AIDS, nephrotic-range proteinuria, and renal failure

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CASE PRESENTATION

A 42-year-old Caucasian woman with AIDS presented with new onset severe lower extremity edema and progressive renal failure. She had a 14-year history of HIV infection acquired from intravenous drug use. At the time of admission, she was being treated with highly active antiretroviral therapy. Her AIDS-defining illness was an episode of pneumocystis pneumonia several years before admission.

One month before admission, she presented with fever and mental status changes associated with cryptococcal fungemia. Blood cultures were positive for *Cryptococcus neoformans* sensitive to amphotericin B and fluconazole. She underwent 2 weeks of therapy with intravenous amphotericin B and 5-fluocytosine, after which she improved clinically and was switched to oral fluconazole. Repeat blood cultures were negative for growth; however, there was persistence of cryptococcal antigenemia. Her serum creatinine at the time of discharge was 2.2 mg/dl (194 μmol/l), increased from baseline creatinine of 0.9 mg/dl (80 μmol/l) 3 months before admission. Review of urinalyses available before admission showed intermittent trace proteinuria without evidence of casts, crystalluria, pyuria, or hematuria. She denied recent travel or exposure to birds and did not have any pets. Medical therapy consisted of lamivudine/ziduvidine (Combivir®), efavirenz (Sustiva®) and fluconazole.

On physical examination, the patient appeared chronically ill and weighed 111 kg. She was afebrile with a blood pressure of 151/80 mm Hg. There was no jugular venous distension, thyromegaly, or lymphadenopathy. Cardiac examination was remarkable for a grade 2/6 holosystolic murmur at the left apex radiating to the axilla. The lungs were clear to auscultation and percussion. There was hepatosplenomegaly. The extremities revealed 2/4 pitting edema and erythema, with no clubbing or splinter hemorrhages. Neurologic examination revealed no meningismus, and normal sensory, motor, and cranial nerve examinations.

Laboratory values on admission were as follows: blood urea nitrogen, 66 mg/dl (24 mmol/l) (normal range 8–18 mg/dl (3–6.5 mmol/l)); serum creatinine, 2.7 mg/dl (239 μmol/l); serum albumin, 2.9 g/dl (29 g/l) (normal range 3–5 g/dl (30–50 g/l)); and 24 h urine protein 5 g. Serum sodium, potassium, bicarbonate, chloride, transaminases, and bilirubin were within normal limits. Urinary indices: random urine sodium 109 mmol/l, random urine creatinine 19 mg/dl, urine osmolality 254 mOsm/kg (254 mmol/kg), and fractional excretion of sodium 11.4%. White blood cell count was 4.5×10^6/l (normal range $4.5–11 \times 10^6$/l), white blood cell count differential: segmented neutrophils 80% (normal range 54–62%), band forms 1% (normal range 3–5%), lymphocytes 9% (normal range 25–33%), and monocytes 10% (normal range 3–7%). Other laboratory values on admission included hemoglobin, 8.6 g/dl (86 g/l) (normal range 13–16 g/dl (130–160 g/l); platelet count, 58×10^9/l (normal range $150–500 \times 10^9$/l); and helper T-lymphocytes (CD4) cell count, 137/mm^3 (normal range 500–1500/mm^3). Peripheral blood smear revealed 2+ red blood cell polychromasia and 2+ schisocytosis. Urinalysis showed specific gravity 1.010, 300 mg/dl of protein, and negative nitrite. Microscopic examination of the urine revealed 50 white blood cells per high-power field, 20 red blood cells per high-power field, negative yeast, no crystalluria, no casts, and negative bacteria. Wright's stain revealed no urinary eosinophils. Urine culture, urine India ink, and urine mucicarmine stains were not performed. Serum complement levels were normal. Hepatitis C antibody was positive. Hepatitis B antibody and surface antigen were negative. Chest radiograph was unremarkable. Echocardiogram showed normal valvular structures without any evidence of vegetations. Magnetic resonance imaging of the brain revealed no mass lesions. Computed tomographic scan of the abdomen revealed no renal masses, perinephric stranding, or fluid collections. Renal ultrasound showed normal-sized kidneys (right kidney 11.5 cm and left kidney 11.8 cm) with preserved cortico-medullary differentiation. There was no evidence of calyectasis or hydronephrosis. Owing to acute renal failure (ARF) and nephrotic-range proteinuria, a renal biopsy was performed.

