Case Report

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Pneumocystis carinii in a patient with hypercalcemia and renal failure secondary to sarcoidosis

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Hyperkalziämie, Nierenversagen und *Pneumocystis carinii* bei einem Patienten mit Sarkoidose

Zusammenfassung. Wir berichten von einem 48 Jahre alten Patienten mit Dyspnoe, massiver Hyperkalziämie und Nierenversagen im Rahmen einer Sarkoidose, die histologisch und zytologisch verifiziert werden konnte. Eine vorübergehende Erniedrigung der CD4+ T-Zellen (282/µl) deutete auf eine Störung des Immunsystems während der akuten Phase der Erkrankung hin. Überraschenderweise wurden zahlreiche *Pneumocystis carinii* „Trophozoiten" mittels Immunfluoreszenz und PCR in der Bronchiallavage nachgewiesen, was auf eine Infektion oder Kolonisation der Lunge hindeutete. Eine HIV-Infektion bestand nicht. Unter Therapie mit Kortikosteroiden gemeinsam mit Trimethoprim-Sulfamethoxazol kam es zu einer raschen Besserung der erhöhten Kalzium- und Kreatininkonzentrationen. Da eine Übertragung von *P. carinii* von asymptomatischen Trägern auf immunsupprimierte Personen möglich ist, könnten Patienten mit Sarkoidose ein bisher noch unerkanntes Reservoir für die Verbreitung von *P. carinii* darstellen.

Schlüsselwörter: Sarkoidose, Pneumocystis carinii, Hyperkalziämie.

Summary. A case of severe dyspnea, hypercalcemia and renal failure secondary to sarcoidosis is reported. The clinical diagnosis of sarcoidosis in a 48-year-old man was confirmed by histology and cytology. Transiently decreased numbers of CD4+ T cells (282/µl) indicated impaired immunity in the absence of HIV-infection during the acute phase of the disease. Surprisingly, numerous "trophozoites" of *Pneumocystis carinii* were detected by immunofluorescence staining and PCR in the bronchoalveolar fluid indicating infection or colonization of the lungs. Corticosteroid therapy was administered together with trimethoprim-sulfamethoxazole and rapidly reduced elevated serum calcium and creatinine concentrations. Since airborne person-to-person transmission of *P. carinii* to susceptible individuals might be possible, patients with sarcoidosis could be a previously unrecognized reservoir for *P. carinii* distribution in hospitals and in the community at large.

Key words: Sarcoidosis, *Pneumocystis carinii*, hypercalcemia.

Case report

A 48-year-old man of Swedish origin was referred to our ward with a six-week history of headaches, mild dyspnea during exercise and moderate weight loss (3 kg). His condition had worsened during summer vacation in southern Europe, where he had appreciated the intense sunlight. The patient treated the headache with increasing doses of Aspirin® (up to 2.5 g per day). Physical examination was unremarkable except for hepato-splenomegaly. Initial laboratory tests (Fig. 1) showed severe hypercalcemia (4.45 mmol/l), hypercalciuria (13.5 mmol/24 h; normal range: 2.5–7.5 mmol/24 h), and impaired renal function (creatinine: 3.21 mg/dl, creatinine-clearance: 22.7 ml/min). A CT-scan of the chest revealed multiple enlarged, partially calcified hilar and mediastinal lymph-nodes and nodular changes in the lungs. No history of exposure to occupational dust was reported. Elevated serum concentrations of angiotensin con-

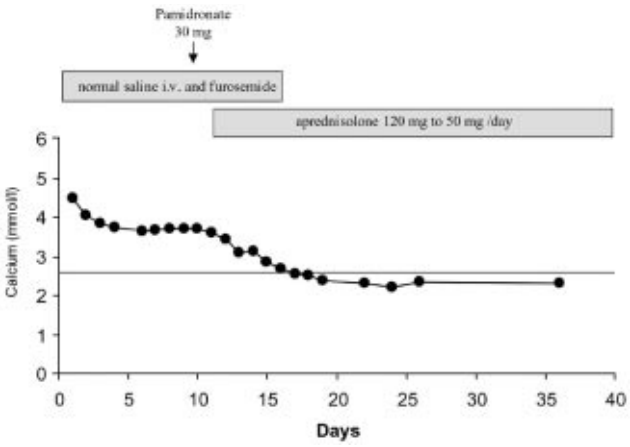

Fig. 1. Time course serum calcium concentrations and therapeutic strategies. The horizontal line indicates the upper normal limit of serum calcium concentration

