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Candida Species Contamination of Preservation Fluid—Outcome of Renal Transplantation in 6 Patients

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ABSTRACT

Background. Fungal infections are a rare but important cause of morbidity and mortality in kidney transplantation. Fungal contamination of the kidney preservation fluid may, sometimes, be the cause of these infections. However, the clinical consequences of fungal contamination of this fluid are not completely understood and literature on this topic is controversial. The purpose of this study was to determine the incidence of preservation fluid contamination by fungi and its clinical consequences.

Methods. From June 2010 to September 2011, a prospective cohort analysis was conducted at our center, enrolling all patients who received a renal allograft and whose perfusion fluid was analyzed for microbiology sterility. Patients with perfusion fluids positive for fungi were further studied: the patients' status was assessed during regular visits and data were recorded, including clinical characteristics, infections, graft function, immunosuppressive regimen and outcomes.

Results. Microbiologic, cultures of 70 kidney perfusion fluids using specific mycologic media, obtained from 74 cadaveric renal transplants (4 fluids were unsuitable for analysis), were evaluated. Six samples were positive for yeasts (8.6%), with 4 isolates of *Candida albicans* and 2 isolates of *Candida glabrata*. Four patients had no evidence of fungal infection during the follow-up period (median 321 days); conversely, 2 patients developed severe mycotic vascular complications leading to transplantectomy.

Conclusions. Perfusion fluid contamination by fungi is an elusive situation that can lead either to an unremarkable clinical course or to graft loss life-threatening situations. Routine culture of kidney perfusion fluid is critical for prompt diagnosis and early implementation of appropriate treatment.

INFECTIOUS COMPLICATIONS are an important cause of morbidity and mortality in patients undergoing renal transplantation.[1] Despite the decreasing incidence, fungal infections still affect 5% of renal transplant recipients.[2,3] Yeast infections generally occur in the first 3 months after transplantation, with a wide range in severity, from simple mucocutaneous infections to life-threatening conditions.[2] Contamination of the preservation fluid is probably an independent factor leading to donor kidney contamination. Exogenous contamination during recovery and handling of the graft has been suggested as a possible source of these infections, with several cases reported.[4–8] Accordingly, Albano et al presented a study that strongly suggests that organ contamination was the consequence of peritoneal donor contamination in at least 9 of 12 cases.[9]

The prevalence of yeast contamination of kidney preservation fluid is variable. Botterel et al have recently published a study with a prevalence of 3.1%, using plating media specific for yeasts.[5] Other publications have reported different frequencies (2%–10%), although some authors did

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not use specific yeast culture media.[10,11] Botterel et al called attention to the fact that only 28.5% cases were identified when routine bacteriologic methods were used as compared to the yield obtained when specific mycologic procedures were used.[5]

The clinical consequences of yeast contamination of preservation fluid are not completely understood and data are conflicting. *Candida* sp have been related to serious complications mainly by compromising the vascular anastomosis with mycotic arteritis and aneurism. Facing lethal events, some authors have recommended preventive nephrectomy when yeast contamination of preservation fluid is identified.[10] Others have suggested a more conservative approach, since factors predisposing patients to adverse events are not yet established and conservative strategies have also achieved acceptable results.[5,11–13]

The purpose of this prospective analysis is to determine the frequency of preservation fluid contamination by fungi and its clinical consequences.

MATERIALS AND METHODS

In this prospective analysis, samples of the preservation fluid from kidney transplantation procedures performed at our center were analyzed, and all patients culture-positive for fungi were selected. Samples were collected immediately prior to the back-table dissection of the kidney. One 5-mL sample of the perfusion fluid was taken from the bag containing the kidney and was immediately inoculated into an aerobic blood culture bottle from BacT/Alert automated system (BioMerieux) and sent to the microbiology laboratory. There, 5 mL of sterile blood was added to the bottle and only then it was loaded in the BacT/Alert equipment that is a system of continuous monitoring of blood cultures for detecting bacteremia and fungemia. In positive bottles, 1 mL of fluid was aseptically removed and platted in a blood-gelose plate incubated at 37°C for 48 hours. *Candida* identification was done by the automated system Vitek2 (bioMerieux). The patient's status was assessed during regular outpatient's clinic visits, and data, including clinical characteristics, infections, graft function, immunosuppressive regimen, and mortality, were recorded. Patients had a minimum follow-up of 114 days.

