

# Effects of Advances in Therapy on the Neuropathology of HIV Infection

## Introduction

Since the emergence of the Human Immunodeficiency Virus (HIV) pandemic in the early 1980's considerable progress has been made in our understanding of this retrovirus and of its effects on the human body, yet despite years of dedicated research there remain many unanswered questions. The introduction first of Zidovudine (AZT) in 1987 and then highly active anti-retroviral therapy (HAART) [Box 1] in 1997 has undoubtedly had a beneficial effect on disease progression but has not eliminated infection. By means of preventing different aspects of viral replication, HAART generally succeeds in reducing plasma HIV load to undetectable levels and partially restores CD4 lymphocyte counts. Thus HIV has increasingly become a chronic infection with relatively long life expectancy. From the point of view of understanding the disease process it is essential to evaluate the effects of therapy on the pathogenesis. This review will present a comparison of the neuropathology of HIV before and after the advent of HAART.

**Box 1: HAART components and drugs in use**

Class of drug	Name of drugs
Nucleoside Reverse Transcriptase Inhibitors	AZT Zidovudine
	DDI Didanosine
	d4T Stavudine
	3TC Lamivudine
	DDC Zalcitabine
	ABC Abacavir
Non-Nucleoside Reverse Transcriptase Inhibitors	NVP Nevirapine
	EFV Efavirenz
Protease Inhibitors	IDV Indinavir
	SQV Saquinavir
	RTV Ritonavir
	NFV Nelfinavir

Soon after the discovery of HIV the central nervous system (CNS) was identified as a major target for virus induced changes and for opportunistic conditions [Box 2]. Cognitive impairment was present in 20-30% of pre-HAART cases, with many going on to develop HIV associated dementia (HAD). HAD presented as a subcortical dementia with cognitive symptoms including impaired memory and concentration, mental slowing and difficulty in multi-tasking. Behavioural difficulties included apathy, withdrawal, irritability and personality changes, while motor changes included clumsiness or slowing of fine movement and gait unsteadiness. To date the exact pathogenesis of HAD remains elusive. Although there have been reports of HIV DNA recovered from neurones<sup>1</sup> the consensus opinion is that direct infection of neurones by HIV does not occur and is therefore not a contributing factor in HAD. Microglia appear to be the only cell type capable of supporting productive HIV infection in the brain. HIV encephalitis (HIVE) is common in infected individuals who develop dementia; however neither HIVE nor brain viral load correlate well with dementia. The pathological features which are most closely linked with dementia are the activation of microglial cells and the influx of macrophages particularly in the basal ganglia.<sup>2</sup> Neurotoxicity and the underlying causes of neurological symptoms are most likely multifactorial in origin.

## Pre-HAART neuropathology in HIV infected subjects

When examined before the onset of AIDS, the brains of HIV infected individuals show relatively minor changes. There is no evidence of HIVE, opportunistic infections or lymphomas, though there is often a low grade lymphocytic leptomeningitis and perivascular lymphocytic cuffs found within the brain.<sup>3</sup> Myelin pallor, gliosis and macrophage activation have also been reported.

With the onset of AIDS, the most significant pathological features in the brain are the presence of HIVE and/or opportunistic conditions in up to 50% of individuals. The immune privileged status of the brain and consequent restricted/limited potential for immune reactions to occur within the brain, coupled with the failure of the peripheral/systemic immune system in AIDS, make the brain a prime site for the development of opportunistic conditions. The major AIDS-related CNS opportunistic pathologies, together with their aetiological agents, are given in Box 2. It is often difficult to assess the changes induced directly by HIV in AIDS patients if confounding opportunistic conditions are also present.

White matter damage is apparent in some subjects, with and without HIVE, varying from minor pallor to widespread breakdown, the latter frequently showing axonal damage in the form of  $\beta$  amyloid precursor protein accumulation in white matter varicosities and axonal bulbs (Figure 1). Examination of brains with no evidence of opportunistic conditions reveals changes in the blood vessels including calcification, vasculitis and infarcts. In AIDS brains with no opportunistic conditions and no HIVE, the perivascular lymphocytic infiltration observed in pre-symptomatic subjects is not usually noted, although in HIVE some lymphocytes are commonly found in the brain parenchyma.

