Cytomegalovirus Pneumonia in AIDS Patients

Value of Cytomegalovirus Culture From BAL Fluid and Correlation With Lung Disease

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Objectives: To verify the value of cytomegalovirus (CMV) cultures of BAL fluid vs postmortem lung histopathology in detecting CMV pneumonia, and to correlate the BAL viral dose with the number of CMV inclusion bodies (CMV-IB) in the lung tissue of AIDS patients.

Design: Retrospective analysis of 434 BALs and 40 autopsies involving 307 AIDS patients; clinical follow-up lasted 10 months.

Patients and methods: The 40 patients who died within 20 days of undergoing BAL were divided on the basis of histopathologic findings into subjects with and without CMV-IB in the lung tissue. The relationship between the BAL viral dose and CMV lung infection was evaluated by counting the early antigen (CMV-EA) positive cells/200 μL of BAL and the number of CMV-IB/mm² of lung tissue.

Results: The predictive value of BAL virus isolation for the diagnosis of CMV pneumonia was 61% for positive and 100% for negative results. The patients with the largest number of CMV-IB had CMV-EA counts from 2 to 840; in those with a moderate and small number, the CMV-EA counts were, respectively, from 11 to 700 and 2 to 300. Among the patients surviving up to 10 months after the BAL index sample, the frequency of recurrent extrapulmonary CMV abnormalities was 27% in those with positive and 7% in those with negative cultures.

Conclusions: BAL CMV cultures from AIDS patients have a very high negative and relatively low positive predictive value for CMV pneumonia. The presence and replication of CMV in the lung may lead to systemic dissemination as suggested by the higher probability of CMV extrapulmonary diseases. Viral titers do not seem to be related to the degree of lung damage.

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Key words: AIDS; bronchoalveolar lavage; CMV culture; lung

Abbreviations: CMV=cytomegalovirus; CMV-EA=cytomegalovirus early antigens; CMV-IB=cytomegalovirus inclusion bodies; MoAb=monoclonal antibody

Vir al isolation from BAL fluid has been shown to be useful for the diagnosis of cytomegalovirus (CMV) pneumonia in bone marrow and lung transplant recipients, but little information is available concerning the same correlation in AIDS patients.

To determine the pathogenicity of the virus in the lung, we compared the pulmonary findings from autopsy and antemortem BAL CMV cultures from 40 AIDS patients with pulmonary signs or symptoms who died within 20 days of undergoing BAL.

Our aims were to verify the value of BAL CMV cultures in detecting CMV pneumonia confirmed by postmortem lung histopathologic study, and to relate BAL viral titers to the number of CMV inclusion bodies (CMV-IB) in lung tissue.

Moreover, we evaluated the frequency of extrapulmonary CMV abnormalities in the patients surviving up to 10 months after the BAL index sample.

Materials and Methods

Study Population

Between January 1, 1993 and December 31, 1995, 307 AIDS patients (diagnosed using Centers for Disease Control and
Prevention criteria with pulmonary signs or symptoms attending the Department of Infectious Diseases, St. Raffaele Scientific Institute, University of Milan, underwent 434 BALs: 40 patients died within 20 days of the procedure; the others either are still alive or died after 20 days or more. Clinical follow-up lasted for 10 months after the lavage.

Specimen Collection

BAL was performed using a video-bronroscope as previously described.7,8 The recovered specimens were pooled and sent for cytology, acid-fast and Gram’s staining, mycobacteria, fungal and CMV cultures, and Pneumocystis carinii detection; the last specimen (presumably the least contaminated by the bronchoscope working channel) was not pooled, but directly sent for semiquantitative bacteriologic and fungal culture.

CMV Isolation From BAL Fluid

Five milliliters of fluid was centrifuged (15 min at 3,000 g) to remove cells, bacteria, and debris. The cells were then washed and directly stained. The supernatant was filtered through a 0.45-μm syringe filter and 200-μL aliquots were used to inoculate multiple MRCS-containing shell vials for spin amplification.7 The cells were stained using a monoclonal antibody (MoAb) to the p72 CMV early antigen (CMV-EA; DuPont de Nemours Speciality Diagnostics; Wilmington, Del) in a fluorescence assay. The apple green-stained cell nuclei indicative of CMV infection were counted and expressed as the number of CMV-EA-positive cells/200 μL of fluid.

