.

Declaration with signature in possession of candidate and supervisor.

AIDS Research and Therapy

Diffusion Tensor Imaging point to ongoing functional impairment in HIV-infected children at age 5, undetectable using standard neurodevelopmental assessments.

--Manuscript Draft--

Manuscript Number:	
Full Title:	Diffusion Tensor Imaging point to ongoing functional impairment in HIV-infected children at age 5, undetectable using standard neurodevelopmental assessments.
Article Type:	Research
Funding Information:	National Institutes of Health (NIH R01HD071664) — Prof Mark F Cotton National Institutes of Health (R21MH096559) — Prof Mark F Cotton Harry Crossley Foundation — Dr Barbara Laughton National Research Foundation (CPR20110614000019421) — Prof Ernesta M Meintjes National Institute of Allergy and Infectious Diseases (U19 AI53217) — Prof Mark F Cotton
Abstract:	Abstract Title: Diffusion Tensor Imaging point to ongoing functional impairment in HIV-infected children at age 5, undetectable using standard neurodevelopmental assessments. Background: Perinatal HIV infection negatively impacts cognitive functioning of children, main domains affected are working memory, processing speed and executive function. Early ART, even when interrupted, improves neurodevelopmental outcomes. Diffusion tension imaging (DTI) is a sensitive tool assessing white matter damage. We hypothesised that white matter measures in regions showing HIV-related alterations will be associated with lower neurodevelopmental scores in specific domains related to the functionality of the affected tracts. Methods: DTI was performed on children in a neurodevelopmental sub study from the Children with HIV Early Antiretroviral (CHER) trial. Voxel-based group comparisons to determine regions where fractional anisotropy and mean diffusion differed between HIV+ and uninfected children were done. Categorical variables were summarised using frequency and percentage frequency distributions overall and by group. Continuous measurements were summarised using means and standard deviation. Variables were compared between the groups using Chi-square tests and ANOVA. Spearman Correlation of DTI parameters in abnormal white matter with directed neurodevelopmental tests were examined. Results: 38 HIV+ children (14 male, mean age 64.7 months) and 11 controls (4 male, mean age 67.7 months) were imaged. 2 Clusters with lower fractional anisotropy and 7 clusters with increased mean diffusion were identified in the HIV+ group. The only neurodevelopmental domain with a trend of difference between the HIV+ children and controls (p=0.08), was Personal Social Quotient which correlated to improved myelination of the forceps minor in the control group. As a combined group there was a negative correlation between visual perception and radial diffusion in the right superior longitudinal fasciculus and left inferior longitudinal fasciculus which may be related to the fact that these tracts, forming part of the visual perception pathway, are at a crucial state of development at age 5. Conclusion: Even directed neurodevelopmental tests will underestimate the degree of microstructural white matter damage detected by DTI. The visual perception deficit detected in the entire study population should be further examined in a larger study.
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Introduction:

It is well established that perinatally acquired HIV infection negatively impacts cognitive functioning. [163] The principal domains affected vary with limited data from countries where HIV is prevalent. A recent meta-analysis of 22 studies (37% from sub-Saharan countries) on vertically infected children aged 6 to 18 years, found that the main cognitive domains affected by HIV are working memory, processing speed and executive functioning. [164]

Studies show that commencing combination antiretroviral therapy (ART) before 6 months in perinatally HIV-infected (HIV+) infants improves neurodevelopmental and clinical outcomes. [165,166,167,168] Concerns that ART might cause neurotoxicity and that adherence may wane, led to planned treatment interruption studies, which showed safety and no effect on short term neurocognition. [169,170] The clear advantage of early time-limited over deferred-continuous therapy for clinical outcomes was demonstrated in the Children with HIV Early Antiretroviral (CHER) trial. [11] Recently, Laughton et al. reported neurodevelopmental outcomes over 5 years in a CHER sub-study. Neurodevelopmental outcomes were similar between the treatment arms (delayed continuous ART or early ART with interruption at 40 or 96 weeks) and uninfected controls. The only exception was visual perception, measured on the Beery Visual Perception subtest, where all HIV+ arms performed significantly worse. This deficit was neither detectable on the Beery visual motor integration test (Beery-VMI) [171] nor the Griffiths Mental Development Scales (GMDS). [172]

Neuroimaging is instrumental in describing HIV effects on brain macro- and microstructure. [82] Specifically, diffusion tensor imaging (DTI) can be used to examine the nature of white matter (WM) damage through quantitative parameters such as fractional anisotropy (FA) and mean diffusivity (MD). [38,40,41,143,173] Loss of axonal integrity decreases FA and increases MD, however, increased FA may also indicate diminished complexity of the axonal matrix due to loss of crossing fibres.[52] We previously published a DTI study on HIV+ children (mean age 5.7 years) from the cohort studied by Laughton et al., and demonstrated WM abnormalities of the projectional fibres of the corticospinal tracts (CST) and also association fibres of the superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF), inferior

frontal occipital fasciculus (IFOF) and uncinate fasciculus (UF). Children on continuous ART from within the first year of life had less WM damage than those randomised to treatment interruption. This was an important finding when considering that Laughton et al. showed that neurodevelopmental outcome at 5 years was not adversely affected by planned treatment interruption. [172,174]

The aim of the current study was to examine associations of FA and MD values in regions shown to be significantly different between HIV+ children and controls in the aforementioned DTI study [174], with directed neurodevelopmental scores. The latter were identified as those tests related to the expected function of involved WM tracts, for example CST and motor development, as opposed to the full battery of neurodevelopmental tests. We hypothesised that WM measures in regions showing HIV-related alterations would be associated with lower neurodevelopmental scores in specific domains related to the functionality of the affected WM tracts.

The study group is uniquely homogeneous in that all the children began ART before 18 months, are from the same socioeconomic background and have a narrow age range.

Materials and methods:

Subjects:

56 Xhosa children enrolled in a neurodevelopmental sub study of the CHER trial [2,11] in Cape Town, South Africa underwent magnetic resonance imaging (MRI) of the brain at 5 years of age. The group comprised HIV+ children who commenced ART early (N=42) and age-matched, HIV-uninfected controls (N=14) from a parallel vaccine study, with informed consent from parents or caregivers. [147]

Inclusion criteria for the neurodevelopmental sub-study were birth weight > 2000g, normal neurological examination at a clinical visit near three months of age and no central nervous system problems or dysmorphic syndromes.

The CHER trial:

The CHER trial (the source of our patient population) was a two-centre study in which HIV+ infants between 6 and 12 weeks of age with CD4 ≥25% were randomized to one of three treatment strategies: ART deferred (ART-Def) until indicated; early limited ART for 40 weeks (ART-40W); or early limited ART for 96 weeks (ART-96W). Continuous ART was initiated in ART-Def when the CD4 declined below 25% in the first year of life and 20% thereafter or for Centres for Disease Control severe stage B or C disease. The same criteria applied to restarting ART in ART-40W and ART-96W. Infants with a CD4% < 25% were enrolled into a separate group (part B), initially to be randomised into ART-40W and ART-96W, but then retained on early continuous ART. The entire cohort comprised 451 HIV+ infants below 12 weeks of age, of which 115 were enrolled in Cape Town. [11]

First-line ART was lopinavir-ritonavir, lamivudine and zidovudine; only one child was on second line therapy comprising Didanosine, Abacavir with Nevirapine. Most mothers participated in the prevention of mother to child transmission program, which included zidovudine antenatally from 32 weeks and for infants a single dose nevirapine at delivery and zidovudine for 7 days.

Neurodevelopmental assessments:

The GMDS extended revised version (2-8 years) was performed at 5 years of age. [129] The GMDS assesses neurodevelopment on the subscales: locomotor, personal-social, hearing and language, eye and hand co-ordination, performance (visuospatial skills including speed and precision) and practical reasoning. A global GMDS score is also obtained. Standardized translations into IsiXhosa and Afrikaans were used. One of two pediatricians conducted the assessments, assisted by a GMDS-trained translator. We converted raw scores into age equivalents using standardized norms and calculated a quotient as a percentage of each child's chronological age, using the United Kingdom norms with a mean of 100 and standard deviation of 15.[129,128] Significant developmental delay was regarded as quotients below 70. The Beery-Buktenica tests of visual-motor integration (Beery-VMI), visual perception and motor coordination (6th edition) were also administered (**Table 5.1**). [175] Standard scores were calculated from raw scores using USA norms. While these developmental tests

are not standardised for South African children, they are often used and considered culturally fair and reliable.[129,130,132,131]

Baseline laboratory and clinical data at enrolment and within 6 months of MRI scan, including CD4, CD8 parameters and viral load (VL) were obtained from the CHER database. VL >750 000 copies/mL were assigned as 750 001 and those <400 copies/mL as 399 (viral suppression).

Ethical approval for the study was obtained from ethics boards of all institutions involved.

MRI Data Acquisition:

The children were imaged on a 3T Siemens Allegra MRI (Erlangen, Germany), without sedation while watching an age-appropriate feature film, using structural T1 imaging followed by 2 DTI acquisitions with opposite phase encoding directions using a twice-refocused spin echo sequence. [148] The 3D echo planar imaging (EPI) navigated [149] multiecho MPRAGE [150] (MEMPR) sequence was acquired in a sagittal orientation with the following parameters: FOV 224×224 mm, 144 slices, TR 2530 ms, TE 1.53/3.19/4.86/6.53 ms, TI 1160 ms, flip angle 7°, voxel size 1.3×1.0×1.0 mm^3. DTI was performed in 30 directions with b-value 1000 s/mm^2, voxel size 2×2×2 mm^3, TR/TE 9500/86 ms, and 4 volumes with b = 0 s/mm^2.

MRIs of children with motion corruption, showing incidental brain abnormalities, interslice instabilities or with an interval of over a year from the GMDS were excluded.

Statistical analysis:

DTI data were previously analysed in FSL (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, Oxford, UK). [174] Locations of clusters showing group differences were identified using the Harvard-Oxford cortical and subcortical and John Hopkins University WM tractography atlases provided in FSL and an MRI atlas of human WM anatomy. [156,176] For each cluster, average FA and MD, and corresponding AD and RD values, were extracted.

Categorical variables were summarised using frequency and percentage frequency distributions overall and by group. Continuous measurements were summarised using means and standard deviation. Variables were compared between the groups using Chi-square tests and ANOVA.

Specific functionality of the WM tracts with clusters of abnormal FA and MD in the HIV+ group compared to controls [174] were identified. We used a directed approach to select neurodevelopmental tests that would closely match this functionality for correlation to minimize the effect of multiple comparisons (no specific adjustments for multiple comparisons were made).

