CASE REPORT

Polymicrobial *Candida* Infection in Prosthetic Aortic Graft Tissue

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Abstract
A case of mixed infection with *C. glabrata, C. krusei* and *C. albicans* in an abdominal aortic graft is described. Variable yeast morphology visualized on tissue Gram stain indicated polymicrobial *Candida* infection resulting in initiation of appropriate antifungal with favorable patient outcome.

Keywords
Polymicrobial Candida infection, Prosthetic aortic graft infection, Deep-seated Candida infection, Pakistan

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Informed Consent
Informed consent has been obtained from the patient (or patient's guardian) for publication of the case report and accompanying images.

Introduction
Aortic graft infections are a recognized complication of abdominal aortic repair surgery.[1] Fungal aortic graft infections are although rare but have been associated with significant morbidity and adverse outcomes.[1,2] Amongst fungi candida is the most frequent causative agent of vascular graft infections.[2] Microbial diagnosis of such cases is challenging and requires invasive sampling in most cases as blood cultures are often negative. Management of vascular graft infections is challenging and requires surgical intervention in most cases with removal of graft and debridement of infected tissue or in situ graft replacement.[2-4] Simultaneous appropriate and prolonged antifungal therapy is also necessary. However, successful outcome with conservative management has also been reported only in selected cases.[5] Despite timely surgical intervention and appropriate antifungal therapy, fungal graft infections have been reported as an independent risk factor for both immediate and long-term mortality in a series of patients who underwent in situ graft replacement and Candida *glabrata* has been associated with the highest mortality.[6]

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Case Report
A 36-year old male with a history of protein S deficiency and transitional cell carcinoma (low grade) presented in the emergency department with right lower leg swelling, redness and mild throbbing abdominal pain. Two days back, he also had fever with shivering and diaphoresis. Six months before presentation, he had undergone transurethral resection of bladder tumor which was followed three months later by aorto-bi-iliac grafting for infrarenal mycotic aneurysm with PTFE graft through transperitoneal approach. General examination revealed swelling and redness over right leg that was extending up to umbilical region. He was afebrile and vitally stable (Blood Pressure: 138/92 mm Hg, Pulse: 80 beats per minutes, respiratory rate: 12 beats per minutes, Oxygen saturation: 95% at room air). On systemic examination, no abdominal tenderness was found and the rest of examination was within normal limits. An ultrasound Doppler showed no evidence of deep venous thrombosis in right lower limb vessels. On the day of admission, the leukocyte count was 12600/mm^3 and blood culture grew *C. albicans*. On subsequent days, the leukocyte count rose to 22300/mm^3 and additional sets of blood cultures sent on day 5 and 7 yielded yeast and E. coli resistant to ceftriaxone and piperacillin-tazobactam, respectively. Computed tomography scan (CT) of abdomen and pelvis was performed to check the status of aortic reconstruction and to identify the source for this unexplained severe limb cellulitis. CT scan revealed specks of air around the graft secondary to aortoduodenal fistula. The graft was passing through the 3rd and 4th part of the duodenum. Infectious diseases consultation was taken, and the patient was started on empiric imipenem and fluconazole, as identification of the yeast in the second blood culture was still not confirmed. The patient underwent exploration on day 15, with resection of infected graft, repair of the duodenal defect and neo-aortic reconstruction from superficial femoral veins grafts harvested from both thighs in pantaloon fashion. The procedure also involved duodenal repair accompanied with gastrostomy and a feeding jejunostomy tube. Per-operatively, multiple adhesions of small bowel along with infected graft were found invading the posterior wall of duodenum resulting in 50-60% defect in the circumference of duodenum. The patient tolerated the procedure well and there were no intraoperative complications. Post-operatively he was shifted to the intensive care unit. Gram

stain of the excised PTFE aortic graft tissue revealed budding yeast cells with two distinct morphologies; one with pseudohyphae and another comparatively small yeast with no pseudohyphae (Fig 1a and 1b). Based on Gram stain which was suggestive of a possible non-*C. albicans* Candida species, antifungal treatment was changed from fluconazole to amphotericin B. The total leucocyte count reduced from 37500 to 13400/mm^3 in two days. After 48 hours of incubation, three *Candida* species, *C. albicans*, *C. glabrata* and *C. krusei* grew from tissue culture. At the same time, blood culture isolate was confirmed as *C. glabrata* and *C. albicans*. Details of isolation of *Candida* species and their sensitivities are shown in (Table 1). Blood culture sent 10 days later remained negative and abdominal drain fluid was also sterile, thus achieving microbiological clearance. Imipenem was stopped. Apart from amphotericin B related nephrotoxicity, managed by dose adjustment and increased hydration, the postoperative course of the patient was unremarkable. The patient was extubated and discharged. The antifungals were stopped after 4 months and at one year follow up patient had no clinical evidence of persistent or new infection.

