Thrombotic Microangiopathy and Cytomegalovirus Disease in Patients Infected with Human Immunodeficiency Virus

Caroline Maslo, Marie-Noëlle Peraldi, Jean-Claude Desenclos, Béatrice Mougenot, Charlotte Cywiner-Golenzer, François-Patrick Chatelot, Christine Jacomet, Eric Rondeau, Willy Rozenbaum, and Jean-Daniel Sraer

Thrombotic microangiopathy (TMA) can occur during the course of human immunodeficiency virus (HIV) infection. Clinical and pathological data for 29 patients with TMA and HIV infection were recorded. In a retrospective case-control study, we analyzed the link between opportunistic infections or drug therapies and TMA. Twenty-five patients (mean CD4+ cell count ± SD, 71.9 ± 18.3/mm³) had renal impairment, and four had neurological dysfunction. In one-half the cases, the disease was progressive with isolated fragmentation anemia appearing several months before the clinical symptoms. The diagnosis of TMA was confirmed by histological examination of kidney biopsy specimens (18 cases). Endothelial cytomegalovirus (CMV) inclusions were associated with TMA in nine of 18 cases, whereas histological examination did not detect CMV in any control specimens (P < .001). The case-control study demonstrated a link between TMA and clinical CMV infection (odds ratio, 3.9; 95% confidence interval, 1.1–14). We conclude that TMA is a late complication of HIV infection and can be associated with systemic CMV infection in this setting.

Twenty-one cases were histologically proven. Additionally and independently, we conducted a retrospective case-control study of 16 consecutive patients with TMA and HIV infection to identify possible risk factors.

Patients and Methods

Patients

Between June 1989 and December 1994, we reviewed the charts of 29 HIV-infected adults with TMA. Twenty-four patients were referred to the Nephrology Unit of Hospital Rothschild (18 cases) or other similar units (six cases) in Paris. Five additional patients without major renal impairment were treated in the Infectious Diseases Unit of Hospital Rothschild. TMA was defined by the association of the following: (1) unexplained hemolytic anemia (hemoglobin level of <100 g/L, low serum haptoglobin level, and serum lactate dehydrogenase level of >1,000 U/L) with >2% schistocytes; (2) peripheral thrombocytopenia (platelet count of <100 × 10⁹/L); and (3) a pathological diagnosis of TMA and/or renal disease (blood creatinine level of >130 mmol/L and/or daily urinary excretion of protein of >1 g) or unexplained neurological dysfunction.

The following examinations were performed for all patients: coagulation tests; bone marrow aspiration or biopsy; determination of peripheral CD4+ lymphocyte count, blood creatinine level, and daily urinary excretion of creatinine and protein; tests for bacteria and parasites in urine and stool samples; indirect funduscopy (performed by an ophthalmologist); and test for cytomegalovirus (CMV) in blood samples. The following tests were performed when indicated: PCR analysis for verotoxin in stools (15 cases), standard examination of CSF
obtained by lumbar puncture and brain MRI (14), bronchoalveolar lavage (10), and PCR analysis for CMV in CSF (6).

Pathological and Immunocytochemical Analysis

Twenty-five tissue specimens from 21 (72.4%) of 29 patients were available. These specimens included kidney samples in 18 cases (13 biopsy specimens and five postmortem specimens), bronchopulmonary biopsy specimens in 3, and postmortem liver specimens in 4. Samples were processed for light microscopy by standard methods. Between 15 and 20 sections of each kidney specimen were examined. Afterward, 11 of 18 paraffin-embedded kidney biopsy samples were subjected to immunocytochemical analysis with monoclonal mouse antibody to CMV (clone E13, IgG1 with κ light chain; Biosoft, Argenque, Varilhes, France), which recognizes a CMV antigen corresponding to a 76-kD nuclear protein present as early as 2 hours after infection.

Briefly, the sections were incubated with the antibody (final concentration, 7.2 μg/mL), and fixed antibody was detected by successive incubation with biotinylated sheep antibody to mouse that was diluted to 1:300 (Amersham, Les Ulys, France), streptavidin-horseradish peroxidase diluted at 1:500 (Dako, Trappes, France), and aminoethyl carbazole. Two sections of each kidney biopsy specimen were studied.

