Case Report: AA-Amyloidosis Caused by Visceral Leishmaniasis in a Human Immunodeficiency Virus-Infected Patient

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Abstract. AA-amyloidosis is not known to complicate the course of visceral leishmaniasis (VL) in humans, even though glomerular and interstitial renal pathology, including AA-amyloidosis, has been observed in animals experimentally infected with leishmanial parasites.1,2 Here, we report a case of nephrotic syndrome caused by AA-amyloidosis that developed in a human immunodeficiency virus (HIV)-infected patient in the setting of drug-unresponsive VL of 10 years’ duration and associated CD4+ lymphopenia. Successful re-induction VL treatment was associated with a sustained reduction of serum AA levels, disappearance of leishmanial serum immune complexes, and CD4+ lymphocyte increase. These findings suggest that VL was the underlying etiology of AA-amyloidosis.

INTRODUCTION

AA-amyloidosis is not known to complicate the course of visceral leishmaniasis (VL) in humans, even though glomerular and interstitial renal pathology, including AA-amyloidosis, has been observed in animals experimentally infected with leishmanial parasites.1,2 Here, we report a case of nephrotic syndrome caused by AA-amyloidosis that developed in a human immunodeficiency virus (HIV)-infected patient in the setting of drug-unresponsive VL of 10 years’ duration and associated CD4+ lymphopenia. Successful re-induction VL treatment was associated with a sustained reduction of serum AA levels, disappearance of leishmanial serum immune complexes, and CD4+ lymphocyte increase. These findings suggest that VL was the underlying etiology of AA-amyloidosis.

METHODS

Detection of leishmanial immune complexes in serum cryoprecipitate. Two hundred microliters (200 µL) of serum were kept at 4°C for 2 hours; cryoglobulins were then pelleted by centrifugation at 14,000g for 10 min at 4°C. After discarding the supernatant, the pellet was suspended in cold phosphate buffered saline (PBS) and centrifuged again using the same conditions. After three washing steps, the final pellet was dissolved in 200 µL of PBS at 37°C and 50 µL of this solution analyzed for presence of leishmanial antibodies by means of immunoblot. This showed the presence of antibodies directed against seven leishmanial antigens whose molecular weights were 14, 16, 30, 46, 65, 70, and 78 kD, respectively. The immunoblot image is available on request from C.M.

Molecular identification of Leishmania species. Species identification was performed by sequencing portions of the leishmanial DNA polymerase and RNA polymerase genes, according to the original report by Croan and others. A 924-bp region of the DNA polymerase gene (Genbank accession no. AF009147; nucleotides 1–924) and a 1,293-bp region of the RNA polymerase gene (Genbank accession no. XM001467548; nucleotides 2413–3706) were sequenced by using an Applied Biosystems (Courtaboef, France) 3130 genetic analyzer and Seqscape analysis software.

CASE REPORT

In 1996, a 22-year-old man presented with fever, night sweats, weight loss, and hepatosplenomegaly. He had used intravenous drugs, without ever practicing “skin popping,” in Portugal before immigrating to Switzerland in 1993. He denied any drug use since then and had a full-time job. Laboratory evaluation showed pancytopenia (hemoglobin, 110 g/L; platelets, 81 G/L; white blood cells, 3 G/L with 72% neutrophils and 18% lymphocytes). Hepatic enzymes and serum creatinine were normal. The HIV serology was positive. The CD4+ count was 8 cells/mm³ and HIV viremia was 568,000 RNA copies/mL. The patient had prior exposure to hepatitis B (HBsAg negative; anti-HBc, and anti-HBs positive) and hepatitis C virus (anti-HCV positive, HCV RNA negative). Bone marrow biopsy showed Leishmania amastigotes and a diagnosis of VL was made.

Antiretroviral therapy, including zidovudine, lamivudine, and ritonavir, was started. The HIV viremia rapidly became and has remained undetectable. The CD4+ count plateaued at 160–170 cells/mL, presumably caused by persistent leishmanial bone marrow infiltration. In 1998 saquinavir was added. In 2006, therapy was switched to abacavir, lamivudine, and efavirenz; the CD4+ count remained unchanged.

As VL treatment, the patient received in 1997 and 1999 (when a second bone marrow biopsy showed Leishmania amastigotes) two courses of meglumine antimoniate 20 mg SbV/kg/day intravenously for 28 days each, but pancytopenia persisted. In 2001, 3 + proteinuria was first noted on a urine
dipstick, but was not further evaluated. The patient declined further treatment attempts until March 2005, when, because of persistent pancytopenia, he agreed to a third bone marrow biopsy. This again showed numerous *Leishmania* amastigotes.

