Cytomegalovirus-Induced Alveolar Hemorrhage in Patients with AIDS: A New Clinical Entity?

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We report five cases of alveolar hemorrhage associated with intravascular hemolysis in patients with AIDS. Cytomegalovirus was the only pathogen recovered from the lungs of these patients. There was evidence of multivisceral spread of the virus in all patients, and all had viremia. All had clinical, biological, and pathological features of pulmonary vasculitis, and the conditions of four improved with specific anti-cytomegalovirus therapy.

Cytomegalovirus (CMV) is a common cause of opportunistic disease in HIV-infected patients with severe immunodeficiency. The need for a specific treatment is well established in cases of CMV retinitis or gastrointestinal disease, but treating lung infection is debatable when the virus is recovered only from lung specimens. Although CMV is recovered from 20% to 40% of bronchoalveolar lavage (BAL) fluid specimens from patients with AIDS in large epidemiologic studies [1, 2], this virus is rarely the sole pathogen. According to these studies, no additional morbidity is associated with the presence of CMV in the lungs of patients with AIDS.

In 1993 we observed for the first time a case of alveolar hemorrhage in an HIV-seropositive patient, in which CMV was the sole pathogen and the clinical course was favorable with administration of specific treatment. Since then, we have observed four similar cases over a 1-year period. These cases are reported herein, as we think they could represent a new clinical entity in patients with AIDS and severe immunodeficiency.

Methods

The five patients were admitted to the respiratory unit of our hospital between July 1993 and June 1994. During this period, fiberoptic bronchoscopy and BAL were performed 135 times as part of the diagnostic procedures for HIV-seropositive persons. BAL fluid contained CMV inclusion cells in 16 cases upon cytological examination, and it contained >20% hemosiderin-laden macrophages (siderophages) in 21 cases.

Diagnostic procedures included clinical examination, chest roentgenography, CT of the thorax, arterial blood gas sampling and standard biological tests. Coagulation tests included determinations of partial thromboplastin time, prothrombin time and fibrinogen level. Blood and urine cultures were performed in all cases, on appropriate media for isolation of bacteria, viruses, and fungi (including mycobacteria) (Wampole Isolator, Cranbury, NJ). Cryptococcal antigen testing, serologies for atypical pneumonias, and cultures of sputum for mycobacteria were performed in all cases.

CMV viremia was assessed by immunoperoxidase staining of MRC5 cells with the immediate early monoclonal antibody E13 (Clonatec, Biosoft, Paris), 48 hours after inoculation with the patient's peripheral blood leukocytes. This allowed a semiquantitative evaluation of viremia. Cultures were also examined for the typical cytopathic effect 3 weeks after inoculation.

Fiberoptic bronchoscopy was performed for morphological, histologic, and microbiological purposes. Bronchial mucosae were examined for neoplastic processes, especially Kaposi's sarcoma (KS). Bronchial biopsies, protected brushings, transbronchial biopsies, and BAL were performed according to standard techniques. Specimens were cultured for bacteria, mycobacteria, Legionella species, fungi, and viruses (such as herpesviruses and respiratory syncytial virus). BAL fluid specimens were grown on MRC5 cells for CMV isolation, and viral antigens were detected by immunoperoxidase staining at 24 hours and by cultural evidence of cytopathic effect. Cytological and pathological studies were performed by a pathologist who specializes in lung diseases. Specimens were stained with standard hematoxylin/eosin, Grocott-Gomori methenamine-silver nitrate (for parasites), and Perls' preparations (for siderophages).

Osteomedullar biopsy was performed for our patients at the time of hospitalization if it had not been done within a few weeks before admission.

All patients underwent ophthalmoscopic examination. Depending on the clinical status of the patient, other investigations
were done in order to demonstrate other CMV organ involvement.

CMV pneumonitis was defined according to the criteria of Masur et al. and Emmanuel et al. [3, 4].