The specificity of immunocytochemical analysis was assessed by the negativity of colonic biopsy specimens from five CMV-seronegative and five CMV-seropositive immunocompetent adults. Control samples for histological examination and immunocytochemical analysis were as follows: (1) 7 renal biopsy specimens from HIV-infected patients without TMA who had <80 CD4+ cell/mm3 (acute tubular necrosis, 3 patients; HIV-related nephropathy, 2; glomerulonephritis, 2), (2) 15 postmortem kidney specimens from patients with AIDS and no renal impairment or TMA at the time of death, and (3) 5 renal biopsy specimens from HIV-seronegative patients with typical postdiarrheal HUS.

Case-Control Study

We studied 16 consecutive HIV-infected patients for whom TMA was diagnosed in the Infectious Diseases Unit of Hospital Rothschild between December 1992 and December 1994. Controls were selected randomly from HIV-infected patients who did not have clinical or laboratory signs of TMA and were inpatients or outpatients at the same time that a case was diagnosed. Day 0 was defined as the first day that schistocytes were identified. Potential controls were excluded if they had schistocytes. A total of 64 patients were enrolled in the control group (control-to-case ratio, 4:1). We reviewed the medical charts of each case and control and recorded the following demographic data: age, sex, risk factors for HIV infection, and CD4+ cell count on day 0.

Ongoing opportunistic infections were also recorded. The diagnostic criteria for opportunistic infections were as follows: cryptosporidiosis, detection of parasites in stool; microsporidian infection, detection of parasites in stool; and Mycobacterium avium complex infection, bacteriologic evidence or clinical manifestations and an improved condition during antimycobacterial therapy (presumptive diagnosis).

CMV disease was defined as old or new clinical manifestations of infection (funduscopically evident retinitis, extensive pneumonitis with CMV inclusions in cells in bronchoalveolar lavage fluid and no other pathogens, and response to anti-CMV therapy; CMV encephalitis was defined by consistent neurological and radiological abnormalities and detection of CMV by culture and/or PCR analysis of CSF). The diagnosis of cutaneous and pulmonary Kaposi’s sarcoma was based on clinical criteria.

The following concomitant treatments on day 0 were recorded: co-trimoxazole, pyrimethamine, sulfadiazine, rifabutin, antimycobacterial therapy, broad-spectrum antibiotics, fluorocytosine, ketoconazole, itraconazole, bleomycin, doxorubicin hydrochloride, vinblastine sulfate, vincristine sulfate, zidovudine, didanosine, stavudine, acyclovir, ganciclovir, and foscarnet.

Data for cases and controls were analyzed by calculating the crude odds ratio for each variable. Because the cases and controls differed significantly in terms of the CD4+ cell count, odds ratios were subsequently adjusted for the CD4+ cell count (as a continuous variable) by multiple logistic regression analysis to take into account any confounding effect. Finally, variables that were associated (P < .05) with TMA after adjusting for the CD4+ cell count were tested in a single multiple regression model.

Statistical significance was assessed by the Kruskall-Wallis test for continuous variables, the odds ratio and Cornfield 95% confidence interval for univariate analysis, and the 95% confidence interval of the adjusted odds ratio obtained by multiple logistic regression [8, 9]. Survival rates were calculated on the basis of means determined by the Kaplan-Meier product limit method. Laboratory parameters were analyzed by using a t test.

Results

Clinical Manifestations

The baseline characteristics of the 29 patients are presented in table 1. The mean CD4+ cell count was <80/mm3; 21 patients had very low CD4+ cell counts of <40/mm3, and eight had CD4+ cell counts of >100/mm3 (two of whom had CD4+ cell counts of >300). The mean time ± SD between the onset of AIDS and the diagnosis of TMA was 23.3 ± 16.1 months. There was a wide variety of clinical manifestations. It is interesting that clinical manifestations were preceded by laboratory abnormalities in one-half of the cases: schistocytes were detected before the onset of renal failure or neurological dysfunction (mean interval ± SD, 4.2 ± 2.2 months; range, 1–9 months). Clinical manifestations were as follows.