Pre-HAART, approximately 50% of AIDS cases showed vacuolar myelopathy with myelin pallor and macrophage accumulation in the dorsolateral tracts in the spinal cord.

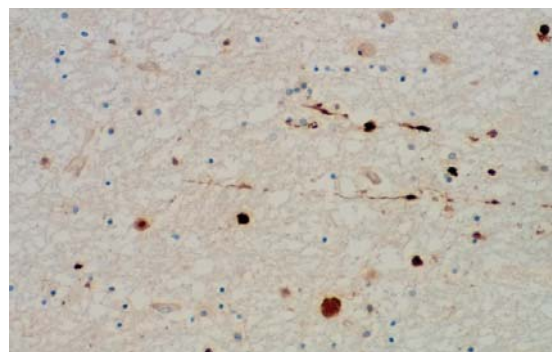


Figure 1: Accumulation of  $\beta$ -amyloid precursor protein ( $\beta$ APP) in white matter varicosities and axonal bulbs. (x100).

## Changes in the incidence of HIVE and opportunistic conditions in the brain since the introduction of HAART

Reports of recent autopsy series show a decrease in HIVE and most opportunistic CNS conditions in subjects treated with HAART [Box 2]. In Edinburgh the incidence of HIVE has fallen by approximately 50%, as has evidence of Cytomegalovirus (CMV) infection in the brain; rates of toxoplasma infection have fallen to almost zero; in contrast progressive multifocal leukoencephalopathy (PML)



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**Box 2: Major CNS pathologies in HIV infected individuals**

Major CNS pathologies	Aetiological agent	Pathological basis	Change in incidence since the introduction of HAART (based on worldwide reports)	References
HIV encephalitis (HIVE) Figure 2	HIV [retrovirus]	Infection of microglia/macrophages leading to formation of multinucleated giant cells and microglial nodules. Immuno reactivity for HIV antigens (p24, gp41).	↓	[13, 14]
Cryptococcal meningo-encephalitis	Cryptococcus neoformans [encapsulated fungus]	Cryptococcus proliferates in the sub-arachnoid space and in the deep grey matter. Immune response in HIV is often sparse with focal collections of macrophages and formation of giant cells.	↓	[7]
CMV encephalitis Figure 3	Cytomegalovirus [herpes virus]	Variable pathology, from isolated cytomegalic cells to severe necrotising hemorrhagic encephalitis. The most common pattern is a diffuse microglial nodular encephalitis in deep grey matter.	↓	[13, 14]
Toxoplasmosis	Toxoplasma gondii [Protozoa]	Focal lesions of varying size often with necrotic centre. Granulomas may be present and microglial nodules may contain encysted organisms or free tachyzoites.	↓	[13, 14]
Primary central nervous system lymphoma (PCNSL) Figure 4	Epstein-Barr virus (EBV) [herpes virus]	Often diffuse multi-focal tumours. Lymphoma cells tend to be distributed around blood vessels forming concentric layers of perivascular cuffs. Malignant cells are of B lymphocyte origin and are invariably infected with EBV and express the viral antigens EBNA-2 and LMP-1	↓	[15]
Progressive multi-focal leukoencephalopathy (PML)	JC virus [polyomavirus]	Foci of myelin pallor in the subcortical white matter or at grey/white matter junction. Viral inclusions in oligodendrocytes and bizarre enlarged astrocytes are usually present. Viral DNA is detectable by in-situ hybridisation.	– (no change)	[13]
VZV encephalitis	Varicella Zoster Virus [Herpes virus]	A variety of neuropathologies may occur including: multifocal encephalitis, ventriculitis, necrotising myelitis and vasculopathy resulting in cerebral infarction.	Pre-HAART VZV was a rare CNS complication. Post-HAART reports suggest the incidence of VZV may be increasing	[14]

and Primary CNS lymphoma (PCNSL) show much smaller changes [Box 3]. Reports from other studies around the world also suggest significant decreases in most of the common CNS opportunistic conditions, particularly those associated with low CD4 counts [Box 2]. It is interesting to note that the overall incidence of conditions such as PCNSL decreased dramatically with the introduction of HAART. However, after stratification on CD4 cell count, the incidence changes little for those who still have low CD4 counts despite therapy. The proportion of patients with low CD4 count, who are at greatest risk of developing lymphoma, has greatly decreased since the introduction of HAART.