Case Reports

*Patient 1.* A 28-year-old man, HIV-positive since 1989, was receiving pyrazinamide and pyrimethamine as primary prophylaxis and had been treated with zidovudine (AZT) and didanosine (ddI). In January 1993 his CD4 cell count was 2/mm³, and cutaneous KS lesions appeared. He had never smoked. Six months before admission, dyspnea, fever, and nodular opacities on chest roentgenograms were noted. Fiberoptic bronchoscopy disclosed minor bronchial KS lesions, and a bronchial brush culture specimen was positive for *Mycobacterium chelonae.* CMV inclusion cells were observed in BAL fluid on cytological examination. Antimycobacterial treatment and a single course of therapy with bleomycin were given in June 1993.

Despite this treatment, extensive interstitial pneumonitis developed in July 1993. Concomitant acute anemia required blood cell transfusions. The patient was pale and mildly confused and had a bilateral cerebellar syndrome. A CT scan of the thorax showed two nodules and widespread interstitial shadowing, but adenomegaly was not apparent in the mediastinum. Fiberoptic bronchoscopy was performed again and showed small foci of bronchial KS; the BAL fluid was hemorrhagic and contained 92% macrophages (77% siderophages). Cytology revealed the presence of numerous CMV inclusion cells, and the BAL fluid culture was positive for CMV. No other pathogen was recovered from the lungs. Transbronchial biopsies showed CMV inclusion cells in the alveolar epithelium, associated with viral inclusions in the endothelial layer of lung capillaries. Alveolar spaces were filled with siderophages and red cells. There was no tumor proliferation.

Biological tests showed a hemoglobin level of 10 g/dL and a haptoglobin level of 6 mg/dL. Blood smears disclosed numerous schistocytes. Coagulation test results were normal. Searches for infectious agents were negative except for the finding of CMV viremia.

The diagnosis was CMV pneumonitis with alveolar hemorrhage and intravascular hemolysis. The patient's symptoms resolved within 3 weeks following initiation of treatment with ganciclovir (5 mg/kg every 12 hours) and antimycobacterial antibiotics.

*Patient 2.* A 29-year-old man was found to be HIV-positive in 1991 and had been treated with AZT and ddI. His CD4 cell count was 1/mm³. He had never smoked. In November 1992 CMV colitis developed, for which he received ganciclovir. Despite this treatment, the patient's symptoms did not diminish, and foscavir therapy was started in March 1993. In July 1993, while he was receiving half-dose maintenance therapy, he became febrile and abdominal symptoms reappeared. CMV retinitis was diagnosed. His condition did not respond to therapy with full-dose foscavir, and dyspnea developed. A chest roentgenogram showed widespread interstitial shadowing. Fiberoptic bronchoscopy did not reveal any endobronchial KS lesions. BAL fluid was hemorrhagic, and cytological examination showed 87% macrophages (17% siderophages) and numerous CMV inclusion cells. No other opportunistic agent was recovered from the lungs. Siderophages were seen within alveolar spaces of transbronchial biopsy specimens. CMV giant cells were disseminated on the epithelial layer and within the lumen of the lung capillaries. Sparse polymorphonuclear clusters infiltrated the walls of small vessels.

The blood cell count revealed a hemoglobin level of 7.6 g/dL and a haptoglobin level of 200 mg/dL. Schistocytes were found in blood smears. Coagulation test results were normal. CMV viremia testing was positive.

The diagnosis was CMV pneumonitis with alveolar hemorrhage, for which he was treated with full-dose foscavir. His respiratory status worsened, and he died 9 days later despite respiratory support. An autopsy could not be performed.

*Patient 3.* A 28-year-old woman had been observed since 1988 because of seropositivity for HIV. She had been treated with AZT and ddI. In September 1993 the CD4 T-cell count was 2/mm³. She received co-trimoxazole as primary prophylaxis. She had never smoked.

