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RESEARCH ARTICLE

Distribution of Brain HIV Load in AIDS

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Approximately one quarter of patients with AIDS develop severe cognitive deficits called HIV-associated dementia complex (ADC). There is some controversy regarding the importance of viral load in mediating neurologic disease. With the advent of sensitive, quantitative and reproducible RNA assays for HIV load in plasma and CSF, we quantified viral load in brains from 10 autopsied HIV-infected subjects and 2 non-infected controls. The new quantitative HIV RNA assays showed general agreement with previously used semi-quantitative immunocytochemical assessments of HIV envelope protein, and were performed without professional subjective interpretation. All cases with very high levels of HIV in the CSF, had high overall levels in the brain, suggesting that CSF viral loads exceeding 10^6 copies per mL may be a surrogate marker of high viral load in the brain. Levels of virus in the spleen showed no clear association with those found in the brain. HIV RNA was not uniformly distributed throughout the brain. Selective regions, including basal ganglia and hippocampus, showed higher levels of virus than the cerebellar cortex and mid-frontal cortical gray matter. Assessment of overall brain viral load requires careful attention to regional quantitation.

Introduction

Approximately one quarter of AIDS patients develop severe cognitive deficits called HIV-associated dementia complex (ADC) (2). With the advent of "triple drug" therapy, it is unclear whether reduction of systemic viral load will decrease the incidence of neurologic disease. In agreement with the original pathologic descriptions (19), we have found that ADC appears to result from chronic HIV encephalitis (24). Nevertheless, there is some controversy regarding the importance of viral load in mediating neurologic disease (1, 7, 8, 12, 21, 24). Part of this controversy results from the different techniques used to assess neuropathology associated with AIDS. Subjective evaluation of brain tissues by neuropathologists is heavily dependent upon the techniques they employ to examine the brain. Some groups have used routine histology which has limited sensitivity in detecting viral encephalitis. In the past (1) we evaluated a variety of techniques to assess CNS HIV load and have found the laborious immunocytochemical approach to be a sensitive, albeit semi-quantitative, means of assessing viral tissue load.

With the recent availability of the RNA assays to measure HIV load in plasma and cerebrospinal fluid (CSF) (4, 16, 23), several groups have examined whether this technique could be used for measuring viral load in tissues (4, 5, 9, 10, 16, 17, 22). In the current study, we quantified brain viral load in autopsy tissues from patients with AIDS using two quantitative HIV RNA assays.

Materials and Methods

Tissues used were from 10 AIDS and 2 non-HIV-infected autopsies all having post-mortem intervals of less than 24 hours and without evidence of opportunistic CNS infection. CSF, CNS and spleen tissues were collected and stored at -80°C. Serum samples were not

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Case	PMT	MNGC	gp41Sum	Ammon Horn		Caudate		Cerebellar Cortex		Globus Pallidus		
				Quantiplex	Amplicor	Quantiplex	Amplicor	Quantiplex	Amplicor	Quantiplex	Amplicor	
1	5	+	5	THTQ	THTQ	THTQ	THTQ	$2.49x10^5$	$1.13x10^6$	ND	ND	
2	4	+	4	$2.00x10^7$	$1.64x10^7$	$2.48x10^7$	$1.73x10^8$	$3.89x10^4$	$3.96x10^5$	$2.76x10^7$	$1.05x10^8$	
3	24	+	3	THTQ	THTQ	$2.94x10^7$	$4.53x10^7$	$2.11x10^6$	$5.11x10^6$	$2.50x10^6$	$6.52x10^6$	
4	24	+	3	$6.18x10^4$	$5.86x10^5$	$2.35x10^7$	$1.81x10^7$	ND	ND	$5.22x10^6$	$6.24x10^6$	
5	18	-	2	$2.42x10^7$	$1.50x10^7$	$1.75x10^7$	$4.42x10^7$	TLTQ	$9.86x10^4$	TLTQ	$1.94x10^4$	
6	24	-	2	$1.00x10^3$	$6.54x10^3$	TLTQ	$2.32x10^3$	TLTQ	$6.98x10^3$	TLTQ	$3.14x10^3$	
7	5	-	0	$4.04x10^5$	$2.39x10^4$	$7.68x10^4$	$5.29x10^4$	$4.25x10^4$	$3.88x10^4$	TLTQ	$5.52x10^4$	
8	5	-	0	TLTQ	$8.80x10^4$	$4.75x10^4$	$3.31x10^5$	TLTQ	$6.45x10^4$	$1.14x10^5$	$2.52x10^5$	
9	9	-	0	TLTQ	$3.89x10^3$	TLTQ	$2.10x10^3$	TLTQ	$1.38x10^3$	TLTQ	$1.88x10^4$	
10	16	-	0	TLTQ	$1.80x10^3$	TLTQ	$1.51x10^4$	TLTQ	$7.77x10^4$	$2.15x10^4$	$1.66x10^4$	
11	10	-	0	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	
12	6	-	0	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	

