Influence of HAART on HIV-Related CNS Disease and Neuroinflammation

I. C. Anthony, PhD, S. N. Ramage, BSc, F. W. Carnie, P. Simmonds, PhD, MRCPath, and J. E. Bell, MD, FRCPath

Abstract

Neuroinflammation has an established link with AIDS-related dementia but has not been investigated in the post-highly active antiretroviral therapy (HAART) era. In this autopsy study we examined post-HAART cases in Edinburgh for the presence of HIV-related pathology and in well-treated cases for evidence of neuroinflammation. We focused on basal ganglia and the hippocampus, 2 key areas of the brain for cognitive functioning and compared pre- and post-HAART cases for neuroinflammatory status. We find evidence, post-HAART, that there is a high level of microglial/macrophage activation that is comparable with the levels seen, pre-HAART, in HIV encephalitis (HIVE) and AIDS cases. This result was maximal in the hippocampus where microglial/macrophage upregulation in the HAART-treated group exceeded that seen in HIVE. In the basal ganglia, HAART-treated cases showed significantly higher levels of CD68-positive microglia/macrophages than in control brains (p = 0.004), and in the hippocampus levels were significantly higher than those seen in control cases, pre-HAART AIDS, and presymptomatic brains (p = 0.01). However, lymphocyte levels in the areas examined were low in HAART-treated cases. We conclude that there is a surprising degree of ongoing neuroinflammation in HAART-treated patients, particularly in the hippocampus. This may pose a threat for the future health of individuals maintained long-term on HAART therapy.

Key Words: HAART, HIV, Lymphocytes, Microglia, Neuroinflammation.

INTRODUCTION

Approximately 20 years ago at the outset of the AIDS epidemic, the emergence of neurological disorders and progressive cognitive decline focused attention on the central nervous system (CNS) (1, 2). Initial autopsy studies showed a variety of CNS pathology in infected patients; opportunistic infections (e.g., cytomegalovirus [CMV], toxoplasma, progressive multifocal leukoencephalopathy [PML], varicella-zoster virus [VZV], Epstein-Barr virus, and JC virus), tumors (particularly primary lymphomas), and HIV-related changes (including HIVE and HIV leukoencephalopathy) (3–7). The introduction of highly active antiretroviral therapy (HAART) in the late 1990s has had a marked influence on the progression of AIDS in those countries able to afford the treatment. AIDS has become a chronic disease in that treatment-compliant patients now have far greater life expectancy than those infected in the early years of the epidemic. The clinical benefits of HAART are clear, with patients showing decreased plasma viral load and an increase in circulating T lymphocytes (8). The restoration of the immune system by HAART has led to a decrease in the incidence and effects of opportunistic infections in the CNS (9, 10). However, although HAART appears to control viral replication it does not eradicate the virus even after many years, and viral reservoirs undoubtedly remain, including the CNS (11). This may be due in part to the poor penetration of the CNS by some drugs used in HAART (12, 13).

AIDS-related dementia was reported in at least 20% of AIDS cases in the pre-HAART era (1), and to date the exact pathogenesis remains elusive. Several studies have reported links between dementia and activation of microglial cells and/or influx of macrophage/monocytes (14–17). In 1995 Glass et al reported that the degree of macrophage/microglial staining showed a better correlation with HIV dementia than did the presence of HIV infected cells within the brain (15). Similarly, Fischer-Smith et al reported an accumulation of perivascular CD14+/CD16+ cells in the brains of patients with AIDS dementia (18).

Numerous studies from the pre-HAART era suggest that activated microglia/macrophages play a key role in the pathogenesis of AIDS dementia (14–17). However, there have been few neuropathological studies reported since the advent of HAART. These reports have focused on general neuropathological features with no in-depth assessment of changes at the cellular level (9, 10, 19, 20). In particular the status of microglia/macrophages has not been investigated in these patients despite the fact that minor cognitive impairment remains a problem in the post-HAART era (21).

