Pulmonary Cytomegalovirus Infection in Immunocompromised Patients*

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Background: Cytomegalovirus (CMV) infection and CMV disease are frequent complications in immunocompromised patients. In this study, the incidence of pulmonary CMV infection was analyzed in different groups of immunocompromised patients and the diagnostic value of immunostaining with anti-CMV antibodies in BAL cells was evaluated in regard to the diagnosis of CMV pneumonitis.

Methods: Five hundred eighty consecutive BAL procedures were analyzed prospectively in 442 immunocompromised and 126 nonimmunocompromised control subjects. CMV culture in BAL fluid was performed by shell vial assay and immunostaining using three monoclonal anti-CMV antibodies.

Results: The incidence of culture results positive for CMV in the BAL fluid varied from 20 to 30% in HIV-positive patients, in patients following stem cell or renal transplantation, and in patients with autoimmune disease or lung fibrosis treated with immunosuppressive agents. CMV was cultured from 4.4% of BALs in patients treated with high-dose chemotherapy and from 2.4% of control subjects. CMV disease developed in 37 patients; in 18 of these patients, CMV pneumonitis was present. The results of CMV immunostaining were positive in a total of 22 BALs, all in patients with CMV disease. The sensitivity, specificity, and positive and negative predictive values of positive CMV immunostaining results for the diagnosis of CMV pneumonitis were 88.9%, 98.6%, 72.7%, and 99.5%, respectively.

Conclusion: The incidence of pulmonary CMV infection is similar in different groups of immunocompromised patients except for patients following high-dose chemotherapy. CMV immunostaining in the BAL fluid is a very helpful method to diagnose CMV pneumonitis in these patients.

Key words: BAL; cytomegalovirus infection; cytomegalovirus immunostaining

Abbreviation: CMV = cytomegalovirus

Evidence of current or past cytomegalovirus (CMV) infection, as determined by positive CMV serology results, is common in the general population. Symptomatic CMV infection is rare in immunocompetent people but is one of the most important opportunistic infections in immunocompromised patients. The incidence of CMV pneumonitis varies according to the underlying disease and is associated with considerable morbidity and mortality. In allogeneic bone marrow transplant recipients, CMV pneumonitis is frequent and is associated with a high mortality rate, especially when the diagnosis cannot be made early and the initiation of treatment is delayed. Following solid-organ transplantation, the incidence of CMV pneumonitis depends on the type of allograft and the immunosuppressive regimen. In contrast, the clinical importance of pulmonary CMV infection in HIV patients is controversial, and CMV often can be found in the BAL fluid of these patients without evidence of CMV pneumonitis.

The diagnosis of CMV pneumonitis is usually based on symptoms of fever, dyspnea, hypoxemia, and diffuse infiltrates on chest radiograph in combination with the detection of CMV in the BAL fluid. A definitive diagnosis of CMV pneumonitis can be made histologically showing cytomegalic cells associated with inflammatory reactions and tissue destruction in transbronchial or open-lung biopsy speci-
mens. However, transbronchial biopsies are performed rarely because of the risk of bleeding, especially in thrombocytopenic patients.

Immunostaining with anti-CMV antibodies in BAL fluid has been shown to be helpful for the diagnosis of CMV pneumonitis in selected groups of patients.\textsuperscript{17-20} In this study, the incidence of CMV detection in the BAL fluid of different groups of immunocompromised patients was determined and the diagnostic value of CMV immunostaining for detection of CMV pneumonitis was evaluated.

**Materials and Methods**

**Patient Population**

Over a 4-year period, 442 BAL procedures were performed in immunocompromised patients, and the diagnostic value of different methods for CMV detection was prospectively evaluated (ie, CMV culture, cytology, and CMV immunostaining). Similar diagnostic methods were used in a total of 126 BAL procedures performed in immunocompetent patients, who were considered to be a nonimmunocompromised control group. The immunocompromised BAL group consisted of the following patients: HIV-positive patients, 227; allogeneic bone marrow or peripheral stem cell transplant recipients, 69; patients undergoing high-dose chemotherapy for hematologic diseases, 68; solid-organ transplant recipients, 49 (renal, 47; heart, 1; lung, 1); and patients with autoimmune disease or lung fibrosis receiving immunosuppressive therapy, 29. The nonimmunocompromised group included the following patients: patients with persistent pulmonary infiltrates receiving antibiotic treatment, 54; patients with sarcoidosis, 30; patients with idiopathic lung fibrosis, 17; patients with autoimmune disease with pulmonary involvement, 12; patients with exogenous allergic alveolitis, 10; and patients with bronchiolitis obliterans-organizing pneumonia, 3. The patients of the nonimmunocompromised group were not treated with immunosuppressive agents at the time of bronchoscopy.