RESULTS

Eighty-nine kidney transplants (74 cadaveric and 15 living donors) were performed in 88 recipients, at the Renal Transplantation Department of Hospital de Santa Cruz, Lisbon, Portugal, between June 2010 and September 2011. Seventy samples of kidney preservation fluids (all but 4, whose samples were unsuitable for culture) were collected from the 74 cadaveric renal allografts. Six of these samples (incidence of 8.6%) were positive for yeasts: 4 grew *Candida albicans* and 2 grew *Candida glabrata*. Samples from patient 2 and 3 also showed the presence of *Streptococcus gordoni* and *Staphylococcus epidermidis*. The incidence of bacterial contamination in the study was 67.1%.

The characteristics of the donor, recipient, histocompatibility, and immunosuppression are shown in Table 1. Note that allografts from patient 5 and 6 were collected from the same donor. However, allografts from the 2 patients with vascular complications (4 and 6) came from different donors. Microbiologic data for the preservation fluid samples, posttransplantation data, and outcome are presented in Table 2.

All patients received antibiotic prophylaxis at the moment of transplantation with cephazoline (2 g intravenous, single dose). Induction immunosuppression was not uniform among the patients. Every patient received maintenance immunosuppression with tacrolimus, mycophenolate mofetil, and prednisone adjusted to weight.

Four of the 6 patients who received kidneys from positive isolates showed no clinical signs of fungal infection during the follow-up period (median 321 days; range 114–528 days).

Patients with *C albicans* presented higher hospitalization periods when compared to those with *C glabrata* (11 vs 21.5 days). Three of the 4 recipients with *C albicans* presented delayed graft function. Two patients (recipients 4 and 6), both with *C albicans* isolation in the preservation fluid, developed serious vascular complications.

Recipient 4 was admitted 37 days after transplantation with severe abdominal pain and hypotension shortly followed by cardiorespiratory arrest. Successful resuscitation was followed by emergency surgery, which revealed the rupture of an aneurysmal dilatation of the renal artery and a large hemoperitoneum, requiring transplantectomy and iliofemoral bypass. Blood and urine cultures were negative before and after the incident. Histologic examination of the graft specimen showed tubular necrosis and inflammatory interstitial infiltration. The renal artery presented internal elastic lamina duplication and atheroma (the aneurysmatic portion of the artery was not available either for histologic or for microbiology exam). The patient was treated with fluconazole 100 mg (first intravenously and then orally) for 20 days and was discharged on hemodialysis. The patient-recipient pair was also transplanted in our unit. His perfusion fluid cultures were negative and did not revealed any complications.

Patient 6 had a prolonged hospitalization after transplantation due to delayed graft function and bacteremia caused by methicillin-resistants *epidermidis.* The patient was discharged with no signs of infection and with a serum creatinine value of 1.5 mg/dL. This patient was readmitted 1 week later with an asymptomatic increase in serum creatinine level to 5.09 mg/dL. Doppler ultrasound was performed and showed a low resistive index (0.6–0.65). The computed tomography (CT) scan and angiography performed revealed an aneurysmatic dilatation in one of the allograft arteries, responsible for the compression of the second allograft artery, limiting its blood flow. Intravascular stent grafting was tried but placement was unsuccessful. Surgical aneurysm repair was performed and tissue culture revealed the presence of *C albicans*. Liposomic amphotericin B therapy was initiated at a dose of 5 mg/kg/d and maintained for 27 days and then switched to voriconazole 200 mg twice a day. These measures were not successful and

Table 1. Characteristic of the Donor, Recipient, Transplantation, and Immunosuppression

