



Incidence and Outcome of Documented Fungal Endocarditis

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ABSTRACT

Background: Fungal endocarditis, the most severe form of infective endocarditis, is characterized by excessive mortality and morbidity.

Objectives: The present study aimed to analyze the characteristics of fungal endocarditis to improve the management of these patients.

Materials and Methods: In this cross-sectional study, vegetations on the mitral or tricuspid valves and embolic material surgically removed from the patients with suspected infective endocarditis between December 2009 and November 2011 were examined for fungal infection by direct smear and culture, and the susceptibility patterns of the isolated species were determined. Then, blood samples were cultured on BACTEC media and real-time PCR was done with blood and tissue samples.

Results: Of the 31 patients with suspected infective endocarditis who did not respond to antibacterial therapy, 11 had confirmed fungal endocarditis. The most frequent predisposing risk factors were previous surgery and drug abuse. The organisms isolated were *Aspergillus spp.* and *Candida albicans*. Resistance to amphotericin B and itraconazole was observed in *Aspergillus* species, and to fluconazole in *Candida albicans*. Positive PCR results were obtained in blood and tissue samples.

Conclusions: Fungal endocarditis should be considered in the patients not responsive to antimicrobials. Moreover, management of these patients can be improved with molecular diagnostic methods and by determining the susceptibility patterns of the etiologic agents.

► Implication for health policy/practice/research/medical education:

The incidence rate of fungal endocarditis has increased in the last decades which can lead to a high mortality rate. To develop more efficient management of the respective patients, it is necessary to investigate the incidence rate using laboratory methods as well as the susceptibility pattern of isolated species.

1. Background

Infective Fungal Endocarditis (FE) is a severe opportunistic infection with a high mortality rate of about 50% (1), despite aggressive treatment. The clinical manifestations may be similar to those of bacterial infections; i.e., fever, major peripheral embolization on echocardiography, focal or generalized neurological features, dyspnea, chest pain, changing or new heart murmurs, heart failure, cough, abdominal pain, finger clubbing, hepatomegaly, malaise, and skin petechiae (2). However, the presence of valve or

prosthesis destruction and large vegetations can lead to diagnosis of FE. The etiologic agents are opportunistic fungi, such as *Candida* species and filamentous fungi, the most important of which being *Aspergillus* species which are not usually recovered from blood cultures. Nevertheless, their isolation should be attempted from surgically removed emboli, diseased valves, or infected foreign bodies (3).

2. Objectives

Because FE is life-threatening and its epidemiology, diagnosis, and treatment are challenging, the present study aims to obtain information that can improve the management of these infections by analyzing cases, comparing different

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diagnostic methods, and determining the susceptibility patterns of the isolated species.

3. Materials and Methods

From December 2009 through November 2011, all the patients with suspected infective endocarditis according to Duke criteria (4), visited at two large university centers (Nemazi Hospital and Shahid Faghihi Hospital) in southern Iran, were enrolled into this study. None of the patients responded to antibacterial agents and all were operated for the removal of infected tissues. The patients who were responsive to antibacterial agents and did not need surgery were excluded from the study. Vegetations on the mitral or tricuspid valves and embolic materials that were surgically removed were immediately sent to the mycological lab and examined for fungal infection. In doing so, direct tissue smears with potassium hydroxide were prepared and cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany). Multiple blood cultures were prepared during the hospital stay with BACTEC medium (Becton-Dickinson, Sparks, MD, USA). Besides, the clinical and microbiological data were collected from the patients' medical records.

Identification of the filamentous fungi isolated in the culture was based on morphologic characteristics of the colony, and the smears were prepared with lactophenol cotton blue. To identify the yeasts, after two passages on potato dextrose agar (Oxoid Ltd, Basingstoke, Hampshire, UK), the API kit (Biomérieux, France) was used according to the manufacturer's instructions. Once the samples were removed from the operating room, they were promptly examined microscopically and antifungal therapy was initiated.

Inoculum suspensions of the yeasts were adjusted to a turbidity of 0.5 McFarland Standard (10^6 cells/mL) and were used to determine susceptibility patterns. Also, the conidia of *Aspergillus spp.* were mixed with 0.05% Tween 20 in sterile saline, and the turbidities of the supernatants were spectrophotometrically adjusted to optical densities ranging from 0.09 to 0.11 (WPA, bioWave, S2100 Diode Array Spectrophotometer, Cambridge, UK) at 530 nm. The E-test was done according to the manufacturer's instructions. Also, two Clinical Laboratory Standards Institute quality control strains, namely *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC6258, were tested in the same

manner (5). Afterwards, appropriate antifungal treatment was continued on the basis of the susceptibility pattern of each isolated species.