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Kidney International (2006) **69,** 2107–2110. doi:10.1038/sj.ki.5000408; Published online 26 April 2006

KIDNEY BIOPSY FINDINGS

Light microscopic examination disclosed one core of renal cortex containing 14 glomeruli, none of which were sclerotic. The glomerular capillary lumina were massively dilated and occluded by abundant fungal organisms (sometimes numbering over 20 per glomerulus) surrounded by macrophage reaction (Figure 1a). The fungi consisted of rounded yeast forms ranging from 5 to 10 μm in thickness, with a thick capsule and rare budding forms. The yeast stained periodic acid Schiff-positive, silver-positive, and mucicarmine-positive (Figure 1b), consistent with *Cryptococcus neoformans*. The macrophage reaction included foamy histiocytes and scattered giant cells. In some glomeruli, cryptococcal organisms were also seen free within the urinary space associated with extracapillary macrophage reaction. No glomeruli with collapsing sclerosis, segmental sclerosis, or intravascular fibrin thrombi were seen. Cryptococcal organisms were also identified focally within tubular lumina, tubular epithelial cells, and the interstitium, producing 'bubbly' zones of interstitial clearing with granulomatous reaction (Figure 1c). Proximal tubular cells contained focal intracytoplasmic protein and lipid resorption droplets. There were scattered proteinaceous casts and red blood cell casts. Tubular atrophy and interstitial fibrosis involved approximately 15% of the cortex. Approximately 30% of the cortex displayed interstitial expansion by edema and mild to moderate inflammatory infiltrates of mononuclear leukocytes. Arteries and arterioles were unremarkable.

Ten glomeruli were studied by immunofluorescence and showed 2+ luminal positivity for C3 in the distribution of the yeast capsules (Figure 1d) and 3+ positivity for fibrinogen. There was also weak segmental granular capillary wall positivity for C3 and granular mesangial positivity for immunoglobulin IgM (1–2+), κ (1+), and λ (1+). Staining for IgG, IgA, C1q, and albumin was negative.

By electron microscopy, many glomerular capillary lumina were occluded by fungal organisms surrounded by macrophages. Fungi were also identified in the tubular lumina and interstitium (Figure 1e). The fungi exhibited a thick spherical capsule with radially oriented fibrillar substructure typical of *Cryptococcus neoformans* (Figure 1f). Some of the intracapillary macrophages formed foam cells. The glomerular endothelium appeared swollen with obliteration of fenestrations. Scattered endothelial tubuloreticular inclusions were identified. There was focal subendothelial accumulation of fibrin tactoids involving several capillaries with organisms. Approximately 5% of capillaries sampled also had sparse small subepithelial or intramembranous electron-dense deposits, some of which were separated by short basement membrane spikes. Foot process effacement involved approximately 60% of the glomerular capillary surface area, predominantly overlying capillaries distended by intraluminal fungi and macrophages.

FINAL DIAGNOSIS

Cryptococcal pyelonephritis, severe, involving glomerular and tubulointerstitial compartments.

CLINICAL FOLLOW-UP

The patient was maintained on highly active antiretroviral therapy therapy. After having received 2 weeks of liposomal amphotericin B and 5-fluocytosine, she was continued on 400 mg of fluconazole daily. Her serum creatinine declined and then stabilized at 2.3 mg/dl (203 μmol/l). She continued to have proteinuria in the range of 2.0–2.5 g/24 h. Repeat urinalysis revealed specific gravity of 1.010, 300 mg/dl proteinuria, negative leukocytes, positive blood, and negative nitrite. Microscopic examination of the urine showed three to five red blood cells per high-power field. Repeat blood cultures were negative. Five months post-discharge, she died at home of a cardiopulmonary arrest.