In this study we set out to assess the neuroinflammatory status of HIV-infected individuals who had been well treated with HAART and who had no signs of specific CNS pathology at autopsy. We compared microglia/macrophages in 3 groups of pre-HAART cases (presymptomatic HIV-positive, AIDS...
with no CNS pathology, and HIVE) with HAART-treated cases and with HIV negative controls. We have studied basal ganglia and hippocampus. Macrophage staining in the basal ganglia has been shown to correlate with AIDS dementia in pre-HAART cases (15). The hippocampus was chosen since recent reports of clinical examination and neuroimaging in HAART-treated individuals have suggested that alterations in the hippocampus are more pronounced than was observed pre-HAART (22).

**MATERIAL AND METHODS**

Reviewing all the HIV/AIDS autopsies performed in Edinburgh since 1986 (n = 270; pre-HAART, n = 228; post-HAART, n = 42) we have conducted a study of the change in prevalence of HIV-associated CNS disease since the introduction of HAART.

In order to investigate the baseline microglial/macrophage status in the HAART-treated group we selected 10 cases that had shown good compliance with therapy for at least 18 months. HAART therapy consisted of a protease inhibitor with one or more reverse transcriptase inhibitors. Actual drug combinations varied between these patients. All HAART-treated cases had blood viral load below detectable limits (50 copies/ml) throughout their treatment regime. In 6 of these cases treatment was withdrawn just prior to death, which resulted in a small rise in viral load before death occurred. HAART cases were compared with three groups of cases from the pre-HAART era and with HIV negative controls. Basal ganglia and hippocampus sections were studied for all cases. Pre-HAART cases fell into one of 3 groups: presymptomatic HIV-positive but with no evidence or history of AIDS defining illness other than a low CD4 count in some cases (n = 11), AIDS (n = 17), and HIVE (n = 17). In addition, a control group of HIV-negative normal individuals was included (n = 9). Cases were excluded if evidence of an opportunistic condition was found in the CNS. Pre-HAART cases were either untreated or had received monotherapy (zidovudine) or occasionally dual therapy (usually zidovudine and didanosine).

Table 1 summarizes clinical data for each group. As part of the routine diagnostic autopsy undertaken for each HIV-positive case, p24 immunohistochemistry was performed on multiple brain areas. These results, combined with histological examination of the sections, were used to classify HIV-positive cases as HIVE or non-HIVE. In the HAART-treated cases there was no evidence of p24 positivity in any region of the brain. Since the post-HAART group included both drug abusers and non-drug abusers, both risk groups were included in our comparison groups since we have previously reported that drug abuse itself has a subtle upregulatory effect on microglia/macrophages and lymphocytes in the brain (23–25). All tissues were obtained from the MRC Edinburgh HIV brain bank and ethical approval for this study was obtained from the Lothian Ethics of Research Committee.

Five-µm sections were cut from formalin-fixed paraffin-embedded blocks and transferred to Superfast plus slides. Immunohistochemistry was performed using the antibodies and pre-treatments shown in Table 2. All antibodies were incubated at room temperature for 30 minutes. A standard ABC or tyramide signal amplification (TSA) was used for the tertiary immunohistochemical step. TSA was used for detection of antigens normally expressed at low levels. Diaminobenzidine (DAB) was used as the visualizing agent for all antibodies.

Sections stained with CD68 and MHC II were quantified using an Image Pro Plus image analysis system (Media Cybernetics, Wokingham, UK). Sections stained for CD8, CD45, Ki-67, and PCNA were subjectively graded on a scale from 0-5 (0 = no positive cells; 1 = 1 to 5 cells; 2 = 5 to 10 cells; 3 = 10 to 20 cells; 4 = 20+ cells with focal patches of high positivity; and 5 = 20+ cells with high positivity across the whole section). Image analysis was not used for CD8-stained sections, as the system is unable to differentiate intravascular from perivascular and parenchymal lymphocytes. Similarly, the system could not differentiate CD45-positive lymphocytes from microglia and therefore these sections were scored manually. CD14, Ki-67, and PCNA-stained sections generally showed low levels of staining that did not warrant the protracted quantitative analysis afforded to CD68 and MHC II, the staining for which is widespread. Glial fibrillary acidic protein (GFAP)-stained slides were assessed by counting the number of positive cells in a grid (0.75 mm²). The area examined crossed the grey/white matter interface in the hippocampus. ANOVA was used to analyze differences between groups. If significant differences were found the Student t-test was used to determine where the differences occurred.

