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# Cerebrospinal fluid viral load is related to cortical atrophy and not to intracerebral calcifications in children with symptomatic HIV disease

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**The relationships between viral load in plasma and cerebrospinal fluid (CSF) and computed tomography (CT) brain scan abnormalities were studied in 39 children between 0.5 and 13 years of age with symptomatic HIV-1 disease. Quantitative RNA PCR was used to determine HIV-1 RNA levels and a semiquantitative analog rating technique was used to evaluate non-contrast CT brain scans. CSF HIV-1 RNA copy number correlated significantly with CT brain scan ratings for severity of cortical atrophy ($r$=0.36; $P<0.05$) but not with ratings of intracerebral calcifications ($r$=−12; NS). The difference between these two correlations was significant ($P<0.05$). Plasma HIV-1 RNA copy number did not correlate significantly with any CT brain scan ratings or with CSF viral load ($r$=0.05; NS). Severity of cortical atrophy appeared to reflect the level of viral load in the CSF, supporting the notion that active HIV-1 replication in the CNS is at least in part responsible for such brain abnormalities in children. The lack of correlation of intracerebral calcifications with other CT brain scan abnormalities as well as with CSF viral load suggests that this lesion is relatively independent and may reflect a different neuropathologic process.**
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## Introduction

In children, central nervous system (CNS) disease, which may result in severely disabling neurological, cognitive, and behavioral abnormalities, is a significant complication of infection with the human immunodeficiency virus type-1 (HIV-1). The primary cause of these neurological deficits and the underlying neuropathologic damage appears directly related to HIV-1 infection of the CNS. Using PCR techniques, HIV-1 DNA has been identified in brain tissue of pediatric patients with HIV disease (Sharer *et al*, 1996; Sei *et al*, 1994). Moreover, the amount of provirus was much higher in the brain tissues of encephalopathic patients than in non-encephalopathic AIDS patients. In contrast, the levels of proviral DNA in lymph nodes and spleen were similar for both subgroups (Sei *et al*, 1995). Subsequent studies have shown increased levels of HIV-1 RNA PCR in CSF from children with abnormal CNS function compared to those with normal CNS function, while these subgroups did not show differences in their levels of plasma HIV-1 RNA (Sei *et al*, 1996; Pratt *et al*, 1996). These findings further support the notion that HIV-associated CNS disease is largely dependent on the magnitude of viral replication within the CNS (Yarchoan *et al*, 1987). HIV-1 infection within the CNS is largely restricted to macrophages and microglia, without evidence for direct infection of neurons. Neurologic damage is postulated to be caused by the release of various neurotoxic viral and/or host factors (Zheng and Gendelman, 1997). Higher levels of replicating HIV-1 in the CNS could

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result in higher concentrations of neurotoxic viral proteins or could stimulate macrophages and microglia to produce neurotoxic factors (Zheng and Gendelman, 1997).

Brain imaging abnormalities, although sometimes minimal, are common in children with symptomatic HIV disease (Belman *et al*, 1986; Epstein *et al*, 1987; Price *et al*, 1988; Chamberlain *et al*, 1991; Kauffman *et al*, 1992; DeCarli *et al*, 1993). Two major groups of CT brain scan lesions, which are relatively independent from each other, can be demonstrated: (1) Cortical Atrophy, manifested as ventricular enlargement and/or subarachnoid dilatation, and frequently associated with white matter abnormalities; and (2) Intracerebral calcifications, which tend to be bilateral and symmetric, and are seen most frequently in the basal ganglia and sometimes in frontal white matter.