Fever, cough, dyspnea, and bilateral parotitis occurred in September 1993 and were soon associated with abdominal pain, vomiting, and headache despite combined treatment with broad-spectrum antibiotics and nonsteroidal antiinflammatory drugs. A chest roentgenogram showed bilateral interstitial shadowing. The first BAL fluid sample contained 100% siderophages and CMV inclusion cells. A CT scan of the brain was normal and did not show any parotid abscess. CMV viremia testing was positive, and no other pathogen could be identified. She began receiving foscavir therapy, but her condition showed no clinical improvement. After 8 days of treatment, hemorrhagic BAL fluid was recovered when fiberoptic bronchoscopy was repeated, and it consisted of 85% macrophages (100% siderophages). There were no CMV inclusion cells, but CMV was found in BAL fluid cultures.

The blood cell count showed a hemoglobin level of 5.9 g/dL and a haptoglobin level of 2 mg/dL. Numerous schistocytes were found in blood smears. Coagulation test results were normal. Ultrasonography of the abdomen revealed acute atheroembolic cholecystitis and pancreatitis. *Mycobacterium avium* complex grew in two blood cultures after a few days but was never recovered from lung specimens. CMV viremia testing was persistently positive.

The diagnosis was CMV pneumonitis, alveolar hemorrhage, and intravascular hemolysis. CMV cholecystitis, pancreatitis, and parotitis were suspected. The patient began receiving ganciclovir and the fever diminished quickly, as did digestive symptoms, hemolysis, and dyspnea. Dyspnea and hemolysis reappeared when full-dose therapy was interrupted, and a new
BAL specimen was found to be hemorrhagic and to consist of 100% siderophages. The patient's condition improved with renewed full-dose treatment, along with administration of antimycobacterial drugs, and she was discharged.

**Patient 4.** A 40-year-old man was found to be HIV-positive in 1986. He had received AZT and ddI. He had never smoked. In 1991 he was treated for *Pneumocystis carinii* pneumonia and thereafter received secondary prophylaxis with co-trimoxazole. The CD4 T-cell count was 4/mm³. Severe CMV retinitis had been treated since 1992 with both ganciclovir and foscavir.

Disseminated mycobacterial infection was diagnosed in January 1994, for which he received clarithromycin and amikacin, as he was still receiving maintenance therapy with foscavir. Despite this treatment, the patient had cough, exertional dyspnea, and hepatosplenomegaly. A chest roentgenogram showed widespread interstitial shadowing. Micronodules and discrete alveolar opacities were seen on the CT scan of the thorax. Fiberoptic bronchoscopy showed a normal bronchial tree, and the BAL fluid was hemorrhagic and consisted of 90% macrophages, 90% of which were siderophages. There were a few CMV inclusion cells, and the BAL fluid was positive for CMV in culture. No other pathogen was recovered from the lungs. Transbronchial biopsy revealed a few CMV inclusion cells in the epithelial layer, and alveolar spaces were filled with siderophages.

Blood cell counts revealed a hemoglobin level of 7.3 g/dL and a haptoglobin level of 7 mg/dL. Coagulation test results were normal. CMV viremia testing was positive. A bone marrow biopsy showed infection due to *M. avium* complex.

The diagnosis was CMV pneumonitis associated with alveolar hemorrhage and disseminated mycobacterial infection. The patient was treated with rifampin, clarithromycin, and clofazimine. Foscavir was given at full dose. After progressive diminishment of fever and dyspnea, the patient was discharged.

**Patient 5.** A 49-year-old man was found to be HIV-positive in 1991; he started receiving primary prophylaxis with co-trimoxazole at this time and later received AZT and ddI. He was a habitual smoker.