DISCUSSION

ARF is a common complication of HIV infection. Patients may present with a variety of patterns of renal involvement. ARF is usually secondary to pre-renal causes or acute tubular necrosis related to sepsis, hypotension, or nephrotoxic agents.[1,2] Differential diagnosis of ARF owing to nephrotoxic agents includes drug toxicity from antifungal agents such as amphotericin B, acute tubular necrosis due to aminoglycosides, acute interstitial nephritis owing to sulfonamides, nephrotoxicity related to antiretroviral agents employed in highly active antiretroviral therapy therapy, and nephrolithiasis secondary to indinavir. Thrombotic microangiopathy and direct renal parenchymal infections by to opportunistic organisms are other potential causes of ARF in the HIV-infected patient.[2] Renal parenchymal infection by a variety of pathogens has been reported, including fungi (*Candida*, *Cryptococcus*, *Histoplasma, Aspergillus, Pneumocystis*), protozoa (*Toxoplasma*), viruses (cytomegalovirus, adenovirus, polyomavirus), and bacteria (*Mycobacteria*, *Pseudomonas*). When renal failure occurs in association with nephrotic-range proteinuria, differential diagnosis includes HIV-associated nephropathy (focal segmental glomerulosclerosis) and a variety of forms of glomerulonephritis, including acute post-infectious glomerulonephritis, IgA nephropathy, 'lupus-like' immune complex glomerulonephritis, and membranoproliferative or membranous glomerulonephritis related to hepatitis C or hepatitis B infection.[1,3,4]

Cryptococcus neoformans is a ubiquitous yeast with worldwide distribution. The fungus is present in the soil and in pigeon droppings. The respiratory tract is the usual portal of entry. The pulmonary infection is usually asymptomatic, but may cause pneumonia or pulmonary nodules. From the lungs, the fungus may disseminate to other organs, especially the central nervous system, causing meningoencephalitis. Cryptococcal disease mainly affects immunosuppressed individuals (especially patients with AIDS or malignancies and transplant recipients), although it may infect immunocompetent patients as well. The