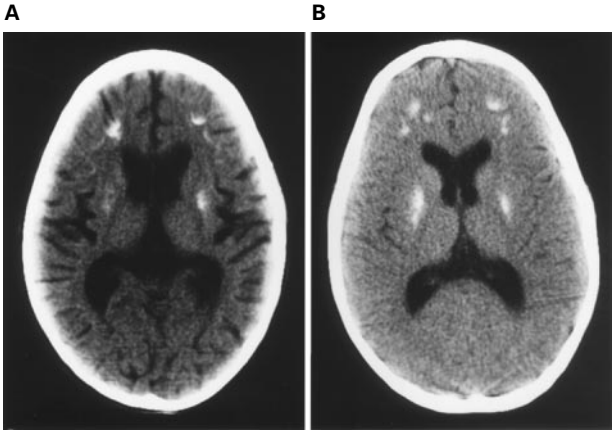


**Figure 1** Serial computed tomography (CT) brain images of a child with symptomatic vertically acquired HIV-1 disease documenting the relative independence of progression and change in intracerebral calcifications and cortical atrophy. (**A**) CT scan when the patient was 4.6 years old showing the presence of bilateral intracerebral calcifications in the basal ganglia and the periventricular white matter of the frontal lobe, and significant ventricular enlargement and sulcal widening. At this time the patient was encephalopathic and immunosuppressed (CD4%=2). (**B**) A follow-up CT scan when the patient was 5.48 years old. In the interim he had been on continuous intravenous infusion of zidovudine and cerebrospinal fluid determination had shown 868 copies/ml. In comparison to scan **A** an improvement in cortical atrophy can be noted, in contrast, the severity of the calcifications has increased, indicating the relative independence of these two lesions.

While most CT brain scan abnormalities are equally common in vertically and transfusion infected children, calcifications are almost exclusively seen in vertically infected patients, even when compared to children who acquired HIV through transfusions in the neonatal period (DeCarli *et al*, 1993; Civitello *et al*, 1994). Thus, the development of intracerebral calcifications may depend in part on when the developing brain gets infected, and may indicate intra-uterine rather than intra- or post-partum infection in these children with vertically acquired HIV infection (Civitello *et al*, 1994).

Despite treatment, calcifications tend to progress, evidenced by increasing density of existing lesions and the occurrence of additional lesions particularly in the periventricular white matter. Progression of calcifications occurs even when other CT brain scan abnormalities improve (see Figure 1). These other abnormalities, ventricular enlargement, subarachnoid dilatation, cerebellar atrophy, and white matter abnormality, tend to show comparable degrees of change (DeCarli *et al*, 1991; Brouwers *et al*, 1994). This pattern of change in imaging variables again suggests that intracerebral calcifications are relatively independent from other CNS abnormalities (DeCarli *et al*, 1993) and that once present, they do not reflect clinical disease progression in the CNS (Brouwers *et al*, 1996).

We have previously demonstrated (Brouwers *et al*, 1995) that greater degrees of CT brain scan abnormalities were significantly related with more advanced stages of HIV disease, as reflected by either lower CD4 measures or elevations of serum p24 antigen. A few other studies have used PCR techniques to associate CSF HIV-1 RNA levels with brain scan abnormalities. Pratt *et al* (1996) found that children with CSF viral loads >10 000 copies/ml were more likely to exhibit brain imaging abnormalities than those with lower viral loads, but these brain scan abnormalities were not further specified. Using the same viral load criterion we

**Table 1** Patient characteristics of the 39 children with symptomatic HIV disease, combined as well as by subgroups according to presence or absence of intracerebral calcifications

| | *All children* | *Calcifications* | *No calcifications* |
|---|---|---|---|
| Gender (male/female) | 26/13 | 14/7 | 12/6 |
| Route of infection (vertical/transf) | 34/5 | 21/0 | 13/5 |
| Age (years) | 3.6 (0.5–12.8) | 2.2 (0.7–5.1) | 5.1 (0.5–12.8) |
| Parental education (years) | 12.9 (10–18) | 12.7 (10–16) | 13.2 (11–18) |
| GIMA[a] | 60.3 (39–122) | 55.1 (39–98) | 66.7 (39–122) |
| Cortical atrophy | 34.9 (0–82) | 39.6 (0–82) | 29.3 (0–73) |
| CD4 per cent | 13.5 (0–52) | 11.2 (1–45) | 16.1 (0–52) |
| (*z*-scores)[b] | −3.03 (−4.63–0.79) | −3.28 (−4.47–0.47) | −2.73 (−4.63–0.79) |

[a]General Index of Mental Abilities based on Bayley MDI, McCarthy GCI, of Wechsler FSIQ. [b]Age corrected *z*-scores for per cent CD4 levels.