Polymerase Chain Reaction (PCR) assays were done with blood and tissue samples as described previously (6-8). Tissue samples were treated with proteinase and lyticase enzymes, and then for DNA extraction, the QIAmp DNA Minikit (Qiagen, Hilden, Germany) was used according to the manufacturer's recommendations. All primers and TaqMan probes were from Metabion (Martinsried, Germany). To avoid contamination, all the samples were handled under sterile conditions in a laminar flow cabinet. The probe and primers for *Aspergillus* used in this study were previously described (6-9) and were able to identify all *Aspergillus* species. To determine the sensitivity of the real-time PCR assay, 100 copies/well of each *Aspergillus fumigatus* and *Candida albicans* DNA were serially diluted and measured. All the statistical analyses were performed using the SPSS statistical software for Windows (Statistical Product and Service Solutions, version15.0, SSPS Inc, Chicago, IL, USA).

3.1. Ethical Considerations

The Ethics Committee of Professor Alborzi Clinical Microbiology Research Center at Shiraz University of Medical Sciences reviewed and approved the study. The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration.

4. Results

In this cross sectional study, out of the 73 patients with infective endocarditis admitted to the two participating hospitals during the study period, 31 had large vegetations and did not respond to antibacterial agents, and were thereby treated surgically. Among these 31 patients, FE was confirmed in 11. The male-to-female ratio was approximately 2:1, and the mean age of the patients was 34.2 ± 1.8 years (range 4 to 67 years). All the patients received antibiotics for at least 1 week but none received any antifungal agents. The characteristics of the patients and the Minimum Inhibitory Concentration (MIC) of different drugs for each of the isolated causal agents have been presented in Table 1.

The isolated organisms were *A. fumigatus* in 2, *C. albicans*

Table 1. Characteristics of Fungal Endocarditis Patients with the MICs of the Isolates (μ /mL)

No	Sex/Age	Etiologic	Amphotericin	Ketoconazole	MIC		Posacoazole	Voriconazole	Caspofungin
					Itraconazole	Fluconazole			
1	F/27	<i>A. niger</i>	0.125	32	1.5		0.047	0.064	0.047
2	M/17	<i>A. flavus</i>	2.0	1.5	0.032		0.25	0.19	0.19
3	M/4	<i>A. flavus</i>	4.0	1.0	0.032		0.125	0.19	0.047
4	M/50	<i>A. fumigatus</i>	2.0	3.0	0.5		0.38	0.75	0.016
5	M/31	<i>C. albicans</i>	0.5	1.5	0.5	32.0	0.094	0.125	0.032
6	F/67	<i>A. niger</i>	0.064	0.125	1.5		0.016	0.023	0.19
7	M/19	<i>A. niger</i>	0.25	0.75	0.125		0.5	0.5	0.016
8	M/42	<i>A. fumigatus</i>	2.0	16.0	3.0		0.38	0.064	0.023
9	M/64	<i>A. niger</i>	0.5	1.5	0.125		0.75	0.125	0.032
10	F/35	<i>A. niger</i>	0.5	2.0	2.0		0.19	0.19	0.064
11	M/21	<i>C. albicans</i>	0.25	1.0	1.0	16.0	0.064	0.64	0.016

Abbreviations: *A.*, *Aspergillus*; *C.*, *Candida*

in 2, *Aspergillus flavus* in 2, and *Aspergillus niger* in 5 patients. One patient in whom the same etiologic agent was identified after more than one operation was considered a single case of endocarditis (case No. 1). Detailed analysis of all the 11 cases revealed that 8 were late cases (onset > 60 days) with onsets between 2 and 48 months after surgery.

The most frequent predisposing risk factor was previous surgery (8 cases) due to a congenital disorder or valve replacement. Besides, two patients were intravenous drug abusers and in 1 patient (No. 4), no predisposing factor was identified. All the patients were treated surgically and with antifungal agents (amphotericin or voriconazole) for a minimum of 6 weeks, and with long-term itraconazole for 6 months (Table 2). After treatment, 1 patient returned with *Aspergillus* pneumonia and positive result on sputum culture.

Fever was present in all the patients and changing heart murmur was recorded in 8 patients. Other clinical signs, such as dyspnea, cough, general body pain, lower extremity pain, and finger clubbing, were detected in 2 cases. White blood cell count was slightly increased to between 4800 and 12300 cells/mL. Moreover, blood culture was positive in only 1 patient infected with *C. albicans*. In addition, blood samples were positive in PCR assays in 10 patients, and tissue samples were positive in all the 11 patients. The mortality rate was 18% (2/11) during the 12-month follow-up period.

MIC for different antifungal agents varied according to the species. Furthermore, resistance to amphotericin B and itraconazole was observed in *Aspergillus* species, and resistance to fluconazole was found in *C. albicans* (Table 1).

5. Discussion

The significance of the present study lies in the inclusion of proven cases of FE with positive tissue culture, a feature that overcomes some of the shortcomings of the earlier

reports in the literature. The gold standard for diagnosis of FE is culture and isolation of etiologic agents from emboli, infected valves, or other materials collected by invasive methods, such as surgery (3).