Table 1. Quantitation of HIV RNA in different regions of the brain, cerebrospinal fluid (CSF) and spleen of 10 AIDS autopsies (cases 1-10) and 2 non-HIV-infected control autopsies (cases 11 and 12). All AIDS autopsies were without significant CNS opportunistic infection and with post-mortem times (PMT) under 24 hours. Histopathologic analysis showed multinucleated giant cells (MNGC) in four of the cases. All brain regions of 2 non-HIV controls contained no-detectable viral RNA. Values are reported as the number of HIV RNA copies per gram of tissue or mL of fluid determined by QUANTIPLEX™ assay or AMPLICOR HIV-1 MONITOR™ assay. Semi-quantitative measurement of HIV gp41 (gp41 Sum) was previously assessed using immunocytochemistry. This assessment requires evaluating 3 CNS regions (mid-frontal cortical gray matter, deep white matter and deep gray matter) for abundance of HIV infected cells (rated as 0, 1, or 2). Summation of the 3 regional scores gives an overall score on a scale of 0 - 6.
THTQ=too high to quantitate, TLTQ=too low to quantitate, ND=not determined

available from these autopsies. Complete neuropathologic gross and microscopic examination were performed on all of the autopsies as previously described (1). Semi-quantitation of HIV load using immunocytochemical assessment of HIV envelope protein gp41 was performed as previously described (14). In brief, immunostaining in the cortical gray matter, cerebral white matter and deep gray matter was assessed on a 3 point scale (0=no cells stained for gp41, 1=less than 2 cells stained for gp41 in an average of five 20x fields, and 2=more than 2 cells stained for gp41 in an average of five 20X fields). To obtain an immunocytochemical index of total brain viral load the 3 regional scores were summed (gp41Sum) to obtain a scale of 0-6.

RNA extraction and measurement. All human tissues were homogenized in TRIzol™ Reagent (GibcoBRL, Life Technologies, Gaithersburg, MD). Sterile glass beads and frozen brain tissue were added to 2 mL tubes along with 1.0 mL of TRIzol™ Reagent per 100 mg of tissue. The mixture was then shaken in a minibead beater for 10 seconds, stored on ice briefly and homogenized again for 10 seconds. This was followed by phase separation with the addition of 0.2 mL of chloroform per mL of TRIzol™ Reagent. The mixture was shaken well and centrifuged at 12,000 Xg for 15 minutes. The upper, aqueous layer was then transferred to a new tube. To the tube containing the aqueous layer, 0.5 mL of isopropanolol was added per mL of TRIzol™ Reagent. The mixture was then split by transferring one half to a fresh tube. One half (corresponding to 50 mg of tissue) was used for HIV RNA determination in the QUANTIPLEX™ bDNA SIGNAL AMPLIFICATION SYSTEM (Chiron Corporation Emeryville, CA) and the other half was used in the AMPLICOR HIV-1 MONITOR™ TEST (Roche Diagnostic Systems, Branchburg, NJ). Following an overnight precipitation at -80°C, the mixture was centrifuged at 12,000 Xg for 10 minutes. The supernatant was discarded and the pellet was washed in 0.5 mL of 75% ethanol, followed by another brief spin for 5 minutes. The ethanol was decanted and pellet air dried for 5 minutes.

For QUANTIPLEX™ quantitation of HIV RNA, 0.44 mL of HIV sample working reagent was added to this pellet. The suspension was vortexed vigorously and incubated at 53°C for 20 minutes followed by an additional 10 seconds of vortexing at the end of incubation to ensure complete solubilization of the RNA pellet. 0.2 mL of the solubilized RNA was placed in duplicate HIV capture wells in a standard QUANTIPLEX™ HIV RNA assay including standards and controls. These were incubated overnight at 53°C before being measured by the luminometer for calculation of HIV equivalents/gram of wet weight tissue as per the manufacturer's specifications. If the duplicate QUANTIPLEX™ samples varied by greater than 35% the test was considered to be invalid.