	1	2	3	4	5	6
Donor						
Age (y)	58	62	66	50	4	
Gender	F	F	F	M	M	
Cause of death	HE	HE	HE	HE	Drowning	
Recipient						
Age (y)	49	57	63	29	17	49
Gender	M	F	M	F	F	M
Transplantation						
HLA mismatch	2	6	6	4	5	2
Anti-HLA (%)	0	0	0	11	0	0
Number arteries/veins	1/1	1/1	1/1	1/1	1/1	2/1
Cold Ischemia (h)	17	17	22	19	15	21
Immunosuppression						
Induction	Basiliximab	ATG	Basiliximab	ATG + IVIG + RIT	Basiliximab	Basiliximab
Maintenance	CNI + MMF + Pred	CNI + MMF + Pred	CNI + MMF + Pred	CNI + MMF + Pred	CNI + MMF + Pred	CNI + MMF + Pred

HE, hemorrhagic stroke; CNI, calcineurin inhibitors; MMF, mycophenolate mofetil; Pred, prednisolone; ATG, antithymocyte globulin; IVIG, Intravenous immunoglobulin; RIT, Rituximab; HLA, human leukocyte antigen.

anastomotic rupture occurred 21 days later (59 days after transplantation), with hypovolemic shock. Transplantectomy was performed with the need for a cross-femoro-femoral bypass. After surgery, the patient became febrile. Blood and urine cultures were negative, antifungal therapy was maintained, and vancomycin and meropenem were added. Thoracic and abdominal CT scans were suggestive of multiple infectious foci spread within the abdomen and lungs. The patient had a prolonged hospitalization period under antibiotic and antifungal therapy, being stable at the moment, under hemodialysis therapy.

Concerning allograft function, at the end of the study, 2 of the 6 patients were dialysis-dependent and the remaining 4 patients had functional allografts, with a median estimated glomerular filtration rate (MDRD) of 49 mL/min/1.73 m^2 (range: 33–88 mL/min/1.73 m^2).

DISCUSSION

Graft handling and exposure to contaminants during organ recovery has been considered the most probable cause for fungal contamination, especially in recovery of multiple-organ transplantations. This hypothesis was reinforced by Albano et al, using genetic analysis to correlate organ and perfusion fluid contamination with peritoneal contamination of the donor.[9,10]

The incidence of yeast contamination is lower than bacterial contamination, but severe adverse events have been reported.[9–11,14–19] During our study period, 6 of the 70 samples (incidence of 8.6%) showed contamination with *Candida spp,* a similar value reported in other series (range 1.7%–9.6%).[5,10–19]

We used an automated broth culture system for microbiologic analysis. Comparison between series is difficult

Table 2. Preservation Fluid Microbiologic Results, Post-transplantation Datas and Outcome

	1	2	3	4	5	6
Preservation fluid testing	*Candida glabrata*	*C glabrata Streptococus*	*Candida glabrata Streptococus epidermidis*	*C albicans*	*C albicans*	*C albicans*
Antifungal treatment	No	No	No	Yes	Prophylaxis	Yes
Hospitalization (d)	12	10	14	27	13	32
Delayed graft function	No	No	No	Yes	Yes	Yes
SCr at hospital discharge (mg/dL)	1.9	1.4	2.2	2.0	2.0	1.5
Periallograft fluid collection	No	No	No	Yes	Yes	No
Angio CT/angiography	Renal artery stricture	—	—	—	—	Renal artery pseudoaneurysm
Outcome						
Follow-up (d)	528	471	487	212	114	114
Last SCr value (mg/dL)	1.7	1.6	1.6	HD	1.0	HD
GFR–MDRD (mL/min/1.73 m^2)	43	33	33	—	88	—
Anastomotic rupture	No	No	No	Yes	No	Yes
Day after transplantation	—	—	—	37	—	59

SCr, serum creatinine; GFR–MDRD, glomerular filtration rate estimated with MDRD formula; HD, hemodialysis; ATN, acute tubular necrosis; CT, computed tomography.

because different techniques and culture conditions were applied. Botterel et al mentioned that the use of broth culture techniques were superior to direct plating methods, although even this method can underestimate the true incidence of yeast contamination. The same author showed that routine bacterial culture media identified only 5 of the 21 fungal contaminants detected when plating in media specific for fungi. This finding may indicate the existence of competition between yeasts and bacteria or reflect the different incubation conditions used.[5]