The incidence of FE has increased in the recent decades, and fungal species now comprise 1 - 10% of all etiologic agents isolated in the patients with infective endocarditis (10) and approximately 10% of the agents responsible for blood stream infections (11). *Candida albicans* is responsible for 24 - 46% of all the cases of FE (1, 2, 12) and for 3.4% of all the cases of prosthetic valve endocarditis, with associated mortality rates ranging from 46.6% to 50% (1, 13). After *Candida*, *Aspergillus* species are the second most frequent cause of fungal infection, accounting for approximately 25% of all the cases of FE in cardiac valve prostheses and the great vessels (2). In this study, *C. albicans* was isolated from 2 patients, both of whom were intravenous drug abusers. Additionally, *Aspergillus* species were mostly found in the patients (9/11) who had previously undergone open heart surgery; therefore, *Aspergillus* endocarditis can be considered a type of nosocomial FE, an ominous condition in surgical patients. During major surgery, colonization of the surgical site by airborne *Aspergillus* conidia can lead to nosocomial *Aspergillus* endocarditis. Accordingly, FE can be considered an emerging infectious disease. Previous surgery and intravenous drug abuse were the most frequent risk factors in the present study. Other risk factors for development of FE included parenteral nutrition, immunosuppression, underlying cardiac abnormalities, prosthetic heart valves, indwelling central venous catheters, prolonged use of broad-spectrum antibiotics, and cardiovascular surgery (1, 2, 14).

Typically, only 50% of blood samples are positive for candidiasis, and *Aspergillus species* are not usually

Table 2. Characteristics of Fungal Endocarditis Patients

No	Onset after Surgery	PCR in Blood	PCR in Tissue	Antifungal Therapy	Predisposing Risk Factors	Outcome
1.	4 month	+	+	Amphotericin B	Previous surgery (4 times)	Clinically well after 12 month return with re-current endocarditis
2.	4 years	+	+	Voriconazole	Previous surgery (3 times)	Clinically well after 12 month
3.	12 month	+	+	Voriconazole	Permanent pacemaker	12 month Clinically well after
4.	Not available	-	+	Amphotericin B	No predisposing risk factors	Clinically well after 12 month
5.	Not available	+	+	Amphotericin B	IV drug abuser since 12 years ago.	Clinically well after 12 month
6.	2 month	+	+	Amphotericin B	Prosthetic valve replacement	Clinically well after 12 month
7.	5 month	+	+	Voriconazole	Previous surgery (1 times)	Clinically well after recurrent <i>Aspergillus</i> pneumonitis
8.	4 month	+	+	Any treatment	Previous surgery (1 times)	Died before using the treatment
9.	6 month	+	+	Voriconazole	Previous surgery (1 times)	Clinically well after 12 month
10.	2 month	+	+	Any treatment	Previous surgery (2 times)	Died before using the treatment
11.	Not available	+	+	Amphotericin B	IV drug abuser	Clinically well after 12 month

recovered from blood cultures (15, 16). In the present study, blood culture was positive in only 1 patient; therefore, blood culture is of little help in diagnosis of FE.

Accurate molecular methods are available for diagnosis of many infections. Peter et al. reported that such methods in tissue samples were as much as 3-fold more sensitive than Gram staining and culture (17). In this connection, Breikopf et al. reported the sensitivity of bacterial broad-range PCR to be 41.2% compared to 7.8% for culture and 11.8% for Gram staining (18). Besides, nested-PCR for detection of proven and probable aspergillosis had a sensitivity of 80% and a specificity of 96.2% (6). This method has also been used for diagnosis of FE (19), and in the present report, PCR was positive in all tissue samples and in 10/11 blood samples. In patient No.9, one blood sample was evaluated for FE prior to surgery and voriconazole was started when the PCR results became positive. He recovered from the infection sooner than the other patients did. Resistance of fungal species to antifungal agents has been reported in many studies (19-21). In the current study, some isolates were found to be sensitive to some antifungal agents. This finding is potentially helpful in determining appropriate treatment.

After surgical debridement and antifungal treatment with amphotericin B or voriconazole in the 11 patients included in the present study, the 12-month survival rate was 82% and the mortality rate was lower than that reported in other studies (13). The results of the present study thus showed that early detection (direct smears examined immediately while surgery was in progress) as well as prompt initiation of appropriate treatment could reduce mortality from FE. We chose the treatments for each patient on the basis of culture results and the susceptibility to antifungal agents of the isolated species. However, because of the late diagnosis of FE, patient No. 8 died 12 hours after surgery and patient No. 10 died after 1 day. The results of this study are potentially useful in designing and implementing future interventions for early detection, prevention, and treatment of FE.

FE is often a secondary infection that develops as a consequence of the management of the FE, especially in the patients who have recently undergone surgery. For treatment to be effective, a rapid diagnosis and appropriate antifungal therapy are essential. PCR assay is a potentially useful diagnostic tool for this infection.

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Authors' Contribution

Parisa Badiee: designing the study, performing the laboratory procedure, data analysis, and writing the manuscript; Ahmad Ali Amirghofran, Mohammad Ghazi Nour, Masih Shafa, and Mohammad Hassan Nemati: performing the heart surgery, sample collection, and review of the article.

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