Microbiological Diagnosis

Tissue sample was processed for fungal culture using standard methodology. Microscopy was performed using Gram stain and 10% potassium hydroxide. *Candida* species was identified using germ tube production, colony morphology on BiGGY Agar (Becton Dickinson), Urea agar (Oxoid), susceptibility to

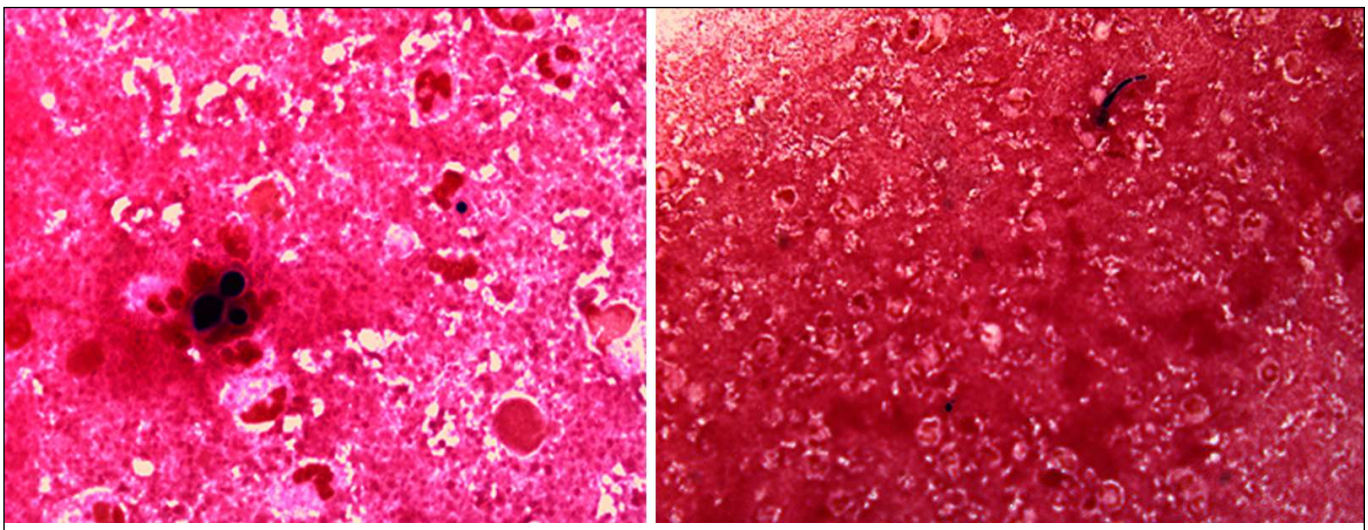

Fig. 1a **Fig. 1b**

Fig 1a and 1b. Gram stain of infected graft showing yeast with two distinct morphologies showing variability in size (Fig. 1a) and presence and absence of pseudohyphae (Fig 1b) marked by arrows.

Table 1. Antifungal susceptibilities of *Candida species* isolated from the patient

Organism	**Specimen**	**Fluconazole** MIC microgram/ ml (Interpretation)	**Voriconazole** MIC microgram/ ml (Interpretation)	**Amphotericin** MIC microgram/ ml (Interpretation)	**Caspofungin** MIC microgram/ ml (Interpretation)
C. albicans	Blood and tissue	0.5 (S)*	<0.008 (S)	<0.12 (S)	0.03 (S)
C. glabrata	Blood and tissue	16 (R)*	0.25 (SDD)*	0.12 (S)	0.03 (S)
C. krusei	Tissue	32 (R)	0.25 (S)	0.25 (S)	0.06 (S)

*S: Sensitive, R: Resistant, SDD: Susceptible dose dependent

cycloheximide (Becton Dickinson), inoculation on Corn Meal Tween 80 agar. Isolated colonies were also evaluated for sugar assimilation on the biochemical test panel API 20C AUX (Biomérieux). Antifungal susceptibility was performed using YeastOne Sensititre plates (Trek Diagnostics Systems).

