

Case Report

Invasive aspergillosis with myocardial involvement after kidney transplantation

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Introduction

Invasive aspergillosis (IA) has emerged as a major life-threatening infectious complication in patients with prolonged neutropenia and in solid-organ transplant recipients [1]. The frequency of IA in kidney transplantation varies between 0.5% and 2.2% [2]. The high case-fatality rate of 62% overall in renal transplant recipients and up to 88% in disseminated disease emphasizes the need for more accurate diagnostic techniques and better therapeutic options [3]. Although the recent introduction of new antifungal agents, such as voriconazole and caspofungin, offers new opportunities in the treatment of IA [4,5], optimal treatment and management of these patients remains problematic and controversial.

We discuss a case of IA with predominant thyroid and myocardial involvement after kidney transplantation, focusing on adequate therapy, duration of treatment and surveillance.

Case

A 49-year-old patient with diabetic nephropathy received a cadaveric renal allograft. Cytomegalovirus status was negative for the recipient and positive for the donor. The initial immunosuppression consisted of cyclosporin (trough levels: 350–450 µg/l), mycophenolate mofetil (2 × 1500 mg daily) and prednisone (50 mg daily). Initial graft function was satisfactory; serum creatinine decreased from 7.8 mg/dl (696 µmol/l)

to 2.4 mg/dl (216 µmol/l) within 9 days. Two biopsy-proven acute rejections, a steroid-resistant interstitial rejection on day 43 and a vascular rejection on day 101, were treated with anti-T-lymphocyte globulin (ATG: 4 mg/kg for 7 days) and anti-CD3 antibodies (OKT3: 5 mg for 7 days), respectively. Additionally, cyclosporin was replaced by tacrolimus on day 102 (trough levels: 12–15 µg/l). Thereafter, serum creatinine concentration remained stable between 1.6 and 2.8 mg/dl (150–250 µmol/l). Four months after transplantation and 10 days after therapy with OKT3, the patient was admitted with fever, chills, myalgia and slight right-sided neck pain. Laboratory tests showed normal white blood cell count ($7.8 \times 10^9/l$ with $7.4 \times 10^9/l$ neutrophils and $0.15 \times 10^9/l$ lymphocytes) and elevated C-reactive protein (CRP) (78 mg/l; normal: <5 mg/l) (Figure 1). Computed tomography (CT) of the chest disclosed a small nodular lesion (Ø 1.2 cm) in the left lower lobe and an inhomogeneous enlargement of the right thyroid lobe. Fine needle aspiration of the thyroid yielded *Aspergillus fumigatus* (minimum inhibitory concentration: amphotericin B 0.5 mg/l, itraconazole 0.25 mg/l). *Aspergillus* antigen (galactomannan test) was positive (index: 2.2; normal: <1.0). IA with involvement of thyroid and lung was diagnosed. Hemithyroidectomy was performed, tacrolimus trough level lowered (from 10–15 to 7–10 µg/l), mycophenolate mofetil reduced (from 3000 to 2000 mg daily) and prednisone tapered within 4 weeks. Antifungal therapy with amphotericin B lipid complex (5 mg/kg daily) was started. The patient remained febrile and CRP did not decrease. Echocardiography and magnetic resonance imaging (MRI) of the heart disclosed myocardial abscesses and fibrous pericarditis, suggesting cardiac involvement of IA (Figure 2). Caspofungin (50 mg daily) and flucytosin (4 × 3500 mg daily) were added to amphotericin B. Two weeks later, flucytosin was stopped due to leukopenia ($2.7 \times 10^9/l$, 78% neutrophils). Eleven weeks after the start of antifungal combination therapy, the myocardial and pulmonary lesions vanished, CRP normalized and *Aspergillus* antigen became negative. The patient was discharged