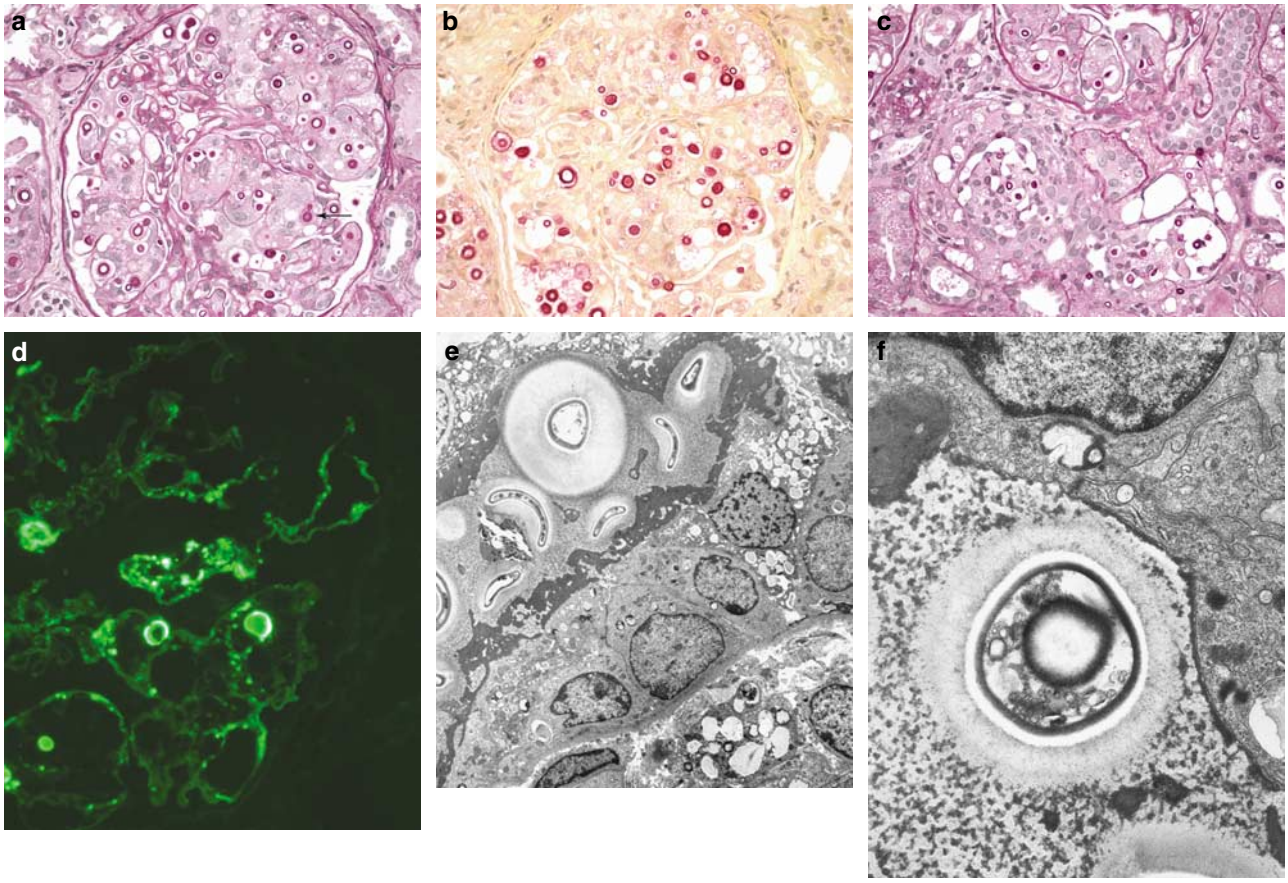

Figure 1 | Renal biopsy findings. (**a**) Glomerular capillaries are massively distended by abundant intracapillary yeast forms surrounded by numerous histiocytes. The yeast forms have a thick periodic acid Schiff-positive cell wall. A rare budding form is present (arrow) (original magnification, $\times$ 400). (**b**) Mucicarmine stain highlights the characteristic mucinous capsule of the *Cryptococcus* (original magnification, $\times$ 400). (**c**) Tubules are splayed apart by an interstitial granuloma containing swollen histiocytes and abundant yeast forms. The interstitium has focal clearing, imparting a 'bubbly' appearance. A portion of a glomerular tuft is seen at the top of the field (periodic acid Schiff, original magnification, $\times$ 200). (**d**) There is high-intensity immunofluorescence positivity for C3 outlining yeast capsules within glomerular capillary lumina. Focal granular capillary wall positivity is also seen in the glomerular basement membrane (original magnification, $\times$ 600). (**e**) A low-power electron micrograph shows an intra-tubular 'cast' of aggregated cryptococcal organisms in a granular matrix (original magnification, $\times$ 2000) (**f**). A high-power electron micrograph of a single, intra-tubular cryptococcal organism shows a thick, clear capsule surrounding the electron-dense cell wall (original magnification, $\times$ 10 000).

cryptococcal yeast measures 4–6 μm in diameter and stains eosinophilic in tissue sections. It is surrounded by a halo corresponding to its mucinous capsule, which can be revealed with the mucicarmine stain. Human infection with capsule-deficient *Cryptococcus* has been described. The capsule-deficient *Cryptococcus* is mucicarmine-negative, but can be identified with the Fontana–Masson silver stain. The nature of the tissue response to cryptococcal infection depends on the immune status of the patient. Immunocompetent patients develop a chronic granulomatous reaction composed of lymphocytes, macrophages, and foreign-body giant cells, whereas immunocompromised patients may have extensive fungal tissue invasion with minimal inflammatory reaction.

Autopsy rates of renal involvement in disseminated cryptococcosis are high, ranging from 26 to 45%.[5,6] In a post-mortem study of patients with AIDS, cryptococcal infection with multi-organ involvement was noted in 23 of 252 (9.1%) patients, of which 26% had renal involvement.[7] The renal infection is often clinically silent. Renal dysfunction occurring during disseminated fungal infection is more frequently due to nephrotoxicity of amphotericin B or sepsis than direct renal parenchymal infection. Very rarely, cryptococcal pyelonephritis can be the first manifestation of the systemic disease.[8]

The major histologic manifestations of renal cryptococcosis include histologically identifiable renal infiltration by fungal organisms, severe interstitial inflammation, granuloma formation, and tubulitis.[9,10] Cryptococcal localization in glomeruli has occasionally been described.[5,10] Cryptococcal pyelonephritis may lead to papillary necrosis.[8] Urine cultures and staining of the spun urine with mucicarmine or India ink stains are helpful diagnostic tests.