Spearman correlation was used to test for relationships between directed developmental scores and FA and MD values in affected regions. Correlations were performed as a combined group (HIV+ and controls) as well as controls and HIV+ groups separately.

Results:

Seven of 56 children assessed were excluded: one HIV+ child whose structural image was motion corrupted, one control child with incidental periventricular leukoencephalopathy, two HIV+ children with data interslice instabilities, 2 control children in whom GMDS at age 5 were not performed, 1 HIV+ child with a period of more than 1 year and 2 months between the Griffiths analysis and the MRI scan. We therefore present data for 38 HIV+ children (mean ± sd = 5.4 ± 0.3 years; 14 boys) and 11 healthy controls (mean ± sd = 5.6 ± 0.5 years; 4 boys, 9 HIV exposed). (**Table 5.1**)

The HIV+ and control groups did not differ for demographic variables (all *p*'s > 0.1); or on the interval between scan and Griffiths, which was 123.8 days on average. The groups also did not differ on any of the Beery Buktenica Scales (all *p*'s > 0.2) or the GMDS (all *p*'s > 0.5), except for Personal Social Quotients that tended to be lower in HIV+ children. **Table 5.2** describes the GMDS and Beery Buktenica scales.

Correlation of FA and MD with WM-directed neurodevelopmental tests:

Previously, we found lower FA in CST, and higher MD in ILF, SLF, CST, IFOF, forceps minor and UF, in HIV+ children than controls. (**Figure 4.1, 4.2**) [174]

Table 5.3 describes the clusters and neurodevelopmental tests selected for correlation. Overall, the Beery visual perception was negatively correlated with RD in the right temporal SLF (r = -0.31, p = 0.03) and left putamen region of the ILF (r = -0.29, p = 0.05). In the left forceps minor, higher AD was related to increases in Practical Reasoning scores (r = 0.32, p = 0.03,), while RD in the same region showed a strong negative correlation with Personal-Social scores in the controls *only* (r = -0.62, p = 0.05). In the HIV+ group, negative correlations were found between Performance subscale scores and RD in the right UF (r = -0.32, p = 0.05), and between Beery Motor Coordination and AD in the brainstem in the CST (r = -0.33, p = 0.05). No other significant associations were found between WM measures in affected regions and neurodevelopmental scores (all p's > 0.1). (**See figure 4.3a-f).**

Discussion:

In this study, we aimed specifically to examine the potential role of HIV-related WM alterations on neurodevelopmental outcomes, by examining correlations of WM measures with scores on functional domains where affected tracts play a critical role. When comparing neurodevelopmental performance between HIV+ and uninfected groups, only the Personal-Social Quotient showed a trend of being lower in HIV+ children. However, this finding does not appear to be attributable to observed WM deficits, as WM measures from clusters in neither the IFOF nor the forceps minor showed association with Personal-Social scores. *Only* among controls, did we find association of increased RD in the left forceps minor with poorer Personal-Social Scores. Overall, increased RD in the SLF and ILF was associated with poorer performance on Beery visual perception, and decreased AD in forceps minor with poorer Practical Reasoning. In HIV+ children, increased RD in the right UF was associated with lower Performance scores, and increased AD in the brainstem region of the CST with poorer Beery motor coordination.

In view of the increases in RD and AD observed in HIV+ children in these regions the associations point to impairment in visual perception, motor coordination and performance (which essentially tests visuospatial skills), all of which have been described in HIV+ children. [177]

In contrast to most previous studies that demonstrated clear differences between HIV+ and uninfected children on various functional domains, [95,178,179] children in our study performed similarly at this age on all administered tests. Even in the larger group from the CHER neurodevelopmental sub-study, only visual perception deficits were detected in the HIV+ children at age 5 years.[172] Our findings support those of a recent meta-analysis which concluded that both general intellectual functioning and motor coordination are less impaired in HIV+ children than previously believed. [164] Various factors may explain the different study outcomes, including differences in methodologies, study populations and treatment regimens. The sample studied here comprised children of similar age and socio-economic background with a very well documented treatment history. All had commenced treatment by 18 months and achieved viral suppression by 49 months (median age at first viral suppression was 10 months). In view of the homogeneity and early treatment of these children, it is perhaps not surprising that their neurodevelopmental scores were minimally impaired compared to controls. Alternatively, it is possible that the battery of tests selected are not sensitive enough to detect the subtle impairments evident at this age.

It is known that WM integrity is correlated with cognitive performance in a fibre specific manner. [180] For example, in stroke patients, the degree of CST injury, defined by DTI, correlates with motor impairment. [57,181–183] The SLF plays an important role in higher brain functions particularly language, [184,185] spatial awareness and symmetric processing. [186] The ILF is involved in visual memory [187,188] and the UF in the formation and retrieval of memories.[180,189] Studies in patients with multiple sclerosis also found a relationship between working memory performance and fibres of the SLF and IFOF. [190] The negative correlation found here between visual perception and RD in the SLF and ILF, provides additional evidence that these tracts have a role in interpreting visual information. Although in our small sub-sample, we did not detect group differences on Beery Visual Perception test, this domain did show HIV-related deficits in the larger sample assessed in the CHER neurodevelopmental sub-study.

[172] Notably, the control group in our sub-sample comprised largely HIV-exposed uninfected children (HEU), who may also be affected by perinatal HIV and ART exposure and explain our failure to detect developmental differences on this domain. Visual perception, encompassing the appreciation of an object's qualities and its location in space, is dependent on the processing of visual information in the inferior temporal and posterior parietal cortices, respectively. [191] If perception is incorrect or altered in any way, problems with reading, spelling, handwriting, mathematics and comprehension can occur.

Ventral (occipitotemporal) and dorsal (occipitoparietal) visual pathways exist which are functionally specialized. Dorsal stream functions are related to spatial processing and control of visually guided actions and ventral stream functions to perceptual identification. [191,192] The most important WM trajectories of the ventral stream are the ILF and the IFOF [192] also described as intrahemispheric visual association WM tracts, as well as the UF. [193] It is striking that all three of these tracts demonstrated abnormalities in our HIV+ children.

The ventral visual stream is almost adult-like at 5-7 years of age, with DTI metrics demonstrating a rapid increase in FA and decrease in MD in the ILF between ages 5 – 7 years, [192] placing the children in our study at a critical age in maturation of WM tracts for visual perception. HIV-associated WM damage, described as being predominantly altered myelination, [174] may well account for the abnormalities in visual perception identified by Laughton et al., although not clearly demonstrated in the Beery Visual perception test in this group.

Increased AD in the left forceps minor was associated with improved practical reasoning for the group as a whole. This would imply that HIV+ children may have better practical reasoning skills, even though we failed to detect a group difference for this domain in the current sample and the larger CHER sub study. The Practical Reasoning subscale assesses earliest arithmetic comprehension and the ability to solve very basic practical problems. The forceps minor connects the lateral and medial surfaces of the frontal lobes and crosses the midline via the genu of the corpus callosum. It is an interhemispheric sensory and auditory connection pathway involved in emotional functions and behavioural control. [194,195] It may play an important role in

mathematical skills as indicated in a study where children with increased mathematical ability demonstrated higher FA in WM tracts, particularly the forceps minor and major tracts connecting the frontal lobes with basal ganglia and parietal regions. [196] In our study however, we did not demonstrate higher FA but rather increased MD in the forceps minor. This apparent contradiction thus remains unexplained.

The only domain where controls tended to score higher than the HIV+ group was the Personal Social subscale. The strong negative correlation between RD in left forceps minor and Personal Social Quotient among controls suggest that increased myelination (characterised by RD reductions) in this tract may relate to improved personal and social development. HIV-related white matter alterations in this region may be responsible for the fact that this relation is not evident in HIV+ children.

Among HIV+ children, increased RD in the right frontal UF is associated with lower scores on the Performance subscale, which assesses visuospatial skills, speed and precision. The functionality of the UF is still under debate but several studies suggest at least the following: it is a long-range association fibre connecting the frontal and temporal lobes, (the amygdala in the temporal lobe with the orbitofrontal cortex) and is involved in various types of memory, language and social-emotional processing. [197] The domain of working memory was identified as being affected by HIV in the meta-analysis by Phillips and colleagues. [164] It could be that this component of visuospatial processing leads to impaired performance only in the children with the highest RD's, who were from the HIV+ group. Unfortunately working memory was not assessed as a separate domain in our study.

Increased AD in the brainstem region of the CST is associated with poorer Beery motor coordination test scores in the HIV+ children. Three clusters with abnormal FA and MD in the CST compared to controls were found in the HIV+ group, however no performance differences were found in either the GMDS motor function or the Beery motor coordination. Locomotor deficits were present in this group at a younger age [172] therefore these findings may suggest that conventional neurodevelopmental assessments at this age are not sensitive enough to detect persistent deficits.

Notably, the WM deficits in the UF and brainstem region of the CST (regions that demonstrate association with performance measures in the HIV+ group only) were not evident when the children in the current study were re-assessed at 7 years[198], indicating that these deficits may represent a developmental delay that resolves at later ages. In contrast, WM alterations in the ILF and forceps minor persist at age 7 years, suggesting that effects on visual perception may be more long-term or even permanent.

Limitations of this study are the small sample size of controls and the large number of HEU children (9 out of 11) in this group. The secondary effects of HIV and ART exposure in the HEU controls may have influenced neurodevelopmental scores and decreased our ability to detect neurodevelopmental differences between the two groups. However, in a study by Boivin et al. maternal triple antiretroviral exposure both ante- and post-partum did not result in developmental risks for the HIV- exposed and uninfected children through age 60 months compared to unexposed, uninfected children 199. On the other hand, the study group is uniquely homogenous for age, timing of ART and socioeconomic background.

Conclusion:

Although the detrimental effect of HIV on WM is ameliorated by early ART, regional WM alterations on DTI MRI remain and show association at age 5 years with specific functional domains, including visual perception, performance and motor coordination. In view of the visual perception deficit reported in these children at this age, the effect of HIV on the visual perception pathway should be further examined in a larger study group. Our findings suggest that brain imaging is more sensitive for subtle alterations from HIV and/or ART than standard neurodevelopmental tests. Alternatively, more sensitive neurodevelopmental tests should be developed.