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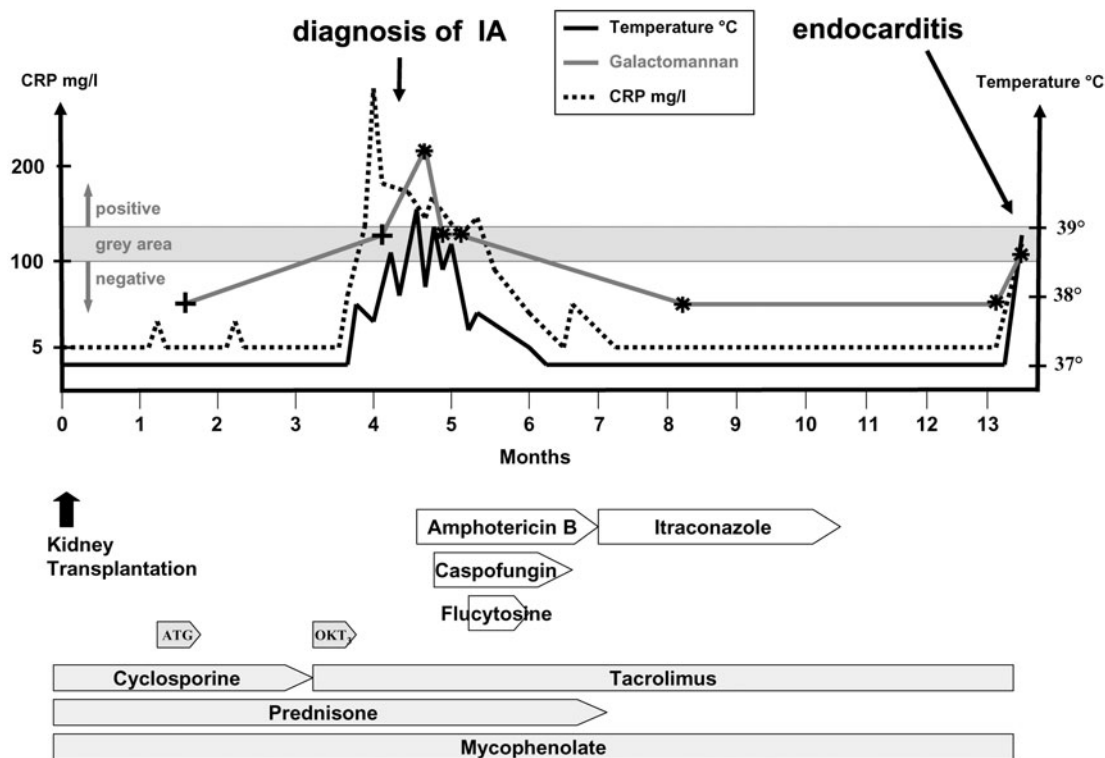


Fig. 1. Clinical and laboratory course of IA. Galactomannan test performed retrospectively and galactomannan test performed prospectively.

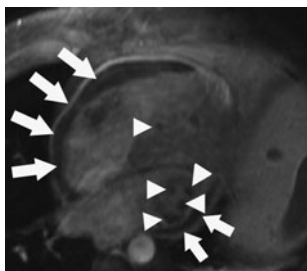


Fig. 2. Contrast-enhanced fat-suppressed T1-weighted turbo fast low-angle shot (FLASH) image in the horizontal long axis of the heart shows fibrous pericarditis (arrows) and intramyocardial abscesses (arrowheads).

on oral itraconazole (3×200 mg daily) for an additional 4 months. Due to the pharmacokinetic interaction between itraconazole and tacrolimus, we reduced the dosage of tacrolimus from 2 to 0.5 mg twice daily. However, in spite of the reduction, trough levels increased from 9 to 25 $\mu\text{g/l}$. Therefore, we paused tacrolimus for 8 days and continued with a dosage of 0.5 mg once daily every other day to every third day, achieving trough levels between 10 and 13 $\mu\text{g/l}$. The increase of tacrolimus trough levels was accompanied by a temporary increase of the serum creatinine from 2.4 mg/dl (220 $\mu\text{mol/l}$) to a peak value of 4.4 mg/dl (389 $\mu\text{mol/l}$). The further clinical course was uneventful until a slight increase of CRP (range: 12–38 mg/l) was detected 6 weeks after stopping itraconazole. Physical examination was unremarkable

and, in particular, no cardiac murmur was heard. MRI of the heart, CT scan of the chest and galactomannan test were normal. One month later, the patient died unexpectedly of cardiac shock. Autopsy revealed IA with endocarditis and rupture of the aortic valve, myocardial fungal granuloma (Figure 3) and involvement of lung and prostata. Culture of the aortic valve yielded *A. fumigatus*.

Discussion

We report an unusual presentation of IA with predominant myocardial involvement 4 months after renal transplantation. Major risk factors for IA include prolonged neutropenia, chronic granulomatous disease and the use of severe immunosuppressive regimens and steroids [6]. Our patient was not neutropenic, but severely immunosuppressed and lymphopenic ($<0.2 \times 10^9/\text{l}$). Severe long-lasting T-cell immunosuppression was caused by the treatment with ATG and OKT3, leading to T-cell depletion and functional T-cell alteration, by the use of tacrolimus in preventing adequate cytokine release and T-cell activation and by mycophenolate mofetil as an antiproliferative agent [7,8]. Additionally, the activity of macrophages and neutrophils, which are important in the phagocytosis of *A. fumigatus*, was altered by steroids [1]. As addressed by Singh et al. [9], antifungal attributes of immunosuppressive drugs also are involved in the pathogenesis of opportunistic mycosis. *In vitro* data

