Candida albicans Endocarditis Associated with a Contaminated Aortic Valve Allograft: Implications for Regulation of Allograft Processing

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A patient developed Candida albicans endocarditis and fungemia after undergoing aortic valve replacement with an allograft. The allograft had been found during tissue bank processing to be contaminated with C. albicans, but it was culture-negative for C. albicans after routine disinfection with an antifungal-containing antimicrobial solution. Comparison of the preimplantation and post-implantation C. albicans isolates revealed remarkable genetic similarity, but antifungal susceptibility testing showed that the postimplantation isolate was more resistant to fluconazole and amphotericin B than the preimplantation isolate, suggesting emergence of resistance after disinfection. Implantation of a contaminated heart valve allograft can occur despite disinfection during processing and can result in endocarditis in the recipient. Antimicrobial disinfection protocols that include antifungal drugs may be ineffective. Current U.S. Food and Drug Administration regulations do not require companies to specify details concerning allograft processing. Additional measures may be required to prevent tissue bank release of allografts contaminated with C. albicans or other pathogens.

Since the first aortic valve allograft replacement was described in 1962, the clinical use of allografts has been limited by their supply and durability [1]. Efforts to increase the supply of usable valve allografts have resulted in the development of new methods of valve collection, disinfection, and cryopreservation that have been associated with varying rates of allograft contamination [2].

Contamination of a valve allograft can lead to endocarditis in the recipient, an especially serious and often fatal condition [3]. Fungal endocarditis, although more unusual than bacterial endocarditis, is associated with a particularly high mortality rate. In this article, we describe the development of fungal endocarditis associated with the implantation of a contaminated aortic valve allograft.

Case Report

A 61-year-old man underwent allograft aortic valve (CryoLife, Marietta, GA) replacement for long-standing aortic insufficiency. There were no immediate postoperative complications, and the patient was discharged 5 days later. Sixteen days after surgery, the patient was readmitted because of fever (temperature, 104°F), nausea, and diarrhea. A physical examination revealed abdominal tenderness. The patient’s WBC count was 6,800/mm³. Cultures of blood drawn on admission were positive for Candida albicans, and therapy with intravenous amphotericin B (Bristol-Myers Squibb, Princeton, NJ) and oral flucytosine (Hoffmann-LaRoche, Nutley, NJ) was started.

Fungal endocarditis was suspected, and transesophageal echocardiography revealed aortic valve allograft dehiscence; the patient underwent replacement of the infected aortic valve allograft with another allograft from the same supplier. Intraoperative examination confirmed dehiscence of the aortic valve from the septum; in addition, an intramyocardial abscess and multiple vegetations were found in and around the suture line. Photomicroscopic examination of smears of a swab specimen of the valve surface prepared with potassium hydroxide (KOH) revealed yeast elements, and culture of the valve tissue yielded C. albicans. The patient defervesced postoperatively, received intravenous amphotericin B for a total of 8 weeks, and has remained symptom-free since treatment.

A review of the aortic valve allograft harvest and processing history revealed that during valve processing, culture of a tissue sample from the allograft valve was positive for C. albicans.
Methods

All *C. albicans* isolates were identified by routine methods. Antifungal susceptibility testing by broth macrodilution was performed by the authors according to the methods of the National Committee for Clinical Laboratory Standards [4]. Pulsed-field gel electrophoresis and gene probe with use of the Ca3 probe were performed as previously described [5].

Isolate genotyping and antifungal susceptibility testing. The *C. albicans* isolates obtained from valve tissue during processing and at the time of removal of the valve from the recipient were highly similar according to DNA fingerprinting using Southern blot hybridization with the DNA probe Ca3 [5, 6] (figure 1). The two isolates differed by a single, high-molecular-weight band, which represents a hypervariable segment that hybridizes with the C1 sequence of the Ca3 probe [5, 7]. A dendrogram in which the two isolates were compared with 20 isolates randomly chosen from the Dendron database [6] demonstrates their high degree of relatedness (figure 2).

Antifungal susceptibility testing revealed that the isolate obtained from the valve post-implantation was more resistant to fluconazole and amphotericin B than was the isolate obtained during allograft processing (table 1). The two isolates showed equivalent susceptibility to flucytosine, ketoconazole (Janssen Pharmaceutica, Titusville, NJ), and itraconazole (Janssen Pharmaceutica).

Discussion

The allograft heart valve is harvested from a brain-dead or postmortem donor, processed in an antimicrobial disinfection solution, cryopreserved, and stored until the valve is requested for implantation. Tissue samples obtained for sterility testing are cultured for bacteria, fungi, and acid-fast bacilli at multiple stages during harvesting and processing to rule out microbial contamination of the valve. Tissue samples are obtained by trimming of the valve at harvesting, and the valve “trimmings” are cultured before and after antimicrobial disinfection [8].