Immediate Early (pp65) Antigenemia in Polymorphonuclear Leukocytes

Seven milliliters of whole blood was collected in edetic acid tubes. After dextran separation (30 min at 37°C), the polymorphonuclear leukocytes were collected from the upper phase, washed twice, and counted; 2×10⁶ cells were used to prepare adequately fixed and permeabilized cytospin slides. The presence of the immediate early pp65 CMV antigen was evaluated using a specific MoAb (Biosoft-Argene Clone 1C3; Varilhes, France) and immunofluorescence staining. Antigenemia was quantified by counting the positive polymorphonuclear nuclei.5,6

Histopathology Staining

The patients who died within 20 days of undergoing BAL were enrolled in a retrospective histopathologic search for the presence of typical intranuclear or intracytoplasmic CMV-IB in all tissues and other opportunistic lung pathogens. Autopsy evidence of CMV infection was considered to be the presence of typical intranuclear or intracytoplasmic CMV-IB at hematoxylin-eosin staining, confirmed by means of CMV immunoperoxidase staining (DAKO Patts A/S, diluted 1:25).

The number of CMV-positive slides reviewed was 3 to 6 for lung and 2 to 10 for extrapulmonary tissue. All of the lung, lymph node, spleen, adrenal, and other tissue sections macroscopically indicated as being of interest were examined using the same staining methods.

The quantification of lung CMV was expressed as the mean number of CMV-IB per square millimeter of lung tissue obtained after evaluating six microscopic fields X slide (10/0.22 objective with a millimetric grid at Zeiss standard 25 microscopy).

Statistical Analysis

The histopathologic results were taken as the reference test. The sensitivity, specificity, and positive and negative predictive values are based on their standard definitions using a two-by-two table.

Results

Comparative Analysis of BAL Fluid Virus Isolation and CMV Antigenemia vs Autopsy Findings

During the 36 months of the study, 40 patients who died within 20 days of undergoing BAL underwent autopsies: the CMV-IB search revealed CMV in the lung tissue of 11, all of whom died of pneumonia; CMV was detected in the extrapulmonary tissue of 12 of the remaining 29 subjects.

CMV was isolated in the BAL specimens of the 11 subjects with and 7 of 29 without lung tissue CMV; pp65 CMV antigenemia was detected in, respectively, 8 of 11 (73%) and 5 of 29 (17%) cases.

At autopsy, the other CMV-infected sites were the adrenal glands (37.5%), the GI tract (7.5%), and the CNS (5%).

Before BAL, 13 of 40 subjects had received anti-CMV therapy, 9 of whom had CMV-positive BAL cultures; 27 of 40 subjects did not receive anti-CMV therapy, 9 of whom had CMV-positive BAL cultures.

Autopsy revealed that CMV was the only causative agent of pulmonary infection in 5 of 11 patients who died of pneumonia (45%); 5 patients had a large and 6 had a small number of CMV-IB (Table 1).

P carinii and Aspergillus species were detected in, respectively, 4 (36%) and 2 (18%) of the 11 patients, 1 of whom also had Kaposi’s sarcoma.

The lung pathogens observed in the patients without lung tissue CMV were P carinii (41%), Aspergillus species (10%), and acid-fast bacteria (10%); one case of bacteria and one of Candida were also observed, as well as four cases of Kaposi’s sarcoma, three of lymphoma and one of adenocarcinoma.

CMV was cytologically detected in only 1 of the 11 patients with lung tissue CMV.