**TABLE 1. Clinical Details in Different Patient Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Age in Years (range)</th>
<th>Sex (male:female)</th>
<th>Drug Abusers (ratio of abusers: non-abusers)</th>
<th>Percentage of Hepatitis B/C-Positive Cases</th>
<th>Patients with Cognitive Impairment or Dementia</th>
<th>Length of Time on HAART (months)</th>
<th>Mean Final CD4 Count Cells/l (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 9)</td>
<td>31 (18–45)</td>
<td>6:3</td>
<td>0:9</td>
<td>0%/0%</td>
<td>None</td>
<td>0</td>
<td>Normal (assumed)</td>
</tr>
<tr>
<td>Presymptomatic (n = 11)</td>
<td>32 (26–40)</td>
<td>8:3</td>
<td>11:0</td>
<td>27%/18%</td>
<td>None</td>
<td>0</td>
<td>321 (167–496)</td>
</tr>
<tr>
<td>AIDS no CNS pathology (n = 17)</td>
<td>40 (25–60)</td>
<td>13:4</td>
<td>9:8</td>
<td>29%/29%</td>
<td>5</td>
<td>0</td>
<td>49 (3–160)</td>
</tr>
<tr>
<td>HIVE (n = 17)</td>
<td>33 (22–49)</td>
<td>14:3</td>
<td>9:8</td>
<td>35%/12%</td>
<td>10</td>
<td>0</td>
<td>53 (1–137)</td>
</tr>
<tr>
<td>HAART-treated (n = 10)</td>
<td>45 (33–62)</td>
<td>6:4</td>
<td>8:2</td>
<td>10%/80%</td>
<td>None</td>
<td>18+*</td>
<td>221 (44–368)**</td>
</tr>
</tbody>
</table>

* Six cases were treated with AZT prior to HAART.
** Three developed AIDS defining illnesses before being treated with HAART. The rest were presymptomatic but some had low CD4 counts.
RESULTS

Pre-HAART/Post-HAART Comparison of Brain Pathology

Analysis of 228 pre-HAART and 42 post-HAART autopsy cases in the Edinburgh HIV cohort showed considerable changes in the prevalence of a number of HIV-associated brain disorders (Fig. 1). There was a decrease of 48% in CMV, 100% decrease in toxoplasma, and 67% decrease in HIVE. However, the prevalence of both lymphoma and PML remained stable. This study included both drug abusers and non-drug abusers (72% drug abusers). Treatment was available to all patients; however, the treatment which individual patients received/accepted varied widely. Pre-HAART cases received either no treatment or monotherapy (usually AZT); in occasional cases dual therapy was administered. Post-HAART cases all received triple therapy but for varying periods (consisting of at least three drugs with a minimum of one reverse transcriptase inhibitor and one protease inhibitor).

For the more detailed study we selected 10 of the 42 post-HAART patients as described in Material and Methods. All 10 cases had a plasma viral load below detectable limits for the duration of their therapy. Six of the 10 had been treated with AZT prior to the introduction of HAART and three of these had been treated for an AIDS defining illness before receiving HAART. None of the HAART-treated cases displayed signs of cognitive impairment although 2 suffered from depression. The clinical data for this group of patients are shown in Table 1. We confirmed that these cases were negative for HIVE and opportunistic conditions and were HIV p24-negative. Occasional perivascular macrophages were noted in the white matter, together with subtle perivascular lymphocyte infiltration of the central white matter. Very rare microglial nodules were also observed in some cases. In summary, the changes seen in these HAART-treated brains appeared to resemble those seen in pre-HAART, presymptomatic brains rather than the changes of late stage AIDS.

Investigation of Microglial Markers

CD68

Expression of CD68 in the basal ganglia of HAART cases was comparable to that seen in presymptomatic, AIDS, and HIVE cases and significantly greater than non-HIV controls (p = 0.004) (Fig. 2). Figure 3 shows that CD68 levels in the hippocampus of HAART cases are comparable with those seen in HIVE and significantly greater than those seen in AIDS, pre-symptomatic, or control brains (p = 0.01). In the hippocampus, HAART cases showed higher levels of CD68 expression than in the basal ganglia. Figures 2 and 3 compare grey and white matter for CD68 positivity and show that white matter has marginally greater levels of staining. Figure 4 shows comparative photographs of CD68 in different groups.