Table 5.1: Demographics and neurodevelopmental scores of the HIV+ group and controls

	HIV+	Controls	*p*
N	**38**	**11 ***	
Gender (M/F)	14/24 (37%/63%)	4/7 (36%/64%)	0.97 (χ^2)
Age at Griffiths (months) range	60.9 (1.3) 58.0 – 64.5	63.3 (4.8) 59.5 – 71.0	0.12
Age at scan (months) range	64.7 (3.5) 58.8 – 74.4	67.7 (5.5) 61.2 – 74.4	0.11
Time between Griffiths and scan (days) range	119.2 (104.3) 0 – 386	128.3 (131.8) 21- 392	0.81
Age starting ART (weeks) range	18 (16.8) 7.0 – 75.7	NA	
Cumulative Time on ART (weeks)	234 (50.8)	NA	
CD4 Baseline Nadir At scan	 1965.4 (939.8) 703.2 (391.6) 1132.7 (480.7)	NA	
CD4% Baseline Nadir At scan	 34.5 (10) 20.4 (6.5) 35.2 (8.2)	NA	
CD8 Baseline Nadir At scan	 1693.3 (899.3) 575.4 (329.1) 1025.7 (536.9)	NA	
Griffiths Q scores Locomotor Personal Social Language Eye Hand Coordination Performance Practical Reasoning General	 96.1 (16.2) 90.8 (9.4) 75.4 (10.7) 85.4 (9.0) 75.4 (10.5) 76.7 (8.6) 83.5 (6.5)	 93.0 (11.9) 96.4 (9.0) 77.7 (10.7) 84.3 (12.2) 78.0 (19.5) 75.2 (11.4) 84.1 (8.3)	 0.57 0.08 0.53 0.74 0.57 0.65 0.81
Beery-Buktenica Visual Motor Integration Visual Perception Motor Co-ordination	 91 (9.1) 76.7 (14.7) 94.8 (8.3)	 87.4 (7.2) 83.1 (13.7) 93.2 (10.4)	 0.23 0.22 0.59

Values: mean (Standard Deviation) unless otherwise stated.
* 9 HIV exposed and uninfected, 2 unexposed

Table 5.2: Description of abilities assessed with the GMDS and Beery-Buktenica test

Griffiths Mental Development Scales	
Subscale	**Description of abilities assessed**
Locomotor	Balance and stability – jumping over hurdles, balancing on one leg, skipping and running.
Personal-Social	Self-care including dressing, washing, tying shoe laces and being able to provide full name and address.
Hearing and Language	Receptive and expressive language is assessed. Naming objects and describing their use. Children are required to freely talk about a large/busy picture where vocabulary, sentence structure, pronouns and descriptive words are assessed. Auditory short-term recall with repetition. Naming colors, similarities opposites and descriptive.
Eye and Hand Co-ordination	Free Drawing of a person and a house. Copying geometric shapes. Writing name and copying letters. Cutting and folding paper and threading beads.
Performance	Visuo-spatial skills including speed and precision. Completing form boards and block patterns which are timed.
Practical Reasoning	Closest to arithmetical reasoning: counting blocks, knowing days of the week, high/low, long/short, heavy/light, middle and concept of speed. Short-term memory of items shown. Arranging sequences of cards to tell a story.
General Griffiths Quotient:	Average of the 6 subtests above.
Beery-Buktenica test of Visual Motor Integration:	
Beery VMI	Child is required to copy various geometric forms and draw them below the example figure.
Beery Motor Co-ordination	Draw the same geometric forms by joining dots and keeping within the guidelines. Draw as many as can within a time limit.
Beery Visual Perception	Identify shape out of a few that matches the example. Do as many as can within a time limit.

Table 5.3: WM tracts in which clusters showing FA reductions and MD increases in HIV+ children compared to controls are located, the function of the implicated tracts and neurodevelopmental tests that assess said function.

Tract	Tract function [200]	Griffiths Mental Development Subscale used	Beery-Buktenica Test of Visual Motor Integration
Corticospinal Tract	Descending projection fibres connecting motor area to the spinal cord. Arise from motor cortex of pre- and postcentral gyrus.	Locomotor	Beery Motor Co-ordination
Superior Longitudinal Fasciculus	Association fibres – unite different cortical areas within the same hemisphere. Bidirectional bundles connecting the frontal lobe to the parietal, temporal and occipital lobes. Function: integration of auditory and speech nuclei, spatial awareness and symmetric processing. Interruption decreases the ability to repeat spoken language and can also cause unilateral neglect.	Hearing and Language Eye and Hand Coordination Performance	Beery –VMI Beery – Visual Perception
Inferior Longitudinal Fasciculus	Connects the cortices of the anterior temporal and posterior occipital lobe and joins the inferior aspect of the SLF. Function: visual emotion and visual memory. Interruption may result in unilateral visual neglect, visual amnesia, hallucinations, and visual hypo emotionality.	Eye and Hand Coordination Performance Practical Reasoning	Beery – VMI Beery- Visual Perception
Inferior Fronto Occipital Fasciculus	Connects the ipsilateral frontal and occipital, posterior parietal and temporal lobes. Function: integration of auditory and visual association cortices with the prefrontal cortex.	Personal-Social Language Practical Reasoning	Beery – VMI Beery- Visual Perception
Forceps Minor	The forceps minor is the anterior part of the corpus callosum, it connects the homologous regions of the anterior frontal lobes between two hemispheres. Among the regions included are the front polar cortex which has been shown to be important for cognitive behavioural control, decision making, and attention control.	Personal-Social Language Performance Practical Reasoning	
Uncinate Fasciculus	Connects the orbital and inferior frontal gyri rectus to the anterior temporal lobe. It has the longest period of development in terms of FA and is the only WM tract that continues to develop beyond 30 yrs. Part of the limbic system. Integrity of the tract has been related to proficiency in auditory-verbal memory and declarative memory.	Language Performance Practical Reasoning	

Key to abbreviations:
R: right, L: left, CST: corticospinal tract, SLF: superior longitudinal fasciculus, ILF: inferior longitudinal fasciculus, IFOF: inferior fronto occipital fasciculus, UF; uncinate fasciculus.

Figure 4.1: Represent the two clusters in the right corticospinal tract, where FA was lower in HIV+ children when compared to controls. (1 = right internal capsule, 2 = right parietal lobe)

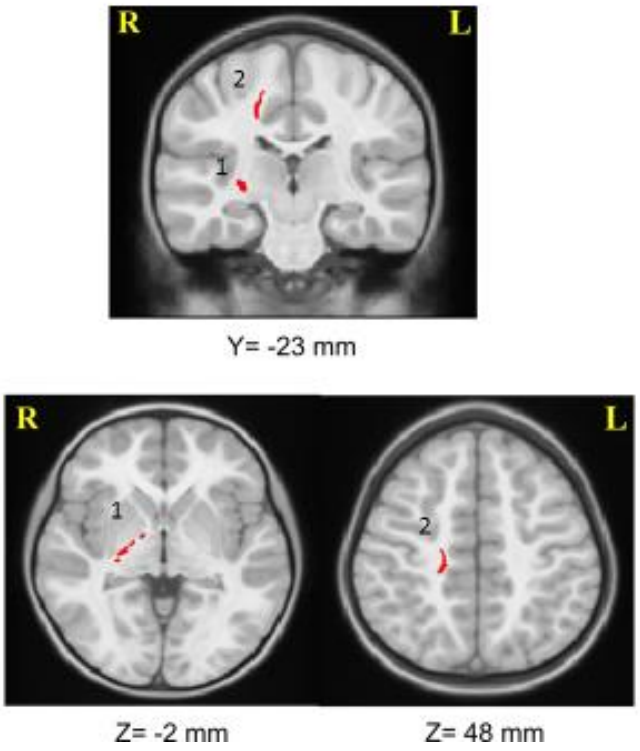
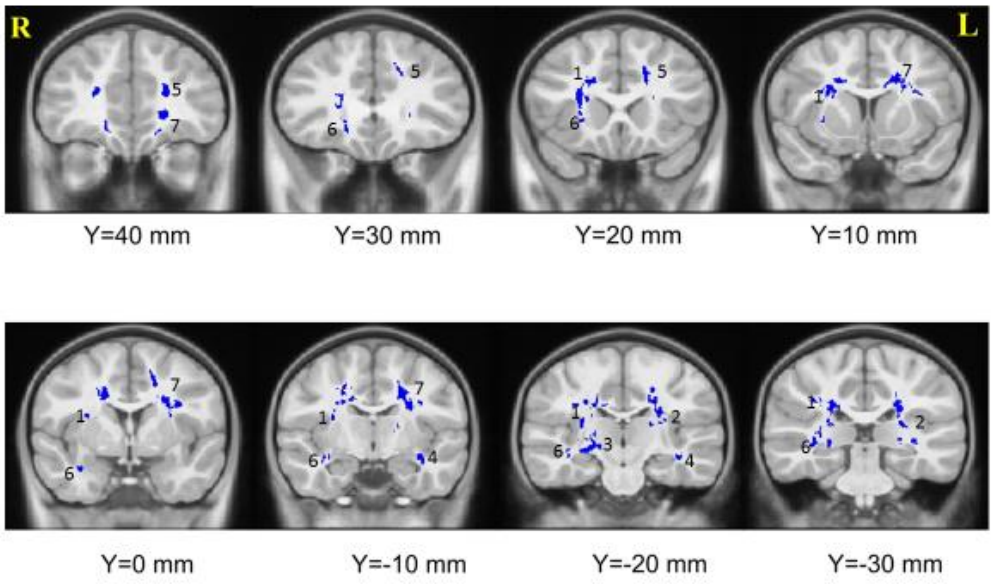