Correlation Between Antemortem BAL Fluid CMV Cultures and Extrapulmonary Pathologic Findings During Life

The presence of extrapulmonary pathologic findings in all of the 307 studied patients was evaluated by dividing the CMV diseases diagnosed before and after BAL (Table 2). The frequency of the abnormalities diagnosed before BAL was the same in the patients with a positive or negative index, but that of post-BAL-diagnosed diseases was four times greater in those with positive BAL cultures.
Table 1—Quantification of Lung Infection and Its Correlation With Extrapulmonary CMV Infection, Lung Co-pathogens, Anti-CMV Therapy, and the Quantification of CMV in BAL Fluid

<table>
<thead>
<tr>
<th>Patients</th>
<th>No. of CMV-IB per mm² of Lung Tissue</th>
<th>No. of CMV-EA per 200 µL of BAL</th>
<th>Extrapulmonary CMV Infection</th>
<th>Anti-CMV Therapy* After BAL</th>
<th>Lung Co-pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>2</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>2</td>
<td>Adenalinis</td>
<td>Yes</td>
<td>P carinii</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>Pos¹</td>
<td>Adenalinis, gastritis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>840</td>
<td>No</td>
<td>Yes</td>
<td>P carinii, Aspergillus</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>80</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>51</td>
<td>No</td>
<td>Yes</td>
<td>P carinii</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>700</td>
<td>No</td>
<td>Yes</td>
<td>Aspergillus</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>7</td>
<td>No</td>
<td>Yes</td>
<td>P carinii</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>300</td>
<td>Adenalinis, encephalitis</td>
<td>No</td>
<td>Kaposi’s sarcoma</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>250</td>
<td>Adenalinis, encephalitis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>2</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*Three to 10 days of induction therapy.

¹This sample was positive but the quantification was not evaluated.

BAL Fluid CMV Titers and Lung Infection

No correlation was found between the numbers of lung CMV-IB and BAL fluid CMV-EA-positive cells (Table 1).

Although anti-CMV induction therapy was given only for 3 to 10 days (not long enough to be effective), it did not seem to affect the histopathologic result: the patients with a high number of CMV-IB all received induction therapy, whereas most of those with a low number did not.

Statistical Evaluation

All of the 11 patients with postmortem lung CMV-positive findings had a positive BAL fluid culture; of the remaining 29 patients, only 24% had a positive culture.

The prevalence of CMV infection in our sample was 26.5%.

The BAL CMV culture was positive in 8 of 13 patients who had previously received anti-CMV drugs and in 9 of 27 previously untreated patients.

Table 3 shows the sensitivity, specificity, and positive and negative predictive values for the diagnosis of CMV pneumonia in the patients as a whole, as well as the effect of anti-CMV drugs.

Table 2—Correlation Between Antemortem CMV Cultures From BAL and CMV Extrapulmonary Pathologic Findings During Life

<table>
<thead>
<tr>
<th>CMV Diseases</th>
<th>CMV-Positive</th>
<th>CMV-Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before BAL</td>
<td>7/98 (7)</td>
<td>11/209 (5)</td>
</tr>
<tr>
<td>After BAL</td>
<td>18/67 (27)</td>
<td>11/161 (7)</td>
</tr>
</tbody>
</table>

DISCUSSION

The available data concerning the clinical significance of CMV isolation from the BAL fluid of AIDS patients are few and conflicting.⁹ The significance of culturing CMV from respiratory specimens in the absence of demonstrable tissue damage in HIV-1-infected patients (or other immunocompromised subjects) is uncertain, particularly in relation to therapeutic decision. The presence of BAL CMV has so far been shown to be closely related to the presence of active or soon-to-be-active clinical disease only in lung or heart-lung transplanted patients.¹⁰

In our experience, the sensitivity and the negative predictive value of BAL CMV cultures for the diagnosis of CMV pneumonia are good, while the specificity and positive predictive value are relatively low. Our data show that pre-BAL anti-CMV treatment has no effect on the predictive value of BAL CMV cultures; negative virus isolation may exclude CMV pneumonia, but positive isolation may represent a “nondiagnostic” test result as recently described by Mann et al.⁹

Two major risks underlie the management of CMV-positive BAL cultures in AIDS patients: (1) causing unnecessary toxic reactions in patients without CMV pneumonia; (2) failing to identify appropriate treatment for non-CMV pneumonia, particularly given the frequent detection of multiple microorganisms in BAL fluid.