The other half of the RNA pellet from the CNS extraction was analyzed with the AMPLICOR HIV-1 MONITOR™ assay according to manufacturer's instruc-

cont.	Mid-Frontal Cortex		Putamen		Substantia Nigra		Global Mean in Brain		Cerebrospinal Fluid		Spleen	
	Quantiplex	Amplicor	Quantiplex	Amplicor	Quantiplex	Amplicor	Quantiplex	Amplicor	Quantiplex	Amplicor	Quantiplex	Amplicor
1	$5.04x10^6$	$1.79x10^6$	ND	ND	$5.33x10^7$	THTQ	$1.19x10^7$	$2.77x10^7$	THTQ	$1.66x10^6$	$1.14x10^7$	$1.27x10^7$
2	$3.74x10^4$	$4.44x10^4$	THTQ	THTQ	$5.13x10^6$	$1.75x10^7$	$3.48x10^6$	$1.09x10^7$	$2.15x10^4$	$2.10x10^4$	$3.66x10^6$	ND
3	$5.46x10^5$	$1.10x10^6$	$1.73x10^6$	$3.50x10^6$	$1.74x10^6$	$3.45x10^6$	$3.96x10^6$	$8.78x10^6$	THTQ	$1.97x10^6$	$6.28x10^6$	THTQ
4	$6.10x10^5$	$1.65x10^6$	$3.20x10^5$	$7.30x10^5$	ND	ND	$1.08x10^6$	$2.40x10^6$	THTQ	$2.10x10^6$	$4.32x10^5$	$8.85x10^5$
5	TLTQ	$3.82x10^4$	TLTQ	$5.96x10^4$	TLTQ	$1.63x10^5$	$1.71x10^4$	$3.35x10^5$	$1.23x10^4$	$2.69x10^3$	$3.17x10^6$	$1.11x10^7$
6	TLTQ	$4.20x10^3$	TLTQ	$4.54x10^3$	TLTQ	$3.02x10^3$	$1.00x10^3$	$4.09x10^3$	TLTQ	TLTQ	$5.08x10^5$	ND
7	TLTQ	$9.98x10^3$	TLTQ	$1.68x10^4$	$6.12x10^5$	$5.55x10^4$	$1.87x10^4$	$3.06x10^4$	$1.52x10^3$	TLTQ	$1.62x10^6$	$1.73x10^6$
8	TLTQ	$2.83x10^5$	$8.16x10^4$	$1.12x10^5$	TLTQ	$6.78x10^4$	$6.40x10^3$	$1.94x10^5$	$2.03x10^4$	$2.54x10^4$	$4.54x10^6$	ND
9	TLTQ	$1.29x10^4$	$2.16x10^4$	$9.06x10^3$	TLTQ	$2.54x10^3$	$1.55x10^3$	$4.85x10^3$	TLTQ	$4.51x10^3$	$4.58x10^6$	$1.76x10^6$
10	$2.54x10^4$	$9.94x10^3$	TLTQ	$7.20x10^3$	TLTQ	$6.16x10^3$	$2.46x10^3$	$1.06x10^4$	$1.51x10^3$	$3.70x10^4$	$1.22x10^7$	$1.05x10^8$
11	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ			ND	ND	ND	ND
12	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ	TLTQ			ND	ND	ND	ND

tions (18). Briefly a Quantitation Standard (QS) RNA was included in the AMPLICOR HIV-1 MONITOR™ assay to control for sample processing and amplification efficiency. CNS RNA was resuspended in Working Lysis Reagent which contains a known amount of the QS RNA, followed by precipitation of the RNA with isopropanol. The prepared sample was added to the Master Mix reagent for reverse transcription and PCR amplification. Following amplification the HIV and QS reaction products were detected and quantified by hybridization to HIV-1 and QS-specific oligonucleotide probes in a colormetric, microwell plate assay. The amount of HIV-1 RNA was then calculated from the ratio of the total optical density for HIV to the total optical density for QS and the input number of QS RNA molecules.

Spleen tissues were handled in an identical fashion. Measurement of HIV RNA in CSF was performed by centrifuging the CSF at 23,500 Xg for 1 hour @ 4°C, discarding the supernatant and quantitating RNA in the resultant pellet as described above.