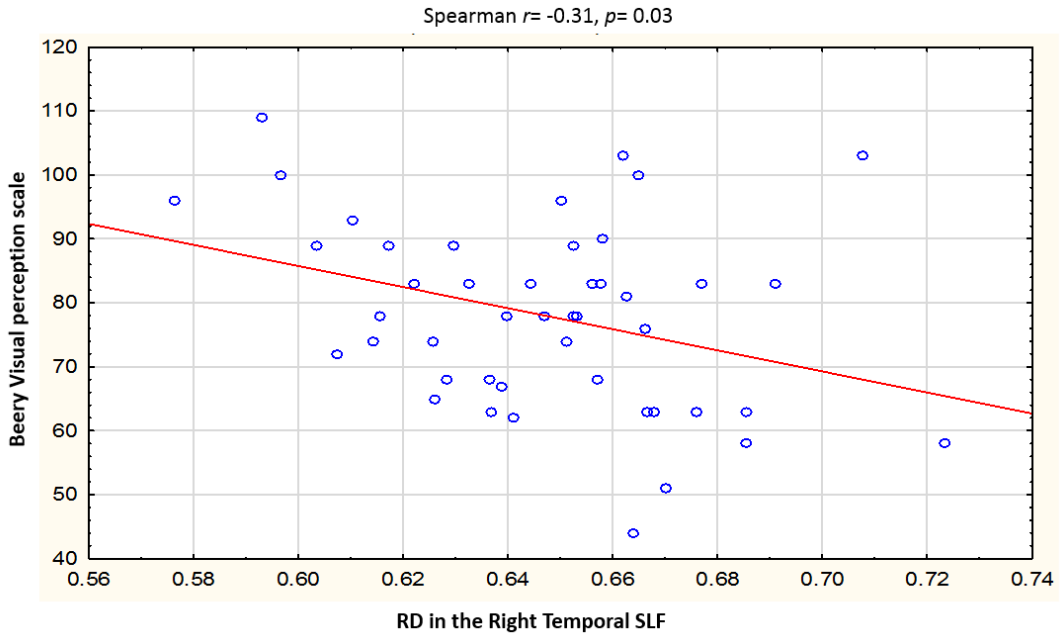
Figure 4.2: Represent the seven clusters with higher MD in HIV+ children compared to controls. (1 = right SLF, 2 = left ILF, 3 = right CST, 4 = left IFOF, 5 = left forceps minor, 6 = right UF, 7 = left forceps minor)



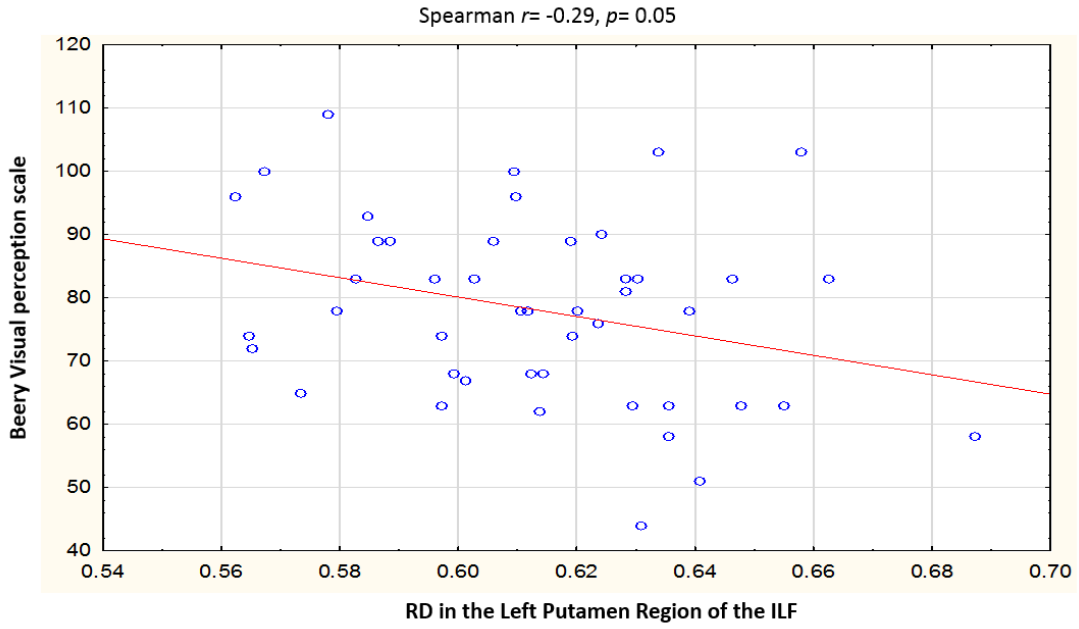
Figures 4.3a-f: Correlations of FA and MD with WM-directed neurodevelopmental tests.

4.3a and b: Increased RD in the (a) right temporal SLF and (b) left putamen region of the ILF were associated with poorer performance on Beery visual perception found in patients and controls.

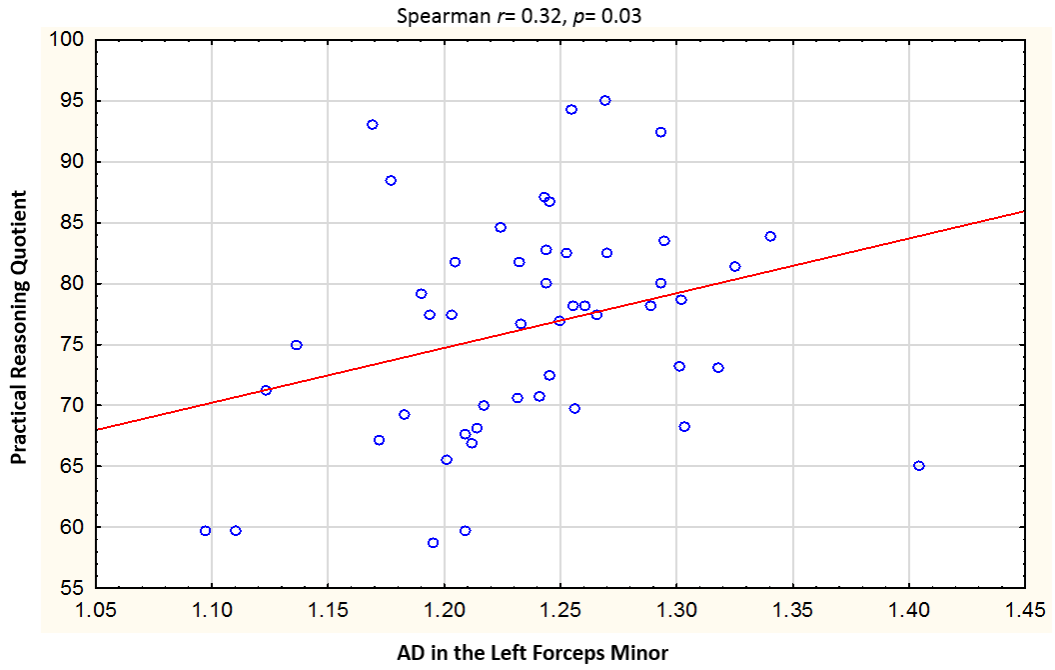
a.



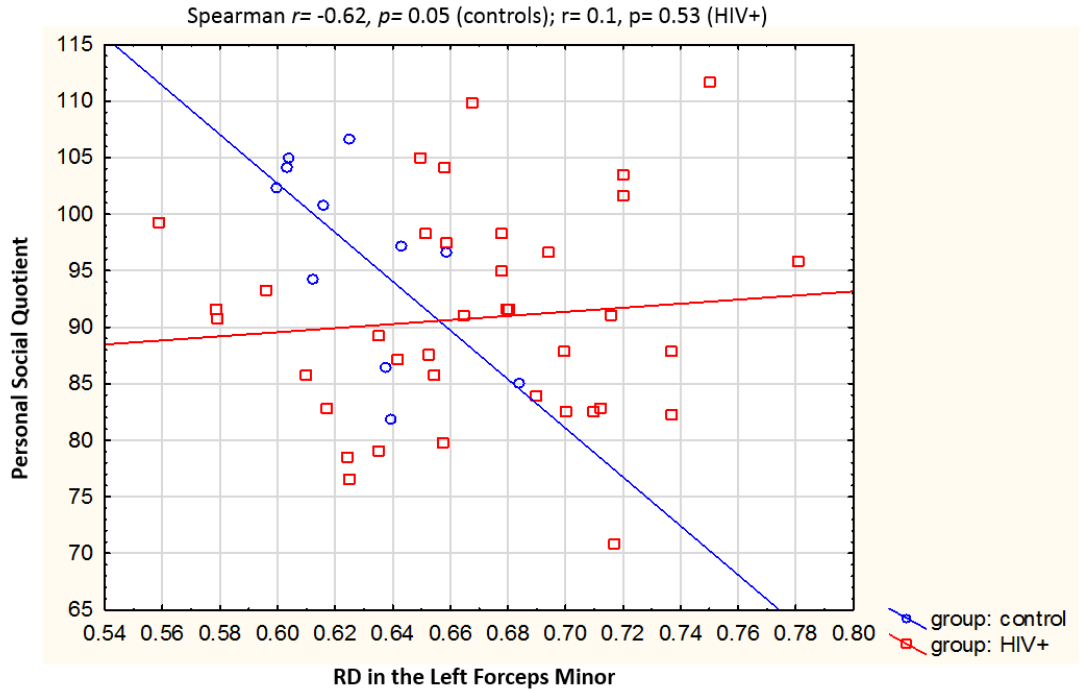
b.



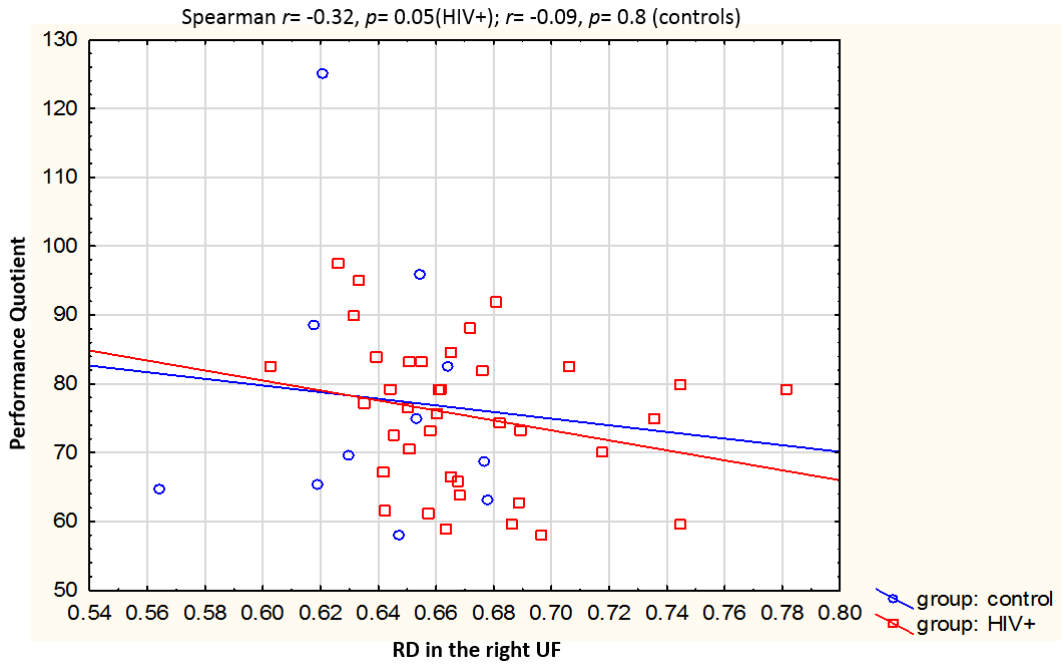
4.3c: Higher AD in the left forceps minor was related to better practical reasoning found in patients and controls.