Renal Impairment. Twenty-five of 29 patients had kidney failure. Four (16%) of these 25 patients had a systolic blood pressure of ≥150 mm Hg and a diastolic blood pressure of
Table 1. Baseline characteristics of 29 patients with HIV infection and TMA at the time of enrollment into the study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male-to-female ratio</td>
<td>27:2</td>
</tr>
<tr>
<td>Mean age ± SD in y (range)</td>
<td>35.9 ± 8.5 (20–56)</td>
</tr>
<tr>
<td>No. of Caucasians</td>
<td>27</td>
</tr>
<tr>
<td>No. of Africans</td>
<td>2</td>
</tr>
<tr>
<td>No. with risk factor for HIV infection</td>
<td></td>
</tr>
<tr>
<td>Homosexuality</td>
<td>24</td>
</tr>
<tr>
<td>Heterosexuality</td>
<td>4</td>
</tr>
<tr>
<td>Intravenous drug abuse</td>
<td>1</td>
</tr>
<tr>
<td>No. with HIV infection status</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic infection</td>
<td>5</td>
</tr>
<tr>
<td>AIDS</td>
<td>23</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
</tr>
<tr>
<td>Mean time ± SD from the onset of AIDS to the diagnosis of TMA in mo (range)</td>
<td>23.3 ± 16.1 (1–59)</td>
</tr>
<tr>
<td>Mean CD4&lt;sup&gt;+&lt;/sup&gt; cell count ± SD in mm&lt;sup&gt;3&lt;/sup&gt; (range)</td>
<td>71.9 ± 18.3 (1–150)</td>
</tr>
</tbody>
</table>

NOTE. TMA = thrombotic microangiopathy.

Table 2. Laboratory findings for 29 patients with HIV infection and TMA at the time of enrollment into the study.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Value</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean hemoglobin level ± SD in g/L (range)</td>
<td>74 ± 28 (49–87)</td>
<td>130–160</td>
</tr>
<tr>
<td>Mean reticulocyte count ± SD in × 10&lt;sup&gt;9&lt;/sup&gt;/L (range)</td>
<td>79.3 ± 53.1 (11–251)</td>
<td>50–120</td>
</tr>
<tr>
<td>Percent with schistocytes at entry into study</td>
<td>&gt;2% in all patients</td>
<td>0</td>
</tr>
<tr>
<td>Percent with schistocytes 3 mo before study entry</td>
<td>&gt;2% in 15 of 29 patients</td>
<td>0</td>
</tr>
<tr>
<td>Direct antiglobulin test</td>
<td>Positive for 1 of 26 patients</td>
<td>Negative</td>
</tr>
<tr>
<td>Mean platelet count ± SD in × 10&lt;sup&gt;12&lt;/sup&gt;/L (range)</td>
<td>87.2 ± 58.9 (17–284)</td>
<td>150–400</td>
</tr>
<tr>
<td>Mean lactate dehydrogenase level ± SD in U/L (range)</td>
<td>1,660.7 ± 990.9 (416–4,270)</td>
<td>&lt;420</td>
</tr>
<tr>
<td>Mean haptoglobin level ± SD in g/L (range)</td>
<td>0.18 ± 0.26 (0.04–1.06)</td>
<td>0.4–3.5</td>
</tr>
<tr>
<td>Mean blood bilirubin level ± SD in mmol/L (range)</td>
<td>42.7 ± 18.5 (20–82)</td>
<td>&lt;17</td>
</tr>
<tr>
<td>Mean prothrombin time ± SD in %</td>
<td>90 ± 20</td>
<td>80 ± 20</td>
</tr>
<tr>
<td>Mean partial thromboplastin time ± SD in s</td>
<td>33 ± 10</td>
<td>25–35</td>
</tr>
<tr>
<td>Mean fibrinogen level ± SD in g/L</td>
<td>4.04 ± 1.2</td>
<td>2–4</td>
</tr>
<tr>
<td>Mean level at fibrin degradation products ± SD in mg/mL (range)</td>
<td>80.1 ± 50.2 (0–160)</td>
<td>0</td>
</tr>
<tr>
<td>Mean level of D-dimers ± SD in ng/mL (range)</td>
<td>16.6 ± 4.1 (0–64)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Mean serum creatinine level ± SD in μmol/L (range)</td>
<td>321.0 ± 194.2 (46–845)</td>
<td>60–110</td>
</tr>
<tr>
<td>Mean creatinine clearance ± SD in ml/min (range)</td>
<td>20.5 ± 18.6 (2.6–75)</td>
<td>100 ± 20</td>
</tr>
</tbody>
</table>

NOTE. TMA = thrombotic microangiopathy.
Figure 1. Stain of a kidney biopsy specimen from an HIV-infected patient with thrombotic microangiopathy that reveals a cytomegalovirus inclusion. An enlarged cell with a typical intranuclear inclusion in a glomerular capillary loop is present, and there is evidence of a recent fibrin thrombus in a small artery (Masson stain; original magnification, ×312).