All mean values were assessed after log transformation. For statistical analyses, Spearman rank correlations were performed in a non-parametric fashion. Differences in the HIV RNA load were assessed by the unpaired Student t test. Two-tailed P values of <0.05 were considered significant.

Results

Comparison of measurements of HIV RNA using QUANTIPLEX™ and AMPLICOR HIV-1 MONITOR™ assays. Table 1 displays the number of HIV RNA copies per gram of tissue or milliliter of CSF. To avoid variations in micro-regional tissue pathology, the RNA extracted from a single piece of tissue was measured with both assays. This limited the number of assays that could be run on each specimen. RNA levels could not be determined (ND) for some samples because the results of duplicate QUANTIPLEX™ assays exceeded manufacturer's specifications of variation or the pellet was lost in processing. The detection limits of the two assays are not identical and vary from run to run. For tissues from the non-HIV controls and 33 of the 180 remaining assays (18%), HIV RNA was too low to quantitate (TLTQ). For 13 of the assays (7%), the tissue HIV RNA levels were too high to quantitate (THTQ). Additional tissue specimens from these regions were not available to repeat quantitation. Comparing viral quantitation for the 42 samples where both assays were within lower and upper detection limits showed reasonable agreement between the assays (Figure 1) (Spearman rank correlation $r=0.94$, $P<0.0001$). Those samples exceeding thresholds of one assay or the other were fairly close to the threshold of the other assay (Table 1). For all subsequent logarithmic graphing we have displayed all values less than 1000 copies per gram (or mL) or too low to quantitate as equal to 1000. For graphing the 13 assays where virus levels were too high to quantitate, the values were set at the upper thresholds for each assay (to $6x10^7$ for the QUANTIPLEX™ assay and to $2x10^8$ for the AMPLICOR HIV-1 MONITOR™ assay).

Comparison of overall HIV RNA load to semi-quantitative immunocytochemical assessment of HIV envelope gp41. To obtain an index of the overall brain viral load, we calculated a geometric mean of all of the CNS regions assessed for each case using each assay. Comparison of the mean brain HIV RNA using the two different assays showed good concordance (Figure 2). There was also good agreement between the RNA assessment of HIV load and the previously used semi-quantitative assays of HIV envelope protein gp41 (Table and Figure 2). For example, cases 1-4 with histopatho-

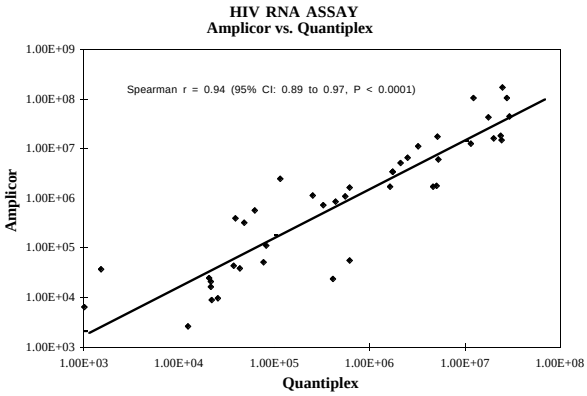

Figure 1. Comparison of brain, spleen and CSF HIV RNA values using two quantitative methods. RNA was extracted from tissues or pelleted from CSF as described in the methods. RNA was split into two equal samples and quantified with the QUANTIPLEX™ and AMPLICOR HIV-1 MONITOR™ assays. For each quantitation the sample was again divided in half and run in duplicate as described in the methods. This scattergram compares the HIV RNA quantitated on each sample using the two assays. The assay couples with incomplete numeric values (i.e. not determined, too low to quantitative or too high to quantitate) were excluded from this scattergram (n=42). Spearman rank correlation coefficient r=0.94.