**BAL**

Indications for BAL were fever, respiratory symptoms, and/or infiltrates seen on the chest radiograph. To obtain BAL fluid, 150 to 300 mL of 0.9% NaCl was instilled into the middle lobe or the segment of the most prominent radiologic infiltrate.\textsuperscript{22} BAL fluid was investigated routinely for bacterial, mycobacterial, and fungal growth. Ten milliliters of BAL fluid was sent to the laboratory for bacterial culture, and another 10 mL was sent for virus culture. Conventional cytology was performed with the remaining fluid. Following centrifugation, the cell pellet was resuspended in cell culture medium and smears were prepared. Routine staining of one or several anti-CMV antibodies was considered to be diagnostic for CMV. Immunostaining for CMV was performed blindly without knowledge of the results of cytology and immunostaining in the BAL fluid.

**Clinical Data**

Medical records and chest radiograph findings of all patients with a culture positive for CMV in their BAL fluid were analyzed retrospectively for the presence of CMV disease within a period of 3 months before and after BAL was performed. Clinical evaluations for the presence of CMV disease were performed blindly without knowledge of the results of cytology and immunostaining in the BAL fluid.

**Definitions**

Pulmonary CMV infection was defined as a positive CMV culture result in the BAL fluid, irrespective of symptoms or signs of disease. CMV disease was defined as CMV infection combined with symptoms and/or tissue invasion of CMV, including CMV pneumonitis, CMV retinitis, CMV hepatitis, and CMV syndrome. CMV pneumonitis was defined as the detection of CMV by culture combined with the presence of fever, respiratory symptoms (eg, dyspnea, hypoxemia, and reduced diffusion capacity in pulmonary function tests), and interstitial infiltrates on chest radiograph. Immunostaining with a monoclonal antibody against CMV in the BAL fluid and viral inclusion bodies in BAL cells detected by conventional cytology were not used as diagnostic markers for the definition of CMV pneumonitis. CMV syndrome was defined as fever combined with at least one of the following symptoms: elevated liver enzymes (aspartate aminotransferase and alanine aminotransferase at least twofold); leukopenia (leukocyte count, $<3 \times 10^9/L$); thrombopenia (platelet count, $<50 \times 10^9/L$) in the absence of other infections; and graft rejection. CMV hepatitis was present if there were elevated levels of liver enzymes and if CMV could be detected in a liver biopsy specimen. CMV retinitis was defined by characteristic funduscopic lesions. GI CMV disease included GI symptoms combined with biopsy specimen-proven CMV tissue invasion.

**Results**

**General Microbiological Findings**

Microbiological findings are summarized in Table 1. Bacteria were found in 31.9% of immunocompromised patients (141 of 442 patients) and in 37.3% of nonimmunocompromised patients (47 of 126 patients). *Legionella pneumophila* was documented in three patients. *Mycobacterium tuberculosis* was found in 5.7% of HIV-positive patients (13 of 227 patients) and in one patient with idiopathic pulmonary fibrosis who had been treated with steroids. Atypical mycobacteria could be cultured in 10.6% of BAL procedures (24 of 227 procedures) in the HIV-positive group. Fungi, including Candida spp, were grown in the BAL fluid of 12.9% of patients (73 of 568 patients). *Aspergillus fumigatus* was detected performed blindly without knowledge of the clinical findings and the results of CMV cell cultures. CMV culture was performed by shell vial assay, spinning the BAL fluid onto human embryonic fibroblasts and determining the presence of CMV immediate early antigens by immunofluorescence and the presence of CMV DNA by *in situ* hybridization after 1 day and 5 days, respectively.\textsuperscript{22}
in one bilateral lung transplant recipient with bronchial stenosis, in a renal transplant recipient, and in an HIV-positive patient with concomitant bacterial and herpes simplex virus infection. Cryptococcus neoformans was found in two patients and Nocardia sp was found in another patient. P. carinii was detected in the BAL fluid of 18.1% of HIV-positive patients (41 of 227 patients), in 16.3% of solid-organ transplant recipients (8 of 49 patients), and in 17.2% of patients with lung fibrosis/autoimmune disease who were receiving immunosuppressive treatment (5 of 29 patients). There was one case of P. carinii in the chemotherapy group and another two cases in the stem cell transplantation group. Pulmonary Kaposi’s sarcoma associated with herpes virus 8 infection was present in 22 HIV-positive patients and in a renal transplant recipient.23