In September 1993 he began receiving bleomycin for extensive cutaneous KS. His CD4 T-cell count was 1/mm³. Fever and dyspnea appeared 1 month later. A chest roentgenogram showed micronodules in the right lower lobe. He was treated with broad-spectrum antibiotics and then with antituberculous quadritherapy, but his condition did not improve. The patient had dyspnea, cough, bilateral crackles, hepatosplenomegaly, and widespread cutaneous KS. Mild confusion and deterioration in mental status were noted. Another chest roentgenogram showed bilateral interstitial infiltrates. Fiberoptic bronchoscopy showed that the bronchi appeared normal. The BAL fluid contained 90% macrophages (60% siderophages). Numerous CMV inclusion cells were present, and no other pathogen was found. Transbronchial biopsy showed evidence of alveolar hemorrhage and numerous CMV giant cells in the alveolar epithelium and in the endothelium of lung capillaries. Similar features were observed within the lumen of bronchial capillaries. Some of the lung and bronchial capillaries were occluded by fibrin thrombi, with sparse polymuclear infiltrates.

The blood cell count showed a hemoglobin level of 7.9 g/dL and a haptoglobin level of 7 mg/dL. Numerous schistocytes were recovered from blood smears. Coagulation test results were normal. CMV retinitis was diagnosed. MRI of the brain revealed no abnormalities. CMV viremia testing was positive. No other microbiological agents were found.

The diagnosis was CMV pneumonitis with alveolar hemorrhage, CMV retinitis, and intravascular hemolysis. The patient was treated with ganciclovir (5 mg/kg every 12 hours) and was soon eupneic and afebrile, and the chest roentgenogram became clear. He was discharged after 15 days and received maintenance therapy, along with co-trimoxazole prophylaxis and vinblastine.

In June 1994 the patient presented with respiratory distress associated with renal failure and hemolysis. A kidney biopsy was performed, and thrombotic microangiopathy was diagnosed. Respiratory support, plasmapheresis, and effective antiviral therapy were initiated, but the patient died 2 weeks later. An autopsy could not be performed, except for a kidney biopsy; the specimen was studied with specific CMV immunofluorescence staining. Endothelial cells were clearly positively stained, within occluded glomerular capillaries and medullary vessels.

**Discussion**

These five patients had similar clinical characteristics (see tables 1 and 2) at the time of admission to our institute, which were suggestive of a CMV-induced alveolar hemorrhage that has not been previously reported in cases of AIDS.

All patients were infected with HIV and were severely immunodeficient, as evidenced by their low CD4 cell counts (<50/mm³). They were receiving prophylaxis for the main opportunistic infections. Clinical findings were characterized by subacute respiratory failure (1–3 months in duration) with dyspnea, new nodular or interstitial infiltrates evident on chest roentgenograms, and hypoxemia. This respiratory syndrome was concomitant with an alveolar hemorrhage defined by the macroscopic appearance of BAL fluid (n = 4) and/or the finding of numerous siderophages on cytological examination (n = 5). This alveolar hemorrhage was not related to pulmonary KS, as has been previously reported [5]. Indeed, in previous descriptions, alveolar hemorrhage usually has been occult, and KS lesions were not observed during fiberoptic bronchoscopy in our patients (except one with minimal injury). This alveolar hemorrhage could not be related to thrombocytopenia, coagulation abnormalities, bacterial or fungal infection, smoking history, or renal or left ventricular failure, as it has been previously in descriptions of immunocompromised hosts [6, 7].

Anemia occurred in all cases as a result of an alveolar hemorrhage and was due also to hemolysis of the microangiopathic type (schistocyte formation) in four patients. The presence of...
Table 1. Data regarding five patients with AIDS who had CMV-induced alveolar hemorrhage.