MHC II

MHC II was expressed overwhelmingly in microglia/macrophages. Endothelial cells and astrocytes also expressed MHC II at low levels in most cases. In the basal ganglia HAART cases showed levels of positivity almost as high as the

![Changes in prevalence of brain pathology in the Edinburgh HIV cohort since the introduction of HAART](image)

**TABLE 2. Antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Manufacturer</th>
<th>Pretreatment</th>
<th>Primary Antibody Concentration</th>
<th>Secondary Antibody</th>
<th>ABC or TSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>DAKO (Ely, Cambridgeshire, UK)</td>
<td>EDTA microwave</td>
<td>1/75</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>ABC</td>
</tr>
<tr>
<td>CD8</td>
<td>DAKO</td>
<td>EDTA microwave</td>
<td>1/50</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>ABC</td>
</tr>
<tr>
<td>CD14</td>
<td>Novocastra (Newcastle, UK)</td>
<td>EDTA microwave</td>
<td>1/100</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>ABC</td>
</tr>
<tr>
<td>CD20</td>
<td>DAKO</td>
<td>Citric acid microwave</td>
<td>1/150</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>ABC</td>
</tr>
<tr>
<td>CD68</td>
<td>DAKO</td>
<td>Trypsin</td>
<td>1/150</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>ABC</td>
</tr>
<tr>
<td>GPAP</td>
<td>Serotec (Oxford, UK)</td>
<td>None</td>
<td>1/20</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>ABC</td>
</tr>
<tr>
<td>Ki-67</td>
<td>DAKO</td>
<td>Citric acid microwave</td>
<td>1/200</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>TSA</td>
</tr>
<tr>
<td>MHC class II</td>
<td>DAKO</td>
<td>Citric acid microwave</td>
<td>1/100</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>ABC</td>
</tr>
<tr>
<td>HIV p24</td>
<td>Novocastra</td>
<td>EDTA microwave</td>
<td>1/200</td>
<td>Rabbit anti-mouse biotinylated (DAKO)</td>
<td>TSA</td>
</tr>
</tbody>
</table>

ABC, avidin-biotin-peroxidase complex; TSA, tyramide signal amplification.

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HIV and AIDS groups (Fig. 5), whereas in the hippocampus the positivity was higher in the HAART cases than in AIDS brains and comparable with HIVE (Fig. 6). Figures 5 and 6 also compare MHC II positivity in grey and white matter for each of the groups and confirm that this is generally more prevalent in the white matter. Although the trends are clear there was no statistical differences between groups.

Analysis of HAART Subgroups

Comparison of the 6 cases who had received only HAART with those who had received AZT monotherapy prior to receiving HAART revealed no significant differences in terms of CD68 or MHC II staining. The history of a non-CNS AIDS defining illness made no difference to levels of microglial/macrophage positivity.

CD14

Expression of CD14 was generally confined to perivascular microglia, with the exception of the HIVE cases where 8 of 17 showed strong parenchymal staining in addition to perivascular staining (Fig. 7). HAART-treated cases resembled other non-HIVE groups in this study in that parenchymal microglia (though not perivascular microglia) were negative for CD14 (Fig. 7).

Investigation of Cell Proliferation

Ki-67

Ki-67 positivity was found only in occasional endothelial cells in 3 HIVE cases and one presymptomatic case. HAART-treated cases were entirely negative as were the control brains.

Investigation of Lymphocyte Markers

CD8

CD8-positive lymphocytes were detected both in the perivascular spaces and in the parenchyma of all groups. Figure 8 shows the distribution in the groups and confirms the expected increased numbers in presymptomatic brains. The HIVE group had levels of CD8 lymphocytes comparable with pre-symptomatic brains. In contrast, the infiltrate in HAART-treated cases was lower than in HIVE cases and comparable with AIDS and control brains.

CD3

The results for CD3 expression closely approximated those for CD8, suggesting that very few lymphocytes in these cases were of the CD4 phenotype.