(Sei *et al*, 1996) reported greater brain atrophy for a subgroup with higher viral load (>10 000 copies/ml), although this group was very small ($n$=5), in comparison to the subgroup with lower viral load ($n$=25).

Based on our review of the various brain imaging abnormalities in children with HIV-infection however, we hypothesize that atrophy and white matter abnormalities reflect the current state of HIV activity within the CNS. Intracerebral calcifications are hypothesized to reflect how and when the CNS became infected in children, but not necessarily the current state of HIV infection in the brain.

This study expands the viral load data included in an earlier study (Sei *et al*, 1996). The purpose is to evaluate in greater depth the relation between CSF and plasma viral load measures and semiquantitative ratings of different types of CT brain abnormalities as well as immune function reflected by CD4 measures.

## Results

*General sample description*

*Demographics* Thirty-nine children, ranging in age from 6 months to 12 years (mean=3.6±0.5; median 2.3 years old) were studied (see also Table 1). They acquired their HIV-1 infection from an HIV-positive mother (i.e. vertically; $n$=34) or via blood-products ($n$=5).

*Immunology* Immune function at the time of lumbar puncture indicated a mean CD4% level of 13.5±2.2% (range 0–52%, median 7%) and a mean age-adjusted $z$-score of −3.03±0.24 indicating that the majority of the children were severely immunosuppressed (see also Table 1).

*Virology* CSF of 39 patients and plasma of 31 patients were available for the determination of viral load using HIV-1 RNA PCR. HIV-1 was detected in the CSF of 31 of 39 patients (80%) and the mean log(10) copy number was 2.2923 (maximum of 222 904). In the plasma HIV-1 was detected in all 31 patients and the mean log(10) copy number was 5.0112 (maximum of 20 945 464).

*Brain imaging* All but one of the children in this sample were rated as having at least one CT brain scan abnormality. The most frequent lesion was cortical atrophy (i.e. ventricular dilatation and/or subarachnoid enlargement), which was noted in some form in 37 of 39 (mean rating 34.9±3.9; range 0–82, median 35.5). Twenty-one subjects had calcifications in basal ganglia, and/or in the frontal white matter (mean severity 14.4±3.1, range 0–61; median 6). All the patients with calcifications had vertically acquired HIV-infection, calcifications were not noted in any of the five transfusion infected children. The mean overall severity rating of the CT brain scan abnormalities was 40.7±3.6 (range 3–87; median 41) indicating on average a moderate degree of abnormality.

*Correlations with brain scan abnormalities*

*Correlations among CT brain scan abnormalities* Ventricular enlargement and sulcal widening were highly correlated ($r$=0.855; $P<0.0001$) and could not be considered independent variables and thus were combined into one variable: Cortical atrophy. This combined variable also correlated with cerebellar atrophy ($r$=0.425; $P<0.01$) but not with white matter abnormalities and calcifications. In addition cerebellar atrophy correlated with white matter abnormalities ($r$=0.374; $P<0.05$) but not with calcifications. Calcification severity therefore did not show any significant correlation with any of the other CT brain-scan variables ($r$ range: 0.033–0.202).