Discussion

This case report describes a 36-year old male patient who was admitted with fungal aortic graft infection. The unique finding in this report is simultaneous invasive infection with three *Candida* species (*Candida albicans, Candida glabrata* and *Candida krusei*). The most interesting observation was an early detection of mixed infection on microscopy with timely initiation of an appropriate antifungal agent. It is important to highlight that although acquired resistance to fluconazole is rare in *Candida albicans, Candida krusei* is inherently resistant to fluconazole and acquired resistance to fluconazole in *Candida glabrata* is common.[7] Therefore, our patient who had been on fluconazole initially had persistent candidemia and was treated successfully when amphotericin B was started. Vascular graft infections are difficult to treat and they require a combined medical and surgical approach. The significance of fungal etiology in these infections could not be ignored due to high risk of mortality. Candida infections are also associated with a significantly higher risk of mortality even after surgical intervention. There is no fixed criterion of antifungal therapy period as in some cases lifelong therapy has been used, particularly for patients with prosthetic graft infections. In our case a surgical intervention with appropriate antifungal therapy for four months resulted in cure. The source of *Candida* infection in our patient was most probably direct spread from gastrointestinal tract through the erosion of duodenal wall by the aortic graft. Prior exposure to fluconazole and broad-spectrum antibiotics for five days very likely selected two fluconazole-resistant *Candida* species, *C. glabrata* and *C. krusei*.[8,9] It is important to remember that when a deep-seated infection with *Candida* species is diagnosed, potential complications of candidemia should be searched for. In our patient, timely initiation of appropriate antifungal possibly played a role in clearing candidemia and thus the organisms did not seed to other sites.

As mixed *Candida* vascular graft infection is a rare entity, a high index of suspicion, early diagnosis, prompt antifungal and surgical treatment are crucial steps to be taken in order to prevent high mortality associated with it. Methods for early diagnosis of fungal infections such as Fluorescence in situ hybridization (FISH) and multiplex PCR and Luminex flow cytometric multianalyte profiling systems, are expensive and not widely available in resource limited settings. In these settings the role of a skilled microbiologist in the detection of mixed fungal infections is crucial.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical clearance

The study was approved by the Aga Khan University Ethical Review Committee, 1373-Path-ERC-09.

Informed consent

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References

1. Laser A, Baker N, Rectenwald J, Eliason JL, Criado-Pallares E, Upchurch GRJ. 2011. *Graft infection after endovascular abdominal aortic aneurysm repair*. ; 54: 58-63.
2. Doscher W, Krishnasastry KV, Deckoff SL. 1987. *Fungal graft infections: Case report and review of the literature. J Vascular Surg*; 6: 398-402.
3. Leroy O, Meybeck A, Sarraz-Bournet B, d'Elia P, Legout L. 2012. *Vascular graft infections. Curr Opin Infect Dis*; 25: 154-58.
4. Saleem BR, Meerwaldt R, Tielliu IFJ, Verhoeven ELG, van den Dungen JJAM, Zeebregts CJ. 2010. *Conservative treatment of vascular prosthetic graft infection is associated with high mortality. Am J Surg*; 200: 47-52.
5. Blanch M, Berjon J, Vila R, Simeon JM, Romera A, Riera S, Cairols MA. 2010. *The management of aortic stent-graft infection: endograft removal versus conservative treatment. Ann Vascular Surgery*; 24: 554.e1–554.e5.
6. Ali AT, Modrall JG, Hocking J, Valetine RJ, Spencer H, Eidt JF, Clagett GP. 2009. *Long-term results of the treatment of aortic graft infection by in situ replacement with femoral popliteal vein grafts. J Vascular Surgery*; 50: 30-39.
7. Pfaller MA, Diekema DJ. 2007. *Epidemiology of invasive candidiasis: a Persistent Public Health Problem. Clin Microbiol Rev***;** 20: 133-163.
8. Garnacho-Montero J, Díaz-Martín A, García-Cabrera E, Ruiz Pérez de Pipaón M, Hernández-Caballero C, Aznar-Martín J, Cisneros JM, Ortiz-Leyba C. 2010. *Risk factors for fluconazole-resistant candidemia. Antimicrob Agents Chemother*; 54: 3149-3154.
9. Ben-Ami R, Olshtain-Pops K, Krieger M, Oren I, Bishara J, Dan M, Wiener-Well Y, Weinberger M, Zimhony O, Chowers M, Weber G, Potasman I, Chazan B, Kassis I, Shalit I, Block C, Keller N, Kontoyiannis DP, Giladi M and for the Israeli Candidemia Group. 2012. *Antibiotic exposure as a risk factor for fluconazole-resistant candida bloodstream infection.* Antimicrob Agents Chemother; 56: 2518-2523.