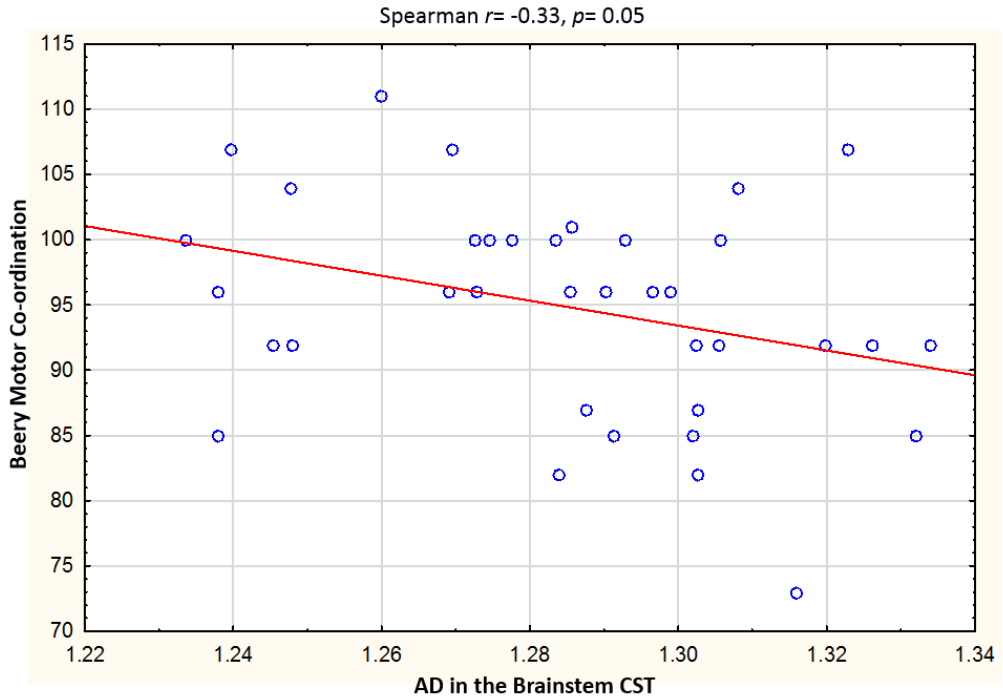
4.3d: In controls only, there is a relationship between decreased RD in the left forceps minor and better personal-social performance.



4.3e: In HIV+ children only, a negative correlation was found between performance and RD in the right UF



4.3f: In HIV+ children only, a negative correlation was found between Beery Motor Coordination and AD in the brainstem in the CST



CONCLUSION

The HIV epidemic has largely been controlled by ART in much of the developed world; however, the same cannot be said of sub-Saharan Africa (SSA), which carries the main HIV disease burden. South Africa alone has 4.5 million people on treatment - more than any other country in the world. [201] There are a large number of children growing up with HIV- disease who are on long-term treatment, placing them at higher risk to develop HIV related brain injury, cognitive impairment and treatment related neurological complications. Although ART has markedly reduced the incidence of HIV encephalopathy, milder forms of HIV-Associated Neurological Dysfunction (HAND) remain prevalent even in those children with low or undetectable HIV viral loads.

Neuroimaging findings in pediatric HIV such as atrophy, basal ganglia calcifications and white matter (WM) T2 hyperintensities have now been well described [94] however the more important microstructural cerebral abnormalities are predominantly identified on specialized MRI techniques such as diffusion tensor imaging (DTI). [174]

In this study, multiple high signal intensity WM lesions were documented on T2 /FLAIR MRI in 50% of HIV-infected patients at age 2 with a predominant frontal and parietal distribution. These patients started ART before 20 weeks of age. No differences in neurodevelopmental scores were found when comparing children with and without WMSA. Neither lesion load nor distribution showed significant correlation with neurodevelopmental scores or neurological examination. There was a trend for association of WMSA and longer time on ART, which initially raised the possibility that WMSA may be related to neurotoxicity of ART.

DTI at age 5 of HIV-infected children and a group of controls demonstrated decreased FA and increased MD in the HIV-infected group in several clusters of WM with symmetrical distribution, predominantly as a result of decreased myelination. Children in the early interrupted ART group, had lower FA compared to those receiving continuous treatment which disputed the ART neurotoxicity argument. The only neurodevelopmental domain with a trend of difference between the HIV+ children and controls was personal social quotient which correlated to improved myelination of the forceps minor in the control group. In addition, as a combined group there was a

negative correlation between visual perception and RD in the right superior longitudinal fasciculus and left inferior longitudinal fasciculus which may be related to the fact that these tracts, forming part of the visual perception pathway, are at a crucial state of development at age 5. HIV-associated WM damage may well account for the abnormalities in visual perception – which was identified in HIV-infected children in the study by Laughton et al., but was not clearly demonstrated in the Beery Visual perception test in the HIV-infected children and controls of our group.

DTI has become a popular method of studying the microstructure of WM and in particular the early changes observed in HIV. From the very first studies which made use of region of interest (ROI) analysis of fractional anisotropy (FA) in the brain of HIV-infected individuals it became clear that conventional MRI underestimates the degree of WM pathology [54,55,202].

ROI methods are dependent on the investigator's choice of region, but using these methods it has emerged that frontoparietal WM and the CC were regions particularly affected in adults [25,49,55]. Generally, there is decreased FA and increased MD which, to a variable degree, has been correlated with clinical and neurocognitive outcomes. Subsequent studies have utilized voxel-wise and whole brain FA analysis and more widespread WM damage has been reported [26,52,54,55,203].
DTI studies in children show similar regional WM changes as adults with lower FA and higher MD and RD in affected white matter compared to normal controls. Diverse groups of children with regards to age, socioeconomic background, treatment history and mode of infection were studied with consistent evidence of WM alterations despite early ART in a large number of these children [38,41,144,174]. DTI can therefore be utilized to quantify the degree of WM damage very early on in the disease.

DTI can be performed on conventional MRI scanners with the most common DWI approach being the pulsed-gradient spin-echo (PGSE) sequence with a single shot echo-planar imaging read out. A minimum of 6 non-collinear encoding directions are required to measure a full diffusion tensor [44,140]. Post processing may be complicated by subject motion and magnetic field inhomogeneities and should be corrected before calculating any subsequent quantitative diffusion maps. Pre and post process packages exist but these require experience for analysis. All these factors contribute

to the current inaccessibility of this specific MRI technique in everyday radiological practice [140,204].

Description in literature of the requirements and expertise needed to post process and interpret imaging such as DTI, fMRI and MRS are daunting with few radiologist signing up to equip themselves [122]. Very few radiological institutions in SSA have the backup and support of MR physicists who have a crucial role in establishing protocols and assist with these tasks. This is on the background that SSA bears 70 percent of the global HIV disease burden but only has 3 percent of the world's health workers and less than one percent of global health spending [205,206]. In resource limited countries problems of basic HIV care such as stock outs of ARV medication, lack of close monitoring of immunological parameters i.e. CD4 and VL and caregivers stopping medication are the reality and primary priority of HIV care [11]. For the near future DTI will be an important research tool only.

Until DTI and post processing is incorporated in standard imaging protocols, ROI measurement of FA may be utilized to assess microstructure of WM using a standard of reference for the clinical interpretation of pediatric DTI images [62].
Suggested ROI's include the CC, frontoparietal WM and within the corticospinal tracts.
Imaging research will continue to examine the immediate and long-term effects of HIV on the pediatric brain and will guide management and ARV treatment regimes. Successful treatment has lessened the severity of HIV related cognitive dysfunction and complications to such an extent that the imaging focus has shifted from structural to functional studies.
At the coalface however, few patients will have access to this technology and the radiologists working in this environment will still be integral to the team in diagnosis of HIV related brain disease.

In this study we clearly demonstrated that DTI offers insight into WM injury that occurs when clinical and developmental tests are relatively normal. This injury may relate to the age of myelination or development of WM structures in the brain but is not shown to be affected by treatment regimen as long as these are initiated early. Long-term effects of HIV on neurodevelopment forms part of on-going research that is done on this group of children who have now been followed up to 11 years of age.

The specific domains in which ongoing deficits have been reported such as visio-spatial would be of particular interest to study in the future.

IMPACT

Published articles directly related to this work:

1. Andronikou S, Ackermann C, Laughton B, Cotton M, Tomazos N, Spottiswoode B,
   Mauff K, Pettifor JM. *Correlating brain volume and callosal thickness with clinical and laboratory indicators of disease severity in children with HIV-related brain disease*. Childs Nerv Syst. 2014 Sep;30(9):1549-57. doi: 10.1007/s00381-014-2434-3. Epub 2014 May 23. PubMed PMID: 24853332.

2. Ackermann C, Andronikou S, Laughton B, Kidd M, Dobbels E, Innes S, van Toorn R, Cotton M*. White matter signal abnormalities in children with suspected HIV-related neurologic disease on early combination antiretroviral therapy*.
   Pediatr Infect Dis J. 2014 Aug;33(8):e207-12. doi: 10.1097/INF.0000000000000288.
   PubMed PMID: 24595047; PubMed Central PMCID: PMC4153800.

3. Andronikou S, Ackermann C, Laughton B, Cotton M, Tomazos N, Spottiswoode B, Mauff K, Pettifor JM. *Corpus callosum thickness on mid-sagittal MRI as a marker of brain volume: a pilot study in children with HIV-related brain disease and controls*. Pediatr Radiol. 2015 Jul;45(7):1016-25. doi: 10.1007/s00247-014-3255-y.
   Epub 2015 Jan 27. PubMed PMID: 25620244.

4. Ackermann C, Andronikou S, Saleh MG, Laughton B, Alhamud AA, van der Kouwe A, Kidd M, Cotton MF, Meintjes EM. *Early Antiretroviral Therapy in HIV-Infected Children Is Associated with Diffuse White Matter Structural Abnormality and Corpus Callosum Sparing*. AJNR Am J Neuroradiol. 2016 Dec;37(12):2363-2369.
   Epub 2016 Aug 18. PubMed PMID: 27538904; PubMed Central PMCID: PMC5161701.