<table>
<thead>
<tr>
<th>Patient no./age (y)</th>
<th>Prophylaxis</th>
<th>CD4 cell count</th>
<th>Radiological findings</th>
<th>Arterial blood gases*</th>
<th>Cytological and pathological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/28</td>
<td>Dapsone, pyrimethamine</td>
<td>2/mm³</td>
<td>Extensive nodular and interstitial pneumonia</td>
<td>PaO₂ = 8.3, PaCO₂ = 4.1</td>
<td>92% M (77% S) Giant cytomegalic endothelial cells</td>
</tr>
<tr>
<td>2/29</td>
<td>Co-trimoxazole</td>
<td>1/mm³</td>
<td>Bilateral interstitial pneumonia</td>
<td>PaO₂ = 8, PaCO₂ = 4.1</td>
<td>87% M (17% S) Giant cytomegalic endothelial cells</td>
</tr>
<tr>
<td>3/28</td>
<td>Co-trimoxazole</td>
<td>2/mm³</td>
<td>Bilateral interstitial pneumonia</td>
<td>PaO₂ = 10.4, PaCO₂ = 4.8</td>
<td>85% M (100% S) No findings</td>
</tr>
<tr>
<td>4/40</td>
<td>Co-trimoxazole</td>
<td>4/mm³</td>
<td>Bilateral interstitial and nodular pneumonia</td>
<td>PaO₂ = 10.9, PaCO₂ = 4.3</td>
<td>90% M (90% S) Giant cytomegalic epithelial cells</td>
</tr>
<tr>
<td>5/49</td>
<td>Co-trimoxazole</td>
<td>1/mm³</td>
<td>Bilateral nodular pneumonia</td>
<td>PaO₂ = 7.5, PaCO₂ = 4.1</td>
<td>90% M (60% S) Giant cytomegalic endothelial cells</td>
</tr>
</tbody>
</table>

* PacO₂ = partial pressure of carbon dioxide (arterial); PaO₂ = partial pressure of oxygen (arterial). Values are kilopascals.
† BAL = bronchoalveolar lavage; M = macrophages; S = siderophages; TBB = transbronchial biopsy. Giant CMV inclusion cells were noted in the BAL fluid in each case.

schistocytes was probably related to CMV vascular injury and/or thrombotic microangiopathy. The latter was diagnosed in only one of our cases (patient 5), but it was not clear whether an abnormal thrombotic phenomenon had occurred in the other four patients. Anecdotal cases of thrombotic microangiopathy in patients with AIDS have been described. In the literature, this disease has not been related to any infectious agent or drug [8, 9].

Finally, we think that in our patients the congestion and narrowing of the capillary lumen by giant endothelial cells may indeed have predisposed them to intravascular hemolysis. No other described cause of anemia in patients with AIDS [10], such as autoimmune anemia, G6PDH deficiency, hemoglobinopathy, or myelofibrosis, was observed in our patients.

This clinical entity could be related to CMV for the following reasons. No parasitic agent (P. carinii, Aspergillus species, Cryptococcus species, or Toxoplasma gondii) or bacterial or viral agent (herpes simplex or respiratory syncytial virus)—except CMV—was found in the lung or blood of these patients. In three of them, disseminated mycobacterial infection was diagnosed. However, mycobacteria were not recovered from the lung at the time of hospitalization, neither from cultures of bronchial brushing specimens or of BAL fluid nor from lung biopsy specimens. Antimycobacterial treatment did not diminish respiratory symptoms. Finally, our five patients met the criteria for diagnosis of CMV pneumonia, as defined below. Four of the five had hypoxemia, diffuse pulmonary infiltrates, and lung biopsy samples consistent with interstitial inflammation with CMV inclusion bodies, in the absence of histologic evidence of other causative processes, as defined by Masur et al. [3]. One patient’s CMV pneumonia was diagnosed on the basis of a compatible clinical presentation associated with the cytological and virologic criteria established by Emmanuel et al. [4].

