Cerebrospinal Fluid Analysis in Human Immunodeficiency Virus Infection*

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ABSTRACT

Cerebrospinal fluid (CSF) analytes were evaluated in 59 human immunodeficiency virus (HIV + ) individuals to assess neurological involvement. Glucose, total protein, cell counts, p24 antigen, CSF:serum albumin/IgG ratios, and oligoclonal bands were measured. Eighty percent of samples showed abnormalities in one or more analyte. In some patient samples, these abnormalities could mimic those of secondary opportunistic infection when none was present. The presence of oligoclonal banding in CSF (31 percent) and disturbances in CSF:serum albumin/IgG ratio (30 percent) were related to decreases in serum CD4+ lymphocytes. Disturbances in CSF:Serum albumin/IgG ratio were also related to severity of non-neurological HIV disease staging. Cerebrospinal fluid oligoclonal bands were distinct from that found in serum in the same subjects. Since immune complexes between immunoglobulins and enzymes are observed in these same patients, these oligoclonal bands may result in artifactualy elevated enzyme results secondary to decreased clearance leading to erroneous clinical decisions. There was no significant relationship between any abnormalities and the presence of neurologic disease as established by a wide variety of other studies. It is important to recognize the limits of CSF interpretation in this patient group.

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Introduction

It is well established that Human Immunodeficiency Virus (HIV) infection results in neurologic disease, both by
direct effects of the virus itself and by permitting secondary opportunistic infections, which include herpes virus, toxoplasmosis, cryptococcosis, syphilis, and others.\textsuperscript{6,8} This creates the need for clinical and laboratory markers which will indicate the presence of HIV related primary neurological disease and of clinically significant secondary infection of the nervous system. Analysis of cerebrospinal fluid (CSF) provides an important method of evaluation of neurological disease. However, studies of CSF in HIV seropositive (HIV + ) patients have indicated that abnormalities in this group may not have the same predictive value they have in a non HIV-infected population.\textsuperscript{2,5} In order to establish the significance of such abnormalities, CSF was evaluated in a cohort of HIV + subjects not selected for the presence of neurological disease. The results of these evaluations were compared with the results of extensive neurological, neuropsychological, clinical neurophysiological, neuroradiological, and other laboratory evaluations.

Materials and Methods

Cerebrospinal fluid evaluation was performed on 59 HIV + subjects. Twenty-eight subjects were classified as asymptomatic, 22 as having AIDS related complex (ARC), and nine as having acquired immunodeficiency syndrome (AIDS). No subject had known active opportunistic infection of the nervous system. The CSF was evaluated for the following parameters: total protein, glucose, cell counts, p24 antigen, serum: CSF albumin/globulin ratio, oligoclonal banding by agarose electrophoresis, Q albumin, cryptococcus polysaccharide antigen, and serological test for syphilis (VDRL). Serum was evaluated for CD4 count, complete blood count, albumin, and IgG.

In addition each subject underwent the following: comprehensive clinical neurological evaluation; extensive neuropsychological evaluation; routine and quantitative electroencephalography (EEG); somatosensory, visual, brainstem auditory and cognitive (P300) evoked potentials; peripheral nerve conduction velocities; magnetic resonance imaging (MRI) of the brain; and evaluation of peripheral lymphocyte function. All statistical analyses were completed with SAS.\textsuperscript{10} Chi square, Pearson product moment, and Spearman correlational analyses of variables were computed as appropriate.

Results

Eighty percent of subjects had one or more abnormalities of CSF. Thirty-six percent had only one abnormality, 17 percent had 2, 10 percent had 3, 7 percent had 4, 8 percent had 5, and 2 percent had 6. The likelihood of finding each different abnormality is shown in table I. There was no significant relationship between overall abnormalities of the CSF and the level of disease progression, abnormalities of the neurological examination, neuropsychological examination, MRI scan, or clinical neurophysiological examination. No subject had elevated titers of cryptococcus antigen or VDRL.

Positive correlations were found between CSF total nucleated cells and both serum white cell counts ($r = 0.39$, $p = 0.002$) and CD4 + levels ($r = 0.34$, $p = 0.009$). A positive correlation was found between the percentage of lymphocytes in the CSF and serum CD4 + lymphocytes ($r = 0.36$, $p = 0.005$). There was an increase in the presence of oligoclonal banding as the serum CD4 + cell count decreased ($r = -0.29$, $p = 0.03$). A positive relationship was found between disturbances of the CSF:serum albumin/globulin ratio and disease staging ($r = 0.25$, $p = 0.05$). A negative relationship was found between serum CD4 counts and the CSF:serum albumin/globulin ratio ($r = -0.25$, $p = 0.05$).
TABLE I

Abnormalities Found in the 59 Cerebrospinal Fluid Samples Evaluated

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percent</th>
<th>ASX</th>
<th>ARC</th>
<th>AIDS</th>
<th>Range</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>49</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Protein</td>
<td>13</td>
<td>22</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>46-102</td>
<td>15-45</td>
</tr>
<tr>
<td>Total nucleated cells</td>
<td>12</td>
<td>20</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>6-136</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>15</td>
<td>25</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>2-92</td>
<td>0</td>
</tr>
<tr>
<td>p24 Antigen</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Oligoclonal banding</td>
<td>18</td>
<td>31</td>
<td>7</td>
<td>7</td>
<td>4</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>CSF: serum Alb/IgG ratio</td>
<td>17</td>
<td>30</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>0.9-2</td>
<td>&lt;0.8</td>
</tr>
</tbody>
</table>

a Other laboratory analyses were within reference ranges.
b Three specimens did not have this study performed.