5. Ackermann C, van Toorn R, Andronikou S*. Human immunodeficiency virus-related cerebral white matter disease in children*. Pediatr Radiol. 2018 Nov 29. doi: 10.1007/s00247-018-4310-x. [Epub ahead of print] PubMed PMID: 30498850.

Presentation of parts of or entire study at meetings:

1. Poster presentation at the 19th Annual Meeting of the Organization for Human Brain Mapping, Seattle 2013: FA as marker for White Matter Abnormalities in Children with HIV on ART.

2. Poster presentation at the 19th Annual Meeting of the Organization for Human Brain Mapping, Seattle 2013: Effects of Motion Corrupted Volumes on DTI Findings between HIV-infected and Healthy Children.

3. Poster presentation at ESPR – 37th postgraduate course and 51st Annual Meeting of the European Society of Paediatric Radiology, Amsterdam 2014: FA as a marker for White Matter Abnormalities in Children with HIV on early ART.

4. Oral presentation Annual Academic day – Stellenbosch University 2014: Quantifiable Imaging Abnormalities of White Matter using FA in HIV infected children compared to controls.

5. Oral presentation JN and WLS Jacobsen Lecture SORSA Durban 2017: Quantifiable Imaging abnormalities of white matter using FA in HIV infected children compared to controls.

6. Oral presentation Marie Grobelaar memorial lecture, Annual Academic day – Stellenbosch University 2018: Quantifiable Imaging abnormalities of white matter using FA in HIV infected children compared to controls.

7. Oral presentation 5th African Society of Radiology (ASR) and 54th Egyptian Society of Radiology conference, Cairo January 2019: Diffusion tensor imaging in paediatric HIV.

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Appendix A
Summary of other imaging studies on the CHER cohort

Neuroimaging of Early Treated Perinatally Infected Children from 5 Years to Adolescence in Cape Town, South Africa

André van der Kouwe1, Mark Cotton2, Marcin Jankiewicz3, Martha Holmes3, Barbara Laughton2, Ernesta Meintjes3 1Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, MA, USA, 2Family Center for Research with Ubuntu, Department of Pediatrics and Child Health, Tygerberg Children's Hospital and Faculty of Health Sciences, Stellenbosch University, Stellenbosch, South Africa, 3Medical Imaging Research Unit, Division of Biomedical Engineering, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

Background:

HIV and TB are the leading causes of natural death in South Africa[1]. HIV treatment and prevention are currently financed primarily (approximately 76%) by the South African (SA) Government while 21% is funded by PEPFAR[2]. HIV treatment constitutes most of the investment by PEPFAR of more than $5 billion in South Africa since 2004[3]. When PEPFAR started, prevention of mother-to-child transmission (PMTCT) of HIV was still experimental. In 2006, the first infants were recruited to the Children with HIV Early antiRetroviral (CHER) study in Soweto and Cape Town. This study aimed to determine whether early limited antiretroviral treatment (ART), soon after primary infection, would be more effective in preventing disease progression than the standard treatment at the time of deferring ART until clinical criteria were met (initially CD4% < 20%). The SA National Department of Health (SANDH; with PEPFAR support) supplied first and second line ARVs. First line ART included zidovudine and lamivudine (supplied by GlaxoSmithKline) and lopinavir-ritonavir (supplied by SANDH). After a year it was observed that early ART reduced the risk of death by 75% compared with deferred ART and the independent Data Safety Monitoring Board recommended that deferment be discontinued. Subsequently it became the standard of care to start ART immediately[4]. The impact of deferring or interrupting ART on neurodevelopment was also of interest. It was observed that early ART improved outcomes in a neurodevelopmental sub-study of the Cape Town cohort[5]. Most of the infant participants co-enrolled on a linked vaccine study that included controls[6]. In 2011, when the Cape Town children reached 5 years of age, we studied them for the first time with magnetic resonance neuroimaging (MRI) at the Cape Universities Brain Imaging Centre (CUBIC), since renamed the Cape Universities Body Imaging Centre. This collaborative work between Stellenbosch University (SUN), the University of Cape Town (UCT) and the Massachusetts General Hospital (MGH), was supported by the Global Brain Disorders Program of the NIH Fogarty International Center (FIC), the NIMH and the NICHD and enabled us to continue to study the children after the CHER study ended. Our projects investigate the longitudinal effects of HIV exposure and/or infection, and ART, on the developing brain, from childhood through adolescence. The FIC program requires capacity building in the low- to middle-income partner country and we developed new MRI techniques together, especially for imaging children.

Methods:

We initially enrolled 114 children from the Cape Town cohort of the CHER study and linked vaccine study. Of these, 77 were HIV infected (42 female, 35 male), 18 were exposed but uninfected (9 female, 9 male), and 19 were unexposed (7 female, 12 male). Participants were assessed clinically at regular intervals. Neuroimaging and

cognitive testing were done at 5, 7, 9 and 11 years. Imaging was performed on a 3 T Siemens Allegra MRI scanner until 9 years and then on a 3 T Siemens Skyra scanner. The research-dedicated 3 T scanner is unique in Sub-Saharan Africa. The scanning protocol included T1-weighted structural scans, diffusion and functional imaging, and spectroscopy. These techniques provide information about possible differences or disruptions in brain structure, white matter connectivity, functional brain networks, and brain metabolism, respectively.

Results:

Early ART appears to be protective against most of the damaging effects of HIV on the brain. HIV encephalopathy was more common in ART-deferred children[5]. Poor immune health at infancy (gauged by CD4/CD8) predicted reduced NAA and choline in the basal ganglia at 5 years[7]. Choline levels in basal ganglia and frontal gray matter continued to differentiate infected and uninfected children at 11 years[8]. The 9 year old children exhibited lower NAA and glutamate in basal ganglia than unexposed control children. Interestingly HIV exposed but uninfected (HEU) children also exhibited these differences. Creatine and choline were also lower in HEU children than control children[9].

White matter signal abnormalities were observed earlier in some infected children in clinical T2 images[10]. Despite early ART, white matter damage (gauged by fractional anisotropy) was observed at 5 years primarily in the corticospinal tracts[11]. This damage persisted at 7 years and new damage appeared[12]. We continue to see white matter differences at 9 years in infected children[13]. Reduced long range and increased short range connectivity was observed in infected children at 7 years using functional connectivity. Poor immune health at infancy correlated with these differences[14]. In an fMRI study of hearing in the same children, group differences (infected vs. controls) in auditory cortex activation were observed on the left side in response to pure audible tones.

Brain morphometric differences were observed at all ages. Infected children had larger nuclei accumbens and putamens bilaterally, and smaller corpora callosa at 5 years[15]. Infected children exhibited larger volume reductions in caudate, putamen and globus pallidus over the range of 5 to 11 years[16]. Although damage was ongoing despite early ART and viral suppression at infancy, early ART reduced this damage. At 7 years, infected children exhibit reduced cortical gyrification and cortical thickness bilaterally in medial occipital cortex[17].

In associated studies, at 11 months of age, children who received early ART did substantially better on Griffiths Mental Development Scales locomotor and general scores than children on deferred ART. Children on early ART did as well as uninfected children except on the locomotor score[5]. Locomotor deficits appeared to resolve at 5 years, however visual perceptual deficits were noted in all HIV treatment groups[18]. Even with relatively early ART, cognitive deficits are evident in infected children at school age, suggesting that immediate ART and additional interventions during infancy/childhood may be helpful[19]. We observed that cortical thickness was associated with neuropsychological outcomes in HIV infected and uninfected children at 5 years. Our analyses of cognitive performance and its relationship to neuroimaging are ongoing.

In technical work, these projects contributed to the development of “volumetric navigators” (vNavs) for real-time tracking and motion correction during high resolution anatomical MRI, necessary to scan children who tend to move in the scanner[20,21]. These methods are currently used in two large multi-center brain imaging studies viz. the Human Connectome Project (HCP) aging and development

studies[22] and the Adolescent Brain Cognitive Development (ABCD) study[23]. Our projects also gave rise to double-echo vNavs for real-time motion and magnetic field correction in spectroscopy, a project originally assigned to a UCT student[20,24]. Motion and shim correction were added to several specialized scan types, including MEGA-SPECIAL for measuring GABA[25], CEST for measuring glycogen[26], and diffusion imaging[27]. A UCT student also developed external hardware for motion tracking in MRI[28].

Conclusion:

While perinatal ART and HIV exposure has a long-term impact on brain development, early ART with possible later interruption is highly effective at suppressing the virus, and clearly neuroprotective. PMTCT therapy is now extremely effective, giving rise to a new population of exposed, uninfected children. We have recruited a new cohort of neonates to evaluate the effects of early ART and HIV exposure without infection on the developing brain. None of the infected mothers enrolled on the study gave birth to infected infants. We will also follow the CHER children and controls through adolescence. The children in this study were born into a world in which PEPFAR had just begun, and they grew up alongside PEPFAR support. The first of these children will reach the age of 15 in 2020, two years after the 15th anniversary of the inception of PEPFAR. Their world would have been very different and their lives and those of many of their peers most likely curtailed if it were not for the generosity and success of PEPFAR.

Acknowledgments:

R21MH096559, R01DC015984 (Peter Torre III), R01HD071664, R01HD085813, R01HD093578, R01HD099846, SANDH, PEPFAR, GSK, The Global Fund.

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APPENDIX B

UNIVERSITEIT•STELLENBOSCH•UNIVERSITY
jou kennisvennoot • your knowledge partner

Ethics Letter

01-Nov-2016
Ackermann, Christelle C

Ethics Reference #: N07/09/208
Title: "Diffusion weighted imaging of the brain in paediatric HIV"

Dear Dr. Christelle Ackermann

Your request for extension/annual renewal of ethics approval dated 20 September 2016 refers.

The Health Research Ethics Committee reviewed and approved the annual progress report you submitted through an expedited review process.

The approval of the research project is extended for a further year.