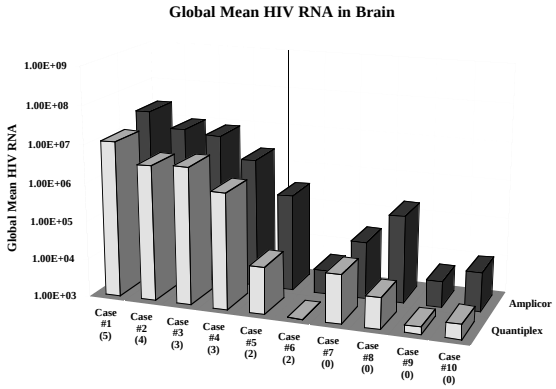

Figure 2. As an index of the overall mean HIV RNA level for each brain, the geometric mean of HIV was determined for 7 CNS regions. The indices derived with the two different assays (QUANTIPLEX™ and AMPLICOR HIV-1 MONITOR™) show reasonable agreement. Cases previously diagnosed with moderate to severe HIV encephalitis on histopathologic criteria (cases 1-4) all have high HIV RNA levels (>10^6 copies / gram of tissue). Cases previously diagnosed with mild to no HIV encephalitis on histopathologic criteria (cases 5 through 10) have lower overall HIV levels. Semi-quantitative immunocytochemical rating of HIV encephalitis (gp41Sum Score) is shown in parentheses.

logically proven moderate to severe HIV encephalitis (gp41Sum scores of 3 or greater), all had high mean brain RNA levels (>10^6 copies per gram). Cases with mild to no histopathologic evidence of HIV encephalitis (gp41Sum scores of 2 or 0, i.e. cases 5-10) had lower and more variable levels of brain viral load as assessed by the RNA assays.

Regional differences in brain HIV RNA load. Regional analysis of HIV RNA load clearly showed that not all regions of the brain were equally involved (Table and Figures 3 and 4). To normalize the data for differences in overall brain viral load, in Figure 3 each regional load was divided by the mean of all 7 brain regions for that case. Both assays showed differential involvement of CNS regions, with high viral load in specific regions (e.g. caudate nucleus and hippocampus) and low viral load in other regions (e.g. cerebellar cortex and mid-frontal cerebral cortex). Of the 7 regions examined, the hippocampus, caudate, and globus pallidus showed the highest levels of HIV RNA, while the mid-frontal cortical gray matter, and cerebellar cortex had lower levels. Some brains with an overall high mean brain load had low levels of HIV in specific regions like the mid-frontal cerebral cortical gray matter, while all brains with high viral concentrations in the mid-frontal cerebral cortex also had high mean brain levels.

To compare regional viral load relative to histopathologic changes, we separated the 10 cases into 2 groups based upon the presence or absence of histopathologic evidence of HIV encephalitis (Figure 4a and 4b). Comparing regional viral loads among the 4 cases with HIV encephalitis showed that caudate had the highest viral load with hippocampus, globus pallidus, putamen and substantia nigra also showing moderately high levels. Cerebellar and mid-frontal cerebral cortex levels were approximately 1 log lower. In cases without HIV encephalitis, viral load in all regions was 2-3 logs lower than in those with HIV encephalitis, but hippocampus and caudate levels were still approximately 1 log higher than all other brain regions.

Comparison of spleen HIV RNA levels to CNS levels. We compared levels of HIV in the spleen to individual CNS regions and to the geometric mean of the 7 brain regions assessed (Table 1). Spleen viral levels were uniformly high and showed no relationship to those levels found in individual CNS regions or to the mean of the 7 brain regions.

Comparison of CSF HIV RNA levels to CNS levels.

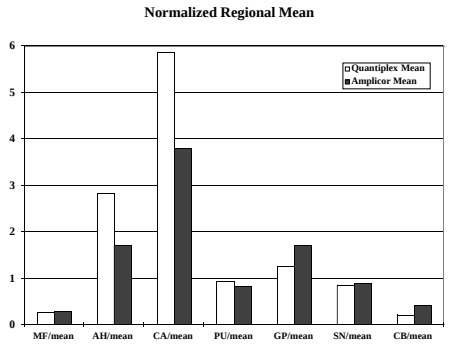

Figure 3. The HIV RNA load was determined for each CNS region using the QUANTIPLEX™ (clear bar) and the AMPLICOR HIV-1 MONITOR™ (dark bar) assays. To normalize the data for differences in overall brain viral load, each regional load was divided by the mean of all 7 brain regions for that case. Both assays showed differential involvement of CNS regions, with high viral load in specific regions (e.g. caudate nucleus and hippocampus) and low viral load in other regions (e.g. cerebellar cortex and mid-frontal cerebral cortex). Region abbreviations: AH=Ammon's Horn, CA=head of caudate, CB=cerebellar cortex, GP=globus pallidus, MF=mid-frontal cortical gray matter, PU=putamen, SN=substantia nigra.