Kidney biopsy specimens; TMA was diagnosed in association with HIV-related nephropathy in one of these cases. Of the lesions in these 18 cases, 2 were predominantly glomerular, 14 were severely arteriolar (2 of which were histologically proven as cortical necrosis), and 2 were both glomerular and arteriolar. In nine (50%) of 18 cases, specific CMV inclusions were found in glomeruli or vessels (figure 1). Standard examination for CMV antigen was positive for six of 11 patients (figure 2), all of whom had CMV inclusions. The CMV-positive nuclei were in endothelial cells.

Histological examination of all control samples did not reveal abnormalities due to CMV, and immunocytochemical analysis of all these samples was negative for CMV. Examination of bronchoalveolar lavage fluid specimens from three patients with respiratory distress secondary to intraalveolar hemorrhage showed CMV inclusions in cells, and histological examination of bronchopulmonary biopsy specimens from these patients demonstrated diagnostic evidence of TMA with endothelial CMV inclusions. TMA lesions were found in postmortem liver specimens from four patients.

Case-Control Study

Cases did not differ from controls in terms of age, sex, risk factors for HIV infection, or duration of AIDS. Mean CD4+ cell counts ± SD were lower in cases than in controls (29.4 ± 40.1/mm³ vs. 107.2 ± 138.8/mm³, respectively; P = .009). CMV disease was the only opportunistic infection associated with TMA (crude OR, 6.1; 95% CI, 1.6–24.8; CD4+ cell count–adjusted OR, 3.9; 95% CI, 1.1–14). There was no difference in the incidence of Kaposi’s sarcoma (cutaneous or pulmonary) in cases and controls. The only drug associated with TMA was fluconazole (crude OR, 5.7; 95% CI, 1.45–24.5; adjusted OR, 3.8; 95% CI, 1.0–14.1).

We further investigated the interaction between CMV infection and fluconazole in a stratified analysis. The odds ratio for the presence of CMV infection and the use of fluconazole (10.4) was much greater than the product of the odds ratio for each exposure alone (5.2); this finding suggests that there is an interaction between CMV infection and fluconazole. However, in a multiple logistic regression model including fluconazole, CMV infection, and CD4+ cell count, an interaction term between fluconazole and CMV infection was not significant (P = .6). In a final logistic regression model, only CMV infection (OR, 4.2; 95% CI, 1.2–15.5) and fluconazole (OR, 4.2; 95% CI, 1.1–15.7) were significantly associated with TMA.

Treatment and Outcome

Twenty-three of the 29 patients received standard therapy for TMA. Seventeen patients received 20 mL of fresh frozen plasma/kg daily for a mean of 10 days (range, 7–15 days); five patients with the most severe forms of TMA were treated for 7 to 14 days with plasma as fluid replacement, and one patient first received fresh frozen plasma for 8 days followed by eight plasma exchanges. Nine patients received other treatments given alone or in combination with fresh frozen plasma and plasma exchanges. These treatments included steroids (1 mg/[kg·d]) in 3 cases, intravenous polyvalent Ig (0.4 g/[kg·d] for 5 days) in 2, aspirin (100 mg/d) in 3, and prostacyclin analogs in 1.

Treatment with fresh frozen plasma and/or plasma exchanges were followed by disappearance of hemolysis, nor-
malization of platelet counts, and improvement in renal function in 57% of the 23 patients; the mean serum creatinine level ± SD (which was 356.4 ± 201.6 μmol/L at study entry) was 231.3 ± 176.1 μmol/L on the last day in the Nephrology Unit (P < .001). Despite improvement in laboratory values, the 50% survival time was only 3 months, and the actuarial survival rate at 2 years was 20%. Four of the five patients who underwent hemodialysis died within 2 months after the diagnosis. Of the six patients who received plasma exchanges, five died during the first 2 months, and one patient required chronic hemodialysis.

Six patients with either severe neurological abnormalities (three) or terminal illness (three) received no treatment and died within 1 month.

**Discussion**

We describe 29 cases of TMA in HIV-infected adults, 21 of which were histologically confirmed. In the remaining eight cases, the diagnosis was based on clear-cut clinical and laboratory findings. The mean CD4+ cell count ± SD was 71.9 ± 18.3/mm³, but 21 patients (72.4%) had very low CD4+ cell counts, thus confirming that TMA occurs more frequently in severely immunosuppressed patients. Most of the presenting symptoms were those commonly associated with classical HUS and TTP. However, some distinctive clinical features warrant special attention.