CD20

Occasional CD20-positive cells were identified in the brain parenchyma in all groups, including the HAART group. In addition the presymptomatic group showed occasional B cells in perivascular lymphocytic cuffs.

Investigation of Astrocytes

GFAP

Table 3 shows the mean number of GFAP-positive cells in each group. All HIV-positive groups showed an increase in the number of GFAP-positive cells compared to controls, with HIVE cases showing the highest levels. HAART-treated cases showed levels comparable with presymptomatic and AIDS cases and below the levels seen in HIVE. No statistically significant difference was found between any of the groups.

DISCUSSION

Retrospective examination of the Edinburgh HIV cohort shows that CNS opportunistic infections have decreased in prevalence since the introduction of HAART, findings that are similar to those in other post-HAART autopsy series (9, 10, 19). In common with Gray et al (9), we find a significant decrease
in the incidence of HIVE, CMV, and toxoplasma, but no change in the incidence of lymphoma or PML in the brains of HAART-treated infected individuals at autopsy. Several new forms of HIV-related neuropathology have been reported in HAART-treated patients (19, 26). Severe leukoencephalopathy has been reported with intense perivascular lymphocytes and gp41-positive macrophage infiltrations with very high brain HIV RNA levels, designated the immune reconstitution syndrome (IRIS) (9, 27). Gray et al have also observed “burnt out” forms of HIVE, VZV encephalitis, and toxoplasmosis in which inflammation was absent and the causal infectious agent could not be detected (19, 26). However, we have found no evidence of burnt out opportunistic conditions or of IRIS in our post-HAART cases.

Previous studies of HAART-treated cases have focused on the conditions described above. There has been no investigation of the microglial/macrophage status in such cases, despite the fact that this is of interest as a likely significant pathological substrate of cognitive impairment in HIV/AIDS. We selected HAART-treated cases that had a history of long-term compliance with therapy and no obvious CNS pathology, in order to investigate the baseline status of microglia/macrophages and lymphocytic infiltrate in the brains of such patients. Changes in these 2 cell populations in the brains of HIV-infected patients have been well documented. In the presymptomatic phase there is upregulation of microglia compared with normal controls and infiltration of T and B lymphocytes (23, 28, 29). In AIDS there is generally a further increase in microglial activity accompanied by a decrease in lymphocytes in most cases (25, 28, 29), while HIVE is characterized by infiltration of monocytes/macrophages and upregulation of microglia (15, 16). Results in this study of the cases dating from the pre-HAART era are entirely in keeping with previous studies and form the background to our investigation of the HAART-treated cases. It should be noted that many of the HIVE cases studied here displayed “mild” rather than florid HIVE. We suggest that the rather low level of microglial activation may be attributable to the fact that these cases are mild and at the same time have no confounding opportunistic conditions present.

This study shows that elevated levels of CD68 and MHC II positivity, representing significant levels of microglial
activation, are present in the brains of HAART-treated cases in both the basal ganglia and hippocampus. These levels are comparable with those seen in HIVE and AIDS brains and exceed those seen in the normal brain. In some cases the levels in post-HAART cases exceed the levels seen in HIVE. CD14-positive cells were confined to the perivascular regions in HAART-treated cases as is the case in all pre-HAART AIDS cases and in some HIVE cases. We find no evidence of cell proliferation in the form of Ki-67 positivity, in either the hippocampus or the basal ganglia, suggesting that the apparent upregulation of microglia/macrophages is not the result of in situ cell division.

We conclude that neuroinflammation in the form of microglial/macrophage activation is a feature of HAART-treated patients and that the status of this cell population has not returned to normal in the context of HAART therapy. These results are surprising given that HAART effectively arrests or returns the patient to the pre-AIDS phase of the disease and therefore we anticipated that the microglia/macrophage status in HAART-treated cases would more closely resemble the findings in presymptomatic cases (pre-HAART).