A confounding result was the presence of positive CMV isolation in 7 of 29 subjects without CMV lung disease, possibly because histology was determined on three to six slides obtained from a macroscopically selected lung section, which may have led to a sampling error. This hypothesis is indirectly sup-
ported by the absence of documented lung CMV infection in the subjects with negative isolation from BAL. Furthermore, the detection of CMV-IB by hematoxylin-eosin staining is not the most sensitive method for detecting CMV infection, which may also explain the low prevalence of lung CMV infection in our series; methods using MoAbs or DNA probes are more sensitive.

Although the positive predictive value of CMV-positive BAL fluid could probably be increased by eliminating sampling errors, it is still >14% diagnostic yield provided by thoracotomy biopsy; we did not perform lung or transbrachial biopsy in order to avoid iatrogenic damage. Cytologic examination of the respiratory tract samples from our cohort led to a diagnostic yield of 9%, similar to the 8% reported by Miles et al. in 60 CMV-positive BAL fluid cultures.

It is thought that CMV infection in AIDS patients is long lasting and that lung damage is caused by cytopathic effects due to the extent of viral replication; in bone marrow and lung transplant recipients, fatal pneumonia may be due to an interplay of acute graft-vs-host disease and CMV infection.

Our study shows that CMV isolation from BAL fluid may indicate bronchopulmonary viral replication, and not viral contamination from the blood as suggested by Miles et al. This hypothesis is supported by the presence of CMV antigenemia in 73% of the patients, and the fourfold greater frequency of extrapulmonary abnormalities during follow-up in the subjects with CMV-positive BAL fluid cultures (27%) than in those with negative cultures (7%). These data suggest that CMV replication in lung is a risk for disseminated infection. Our patients represent a selected and profoundly immunosuppressed population with a very short life expectancy at the time of BAL, but these data may help to guide the clinical treatment of less severely immunocompromised HIV-positive subjects with CMV-positive BAL fluid cultures.

Like those of previous studies in other immunocompromised patients, our data fail to show any correlation between BAL fluid CMV titers and the number of CMV-IB in lung tissue: the patient with the largest number of CMV-IB had only two CMV-EA-positive cells/200 μL of BAL fluid, but the same number was found in one of the subjects with fewer CMV-IB.

We cannot make any statement concerning the relationship between antiviral drug use after BAL and CMV lung damage, because of the short period of treatment and the lack of drug resistance data. Nevertheless, we found that the extent of CMV lung infection does not seem to be affected by antiviral drug therapy: of the subjects receiving ganciclovir or foscarinet when they died, four had a large number of lung tissue CMV-IB, two had a moderate, and one had a small number; this discrepancy was also evident in the untreated patients. Prospective studies will give more information on this aspect.

We can conclude that the presence of CMV in the BAL fluid of AIDS patients has a very high negative predictive value and relatively low positive predictive value for histopathologically diagnosed CMV pneumonia.

This study indicates that CMV in the lungs of AIDS patients may be not only a passenger but also a pathogen, and its replication represents a risk for virus dissemination. In our experience, the BAL viral dose does not seem to be related to the degree of lung infection.

**Table 3—Diagnostic Value of CMV Culture From BAL in the Diagnosis of CMV Pneumonia**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive Predictive Value, %</th>
<th>Negative Predictive Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated with anti-CMV drugs before BAL</td>
<td>100</td>
<td>50</td>
<td>55</td>
<td>100</td>
</tr>
<tr>
<td>Never treated</td>
<td>100</td>
<td>86</td>
<td>67</td>
<td>100</td>
</tr>
<tr>
<td>All</td>
<td>100</td>
<td>76</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>

**References**

3. Miles PB, Baughman RP, Linneman CC. Cytomegalovirus in the bronchoalveolar lavage fluid of patients with AIDS. Chest 1990; 97:1072-76
between immunofluorescent detection of human cytomegalovirus immediate early antigens in polymorphonuclear leukocytes and viremia. J Infect Dis 1989; 160:159-60