verting enzyme (ACE; 235.0 U/l; normal range: 8.0–52 U/l) suggested sarcoidosis associated with hypercalcemia. Parathyroid hormone was suppressed (3.6 pg/ml; normal range: 15–65); parathyroid-hormone related peptide was below the detection limit of the assay (1 pmol/l); 1,25-dihydroxy-cholecalciferol (75 pg/ml; normal range: 15–65 pg/ml) and its precursor 25-hydroxy-cholecalciferol (92 nmol/l; normal range: 30–85 nmol/l) were slightly increased consistent with sarcoidosis and/or exposure to sunlight. LDH was within the normal range; arterial oxygen pressure (PaO_2) was, however, reduced (61 mmHg: normal range: >75 mmHg), indicating respiratory impairment. Thyroid function was normal, serum protein electrophoresis was unremarkable and radiograms did not demonstrate any bone lesion. Ultrasonography of the abdomen confirmed hepato-splenomegaly. In addition, enlarged retroperitoneal lymph nodes were found. No signs of nephrocalcinosis or kidney stones were evident. Hematologic abnormalities included mild lymphocytopenia (0.9 G/l; normal range: 1.0–4.0 G/l) and marked eosinophilia (1.0 G/l; normal range: 0.0–0.4 G/l). Trans-bronchial biopsy of the lung showed non-caseating granulomatous interstitial pneumonia. Bronchoalveolar lavage was performed to rule out infectious causes for dyspnea and weight loss. The bronchoalveolar (BAL) fluid contained an increased proportion of helper T-lymphocytes (CD4/CD8-ratio: 5) consistent with sarcoidosis.

In addition the BAL fluid contained numerous trophozoites of *Pneumocystis carinii*, identified with an immunofluorescence stain and PCR-assay. Immunofluorescence staining (IFA) was performed using a commercial kit employing a monoclonal antibody against a common surface epitope of *P. carinii* f. rat and human (Cellabs, Sydney, Australia) according to the manufacturers instructions. Gene amplification was performed with a PCR based on the specification of Wakefield et al. [1], modified in annealing temperature and cycle duration following DNA preparation (QIAamp DNA mini kit, Qiagen, Germany). No other staining techniques like Grocott or auramin were applied. All other bacteriological tests of the BAL fluid, including PCR assays for Mycobacteria and viruses as well as cultures for other fungi were negative. Repeated assays for antibodies to HIV-1 and 2 (ELISA) and PCR-amplification of retroviral DNA were negative. Analysis of T cell subsets in peripheral blood determined by flow cytometry showed reduced numbers of both CD4+ (282/µl) and CD8+ (238/µl) cells in the presence of lymphopenia (882 lymphocytes/µl; CD4+/CD8+ ratio: 1.2). Serum immunoglobulin concentrations were unremarkable.

Treatment of hypercalcemia included hydration with normal saline and administration of loop diuretics. Because of persistent neurological manifestations (severe headaches, drowsiness) bisphosphonate (pamidronate) was administered. Prednisone treatment, initiated after the diagnosis of sarcoidosis had been confirmed histologically, lead to a rapid improvement: serum concentrations of calcium as well as eosinophilia returned to normal values and creatinine concentrations dropped after a transient rise.

The relatively mild symptoms (dyspnea, weight loss), that may just as well have been caused by sarcoidosis, did not allow a definite diagnosis of *P. carinii* pneumonia (PcP) based on clinical data alone. However, the presence of numerous trophozoites of *P. carinii* in the BAL fluid together with respiratory impairment (reduced arterial oxygen pressure) in the presence of low numbers of CD4+ cells might indicate PcP. Therefore, anti-infectious treatment was administered along with prednisone treatment. Because of the significantly impaired renal function, the dose of antibiotics had to be adjusted [trimethoprim (0.32 g/d)-sulfamethoxazole (1.6 g/d) i.v. for 8 days followed by oral administration of trimethoprim (80 mg/d)-sulfamethoxazole (400 mg/d) for 8 weeks].

Four months after the initiation of prednisone treatment (30 mg/d) the patient was free of symptoms. Laboratory tests revealed persistently elevated serum creatinine concentrations (1.65 mg/dl) in the presence of normal serum calcium concentrations (2.28 mmol/l). No signs of calcification or stones were evident in a CT-scan of the kidneys. A repeated lung biopsy showed the persistence of non-caseating granulomas, but no signs of infection by *P. carinii*, Mycobacteria, or other fungi. ACE activity returned to normal (18.1 U/l). Repeated analyses of T cell subsets in peripheral blood 4 months after discharge revealed normal counts for both CD4+ (652/µl) and CD8+ (498/µl) cells (CD4+/CD8+ ratio: 1.3). Assays for antibodies to HIV-1 and 2 (ELISA) and PCR-amplification of retroviral DNA were still negative.