*Correlations with CD4 per cent levels* More intact immune status, as reflected by higher age-corrected standardized CD4% values, was associated with a lesser degree of cortical atrophy ($r$=0.402; $P<0.05$). Correlations with calcifications ($r$=−0.261), white matter abnormalities ($r$=−0.060), or cerebellar atrophy ($r$=−0.185) were not statistically significant. In this sample immune status also did not

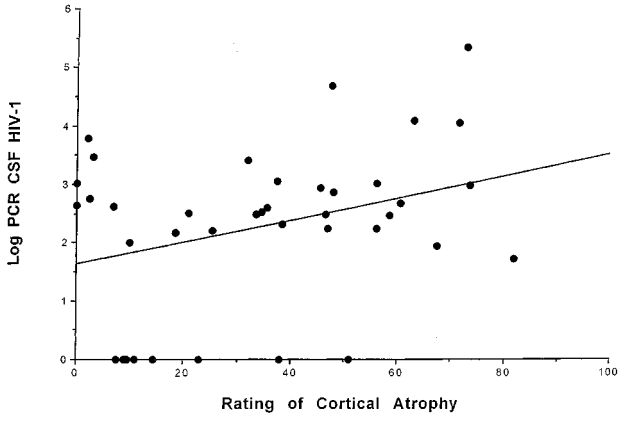


**Figure 2** Relation between viral load, determined by quantitative RNA PCR, in the cerebrospinal fluid and rated degree of cortical atrophy on computed tomography brain scan in children with symptomatic HIV-1 disease. Higher CSF viral load was significantly associated with greater cortical atrophy ($r$=0.36; $P<0.05$).

correlate significantly with viral load (plasma $r=-0.076$; CSF $r=-0.165$).

*Correlations with plasma viral load* No statistically significant correlations of plasma viral load with CT brain scan abnormalities were found (cortical atrophy $r=0.198$; calcifications $r=0.197$; white matter $r=-0.220$; cerebellar atrophy $r=-0.279$). The correlation between plasma and CSF viral load was not statistically significant ($r=0.046$).

*Correlations with CSF viral load* Higher viral load in the CSF was associated with a greater degree of cortical atrophy ($r=0.362$, $P<0.05$). The correlation between CSF viral load and calcifications ($r=-0.120$), white matter abnormalities ($r=-0.003$) or cerebellar atrophy ($r=0.283$) were not statistically significant.

*Comparisons between correlations* The correlations of plasma viral load with cortical atrophy ($r=0.198$) and plasma viral load with intracerebral calcifications ($r=0.197$) were not significantly different. For CSF viral load however there was a significant difference ($t=2.361$; $P<0.05$) between the significant positive correlation with cortical atrophy ($r=0.362$) and the correlation with calcifications ($r=-0.120$).

When the correlation of plasma viral load with atrophy was compared with the correlation of CSF viral load with atrophy, or when the correlation of plasma viral load with calcification severity was compared with the correlation of CSF viral load with calcification severity, no statistically significant differences were found. This may in part be because the correlation between plasma and CSF viral load was very small ($r=0.046$) reducing the reliability of the differences between correlations with CSF or plasma viral load.

*Comparison of patients with and without calcifications*

Twenty-one (54%) of the 39 patients showed evidence of intracerebral calcifications on CT scan. Because of the non-continuous distribution of this CT brain scan abnormalities we investigated whether the presence of calcifications *per se* was associated with differences in viral load, as well as whether severity of calcifications, when present, showed an association.

Patients with calcifications were significantly younger ($2.22\pm0.26$ *versus* $5.09\pm0.98$ years; $P<0.01$) than patients without calcifications. This was largely due to the fact that the five children with transfusion acquired HIV infection did not have calcifications, and were older than the children with vertically acquired infection. If these five subjects were removed from the sample the two groups were comparable in age ($2.22\pm0.26$ *versus* $3.03\pm0.65$). Patients with calcifications also had more severe white matter abnormalities (mean rating of $9.2\pm2.6$ *versus* $1.0\pm1.0$; $P<0.01$) but did not differ significantly in their rating of cortical atrophy ($39.6\pm5.2$ *versus* $29.3\pm5.7$; $P>0.15$) from the patients without calcifications.

With respect to viral load, no significant differences were found between children with or without calcifications for either plasma (means of $5.14\pm0.28$ *versus* $4.87\pm0.30$; $P>0.50$) or CSF (means of $2.28\pm0.33$ *versus* $2.31\pm0.36$; $P>0.90$) viral load measurements. Within the group of children with calcifications, severity of the lesion did not correlate significantly with plasma viral load ($r=0.151$), while more severe calcifications appeared actually associated with lower CSF viral load though not statistically significantly so ($r=-0.302$; $P=0.19$).