In the HIV population, there are only rare reports of cryptococcal renal infection causing proteinuria or ARF. Praditpornsilpa *et al*. studied the renal pathology of 26 HIV-infected Thai patients with proteinuria greater than 1.5 g/day.

On renal biopsy, 17 patients had mesangioproliferative glomerulonephritis, two IgA nephropathy, two membranous glomerulopathy, two diffuse proliferative glomerulonephritis, one tuberculous granulomatous interstitial nephritis, and two cryptococcal interstitial nephritis accompanied by mild mesangial proliferation; none developed HIV-associated nephropathy. The two patients with renal cryptococcosis had serum creatinine values of 1.6 mg/dl (141 μmol/l) and 1.0 mg/dl (88 μmol/l), and 24-h urine protein values of 1.6 and 3.0 g/day, respectively.[11] Treatment protocols for cryptococcal pyelonephritis in AIDS patients are not well defined. Our patient was treated with the same regimen generally recommended for AIDS patients with central nervous system or disseminated cryptococcosis, namely IV amphotericin B (0.7–1.0 mg/kg/day) plus oral flucytosine (100 mg/kg/day) for 2 weeks, followed by oral fluconazole (200–400 mg/day) for life.[12]

The pathogenesis of ARF owing to extensive fungal infections has not been fully elucidated. In an experimental model of renal candidiasis, localization of organisms in glomeruli and peritubular capillaries through adhesion to endothelium occurred within 5 min of injection of viable *Candida albicans* directly into the renal artery, followed within hours by the formation of intracapillary neutrophilic nodules containing yeasts, and within days by fungal invasion into the tubulointerstitial compartment associated with inflammation and abscess formation.[13] In an experimental study of pyelonephritis in rats, interstitial nephritis occurred 3 days after intrarenal injection of *Cryptococcus neoformans* and the fungi persisted for 6–8 weeks. Over subsequent weeks, the interstitial inflammation continued in the absence of identifiable fungal antigens, accompanied by progressive renal scarring.[14] Cryptococcal production of a serine proteinase that cleaves type IV collagen, fibronectin, and laminin likely facilitates tissue invasion.[15] The subsequent interstitial inflammation is probably enhanced by cryptococcal-stimulated release of chemoattractant cytokines such as macrophage inflammatory peptide-1α (MIP-1α), MIP-1β and RANTES by monocytes and T cells.[16]

In the case reported herein, the pathological finding of foot process effacement overlying capillaries containing fungal organisms with surrounding mononuclear cell reaction suggests that proteinuria may develop as a consequence of intraglomerular cryptococcal infection and the intracapillary release of inflammatory mediators. In patients with sepsis, recognition of pathogenic motifs by toll-like receptors on podocytes with upregulation of B7-1 may play a role in the mediation of foot process effacement and the development of proteinuria.[17] The immunofluorescence finding of staining for C3, without co-deposits of immunoglobulin or C1q, in the distribution of the yeast capsules supports complement activation by the alternative pathway. The few subepithelial and intramembranous electron densities in capillaries containing organisms may represent *in situ* immune complex formation following planting of locally released cryptococcal antigen in the course of glomerular capillary infection. The absence of well-developed, regular membranous features argues against hepatitis C-associated membranous glomerulopathy. The glomerular staining for fibrinogen and the ultrastructural demonstration of subendothelial fibrin tactoid formation, indicating cross-linked fibrin, support a concurrent local activation of the coagulation cascade following endothelial injury.

In summary, the diffuse glomerular involvement by cryptococcal infection in this HIV-infected patient is extremely unusual and accounts for the dual presentation with heavy proteinuria and ARF. The possibility of glomerular cryptococcosis should be entertained in HIV-infected patients or other immunocompromised patients who present with proteinuria and renal dysfunction in the clinical setting of disseminated cryptococcal infection.

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