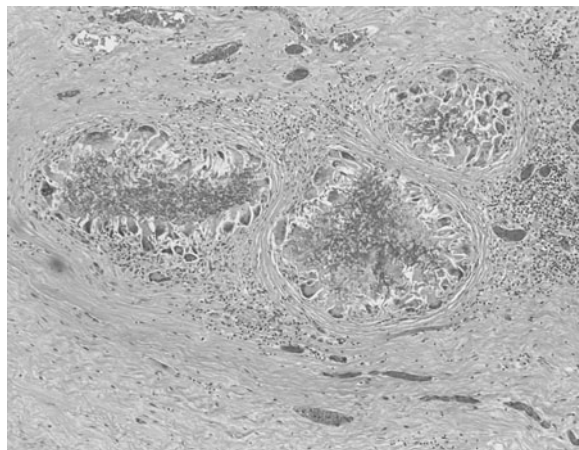


Fig. 3. Multiple granuloma with *Aspergillus* hyphae in fibrotic myocardial tissue. (Haematoxylin and eosin; magnification: $\times 100$).

show a stronger anti-*Aspergillus* activity of tacrolimus in contrast to cyclosporin. These data are supported by animal studies. In a model of IA, mice immunosuppressed with tacrolimus or sirolimus developed microabscesses with low hyphal burden, in contrast with the widespread growth of hyphae in mice treated with cyclosporin [10]. Interestingly, the autopsy of our patient, who received tacrolimus, revealed a pattern of microabscesses in the myocardium. In the clinical setting of transplantation with overt fungal infection, the immunosuppressive effects outweigh the potential antifungal effect of immunosuppressive drugs [9]. Other risk factors for IA, such as proximity to a construction area or cytomegalovirus infection [2,11], did not occur in our patient.

Myocarditis, pericarditis and endocarditis are rare and fatal complications of disseminated aspergillosis, diagnosed in most cases only at autopsy [12]. Early diagnosis and prompt initiation of antifungal therapy for presumed IA are essential to improve the outcome of these patients. However, in contrast to neutropenic patients, for whom empirical antifungal therapy is closely linked to the severity and duration of neutropenia, there is no established clinical or laboratory marker in renal allograft recipients to start empirical therapy. Recently, the detection of circulating galactomannan, which is a component of the cell wall of *Aspergillus* spp., by a sandwich enzyme-linked immunosorbent assay in serum or respiratory specimens has received considerable attention as a valuable method for diagnosing IA [13]. However, the usefulness of the galactomannan test for the early diagnosis of IA remains controversial, because of high specificity (>85%) but varying sensitivity between 29% and 100%, which is dependent on many factors related to the host (underlying disease and immunosuppressive therapy, site of infection, age and exposure to antifungal drugs) and to epidemiological characteristics (prevalence of infection and patient population) [14]. The combination of real-time polymerase chain reaction with automated DNA-extraction and

galactomannan assay was shown recently to improve the reliability of IA diagnosis [15], but these results have to be confirmed in larger trials.

The recent introduction of new antifungal agents, such as voriconazole and caspofungin, offers new opportunities in the treatment of IA. In patients with IA, initial therapy with voriconazole led to better responses and improved survival than the standard approach with amphotericin B [4] and, more recently, caspofungin was shown to be as effective as and better tolerated than liposomal amphotericin B when given as empirical antifungal therapy in patients with persistent fever and neutropenia [5]. Given the different mechanism of action of caspofungin compared with amphotericin B, a combination therapy is promising. A synergistic effect of caspofungin and amphotericin against *Aspergillus* spp. was demonstrated *in vitro* [16]. Case reports showed a favourable outcome for patients with invasive pulmonary aspergillosis refractory to amphotericin, treated with this combination [17,18]. However, the role and efficacy of antifungal combination therapy in patients with IA has not been established yet. Given the high incidence and associated mortality of IA, a prophylactic antifungal treatment with itraconazole [19] would be a reasonable option. However, the threat for emerging resistant strains and the potential drug toxicity and interactions with the immunosuppressive regimen make this approach unattractive for transplant recipients.

Our case report highlights some of the unresolved questions in the management of IA. Initially, the combination therapy with amphotericin B and caspofungin seemed to be effective. The patient recovered and the myocardial and pulmonary lesions vanished radiologically. However, the unexpected death 10 weeks after stopping a 6 month course of antifungal treatment demonstrates that the therapy was only able to suppress but not to eradicate *Aspergillus* infection, as reflected by the histological pattern of myocardial granuloma with central nestling of *Aspergillus* hyphae. Despite a slight increase of CRP 1 month before the patient died, we were not able to diagnose a relapse of IA. Although radiological investigations and *Aspergillus* antigen readings correlated with clinical improvement under therapy, these tools were not helpful for confirming eradication or for detecting the fatal relapse in our case. Therefore, a prolonged, eventually lifelong antifungal suppressive therapy should be considered for selected patients, even in the absence of clinical and laboratory findings suggestive for persistent IA.

Conflict of interest statement. None declared.

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