Approval date: 01 November 2016

Expiry date: 31 October 2017

Where to submit any documentation
Kindly submit **ONE HARD COPY** to Elvira Rohland, RDSD, Room 5007, Teaching Building, and **ONE ELECTRONIC COPY** to ethics@sun.ac.za

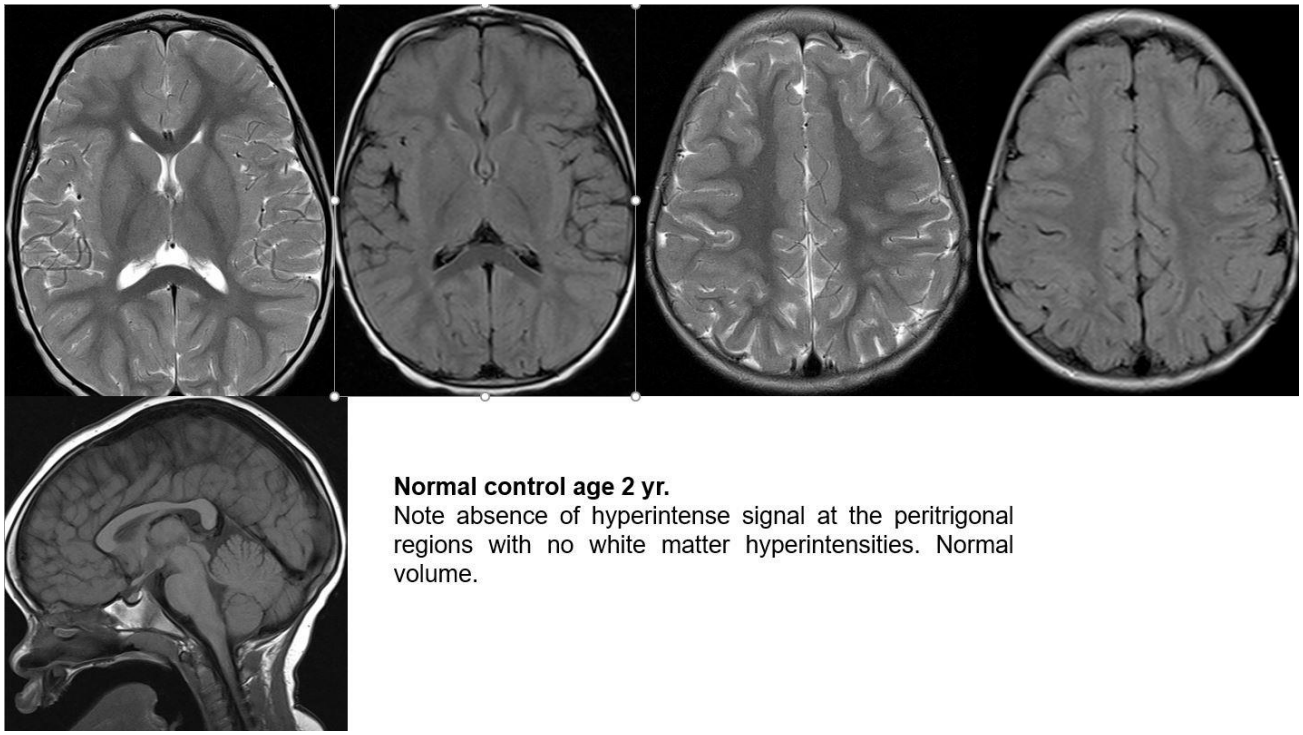
Please remember to use your **protocol number (N07/09/208)** on any documents or correspondence with the HREC concerning your research protocol.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005240 for HREC1
Institutional Review Board (IRB) Number: IRB0005239 for HREC2

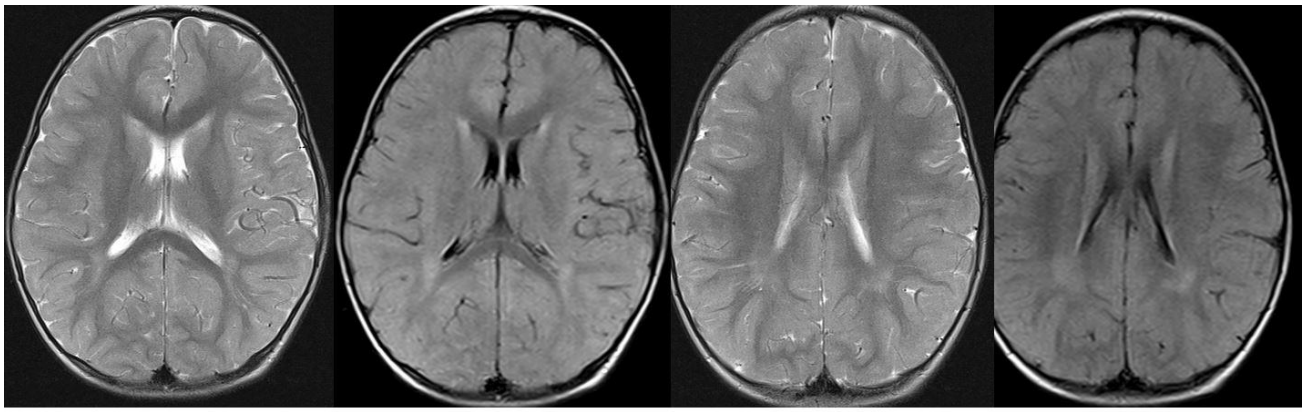
The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Good Clinical Pracices Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Sincerely,
Francis Masiye
REC Coordinator
Health Research Ethics Committee 2

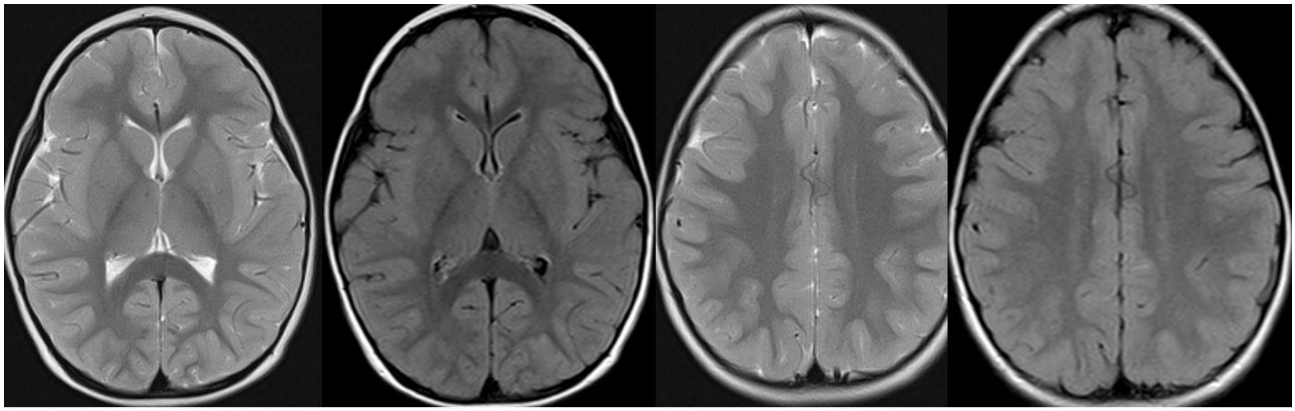
APPENDIX C



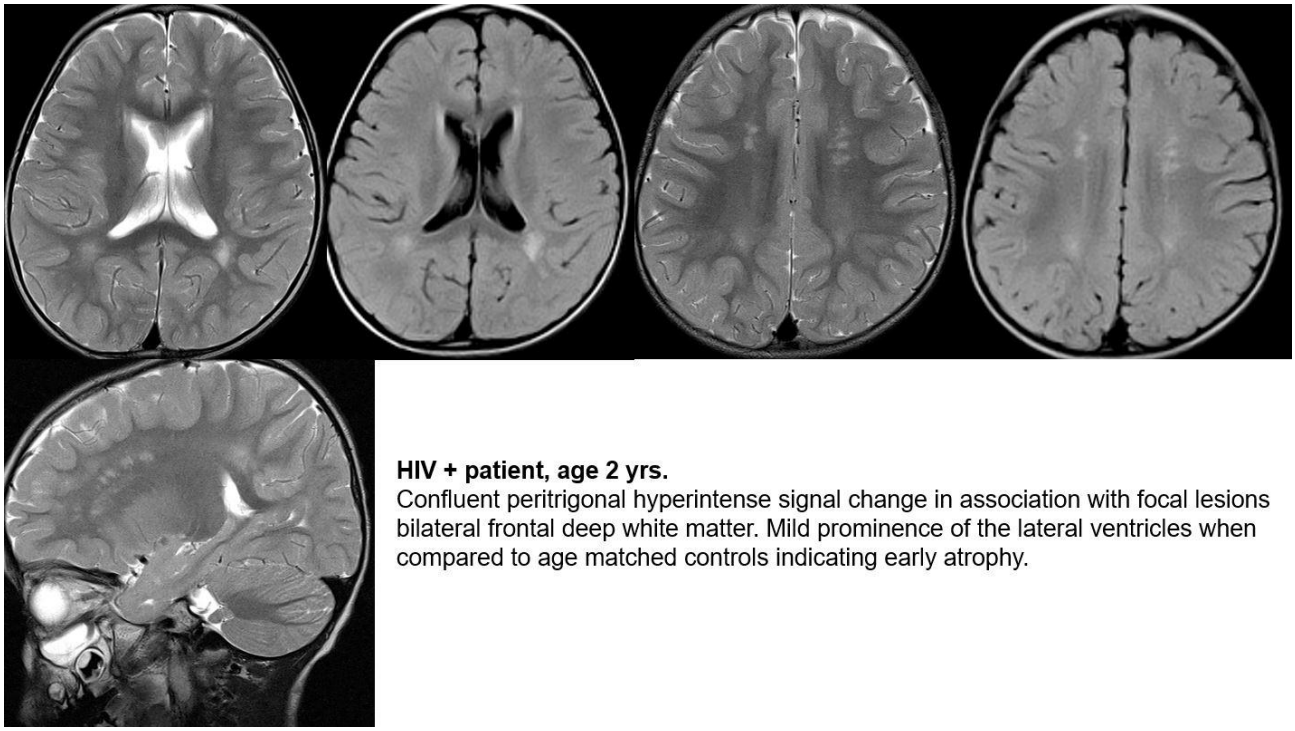
Normal control age 2 yr.
Note absence of hyperintense signal at the peritrigonal regions with no white matter hyperintensities. Normal volume.