Levels of HIV in the CSF were compared to individual brain regions and to an overall brain mean of the 7 regions assessed (Figure 5a). Comparison of mean brain viral load to that measured in the CSF showed no significant correlation when the CSF levels were below 10^5 copies per mL. In all 3 cases where CSF levels exceeded 10^6 copies per mL. the mean brain viral load was substantial (>10^6 copies per gram). Comparing CSF levels of HIV RNA with that found in individual CNS regions showed that HIV load in the CSF reflected that found in the mid-frontal cerebral cortical gray matter (Figure 5b). However, there were cases with low HIV load in the CSF despite high levels of virus in deep brain regions like the caudate.

Discussion

Previous techniques to assess CNS viral load have not been truly quantitative and have been difficult to replicate between laboratories thus complicating cross group comparisons. Recently, substantial advances have been made in the quantification of systemic viral load utilizing assays of HIV RNA in body fluids. Several sensitive and reproducible techniques are now commercially available. These techniques have led to an improved understanding of the pathogenesis of HIV infection that has been nothing less than astounding(11, 20). An example of this is a recent study (16) showing that quantitation of virus in blood at a single time point

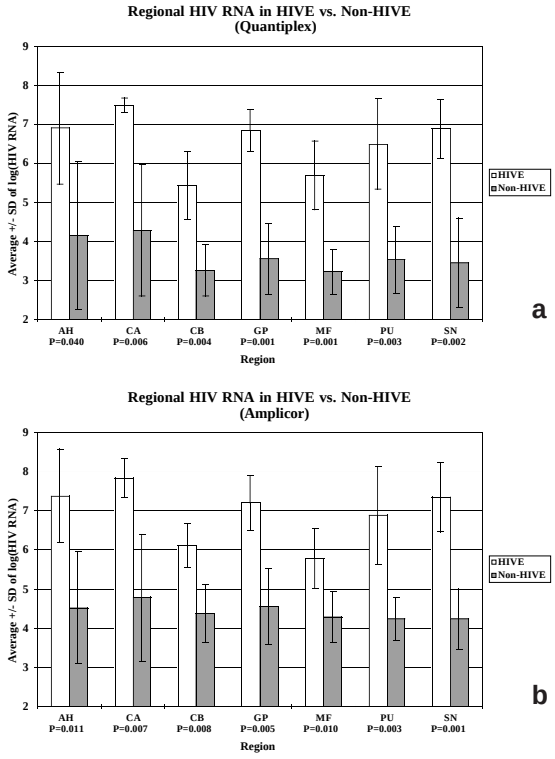

Figure 4. The mean HIV RNA load was determined (a. QUANTIPLEX™, b. AMPLICOR HIV-1 MONITOR™) for each CNS region in cases with and without histopathologic evidence of HIV encephalitis. Cases without HIV encephalitis (dark bars) showed very low levels of virus in most regions except for modest levels in the hippocampus and caudate nucleus. Cases with HIV encephalitis (clear bars) showed high levels in most regions with more modest levels in the cerebellar and mid-frontal cortex. Bars represent standard deviation. Differences in the HIV RNA load were assessed by the unpaired Student t test. Region abbreviations: AH=Ammon's Horn, CA=head of caudate, CB=cerebellar cortex, GP=globus pallidus, MF=mid-frontal cortical gray matter, PU=putamen, SN=substantia nigra.

is an excellent predictor of speed of disease progression. Whether other techniques will give comparable data remains to be seen (9). It is expected that commercially available assays to quantitate viral DNA will be soon available soon for assessing the magnitude of the potential reservoir of non-replicating HIV.

The HIV RNA assays used in our study have several advantages over previous techniques. Most importantly the RNA assays are truly quantitative rather than the previous standard of semi-quantitative assessment by immunocytochemistry (1). Additionally, because the RNA based assays do not require subjective professional interpretation, the results of this and future studies should be readily comparable. Studies examining changes in host gene expression during viral infection