CMV Detection by Culture (Shell Vial Assay)

CMV was detected by the shell vial assay in the BAL fluid of 24.2% of patients in the immunocompromised patient group (107 of 442 patients) and in that of 2.4% of patients in the nonimmunocompromised group (3 of 126 patients; Tables 1, 2). The three CMV-positive patients in the nonimmunocompromised patient group were found in patients with antibiotic-resistant infiltrates. One of these patients had L. pneumoiae infection, and another patient had received steroids prior to bronchoscopy. No CMV could be detected in the BAL fluid of patients with sarcoidosis, idiopathic lung fibrosis, and autoimmune disease if these patients had not been treated with immunosuppressive agents.

The incidence of CMV infection in immunocompromised patients varied according to the underlying diagnosis. CMV could be cultured from the BAL fluid of 24.5 to 29.0% of renal and stem cell transplant recipients, patients with autoimmune disease/lung fibrosis receiving immunosuppressive treatment, and HIV-positive patients. The incidence of CMV infection was 4.4% (3 of 68 patients) in the high-dose chemotherapy group (Table 2).

Table 1—Microbiological Findings in BAL Fluid of 442 Immunocompromised and 126 Nonimmunocompromised Patients

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>No.</th>
<th>Bacteria %</th>
<th>No.</th>
<th>Mycobacteria %</th>
<th>No.</th>
<th>CMV Culture %</th>
<th>No.</th>
<th>Fungi %</th>
<th>No.</th>
<th>Pneumocystis %</th>
<th>No.</th>
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</thead>
<tbody>
<tr>
<td>HIV/AIDS</td>
<td>227</td>
<td>39.7</td>
<td>90</td>
<td>16.3</td>
<td>37</td>
<td>28.2</td>
<td>64</td>
<td>17.6</td>
<td>40</td>
<td>18.1</td>
<td>41</td>
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<tr>
<td>Stem cell transplants</td>
<td>69</td>
<td>15.9</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>29.0</td>
<td>20</td>
<td>5.7</td>
<td>4</td>
<td>2.9</td>
<td>2</td>
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<td>Chemotherapy</td>
<td>68</td>
<td>20.6</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>4.4</td>
<td>3</td>
<td>13.3</td>
<td>9</td>
<td>1.5</td>
<td>1</td>
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<tr>
<td>Renal transplants</td>
<td>49</td>
<td>32.7</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>24.5</td>
<td>12</td>
<td>12.2</td>
<td>6</td>
<td>16.8</td>
<td>8</td>
</tr>
<tr>
<td>Autoimmune disease*</td>
<td>29</td>
<td>34.5</td>
<td>10</td>
<td>3.4</td>
<td>1</td>
<td>27.6</td>
<td>8</td>
<td>20.7</td>
<td>6</td>
<td>17.2</td>
<td>5</td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>442</td>
<td>31.9</td>
<td>141</td>
<td>8.6</td>
<td>38</td>
<td>24.2</td>
<td>107</td>
<td>14.7</td>
<td>65</td>
<td>12.9</td>
<td>57</td>
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<tr>
<td>Infiltrates</td>
<td>54</td>
<td>44.5</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>5.6</td>
<td>3</td>
<td>13.0</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Interstitial lung diseases†</td>
<td>60</td>
<td>30.0</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.7</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Autoimmune disease‡</td>
<td>12</td>
<td>41.7</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Nonimmunocompromised</td>
<td>126</td>
<td>37.3</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>2.4</td>
<td>3</td>
<td>6.3</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Patients with autoimmune disease and lung fibrosis who were receiving immnosuppressive treatment.
†Patients with interstitial lung diseases including sarcoidosis who were not treated with immunosuppressive agents.
‡Patients with autoimmune disease and lung fibrosis who were not treated with immunosuppressive agents.