First, fragmentation anemia and isolated proteinuria were the first signs in one-half the cases, occurring up to 6 months before the onset of clinical signs of TMA. Therefore, persistent unexplained hemolytic anemia with schistocytes and thrombocytopenia call for careful monitoring of renal and neurological functions. Second, the low incidence of high blood pressure (16%), despite extensive arteriolar lesions in the kidneys, is striking. Third, the 50% survival time was only 3 months despite improvement in renal function. This time is lower than those in previous reports [1, 2]; however, the degree of renal impairment at the time of study entry is not comparable with those in the different studies, and the precise immunologic status of the patients was not always given.

Fourteen of 18 patients had a predominantly arteriolar form of HUS. It is interesting that both cases of glomerular lesions, which classically occur more frequently in children with typical HUS and are associated with a good prognosis [10], went into complete remission. PCR analysis for verotoxin was positive for one of these patients.

CMV is not recognized as a potential cause of TMA, although endothelial cells are known to be a common target for CMV [17–20] and CMV-associated vasculitis has frequently been described in allograft recipients and patients with AIDS [21–24]. In a recent study [25], immunocytochemical analysis for a late CMV antigen and in situ hybridization were performed on postmortem kidney samples from 78 HIV-infected patients. CMV-positive cells (intraglomerular or interstitial cells) were detected in 12.8% of the kidney specimens and were usually associated with acute tubular necrosis but never with TMA. Nevertheless, no correlation between CMV infection and glomerular disease was demonstrated in transplant recipients or immunosuppressed patients without AIDS [26, 27].

Two lines of evidence in our study support the link between CMV infection and TMA. First, CMV inclusions were present in nine of 18 kidney biopsy specimens, and these inclusions were always associated with arteriolar lesions. Of the nine patients with renal CMV infections, eight had active retinitis at the time of the diagnosis, and one (who had a CD4+ cell count of 277/mm³) had CMV viremia. CMV antigen was not detected in the control samples from HIV-seronegative patients with TMA or the 15 control biopsy samples from HIV-infected patients without TMA (P < .001).

Three patients had CMV pneumonitis associated with thrombotic microangiopathic bronchopulmonary lesions. All three patients had hemolytic anemia with schistocytes. Renal biopsy confirmed the diagnosis of TMA in all three cases, but CMV inclusions were detected in only two of three kidney biopsy samples.

Immunocytochemical analysis of renal biopsy specimens confirmed the results of standard examinations but did not appear to be more sensitive. Because the number of CMV-positive cells in a given sample was very small, 15 to 20 sections of a sample had to be examined by standard techniques to detect even one CMV inclusion, compared with only two serial sections in immunocytochemical analysis. CMV-infected cells were presumably endothelial cells located in the glomerular capillaries or the interstitial and peritubular vessels.

The second line of evidence supporting the involvement of CMV in TMA is based on the results of the case-control study that was conducted independently; this study identified CMV infection as a risk factor for TMA. Although the number of cases was small, the odds ratio for TMA developing when CMV infection was present was 3.9 after adjustment for the CD4+ cell count.

Classical pathogenic hypotheses for TMA include Kaposi’s sarcoma and antineoplastic drug therapy [1], but these hypotheses were not borne out in our study. However, two patients with Kaposi’s sarcoma had received long-term therapy with IFN-α which has been suspected of being involved in TMA [28]. Verotoxin-producing enteric bacteria are rarely implicated in TMA in HIV-infected patients.

The case-control study identified fluconazole treatment as an independent risk factor for TMA, and the stratified analysis suggested an interaction between CMV infection and flucona-
zole. Fluconazole is an azole derivative widely prescribed to HIV-infected patients as preventive or curative therapy for fungal infections. Hemolytic anemia, thrombocytopenia, and renal impairment had never been linked to fluconazole therapy [29], but it has been documented that CMV infection can promote fungal infections [30, 31].

In conclusion, we believe that CMV infection is an important and previously unrecognized cause of TMA in severely immunocompromised HIV-infected patients. However, our data and the results of the case-control study do not preclude either that cofactors along with CMV contribute to the development of TMA or that all cases of TMA in HIV-infected patients are related to CMV. The overall increase in the incidence of CMV infections in these patients [32], which is due to their prolonged survival, could lead to an increased incidence of TMA.

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References