## Discussion

The purpose of this study was to evaluate the relationship between different types of CT brain scan abnormalities and measures of viral load in children with symptomatic HIV disease. We hypothesized that whereas the degree of cortical atrophy would reflect the current state of HIV activity within the CNS, the presence or severity of intracerebral calcifications would not.

A significant correlation was indeed found between CSF viral load and cortical atrophy. In contrast, the correlation between the presence or severity of intracerebral calcifications and CSF viral load was not significant. In fact, the correlation between cortical atrophy and CSF viral load was significantly larger than the correlation between calcifications and CSF viral load. These findings suggest that the degree of cortical atrophy is associated with the magnitude of active HIV-1 replication in the brain, while calcifications are not. Previous research also has indicated that decreases in the degree of cortical atrophy reflect improvement in CNS functioning associated with the initiation of zidovudine therapy (DeCarli *et al*, 1991), which is presumably due to decreased viral replication in the brain (Yarchoan *et al*, 1987).

The lack of a correlation between intracerebral calcifications and cortical atrophy in the current data replicates earlier findings in which we have argued that these brain abnormalities are independent and may have a different etiology and natural progression (DeCarli *et al*, 1993). As noted before, intracerebral calcifications are almost exclusively seen in vertically infected children. The few transfusion infected children who have developed calcifications acquired HIV through neonatal trans-

fusion and were born prematurely at a lower gestational age (range 25–33 weeks) compared to those transfusion infected children who did not develop calcifications (median of 34 weeks) (Civitello *et al*, 1994). Thus these data seem to indicate that measures associated with intracerebral calcifications, whether it is the first occurrence, occurrence of new sites of lesions, or increased severity of lesions already present are unlikely to be good markers of current status or short term interval change of HIV-1 in the CNS (see also Figure 1). Rather calcifications are more likely a reflection of the timing of HIV-1 invasion into the CNS of such a child.

Recently, Katsetos *et al* (1999) have demonstrated cerebral vasculopathy in five of six children with HIV-associated CNS disease, involving particularly the basal ganglia, and causing secondary mineralization. Particularly CD3 positive T cells immunologically mediate this vasculitis/vasculopathy. A more prominent immune response of these T cells in infants as compared to older children, could be part of the explanation for the selectivity of these abnormalities.

The hypothesis that certain brain abnormalities may have a different etiology and be associated with in-utero infection (DeCarli *et al*, 1993) has been recently further supported on the basis of neurological and virological findings from a French longitudinal study (Tardieu *et al*, 2000).

Our findings further extend earlier reports of a relation between CSF viral load and brain imaging abnormalities (Sei *et al*, 1996; Pratt *et al*, 1996). Our findings indicate that it is not brain abnormalities in general, but cortical atrophy (and not intracerebral calcifications) that sustains the relation between viral load and scan abnormalities. Moreover, our data seem to suggest that the relationship between CSF viral load and cortical atrophy is continuous rather than threshold. These data combined suggest that a degree of cortical atrophy may be a good marker for HIV-1 activity in the CNS.

Further studies are needed to establish whether cortical atrophy also may be an appropriate surrogate marker for CNS drug efficacy in antiretroviral clinical trials. Although the current study did not provide data on the relation between change in atrophy and change in CSF viral load, preliminary results from a recent study have suggested that cortical atrophy may be an appropriate surrogate marker for CNS drug efficacy in antiretroviral clinical trials. In a randomized clinical trial comparing monotherapy of ZDV and ddl with combination therapy ZDV plus ddl, significant differences between treatment arms in the progression of cortical atrophy over a 96 week period reflected the results from the overall study (Raskino *et al*, 1999).