Conventional Cytology and CMV Immunostaining

Inclusion bodies were observed in the BAL fluid of 11 of 442 patients (2.5%) in the immunocompromised patient group, including 4 stem cell transplant recipients, 4 HIV-positive patients, and 3 renal transplant recipients. Twenty-two of the immunocompromised patients had positive immunostaining results for CMV in the BAL fluid (Table 2). In contrast, neither inclusion bodies nor positive immunostaining findings were observed in the BAL fluid of nonimmunocompromised patients. Immunostaining findings were positive in the BAL fluid of 12 of 227 HIV-positive patients (5.3%), 7 of 69 patients (10.1%) following stem cell transplantation, and 3 of 49 solid-organ transplant recipients (6.1%). There were no positive immunostaining findings in patients with autoimmune disease/lung fibrosis and none in patients following high-dose chemotherapy.

The sensitivity of CMV inclusion bodies in regard to positive CMV immunostaining findings was 36.3%, the specificity was 99.3%, the positive predictive value was 72.7%, and the negative predictive value was 96.8%.

CMV Disease (Including CMV Pneumonitis)

A diagnosis of CMV disease, including CMV pneumonitis, was made in 37 patients, as shown in Table 2. There was no CMV disease in patients undergoing high-dose chemotherapy or in patients with autoimmune disease treated with immunosuppressive...
agents. Seven patients (14.3%) developed CMV disease following renal transplantation and 8 patients after stem cell transplantation (11.6%), and all of them showed a positive p65 antigenemia in the peripheral blood. Seven of the eight patients in the stem cell transplantation group experienced graft-vs-host disease. Three of the seven patients who developed CMV disease after renal transplantation were CMV seronegative and had received a graft from a seropositive donor. In the HIV-positive group, CMV disease was present in 22 patients (9.7%). Retinitis was found in six patients, esophagitis was found in five patients, and CMV hepatitis was found in one patient from the HIV-positive group. In five of these cases, patients also had Kaposi’s sarcoma.

CMV pneumonitis was diagnosed in a total of 18 patients. In four HIV-positive patients, CMV pneumonitis was combined with CMV esophagitis or retinitis. CMV syndrome occurred in seven patients; four were stem cell transplant recipients and three were renal transplant recipients. All except one patient with CMV disease following allogenic stem cell transplantation experienced graft-vs-host disease. In three of these patients, bronchiolitis obliterans developed in the later course. Two of four renal transplant recipients with CMV pneumonitis were CMV seronegative before transplantation but had received a graft from a seropositive donor.

Diagnostic Value of CMV Immunostaining

CMV immunostaining was positive in a total of 22 patients, all of them suffering from CMV disease. None of the patients without CMV disease showed a positive CMV immunostaining finding in the BAL fluid, but CMV disease was present in 15 patients without positive immunostaining findings. The sensitivity and specificity of positive immunostaining findings for the diagnosis of CMV disease, including CMV pneumonitis, were 59.5% and 100%, respectively, and the positive and negative predictive values were 100% and 96.4%, respectively (Table 3). Sixteen of 18 patients with CMV pneumonitis revealed positive immunostaining findings in BAL fluid (Table 4). There was no evidence of CMV pneumonitis in six patients with positive immunostaining findings in the BAL. However, all of these six patients with positive immunostaining findings, but without clinical evidence of CMV pneumonitis, had extrapulmonary CMV disease. Sensitivity, specificity, and positive and negative predictive values for the diagnosis of CMV pneumonitis by immunostaining were 88.9%, 98.6%, 72.7%, and 99.5%, respectively.

Discussion

In our study, the incidence of pulmonary CMV infection, defined as a positive CMV culture finding in BAL fluid, was between 24.9% and 29% in all immunocompromised patient groups except in patients treated with high-dose chemotherapy. High-dose chemotherapy with short-term pancytopenia is only a low-risk factor for the development of CMV disease, which compares well with the results of a multicenter analysis of CMV pneumonitis following autologous bone marrow transplantation and a study comparing the incidence of CMV pneumonitis in allogenic and autologous bone marrow transplant recipients.