Discussion

P. carinii organisms are ubiquitous atypical fungi that remain the most common cause of life-threatening opportunistic infections in patients with impaired cellular immunity secondary to HIV-infection or other causes [2]. Recently the traditional concept of reactivation of latent infection has been reevaluated and PcP is now frequently considered to result from de novo acquisition rather than from reactivation of a latent infection [3]. Rodent studies have also shown that immunocompetent hosts, transiently colonized with *P. carinii*, were able to transmit the pathogen to susceptible immunosuppressed hosts [4]. The transmission of *P. carinii* from a patient to healthy health care workers also indicates that airborne human-to-human transmission of *P. carinii* is possible [5]. Therefore, the identification of clinical conditions that might predispose to *P. carinii* colonization should be useful in preventing transmission of *P. carinii* in hospitals and in the community at large. We describe a case of *P. carinii* colonization in the absence of HIV-infection in a patient suffering from severe hypercalcemia and renal insufficiency secondary to sarcoidosis.

Mild to severe hypercalcemia is found in approximately 10% of patients with sarcoidosis [6]. The underlying mechanisms involve high plasma concentrations of 1,25-dihydroxy-vitamin D, caused by extrarenal 1α-hydroxylation of vitamin D in macrophages of sarcoid granulomas [6]. The first step in vitamin D metabolism from 7-dehydrocholesterol to cholecalciferol is enhanced by sunlight leading to increased availability of substrate for 25-hydroxylation in the liver and unrestricted 1-hydroxylation in activated mononuclear cells. In addition, parathyroid-hormone related protein produced in sarcoid granulomas could contribute to the elevation of serum calcium concentrations [7].

Renal involvement in sarcoidosis is rare. When renal disease is present, it may be due to hypercalciuria, hypercalcemia, granulomatous interstitial nephritis, glomerular disease or obstructive uropathy [8]. At admission, our patient had significantly impaired renal function. A transient increase of serum creatinine levels was observed after bisphosphonate therapy with pamidronate. Searching for the reason of the renal involvement, nephrolithiasis, nephrocalcinosis and obstructive uropathy were

ruled out by ultrasonography and CT-scan. We decided not to perform a kidney biopsy because of a normal urinary sediment and a lack of proteinuria indicating the absence of glomerular disease, and because of the rapid improvement of kidney function upon initiation of specific therapy. The significant elevation of serum creatinine in the beginning was most likely due to a functional impairment induced by the vasoconstrictive effects of hypercalcemia and probably by the patient's use of NSAID. A contribution of granulomatous interstitial nephritis however, cannot be ruled out. In case of incomplete recovery (current serum creatinine 1.6 mg/dl), irreversible renal damage due to longstanding disease must be considered.

Opportunistic infections may occur in patients with sarcoidosis [9]. Immunosuppressive therapy and abnormalities in cell-mediated immunity may predispose to these infections [10, 11]. So far an association between colonization of the lung with *P. carinii* and sarcoidosis has been described in two patients [12, 13]. In both cases reduced CD4+ T-cell counts were observed, and one patient fulfilled the criteria of idiopathic CD4+ T lymphopenia [12, 14]. Pneumocystis carinii is the most common cause of pneumonia in patients with AIDS and typically occurs in patients with CD4+ cells depleted to 200 cells/µl or below, but is also occasionally found in individuals with a CD4+ count up to 350 cells/µl [15].

Consistent with pulmonary sarcoidosis, our patient had a normal CD4+/CD8+ ratio in peripheral blood samples, but a markedly increased CD4+/CD8+ ratio in lung samples, which has been attributed to redistribution of helper T cells from blood to sites of disease activity [16]. Absolute numbers of CD4+ and CD8+ cells were decreased in the acute phase of the disease, whereas lymphocyte subsets determined 4 months later revealed normal counts for CD4+ and CD8+ cells. This may indicate a transient impairment of immunity associated with the acute phase of sarcoidosis which might have predisposed to infection or colonization with *P. carinii* f. human. Based on clinical data, the diagnosis of PcP could not be made with certainty. However, the presence of numerous trophozoites of *P. carinii* in the BAL fluid together with respiratory impairment might indicate true PcP. The detection of genomic DNA of *P. carinii* by PCR, an assay which is highly sensitive and much more specific for *P. carinii* f. human than is IFA, confirms the diagnosis of P. carinii infection or colonization [17, 18]. Taken together, a therapeutic (suspected PcP) and/or prophylactic (impaired cell mediated immunity and prednisone treatment) administration of trimethoprim-sulfamethoxazole seemed to be justified.

Interestingly, *Pneumocystis carinii* pneumonia in patients with AIDS is associated with elevated levels of ACE [19] and hypercalcemia [20], indicating possible common defects in macrophage function in sarcoidosis and AIDS.

In conclusion, the available data indicate that, in this patient, an intermittent defect in cell mediated immunity associated with the acute phase of sarcoidosis, hypercalcemia, and renal failure led to infection or colonization of lungs with *P. carinii*. Since airborne person-to-person transmission of the pathogen to susceptible individuals is possible, patients with sarcoidosis could be a previously unrecognized reservoir of a *P. carinii* distribution in hospitals and in the community at large.

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