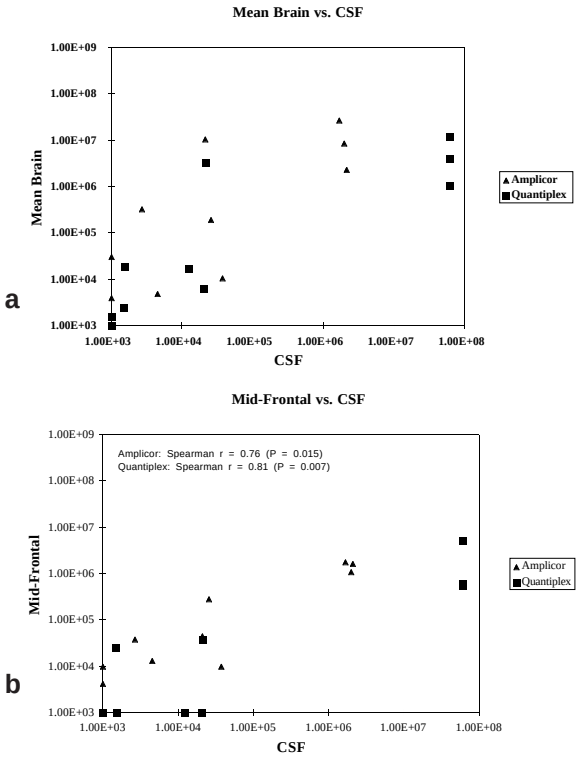

Figure 5. a. Comparison of overall brain mean HIV load to that measured in the CSF shows little correlation when the CSF levels were below 10^5 copies per mL. In all 3 cases where CSF levels exceeded 10^6 copies per mL, the mean brain viral load was high. **b.** Comparison of HIV load in the mid-frontal cortex to that found in the CSF shows a good correlation.

can utilize the same specimen extractions in which virus had been quantified, obviating differences that may be due to microscopic variations in histopathology.

Any extraction technique precludes histopathologic assessment. Additionally, since human brains are not perfused with buffer after death, blood necessarily contaminates the tissue extraction. How significant a confounding variable blood contamination is on tissue RNA extracts depends on 2 factors: the level of virus in the blood and the amount of blood in the tissue. Viral load in plasma varies dramatically between patients, and can exceed 1 x 10^6 copies / mL of plasma. If we assume a very high level of virus in the blood (i.e. 10^6 copies per mL), how much of the tissue based signal could possibly be attributed to blood contamination? Blood volume varies mildly between brain regions (e.g. gray matter versus white matter), however, if we assume 5% of the tissue volume is blood and half of that is plasma, then in 1 gram of brain the maximum viral load signal due to the blood would be: 10^6 x .05 x 0.5 (i.e. 2.5 x 10^4 copies / mL). This is the highest possible contamination and the real contribution, depending upon actual viremia and degree of post-mortem exsanguination, might be 1 or 2 orders of magnitude lower. However, even this high estimate is several orders of magnitude lower than that found in involved regions (e.g. globus pallidus) and would only complicate tissue viral load assessment in relatively uninvolved regions (e.g. cerebellar cortex). Thus the high levels we observed in specific regions of some cases must thus be attributed to local virus rather than blood contamination.

We employed two commercial kits for HIV RNA quantitation and obtained comparable results (Spearman correlation r=0.94) similar to that observed in previous clinical studies(22). Nevertheless, the absolute values of each test differ by a factor of approximately two either because of differences in the standard curves of the different assays, sensitivity, or inherent differences in detecting HIV RNA extracted from tissue samples. Each test targets a different portion of HIV RNA for quantitation; AMPLICOR HIV-1 MONITOR™ assesses the *gag* region while QUANTIPLEX™ assesses the *pol* region. Neither test would be expected to assay singly or multiply spliced transcripts, but both would assess genomic and full length messenger RNA.

The immunodeficiency of AIDS is associated with severe lymphoid depletion. Due to the severe loss of CD4 cells, virus in autopsy spleens might be associated with tissue macrophages as seen in the CNS. This would suggest the hypothesis that CNS viral load would be closely mimicked by that observed in peripheral lymphoid tissue. Our measurements of viral load in these 2 organs show this is clearly not the case. The exact location of viral RNA in the autopsy spleen is not clear, however, our data would suggest the high spleen levels are independent of brain levels. Whether this is also the case in more accessible lymphoid tissue like tonsil is unknown.