The patient then received intravenous liposomal amphotericin B (L-AmB), 3 mg/kg daily for 5 days, followed by secondary prophylaxis every 2 weeks until January 2006 when it was discontinued because of unchanged pancytopenia and CD4+ lymphopenia.

In August 2006, serum creatinine elevation (176 µmol/L) was noted, with nephrotic range proteinuria (6.5 g/24 h), marked hypercholesterolemia (20.5 mmol/L) and hypertriglyceridemia (8.2 mmol/L), hypoalbuminemia (20 g/L), and microscopic hematuria. Renal biopsy showed AA-type amyloidosis, with predominantly glomerular and vascular (mesangial) amyloid deposits, glomerular cryoglobulins, interstitial fibrosis, and deposits of C1q and IgG (Figure 1). The patient had detectable serum cryoglobulins Type III at 2.1 g/dL. In the serum cryoprecipitate, immune complexes containing antibodies directed against seven leishmanial antigens (molecular size, 14–78 kD) were detectable. Bone marrow biopsy again showed large quantities of *Leishmania* amastigotes. In the absence of other identified etiologies, and with persistently undetectable HIV and HCV viremia, the amyloidosis was attributed to chronic inflammation caused by uncontrolled VL.

The serum parasite load was 113,000 copies/mL of leishmanial DNA, and molecular typing revealed *Leishmania infantum* that was consistent with the presumable infection in Portugal. Starting in October 2006, re-induction antileishmanial treatment was given with meglumine antimoniate, 5 days/week, gradually increasing the dose from 5 to 15 mg SbV/kg. After 2 months, combination treatment with alternating doses of intravenous L-AmB (1 mg/kg, 3×/week) and meglumine antimoniate (15 mg/kg, 2×/week) was given for 1 month. Peripheral blood leishmanial DNA rapidly became negative by quantitative polymerase chain reaction (PCR) (Table 1) and spleen size decreased from 18 to 12 cm. In late December 2006, therapy was switched to oral miltefosine 100 mg/day as secondary prophylaxis. Plasma miltefosine levels were measured twice and were within the therapeutic range (15.6 mg/mL and 14.6 mg/mL). The CD4+ count increased progressively, and were within the therapeutic range (15.6 mg/mL and 14.6 mg/mL). The CD4+ count increased progressively, and were within the therapeutic range (15.6 mg/mL and 14.6 mg/mL). The nephrotic syndrome, however, did not improve and at the last follow-up (July 2008) the patient still had heavy proteinuria and a progressive decline in estimated glomerular filtration rate. The patient is now awaiting renal transplantation.

After 15 months of secondary prophylaxis and a CD4+ count > 300 cells/mL for 9 months, antileishmanial treatment...
AA-amyloidosis has rarely been associated with various chronic inflammatory conditions, such as rheumatoid arthritis, bronchiectasis, chronic osteomyelitis, and infections. It is mainly observed with chronic inflammatory conditions, such as rheumatoid arthritis. However, renal function has continued to deteriorate and the patient, while awaiting renal transplantation, has required dialysis since February 2009. From March 08 to June 09 serum leishmanial DNA has remained undetectable without any antileishmanial treatment. CD4+ lymphocyte counts have been stable between 250 and 300 cells/mm³ and the HIV viral load has remained undetectable under antiretroviral treatment. However, renal function has continued to deteriorate and the patient, while awaiting renal transplantation, has required dialysis since February 2009. No cryoglobulins or circulating immune complexes were detected; hence, a direct pathophysiologic link between VL and AA-amyloidosis was not established. Follow-up was not described, except that the patient received L-AmB for 3 weeks and renal function declined requiring hemodialysis. Another HIV-infected patient from Spain with persistent intravenous drug use for > 10 years.

The patient's persistent proteinuria and progressive decline in estimated glomerular filtration rate, despite the favorable parasitic and immunologic response, unfortunately is consistent with the often grim renal prognosis of AA-amyloidosis. Of note, decreased SAA levels were an important marker of improved renal function and survival in 374 patients with AA-amyloidosis of different etiologies. In the patients with a favorable renal outcome, it took a median of 29 months to observe an improvement of the nephrotic syndrome. Thus, whereas VL has been controlled for > 18 months in our patient, the follow-up time might be insufficient to show at least partial renal recovery.