The clinical consequences of fungal contamination of preservation fluid are not completely clarified, since different outcomes have been published in small series. The most frequent severe adverse advents that have been registered are vascular complications, namely mycotic aneurysms and anastomotic rupture.[6–8,20–22]

Using a conservative strategy Botterel et al, and Mantignon et al, Canaud et al showed that in a total 27 recipients, none developed vascular complications.[5,12,13] On the other hand, several authors reported the occurrence of aneurysmatic lesions and anastomotic rupture related to *C albicans* contamination of preservation fluid.[7–9] Veroux et al had also published an interesting case of acute renal failure 30 days after transplantation due to ureteral obstruction by a fungus ball in a patient positive for *C albicans* in perfusion fluid.[23] Our study unveils a large range of events, from patients with no adverse outcome (4 patients) to patients with aneurysmatic development and anastomotic rupture (2 patients) on days 37 and 59 after transplantation (none of them receiving any antifungal prophylactic therapy). In both cases, *C albicans* was involved.

To our knowledge *C albicans* was also the only yeast implicated in other cases with vascular complications.[6–8,21,22] This phenomenon may be explained by the ability of *C albicans* to adhere, invade, and damage human vascular endothelial cells.[24,25]

Our affected patients showed clinical presentations similar to those previously described in the literature: some patients have an abrupt onset with hypovolemic shock requiring emergency surgery, while others have a more indolent course with fever or worsening renal function and imaging techniques revealing structural arterial lesions.[6–9,21,22] The long evolution period could be considered important to allograft salvage, even though Bracale et al have reported disappointing outcomes: from 6 patients with large anastomotic pseudoaneurysm, 5 had open repair and 1 stent grafting. Nevertheless, transplantectomy was necessary in 5 cases.[20]

The establishment of a causal relationship between *C albicans* contamination of perfusion fluid and later vascular complications in the transplant recipient is not always unequivocal. Efforts should be made to establish a microbiologic or histologic link. In case 4, due to the cataclysmic presentation, emergency transplantectomy was performed and the visualized aneurysmal lesion was not properly sampled nor was it sent for microbiologic evaluation. The histopathology only reported inflammatory infiltration of the arterial pedicle. In patient 6 it was possible to perform microbiologic exam of the vascular lesion, which revealed the presence of *C albicans*; the histology showed fibrinoid necrosis of the arterial wall. In both patients, blood and urine cultures done before and after transplantectomy were negative for fungus. Similar situations have also been reported by other authors. Albano et al reported positive cultures in 8 of 11 arterial specimens, but in only 2 of 11 recipients the blood cultures were positive.[9] Renal arteritis cannot be ruled out in the absence of candidemia or candiduria.

The approach to these patients must be stratified and 2 situations must be considered in kidney recipients with perfusion fluid contaminated with fungi: those with and those without signs of arterial allograft lesion, since the prognosis varies dramatically between these 2 groups. Mai et al suggested that nephrectomy should be done in cases in which the preservation fluid is contaminated with *Candida spp*, even though, as seen in our series and in recently published by others, *Candida* contamination not always leads to an unfavorable outcome.[5,8,12,13]

Appropriate antifungal prophylactic therapy has not been determined yet, and centers are applying different approaches, ranging from no therapy to therapy with 2 drugs for at least 3 months.[9,13] When a renal artery aneurysm is detected, the end result is almost invariably allograft removal.[6–9,20]

In Conclusion, perfusion fluid contamination in our sample occurred in 6 of 70 samples (incidence of 8.6%). Based on our current experience and the published literature, we conclude that: (1) kidney perfusion fluid should always be analyzed for sterility; (2) a positive result for *C albicans* should always prompt specific prophylactic therapy and close monitoring with ultrasound and Doppler scan; (3) if vascular injury is detected by ultrasound, confirmation by angiography or CT scan should be performed and allograft removal should be strongly considered.

Perfusion fluid contamination by fungi is an elusive situation that can lead either to an unremarkable clinical course or to graft loss life-threatening situations. Understanding the pathophysiology of fungal renal arteritis is mandatory to create adequate prevention and prophylaxis strategies. Although rare, serious life-threatening vascular complications occur.

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