The incidence of CMV retrieval from the BAL fluid of HIV-infected patients varies between 20%
and 70% in the literature.\textsuperscript{7–10} Except for patients undergoing lung transplantation, only a small amount of data is available concerning the incidence of pulmonary CMV infection in solid-organ transplant recipients and in patients treated with immunosuppressive agents for interstitial lung disease or autoimmune disease. Surprisingly, except in patients following high-dose chemotherapy, there was no significant difference in the incidence of pulmonary CMV infection within the different study groups of immunocompromised patients (Table 2). CMV disease as defined by internationally used criteria\textsuperscript{1,2} was found in patients following solid-organ and stem cell transplantation as well as in patients with AIDS. None of the patients who had undergone immunosuppression for lung fibrosis or autoimmune disease developed CMV disease, despite an incidence of pulmonary CMV infection that was similar to that in other immunocompromised patient groups (Table 2). The risk for pulmonary CMV infection, therefore, might be influenced primarily by the degree of immunosuppression rather than by the underlying disease. In contrast, the risk for developing CMV disease, including CMV pneumonitis, depends on the degree of immunosuppression and the underlying disease. At least in transplant recipients, allogeneic stimulation and associated CMV reactivation may be partly responsible for this difference.\textsuperscript{25}

The diagnostic and prognostic value of pulmonary CMV infection in HIV-positive patients is controversially discussed in the literature.\textsuperscript{7–16} Applying criteria that are identical to those for transplant recipients, the incidence of CMV disease and CMV pneumonitis in HIV-positive patients was 9.7% and 4.4%, respectively, in our study. This rate is lower than in the groups following transplantation, but this difference is not statistically significant. In accordance with most authors, we believe that pulmonary CMV infection, as defined by a positive CMV culture finding in BAL fluid, is of limited clinical importance. However, our results show that positive immunostaining findings for CMV also indicate invasive CMV disease in the HIV patient group. Therefore, HIV-positive patients with positive CMV immunostaining findings in BAL may benefit from antiviral therapy.

The diagnostic value of CMV immunostaining for the diagnosis of CMV pneumonitis is not only good in HIV-positive patients but also in other immunosuppressed patients, as shown by a high sensitivity and specificity. All patients showing a positive immunostaining finding in BAL fluid who did not fulfill the clinical criteria of CMV pneumonitis had extrapulmonary CMV disease. Cases of CMV pneumonitis not accompanied by a positive immunostaining finding were rare. Therefore, positive CMV immunostaining finding in BAL fluid is a useful marker for the presence of invasive pulmonary CMV infection (ie, CMV pneumonitis) and helps to select patients who may be treated with antiviral agents. The differentiation between pulmonary CMV infection and CMV pneumonitis is especially important in the nontransplantation patient groups. In allogenic stem cell transplant recipients, the risk for developing CMV pneumonitis is high in patients with pulmonary CMV infection.\textsuperscript{26} Based on the considerable mortality rate in this patient group, antiviral treatment is usually initiated in patients with positive CMV culture findings in BAL fluid, irrespective of the results of immunostaining or cytology.

Although inclusion bodies are the hallmark of CMV disease in conventional cytology, our data show that immunostaining is clearly superior for the diagnosis of CMV pneumonitis independent of the underlying disease. This is likely due to the fact that full virus replication with a cytopathic effect is needed to show typical inclusion bodies, which corresponds to an advanced stage of CMV infection. Therefore, the sensitivity of positive inclusion bodies in regard to the diagnosis of CMV disease is low. However, an already dormant/latent virus can be cultured with the shell vial technique, explaining the low specificity of this diagnostic approach for the presence of CMV disease. The diagnostic value of quantitative polymerase chain reaction in BAL fluid for the diagnosis of CMV pneumonitis is not yet clear, despite its ability to determine the viral load. However, there is a considerable variability in fluid and cell recovery from BAL fluid, which might influence the quantitative polymerase chain reaction approach.

In conclusion, pulmonary CMV infection is frequent in all groups of immunocompromised patients except among patients following high-dose chemotherapy. Immunostaining with a monoclonal antibody against CMV in BAL fluid is a very helpful method for identifying patients with CMV pneumonitis.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Immunostaining Findings} & \textbf{CMV Pneumonitis} & & \\
 & \textbf{Yes} & \textbf{No} & \textbf{Total} \\
\hline
Positive & 16 & 6 & 22 \\
Negative & 2 & 418 & 420 \\
Total & 18 & 424 & 442 \\
\hline
\end{tabular}
\caption{Diagnostic Value of Immunostaining With Monoclonal Anti-CMV Antibodies for the Diagnosis of CMV Pneumonitis in the BAL Fluid of Immunocompromised Patients}
\end{table}
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