The clinical significance of change in cortical atrophy with therapy in terms of neurobehavioral functioning in pediatric HIV disease, however, has not yet been established. In a previous treatment study (DeCarli *et al*, 1991), most children showed improvements in quantitative measures of cortical atrophy as well as in neurobehavioral functioning, however the magnitudes of change were not correlated. Similarly, in another study evaluating the CNS effects of a number of antiretroviral agents we found no correlation between the magnitude of change in brain imaging variables and neurobehavioral functioning (Brouwers *et al*, 1994). Thus it remains to be demonstrated that measures of cortical atrophy can be substituted for neurobehavioral measures, which could be advantageous in those studies where no valid or reliable assessments of infants and children can be obtained. Similar to our previous studies (Brouwers *et al*, 1994, 1995) we found a significant correlation between the general index of mental abilities (GIMA from either the Bayley, McCarthy, or WISC) and the cortical atrophy rating ($r=-0.56$; $P<0.001$). The correlation between CSF viral load and the GIMA was also significant ($r=-0.39$; $P<0.05$). This last relation, however, was indirect and mediated by the cortical atrophy. That is, when the correlation between CSF viral load and GIMA was statistically adjusted (Baron and Kenny, 1986) for cortical atrophy the correlation was significantly reduced and became non-significant ($r=-0.23$; $P=0.17$). These findings indeed strengthen our argument that under restricted circumstances cortical atrophy could be used as a surrogate for neurobehavioral functioning on a cross-sectional basis.

A second issue is the relative independence between the brain and the rest of the body with respect to HIV disease. We found no correlation between CSF and plasma viral load in this study. Other studies investigating the relation between viral load in plasma and CSF, both in children and adults, have reported conflicting results. Some studies have failed to find a significant relation (Cinque *et al*, 1998; Burchett *et al*, 1998). Other investigators have found significant relations (Lafeuillade *et al*, 1996; Calvez *et al*, 1997), although the magnitude of these have tended to be small (Pratt *et al*, 1996). Even in those studies where CSF and plasma viral load were significantly related, neurobehavioral variables correlated only with CSF and not with plasma viral load (Pratt *et al*, 1996; Robertson *et al*, 1998). Similarly, in this study plasma viral load did not correlate with any of the brain imaging variables. This finding may suggest that the correlation between CSF and plasma viral load is indirect, that is, it reflects the correlation that both variables have with the same underlying variable, the progression of HIV-1 disease, rather than a direct relation between them. We have previously suggested that, in a similar fashion, the

relation between CD4 measures and neurological abnormalities is indirect and provided statistical support for that notion (Brouwers *et al*, 1994, 1995). Other explanations for these differences in findings may be related to (1) the type of antiretroviral therapy the patients were receiving (i.e. differences in the CNS permeability of the therapy and differential effectiveness of the treatment in the systemic and CNS compartments); (2) the stage of the disease (i.e. in the later stages the blood brain barrier may be less intact, thereby creating a greater comparability between the CNS and the rest of the body systems; but see Ellis *et al* (1997); and (3) the overall level and variation of viral load in the sample because with less variation there are less possibilities for correlation.

In summary, brain scan abnormalities are used in clinical trials to evaluate new antiretroviral treatments for HIV infection in children (Englund *et al*, 1997; McKinney *et al*, 1998). This study suggests that rather than using a global measure of brain abnormalities, a measure largely dependent on cortical atrophy should be used to monitor HIV-1 associated structural CNS effects. Measures associated with intracerebral calcifications should not be used in clinical trials as markers of disease progression or endpoint, or as surrogate markers of therapeutic response (Brouwers *et al*, 1996).