Gray et al<sup>4</sup> have also described 'burnt out' forms of HIVE, Varicella Zoster Virus (VZV)

encephalitis and toxoplasmosis in which neither inflammatory infiltrates nor the causal agent could be detected. It is plausible that these 'burnt out' lesions may have resulted from clearance of virus or protozoa by an immune system re-constituted by therapy, with the patients then dying later of an unrelated cause. There have also been reports of subjects who had poorly controlled HIV replication in the brain despite HAART and who displayed intense perivascular infiltration of macrophages and lymphocytes, together with widespread myelin loss and axonal injury, thought to be due to rapid influx of inflammatory cells into the CNS.<sup>5</sup> Vacuolar myelopathy has not been reported in HAART treated individuals.

### Cognitive deficits in HIV (pre and post HAART)

The introduction of AZT led to a decrease in the incidence of HAD and the subsequent introduction of HAART has caused a significant further reduction. However the overall prevalence of HAD appears to be rising, probably as a result of infected individuals living longer with effective therapy.<sup>6</sup> In the pre-HAART era HAD was invariably associated with patients with low blood CD4 counts (<200). Since the introduction of HAART the number of cases who have low CD4 counts and dementia has decreased significantly while the number who develop dementia and have higher CD4 counts (>200) has remained relatively stable.<sup>7</sup> Thus the proportion of dement-

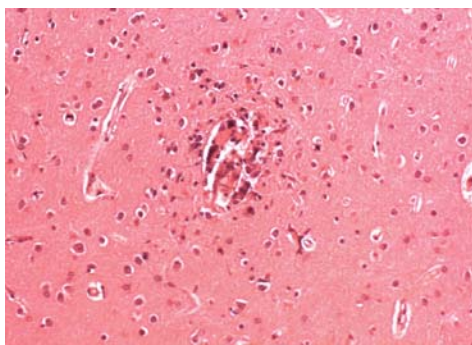


Figure 2: HIVE with giant cells (H&E) (x100)

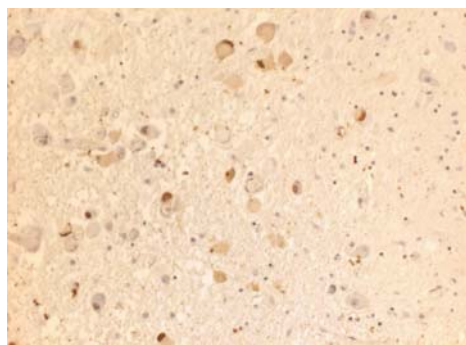


Figure 3: Cytomegalovirus (CMV) encephalitis (x100)

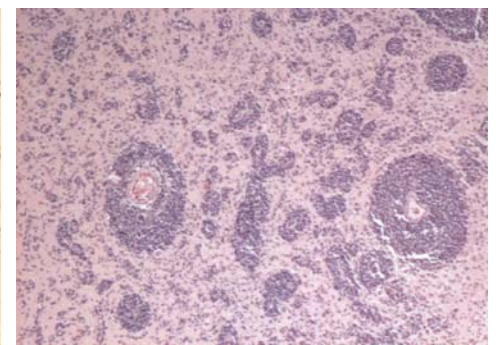


Figure 4: PCNSL - lymphoma cells forming concentric rings surrounding blood vessels. (x40)

**Box 3: Incidence of HIV and CNS opportunistic conditions at autopsy in Edinburgh**

	<b>Pre-HAART (n=228)</b>	<b>Post-HAART (n=44)</b>	<b>% change</b>
<i>HIVE</i>	21.5	6.8	- 68
<i>Lymphoma</i>	5.3	6.8	+ 28
<i>Toxoplasma</i>	4.4	0	- 100
<i>CMV</i>	9.2	4.6	- 50
<i>PML</i>	2.2	2.3	+ 5

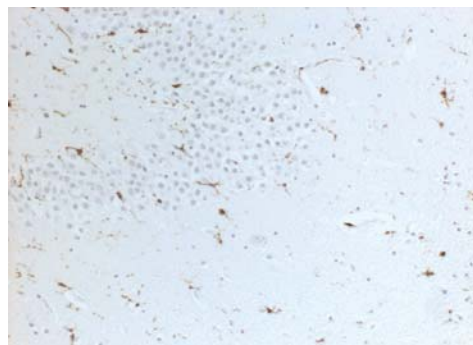


Figure 5: CD68 positive microglia in the hippocampus of a HAART treated subject. (x100)