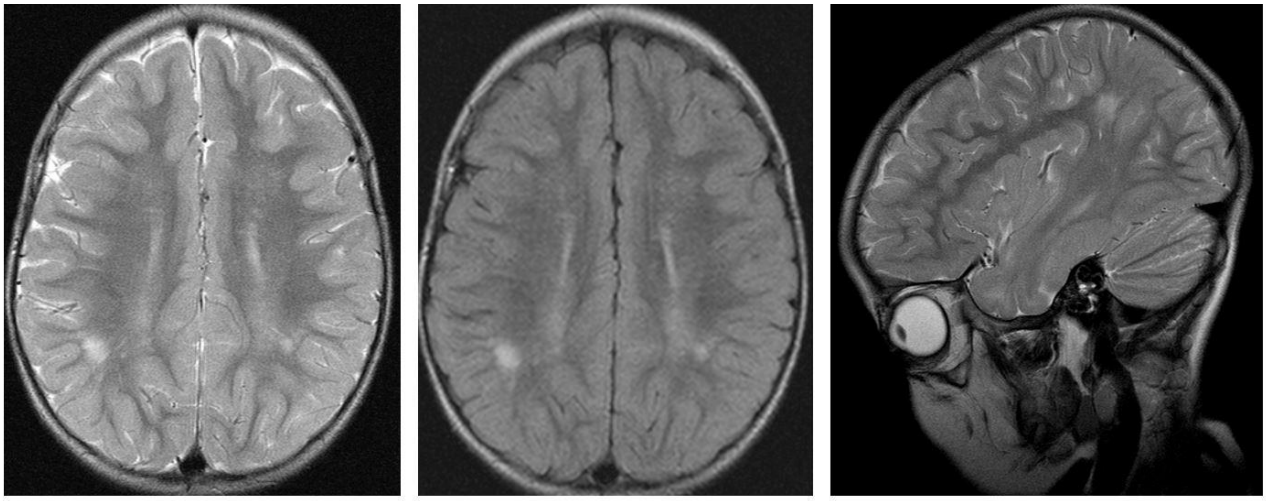
Normal control age 2 yrs.
Note linear to early confluent areas of hyperintensity at the peritrigonal regions, in keeping with perivascular spaces and terminal myelination. These areas can be difficult to interpret in the HIV patient, particularly in this age group.



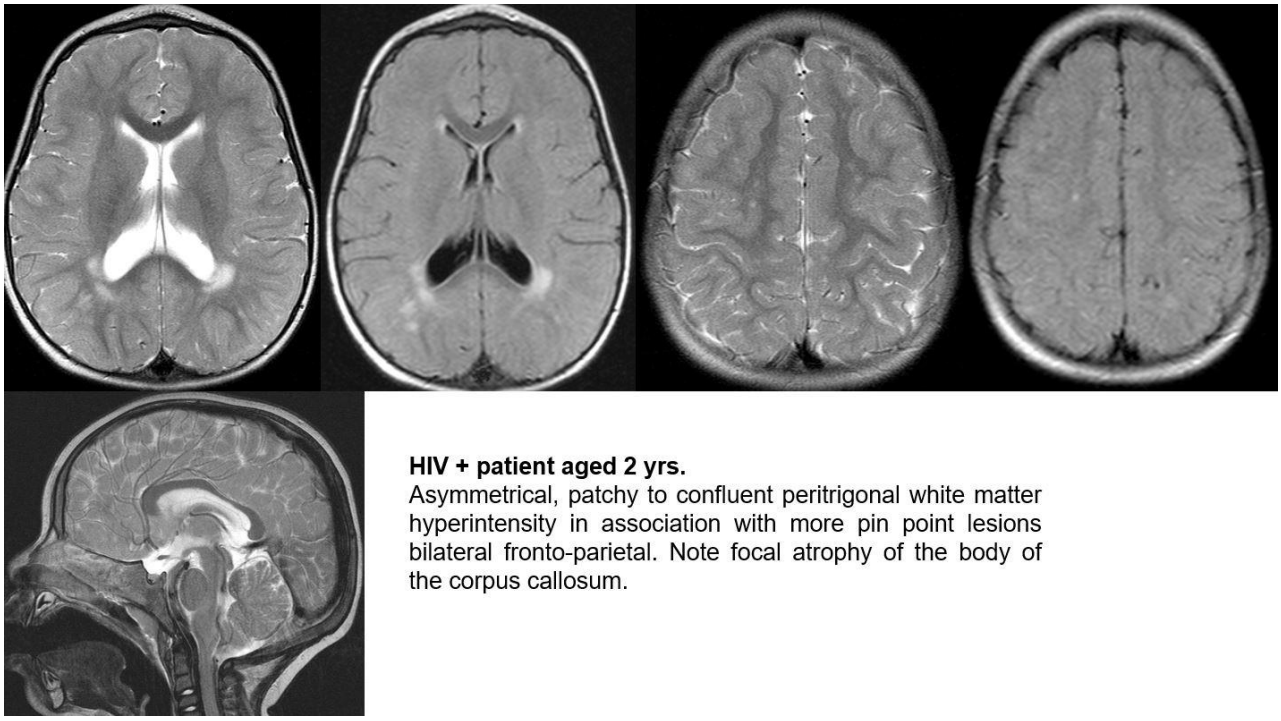
Normal control age 3 yrs.
No abnormal white matter hyperintense signal change. Normal brain volume.



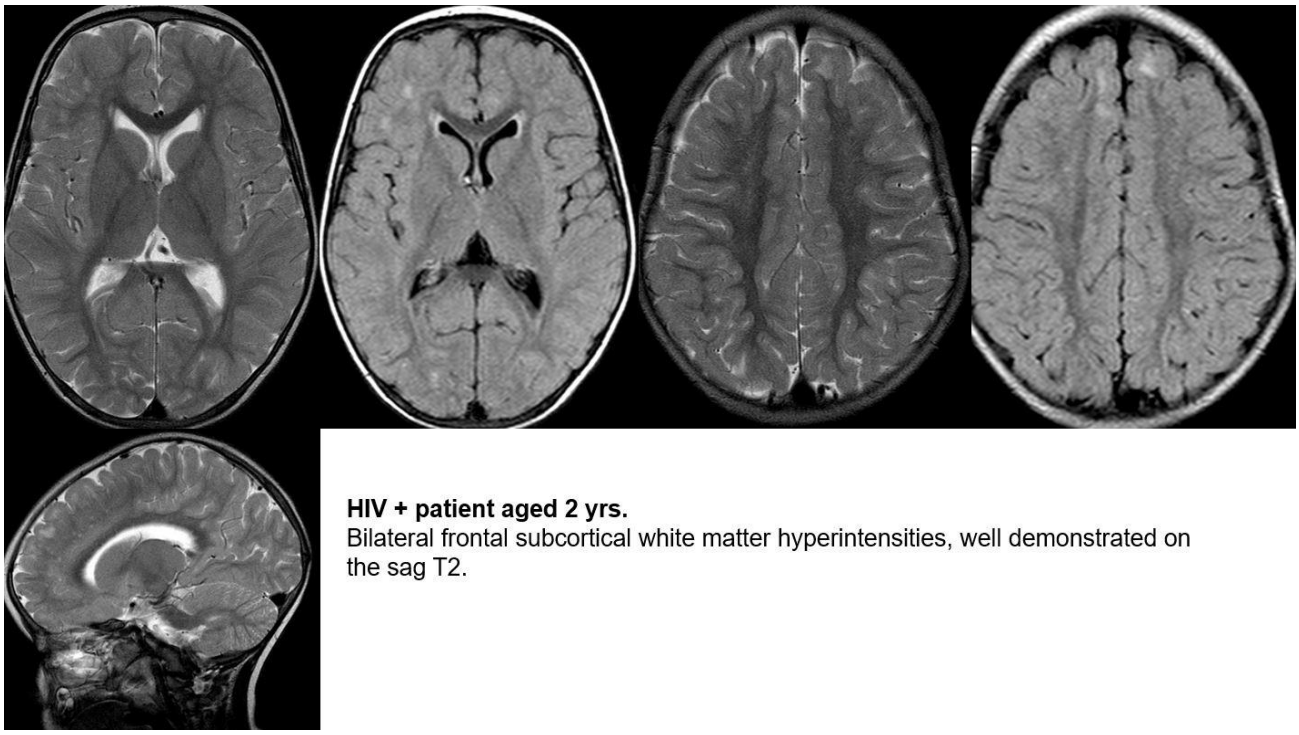
HIV + patient, age 2 yrs.
Confluent peritrigonal hyperintense signal change in association with focal lesions bilateral frontal deep white matter. Mild prominence of the lateral ventricles when compared to age matched controls indicating early atrophy.



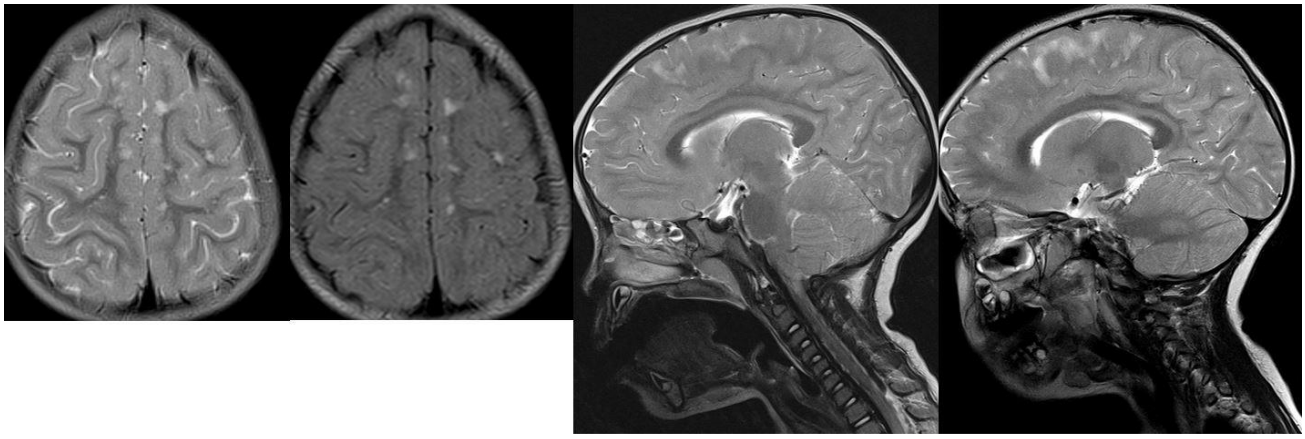
HIV + patient, age 2yrs.
Note the asymmetrical confluent white matter signal change in the posterior parietal regions, extending to the peritrigonal areas which is notable different form the normal peritrigonal white matter hyperintensity seen in terminal myelination and perivascular spaces.



HIV + patient aged 2 yrs.
Asymmetrical, patchy to confluent peritrigonal white matter hyperintensity in association with more pin point lesions bilateral fronto-parietal. Note focal atrophy of the body of the corpus callosum.



HIV + patient aged 2 yrs.
Bilateral frontal subcortical white matter hyperintensities, well demonstrated on the sag T2.



HIV + patient, aged 2 yrs.
Bilateral frontal patchy to confluent subcortical white matter hyperintensities