## Materials and methods

*Subjects*

Forty-one patients with symptomatic HIV disease who were seen at the Pediatric Branch of the National Cancer Institute (NCI) between 10/90 and 9/95 participated in the original study. These patients were evaluated to determine their eligibility for various clinical protocols approved by the Institutional Review Board, or as a part of baseline or follow-up studies associated with these protocols. They underwent comprehensive evaluations, including CT brain imaging, CSF and plasma evaluation, neurobehavioral assessment, and CD4 counts. At the time of the evaluation, children were afebrile and without evidence of another infection. Patients with acute onset of neurological symptoms, who required CSF examination, were not enrolled in this study. Two additional patients were excluded from our original cohort of 41 children described earlier (Sei *et al*, 1996); for one patient no evaluable CT brain scan was available, and one other patient was excluded because he had received cranial irradiation as part of his treatment for cancer, which may also cause CT brain scan abnormalities (Riccardi *et al*, 1985).

*Antiretroviral regimen*

Twenty-eight of 39 patients had been receiving zidovudine, as a single drug ($n$=22) or in combination ($n$=6), for more than 6 weeks at the time of the lumbar puncture. Three of these children were receiving continuous intravenous infusion of zidovudine as a single regimen given at 480 mg/m$^2$/day. The remaining 25 children were on orally administered zidovudine with the doses ranging from 60–180 mg/m$^2$ every 6 h. Eight other patients were receiving monotherapy with either didanosine ($n$=7) or zalcitabine ($n$=1) at the time of lumbar puncture. Six of these eight had previously received oral zidovudine for various periods of time (5 weeks to 2 years, median 9.5 months). Three patients were naive to any antiretroviral treatment.

*Viral load evaluation*

CSF was collected under a normal sterile procedure and an aliquot was sent for routine laboratory testing. The cellular component was removed from the remaining CSF and was stored at $-70^{\circ}$C until used for viral RNA analyses. Plasma samples were processed from anticoagulated whole blood specimens obtained within a week of lumbar punctures (within 24 h in the majority of cases), and subjected to the same virological analyses as the CSF. Quantitation of viral RNA has been described in detail in our previous report (Sei *et al*, 1996).

*Scan evaluation*

A CT brain scan was obtained within close proximity of the date of CSF collection (median 6 days) for 39 children. A neurologist, blind to the clinical status of the patient, rated the scans for the presence and severity of ventricular dilatation, subarachnoid enlargement, white matter abnormalities, intracerebral calcifications, or other lesions, including cerebellar atrophy, using a previously described highly reliable semiquantitative technique (DeCarli *et al*, 1993). The severity of each type of brain scan abnormality was rated on a 100-mm analog scale, from no abnormality (0) to very severe abnormalities (100). In addition, an overall severity rating was made which served as a composite for all observed abnormalities.

*Immunological evaluation*

CD4+ lymphocyte subset percentages were determined by flow cytometry of Ficoll-separated peripheral blood mononuclear cells using an EPICS-Profile flow cytometer with Becton-Dickinson antibodies. To adjust for the rapid physiological changes in normal CD4 levels in the first 4 years of life, formulae developed to correct for these changes and to transform the data to standard ('$z$') scores were utilized as discussed previously (Brouwers *et al*, 1995). The variability in per cent CD4 is significantly smaller compared to the variability in absolute CD4 (Raszka *et al*, 1994) which makes per cent CD4 a better and more reliable immune measure for our current study.

*Statistics*

Measures of virologic activity and immune status were related to the degree and type of CNS abnormality based on CT brain scan ratings. Since the PCR RNA data were significantly positively skewed (skewness of 5.83 and 3.31 for CSF and plasma respectively), a log(10) transformation was applied and a non detectable level was set at log(RNA)=0, which normalized the data (skewness of −0.36 and 0.47 respectively). Because of the wide age-range in this sample, we investigated whether we needed to correct for the effects of age. Age was negatively correlated with Log plasma viral load ($r=-0.39$; $P<0.05$), but not Log CSF viral load ($r=0.10$; NS) with CD4% ($r=-0.37$; $P<0.05$) as well as with the age-adjusted CD4% $z$-score ($r=-0.34$; $P<0.05$), and with the presence of calcifications ($t=2.84$; $P<0.01$). Thus, the data were analyzed with analysis of (co)variance and (partial) correlations where appropriate to adjust for the effect of age, as well as with $t$-tests and the Fisher exact test.

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