Table 2. Extrapulmonary and hematologic findings and outcome for five patients with AIDS who had CMV-induced alveolar hemorrhage.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Extrapulmonary findings</th>
<th>Hematologic findings</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Encephalitis, viremia</td>
<td>Hb, 10 g*; Pl, 295,000; schistocytes; Hapto, 6 mg; LDH, 1,950</td>
<td>Condition improved with ganciclovir and antmycobacterial treatment</td>
</tr>
<tr>
<td>2</td>
<td>Retinitis, viremia</td>
<td>Hb, 7.6 g; Pl, 252,000; schistocytes; Hapto, 200 mg; LDH, 2,064</td>
<td>Died with acute respiratory failure, despite supportive ventilation and treatment with foscavir</td>
</tr>
<tr>
<td>3</td>
<td>Parotitis, cholecystitis, pancreatitis, viremia</td>
<td>Hb, 5.9 g; Pl, 342,000; schistocytes; Hapto, 2 mg; LDH, 1,200</td>
<td>Condition improved with use of ganciclovir alone; relapsed when given half-dose treatment</td>
</tr>
<tr>
<td>4</td>
<td>Retinitis, viremia</td>
<td>Hb, 7.3 g; Pl, 294,000; Hapto, 7 mg; LDH, 400</td>
<td>Condition improved with foscavir and antmycobacterial treatment</td>
</tr>
<tr>
<td>5</td>
<td>Retinitis, viremia</td>
<td>Hb, 7.9 g; Pl, 235,000; schistocytes; Hapto, 7 mg; LDH, 1,500</td>
<td>Condition improved with use of ganciclovir, thrombotic microangiopathy occurred during half-dose treatment</td>
</tr>
</tbody>
</table>

Hapto = haptoglobin level, mg/dL; Hb = hemoglobin level, g/dL; LDH = lactate dehydrogenase, IU/L; Pl = platelet count, per mm³.
* After transfusion of red blood cells.
nary vasculitis is suggested to be responsible for clinical respiratory symptoms in patients with AIDS. This was associated with abatement of anemia and the hemolytic syndrome and negativity for CMV viremia. A relapse of CMV-induced alveolar hemorrhage occurred in two patients when they were given half-dose maintenance treatment.

Although CMV lung infection had been evident in our patients several weeks before their admission, this had not resulted in dose modification or initiation of anti-CMV therapy. This may have been due to the fact that several studies have shown a high frequency of CMV infection in the lungs of patients with AIDS but never demonstrated the need for specific treatment, even when respiratory symptoms were present [1, 2]. On the other hand, some cases of CMV pneumonitis reported in the literature were diagnosed on the basis of very different criteria. Patients were severely immunocompromised, as were ours, but their clinical presentations were not specific and some of them were treated with corticosteroids, co-trimoxazole, or other antibiotics in addition to anti-CMV agents [3, 4, 11–13].

The characteristics of the CMV pneumonitis observed in our cases suggest that CMV probably contributed to respiratory symptoms through vascular injury. The occurrence of alveolar hemorrhage, the presence of schistoocytes, and the finding of viral giant inclusion cells in the endothelial layer of lung capillaries (associated with inflammatory infiltrates in cases 2 and 5) are clinical and pathological features of CMV-associated pulmonary capillaritis. Virological studies have demonstrated the tropism of the virus for endothelial cells, which are a site of virus latency in most immunocompetent hosts, and active replication has also been demonstrated in endothelial cells [14, 15].

Recent clinical studies have supported these findings. First, infected endothelial cells were found in the blood of immunocompromised hosts, in two small cohorts of patients with AIDS and recipients of transplants. Second, the number of these cells correlated with clinical manifestations, and they disappeared upon treatment [15, 16]. Third, acute digestive, dermatologic, or neurological manifestations in immunocompromised patients that have been reported in the literature resulted from CMV vasculitis [17]. Fourth, vasculitis in the respiratory tract of patients with AIDS has been documented in autopsy reports [18]. To our knowledge, our cases are the first in which pulmonary vasculitis is suggested to be responsible for clinical respiratory symptoms in patients with AIDS.

Data from other cases of CMV-induced alveolar hemorrhage in patients with AIDS should be collected in order to confirm the specificity of this clinical entity and the need for anti-CMV treatment in these clinical settings.

References