Microbial contamination is common at harvesting, but fungal contamination is unusual. Common contaminants found before disinfection consist of gastrointestinal and skin flora, including coliforms, viridans group streptococci, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus* species. Pathogens that most commonly cause early onset allograft valve endocarditis include *Staphylococcus* and *Streptococcus* species [2, 9]. Sources of contamination of a valve allograft include the donor, the environment during harvesting and processing, and the operating room during implantation [9].

Fungal endocarditis secondary to extrinsic valve contamination is a rare but potentially fatal complication of allograft valve replacement. The incidence of fungal endocarditis that occurs following surgery for heart valve replacement with allografts is estimated at 0.3%–1.4% [2, 10, 11]. For many of these
were discarded because of contamination, but a minority of contaminating organisms were fungal (6 of 642) [2].

Disinfection of valve allografts through the use of various antimicrobial combinations was first described in the late 1960s [13, 14]. Since that time, antimicrobial agents used for disinfection have been further modified to improve efficacy and valve viability, increasing the supply of usable allografts [2, 12]. However, antifungal agents used for disinfection may damage allograft valve tissue and also may be ineffective. Reports of rates of fungal contamination following disinfection vary widely (1.7%–28.0%), and data indicate that inclusion of antifungal drugs in the disinfection regimen is not effective in eradicating fungal contamination [2, 10]. In some centers, antifungal agents have been removed from the disinfection protocol because of these concerns.

Five tissue banks supply the majority of heart valve allografts in the United States. Four organizations are grouped under an accreditating organization, the American Association of Tissue Banks (AATB), which requires that tissue banks disinfect tissues via a time-specific antibiotic incubation and that facilities establish, validate, and document antibiotic regimens and microbial surveillance methods [15]. AATB-affiliated organizations follow a similar disinfection protocol, using a solution containing four antimicrobials (cefoxitin [Merck & Co.], lincomycin [The Upjohn Co., Kalamazoo, MI], polymyxin B [Burroughs Wellcome, Research Triangle Park, NC], and vancomycin), in which valve tissues are incubated at 2°C–8°C for ~24 hours. All four allograft processing companies affiliated with AATB routinely discard valves with documented fungal contamination of post-harvest trimmings.

In contrast, at Cryolife, a tissue bank that reportedly provides >85% of cryopreserved heart valve allografts in the United States, the disinfection treatment consists of submersion in a solution containing amphotericin B, fluconazole, imipenem, netilmicin, and vancomycin; temperature and duration of disinfection are considered proprietary information and could not be ascertained by the authors.

In the episode described in this article, genetic analyses confirmed that the C. albicans isolate obtained from the valve before disinfection was nearly identical to the isolate obtained after implantation. Isolate exposure to the antifungal drugs used during valve disinfection may have resulted in the emergence of a more-resistant strain, accounting for the decreased antifungal

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<th>Table 1. Antifungal susceptibility of allograft Candida albicans isolates obtained before and after implantation.</th>
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<td>Allograft isolate recovery</td>
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reported cases, the source of contamination was not identified. Treatment is often unsuccessful, and death is frequently the outcome. In one review, >40% (11 of 27) of patients who acquired fungal endocarditis after valve allograft implantation died within 2 weeks of diagnosis [11].

Varying contamination rates measured during allograft heart valve harvesting and processing may reflect differing methods of harvesting, disinfection, and cryopreservation. In order to meet the demand for valve allografts, supply has been increased through harvest from deceased in addition to brain-dead donors [2, 12]. Microbial contamination is quite common at the time of harvesting, particularly in postmortem procurement. In one series, 54% of postmortem heart valves retrieved in open mortuary areas were contaminated at collection; 31 of 642 valves
susceptibility of the postimplantation isolate and the one-band difference between the two isolates on genetic analyses.

The implicated valve was found to be \textit{C. albicans} culture-positive before disinfection. However, cultures of valve trimmings did not yield the organism after disinfection, results leading the tissue bank to conclude that the valve was free of fungal contamination. We are not aware of published reports estimating the rate of false-negative fungal cultures in this setting.

U.S. Food and Drug Administration (FDA) regulations do not require companies that process allografts to specify details concerning the disinfection process, including type of antimicrobials used, temperature and duration of disinfection, sterility testing, or culture findings that necessitate discarding of a valve. Under a proposal published by the FDA for regulation of cellular and tissue-based products, human heart valve allografts would be subject to donor screening and testing, processing, labeling, and registration requirements [16]. Additional regulations may be required to reduce the number of contaminated allografts released for use, including requirements for standardized methods of disinfection, method validation, and compulsory discard lists.

Although many companies do not use antifungal agents for disinfection, discard valves with fungal growth, and have compulsory discard lists for tissue yielding certain pathogens (including \textit{Candida} species) before or after disinfection, these practices are not universally followed. Health care personnel need to remain aware that contamination of allograft valve can occur and that tissue bank disinfection of allografts may be ineffective.

Specific recommendations, such as those presented in a recent article, may be necessary to better standardize processing protocols [2]. As the demand for implantable tissue increases, additional measures should be considered to reduce the risk of infections resulting from contamination of allografts, including those caused by fungal pathogens such as \textit{C. albicans}.

References