Table 1. Time course of laboratory parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sept 06</th>
<th>Dec 06</th>
<th>July 07</th>
<th>Jan 08</th>
<th>July 08</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ lymphocyte count (cells/mm³)</td>
<td>71</td>
<td>160</td>
<td>438</td>
<td>394</td>
<td>400</td>
</tr>
<tr>
<td>Serum creatinine (µg/L)</td>
<td>217</td>
<td>144</td>
<td>199</td>
<td>206</td>
<td>262</td>
</tr>
<tr>
<td>Estimated GFR (mL/min/1.73 m²)</td>
<td>33</td>
<td>52</td>
<td>36</td>
<td>34</td>
<td>24</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>6.5</td>
<td>6.4</td>
<td>ND</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Serum AA level (mg/L)</td>
<td>60</td>
<td>5</td>
<td>&lt; 3</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Serum levels of Leishmania DNA (copies/mL)</td>
<td>113,000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cryoglobulins (g/L)</td>
<td>21</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
</tbody>
</table>

From March 08 to June 09 serum leishmanial DNA has remained undetectable without any antileishmanial treatment. CD4+ lymphocyte counts have been stable between 250 and 300 cells/mm³ and the HIV viral load has remained undetectable under antiretroviral treatment. However, renal function has continued to deteriorate and the patient, while awaiting renal transplantation, has required dialysis since February 2009. ND = not done.

DISCUSSION

Treatment of AA-amyloidosis relies on the control of the underlying inflammatory condition. In the present patient with HIV and L. infantum co-infection, AA-amyloidosis was associated with detectable circulating leishmanial immune complexes. These disappeared and SAA levels declined to normal levels after successful VL treatment with the elimination of circulating Leishmania parasites. This suggests that VL was the underlying etiology of AA-amyloidosis. It might be postulated that HIV-related immunosuppression (despite the successfully suppressed HIV viremia since 1997) maintained an unfavorable balance between the immune response and VL leading to hepatic overproduction of SAA acute phase reactant proteins. A case of AA-amyloidosis in an HIV-VL co-infected, formerly intravenous drug using patient, has been reported in Spanish language. No cryoglobulins or circulating immune complexes were detected; hence, a direct pathophysiologic link between VL and AA-amyloidosis was not established. Follow-up was not described, except that the patient received L-AmB for 3 weeks and renal function declined requiring hemodialysis. Another HIV-infected patient from Spain with persistent intravenous drug use for > 10 years.

AA-amyloidosis (“secondary,” “reactive” amyloidosis) is mainly observed with chronic inflammatory conditions, such as rheumatoid arthritis, bronchiectasis, chronic osteomyelitis, or injection drug use. AA-amyloidosis has rarely been reported as cause of chronic renal failure in HIV-infected patients, mostly in the era before potent antiretroviral therapy became available. In these patients, AA-amyloidosis was typically attributed to subcutaneous injection drug use (“skin popping”), but also uncontrolled HIV viremia, multiple transfusions in a hemophilic patient, or unclear causes. In our patient, injection drug use is an unlikely contributory mechanism, because he had no history of skin popping, subcutaneous abscesses, or endocarditis, and gave a credible history of stopping drug use for > 10 years.

Relapsing, chronic VL is often associated with relatively mild symptoms, as in the present patient. However, the development of AA-amyloidosis in the present patient suggests that effective VL control is important. VL in HIV-infected patients is characterized by frequent post-induction relapses and successful therapy can be a challenge. Carefully monitored co-administration of antileishmanial agents may be required. Quantitative PCR is useful to determine when induction therapy can be switched to secondary prophylaxis and when the latter can be discontinued. Pentavalent antimonials and L-AmB are associated with less frequent and severe increases in serum creatinine levels than other major antileishmanial agents, including paromomycin and pentamidine. Because of the inconsistent efficacy of miltefosine as monotherapy in HIV-infected patients and concerns about resistance development, this drug was selected only after a marked reduction in parasite load was obtained. Oral administration of miltefosine was well suited for prolonged secondary prophylaxis, but no specific dose recommendation in patients with renal failure was available from the manufacturer.

The patient’s persistent proteinuria and progressive decline in estimated glomerular filtration rate, despite the favorable parasitic and immunologic response, unfortunately is consistent with the often grim renal prognosis of AA-amyloidosis. Of note, decreased SAA levels were an important marker of improved renal prognosis and survival in 374 patients with AA-amyloidosis of different etiologies. In the patients with a favorable renal outcome, it took a median of 29 months to observe an improvement of the nephrotic syndrome. Thus, whereas VL has been controlled for > 18 months in our patient, the follow-up time might be insufficient to show at least partial renal recovery.

Received December 1, 2008. Accepted for publication April 22, 2009.

Acknowledgment: The authors are grateful to Dr. Thomas C. Jones for critical review of the manuscript.

REFERENCES