Di Stefano and Brew and colleagues (3, 4) examined the relationship between CSF HIV load and neurologic symptoms. They found that those patients manifesting global cognitive changes had high HIV RNA levels in their CSF. We have recently shown that autopsies with high CSF RNA levels of virus have high CNS tissue viral load as assessed by semi-quantitative immunocytochemistry. The current study shows that this same relationship between CSF and brain tissue holds when brain tissue RNA is quantitatively assessed. Autopsies with greater than 5 X 10^4 copies of HIV RNA per mL of CSF measured by the QUANTIPLEX™ assay or 10^5 copies of HIV RNA measured by the AMPLICOR HIV-1

MONITOR™ assay had HIV encephalitis. This point is a bit surprising as CSF has not traditionally been considered a good marker of CNS disease. Many acute viral infections are associated with meningitis. Quantitative assessment of acute viral meningitides has not been well explored. There is a well known meningitis associated with acute HIV seroconversion. Quantitative assessment of this acute stage is unknown, but the CSF would likely follow the general whole body plasma peak. In the late stages of infection it is difficult to define the origin of CSF HIV. The good correlation of CSF and mid-frontal cortical viral loads might suggest that virus spilling into the extracellular CNS space flows into CSF. However, local production of HIV by meningeal macrophages is also a strong possibility. If the quantitative relationship between CSF and brain exists outside of the perimortem interval, then monitoring CSF might be clinically important for diagnosing and treating CNS disease due to HIV itself. An equally intriguing question is whether treatment efficacy for CNS tissue can be followed by monitoring the CSF. Blood/CSF/brain barriers may lead to dissociation of the CSF and CNS compartments such that treatment apparently efficacious in reducing CSF viral load may not affect tissue virus. This important clinical issue awaits further study.

It is difficult to draw conclusions about the kinetics of viral entry using the static observations inherent in autopsy studies. Nevertheless, when one compares regional viral load in the heavily involved cases with that in the less involved cases (Figures 3 and 4), it appears that virus replicates to high levels in multiple regions simultaneously. Unlike Herpes Simplex virus, HIV appears in multiple CNS regions at a level independent of anatomical connectivity. Whether differential viral load depends upon local factors promoting viral expression in CNS macrophages or depends upon differential monocyte trafficking, remains to be determined.

Devising a solitary index for whole brain viral load is virtually impossible. We chose to assess a geometric mean HIV RNA level in 7 readily definable brain regions, but there are limitations with this index. A minute region of the brain (e.g. substantia nigra) is averaged with cortical gray matter which may make up as much as 25% of the human brain. The cortical gray matter is heterogeneous and there is no reason to suspect that mid-frontal cortex is representative of all the cortex. Unfortunately there is no easy solution to this problem. Even if one were able to extract all the RNA from a human brain, averaging uninvolved regions with critical involved regions might reduce an important signal. Although there are problems with selecting limited brain regions, we sampled neurologically important regions known to be involved in clinical AIDS. Future studies will have to critically assess whether to use a global index of HIV infection or whether selective regional assessment would be more pertinent.

During preparation of this report, 3 studies have appeared examining HIV load in the CNS using RNA based assays. Ellis et al (6) showed elevated levels of HIV RNA in the CSF of neuropsychologically impaired subjects with AIDS. Similarly, McArthur et al(15) found significantly higher levels of HIV RNA in CSF of immunocompromised demented HIV infected patients. Plasma levels of HIV RNA did not correlate with presence or absence of dementia. McArthur et al and Lazarini et al (13) both assessed HIV load in the mid-frontal gyrus. Neither study found a correlation between neurological status and tissue viral loads, leading one group (13) to conclude that "indirect mechanisms may be more significant than viral burden" in mediating neurologic dysfunction. This conclusion is compromised by potentially lengthy intervals between the subject's psychometric evaluation and death, and most importantly inadequate sampling of brain for viral load assessment. As shown in our data, the mid-frontal cortex would be expected to be an insensitive region for analysis. In fact, one of the subjects in the Lazarini et al study had very low levels of HIV RNA in the mid-frontal cortex despite having a histopathologic diagnosis of HIV encephalitis. Detailed neurologic assessment of our cases close to the time of death was not available, however, restrospective analysis of the autopsy clinical summaries showed that only one patient had been diagnosed as demented (case 1).

In summary, assessment of overall brain viral load requires careful attention to regional quantitation. Since HIV does not replicate within CNS neurons, clearly the effect of HIV encephalitis on neuronal function is "indirect." However, quantitative assessment of CNS HIV shows substantial regional loads of HIV which in fact may directly or indirectly account for neurologic damage.

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