Further, these studies reveal an apparent shift in the distribution of neuroinflammation. The most significant findings of the study relate to the hippocampus where we found levels of CD68 positivity highest in the HAART-treated group and elevated above those seen in HIVE (pre-HAART). The difference between the HAART-treated and pre-HAART groups was less marked in the basal ganglia, although CD68 levels were still high. In the pre-HAART era the main focus of HIV-related CNS pathology in most studies was the basal ganglia and pathology in this location was taken as a major contributor to the subcortical dementia manifested by many AIDS patients. Our study suggests that the focus of microglia/macrophage activation in effectively treated patients may be shifting to the hippocampus, an assertion which supports the earlier suggestions of Petito et al that the hippocampus is involved in the pathology of AIDS-related disease (30). Although microglial activation in the hippocampus of HAART-treated subjects was upregulated in comparison to pre-HAART AIDS cases this was not accompanied by a corresponding increase in astrocytosis. Despite the presence of what appears to be a high level of neuroinflammation, no evidence of dementia was observed in the HAART-treated group.

![FIGURE 6. MHC II expression in the hippocampus.](image)

**FIGURE 6.** MHC II expression in the hippocampus.

![FIGURE 7. CD14 expression in the hippocampus of HAART-treated, HIVE, AIDS, and normal brains.](image)

**FIGURE 7.** CD14 expression in the hippocampus of HAART-treated, HIVE, AIDS, and normal brains.
Lymphocytic infiltration of the basal ganglia and hippocampus does not appear to be a major feature of the neuroinflammatory state that we describe in HAART-treated patients. In the areas investigated in detail in this study (basal ganglia and hippocampus) we find no significant increase in lymphocyte numbers in HAART-treated cases compared to pre-HAART AIDS cases and do not see the same level of perivascular infiltration characteristic of presymptomatic cases. It is worth noting that the HAART-treated cases do show some lymphocytic infiltrate in the central white matter (an area not within the scope of this study), reminiscent of presymptomatic brains (28, 31).

Analysis of the HAART-treated sub-groups showed no apparent influence on microglia/macrophages of prior treatment with monotherapy, of a history of drug abuse or of a prior AIDS defining illness.

Assuming that the cases studied here are representative of those who survive with HAART, the question arises as to the likely significance of ongoing neuroinflammation. We recognize that this is an autopsy series and in some respects represents a failure of HAART therapy. However, only one of the patients in this study died from HIV-related causes (8 died of hepatitis C-related pathology and one died of a drug overdose). It is likely that the changes observed in these brains are representative of changes present in surviving treated individuals, with the caveat that hepatitis C requires to be ruled out as a confounding factor in causing neuroinflammation. The increase in microglial activity seen in the hippocampus is of particular concern and may signify a shift in the target areas of HIV in the brain. The reasons for this ongoing inflammation are unclear and merit further investigation. A possible link to HAART therapy itself cannot be ruled out. Ongoing inflammation may also predispose to further CNS damage in those who live longer on therapy and it may be that in the future this will result in the emergence of new types of neurological deficits.

**ACKNOWLEDGMENTS**

We would like to thank Mr. Alan Wilson for his help in collating the patient information and Dr. Colin Smith and Prof. David Graham for providing 2 of the control cases.

**REFERENCES**


**TABLE 3. Astrocyte Cell Counts in the Hippocampus**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Cases</th>
<th>Mean</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9</td>
<td>24.8</td>
<td>±10.8</td>
</tr>
<tr>
<td>Presymptomatic</td>
<td>11</td>
<td>28.0</td>
<td>±7.2</td>
</tr>
<tr>
<td>AIDS</td>
<td>17</td>
<td>28.6</td>
<td>±11.5</td>
</tr>
<tr>
<td>HIV/EAD</td>
<td>17</td>
<td>36.6</td>
<td>±12.2</td>
</tr>
<tr>
<td>HAART</td>
<td>10</td>
<td>27.2</td>
<td>±8.0</td>
</tr>
</tbody>
</table>

Mean = number of GFAP-positive astrocytes per 0.75mm² observed at 200× magnification in the dentate and CA4 region.
20. Masliah E, DeTeresa RM, Mallory ME, Hansen LA. Changes in pathological findings at autopsy in AIDS cases for the last 15 years. AIDS 2000;14:69–74
22. Brew BJ. Evidence for a change in AIDS dementia complex in the era of highly active antiretroviral therapy and the possibility of new forms of AIDS dementia complex. AIDS 2004;18(Suppl. 1):S75–78