ASX = asymptomatic  ARC = acquired immunodeficiency syndrome (AIDS) related complex

Discussion

These results are significant to the practicing clinical chemist, reinforcing the difficulty in interpreting abnormalities of the CSF in HIV infected patients. It should be stressed that the procedures described are standard techniques used by most medical laboratories. Because of this, abnormalities, such as those involving immunoglobulins, are found at a lower rate than are found in similar subjects by others when using more sensitive isoelectric focusing techniques, but are perhaps more relevant to general clinical practice.

Eighty percent of subjects had abnormal values. Some of these abnormalities, such as markedly elevated total nucleated cells or increased polymorphonuclear leukocytes, would suggest the presence of secondary CNS infection. None of these subjects had any evidence of such disease, by clinical examination, culture, antibody testing or subsequent follow-up studies.

In a population which is at high risk for secondary intracranial infection, it is imperative that the clinician and laboratorian understand the limits of CSF evaluation and the potential for overinterpretation of abnormalities of protein and cell counts. When looking for infection in this group, particular stress must be placed on obtaining CSF culture and detecting the presence of known CNS pathogens, including Treponema pallidum and cryptococcus. Although a significant hypoglycorrhachia was not seen in our cohort, the presence of this may increase the likelihood of secondary infection. Other studies of CSF in HIV infected subjects have shown that abnormalities are common. In these, there have been no clear markers which predict the likelihood that a patient is going to develop nervous system abnormalities.

The present study has compared CSF results to those obtained from extensive neurological and neuropsychological testing. Neurological or neuropsychological abnormalities are more likely in the later stages of the disease, and it might have been expected that CSF abnormalities would reflect this. However, overall abnormalities of the CSF were not related to the stage of disease (asymptomatic, ARC or AIDS). Eleven (18 percent) of the subjects in the study had clinical findings which indicated neurological involvement, but there was no clear relationship between the clinical findings and abnormalities in the CSF.

Increased severity of systemic disease, as manifest by a reduction in serum CD4
cells and by disease stage (asymptomatic, ARC or AIDS), was associated with alterations in the CSF:serum albumin/IgG ratio. However, the abnormalities were due to an increase in production of IgG within the central nervous system and not to breakdown of the blood brain barrier, since no patient had an elevated Q albumin index.

Low CD4 cells were also associated with the presence of oligoclonal banding in the CSF. The pattern of oligoclonal banding seen in these subjects appears to be unique (figure 1). Discrete bands were found in both serum and CSF, but there were consistent migration differences between the bands found in CSF and those in serum, again indicating that there was production in the central nervous system rather than leakage from serum to CSF or vice versa. Traditionally, this finding has been accepted as a hallmark of active demyelination. In HIV infection, the increase in IgG may indicate a failure in immune surveillance as the disease progresses, with resulting inappropriate production of antibodies to a variety of different sequestered antigens to normal body components, as well as to specific infectious agents. As has been reported by us¹ and others,⁴ immune complexes involving amylase appear to be frequently observed in HIV positive patients. Similar complexes found by us have been directed to creatine kinase, and inappropriate macro complexes may well be produced to other intracellular antigens, resulting in potentially misleading enzyme findings in blood and, perhaps, CSF.

Our results must not be taken to suggest that CSF examination is not useful in this particular group of subjects; on the contrary, these results clearly demonstrate that CSF analysis is essential in the diagnosis of secondary bacterial, mycobacterial, and fungal infection and may also be indicative of meningeal lymphomatosis. However, it is clear that antibody testing and culture are necessary additions to the evaluation of cellular and chemical elements before these values can be clinically interpreted.

It is unclear at this time whether or not the abnormalities of the CSF found here have predictive value in the eventual

**OLIGOCLONAL BANDING PATTERNS**

![Agarose electrophoresis results on simultaneous CSF and serum samples from three patients infected with HIV, demonstrating oligoclonal bands in the gamma region. The CSF samples were concentrated 80 to 100 fold; sera are unconcentrated.](image-url)
development of the AIDS dementia complex or other neurological or neuropsychological disease. It is our hope that, by careful monitoring of the subtle, early changes in function in these subjects, this question will be answered. It has considerable significance in understanding the pathogenesis of the infection, and, as more treatment options become available, it may be of vital importance in deciding when to initiate therapy aimed at the nervous system.

Acknowledgment

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References