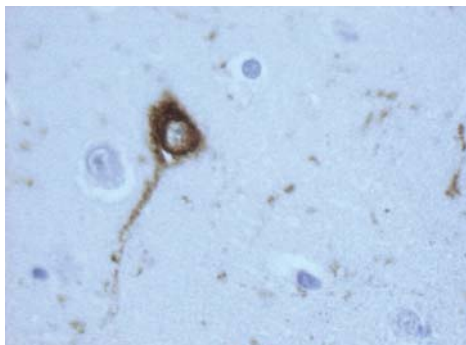


Figure 6: Tau (AT8) in the hippocampus of a HAART treated subject - Neurofibrillary pre-tangle. (x400)

ed subjects with CD4 counts greater than 200 has increased since the introduction of HAART. At the same time, a less severe neuropsychological dysfunction, known as minor cognitive motor disorder (MCMD), has become more common than HAD. Symptoms of MCMD include impaired attention, impaired memory, slowed movement and personality changes. Symptoms in MCMD are milder than HAD and have less impact on daily life.

The reports of Brew and Cysique et al., utilising PET scans and neurocognitive assessment, suggest an increasing involvement of the hippocampus in cognitive dysfunction in HAART treated subjects.<sup>8,9</sup> Early reports in the pre-HAART era linked HAD to basal ganglia dysfunction (i.e. a subcortical dementia).<sup>2</sup> Given the increase in life expectancy of HAART treated subjects, the report by Valcour et al<sup>10</sup> suggesting that older age is associated with increased prevalence of HAD in HIV infected subjects, gives cause for concern for the future of this population.

### Clinical markers of cognitive defects (HIV dementia)

In contrast to observations in pre-HAART individuals in whom there was a relationship between cerebrospinal fluid (CSF) or plasma markers and the neurological status, a recent study by McArthur et al<sup>11</sup> has shown no such relationship in HAART treated individuals. These markers included CSF HIV RNA levels and plasma levels of monocyte chemoattractant protein 1 and tumor necrosis factor alpha. HAART may substantially attenuate the degree of central nervous system HIV infection and immune activation, and in HAART users, the CSF HIV RNA load may fail to discriminate between milder degrees of HAD and MCMD.

### Recent findings in the CNS in HAART treated subjects

Although patients benefit from HAART in terms of control of viral replication and immune reconstitution, the long term effects of both HIV infection and of therapy on the brain remain uncertain.

Analysis of microglia/macrophage levels has shown that levels in the basal ganglia in HAART treated cases are greater than those observed in pre-symptomatic cases before the advent of HAART, though lower than those observed in AIDS cases. However in the hippocampus a large increase in microglial activation is observed in HAART treated subjects, above the levels previously seen in both pre-symptomatic and AIDS cases, despite there being no obvious stimuli such as HIV or opportunistic conditions present within these brains (Figure 5). This degree of neuroinflammation gives cause for concern as to the long term consequences for patients maintained in this state. In the non-HIV infected population neuroinflammation has been linked with Alzheimer's disease. A recent report by Green et al<sup>12</sup> has demonstrated that the deposition of beta-amyloid, one of the key pathological characteristics of Alzheimer's disease, is common in HIV positive patients treated with HAART. In similar studies our group have demonstrated the increased presence of hyperphosphorylated Tau protein, another key pathological characteristic of Alzheimer's disease and other dementias, in the hippocampus of HAART treated subjects (Figure 6).<sup>13</sup>

### Future challenges

While the incidence of dementia may be decreasing there has been limited study of the minor and more subtle neuropathological changes in HAART treated individuals, which may become significant for patients who live longer. In particular the reports of increased deposition of both amyloid and hyperphosphorylated tau in HAART treated individuals require further investigation. To date most pathological studies have focused on the overt major pathologies and associated dementia. As the incidence of these decreases with improved therapy, future studies should aim to determine the pathological basis of the more minor cognitive defects and of psychological symptoms such as depression which contribute significantly to neuromorbidity in HIV infected subjects.

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