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Prognosis in fungemia of unknown origin according to conventional automated microbiology system

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Abstract

Fungemia of unknown origin was investigated using a conventional automated microbiology system (CHROMagar Candida and VITEK 2) to determine which fungal species were involved and clarify what precautions should be taken when treating such patients. A total of 126 patients with fungemia treated at Tokyo Medical University Hospital between June 1, 2013 and November 30, 2017 were enrolled in this retrospective study. The species of pathogen could not be identified in 22 of these patients using the conventional approach. Polymerase chain reaction (PCR), and matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) were subseqently used to identify the fungi in these cases, together with the treatment given and prognosis. *Candida parapsilosis* was the most common species among the unidentified isolates (36%). Relatively rare fungi were also detected, including *Cryptococcus neoformans*, *Cyberlindnera fabianii*, *Lodderomyces elongisporus*, *Saccharomyces cerevisiae*, and *Trichosporon montevideense*. The results of MALDI-TOF MS were consistent with those of PCR for most strains. Micafungin (MCFG) was administered empirically in 73% of cases. Eleven of the 22 patients died within 30 days. Comparisons were made between those who died (death group) and those who did not (survival group). Potentially ineffective combinations of identified fungi and antifungal drugs were found in 4 patients, all of whom were in the survival group. Death was not due to treatment failure, but to poor general condition based on the Sequential Organ Failure Assessment score. Micafungin was used in all 4 cases in which the combination of drug and pathogen was considered inappropriate. All these patients survived, however, due to proper intervention and change in antifungal drug. These results indicate that conventional automated microbiology systems may not be able to identify fungal species in which MCFG is ineffective. This suggests that it is necessary to carefully observe the patient's condition and whether they are responding to the initial treatment when selecting MCFG in cases where pathogen identification is not possible with a conventional automated microbiology system.

Introduction

In recent years, invasive infections such those of the bloodstream by fungal species have become problems with potentially high mortality rates. The use of broad-spectrum antibacterial drugs, central venous catheter

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insertion, and peritonitis are well-known risks of the bloodstream infection by fungal species[1]. Species identification is key to treatment success. Facilities that can perform fungal susceptibility testing are limited, and in a clinical setting, bloodstream infections involving unidentified fungal species are often analyzed by using *Candida* medium and an automated identification system. The goals of this study were to determine which fungi were unidentifiable and identify points of which the physician should be aware of in treating fungemia patients.

Materials and Methods

This retrospective study included a total of 126 fungemia patients treated at the Tokyo Medical University hospital between June 1, 2013 and November 30, 2017. The species involved could not be identified using an automated identification system and selective media in 22 of these patients. Therefore, they were subsequently recorded in the clinical records as *Candida* sp. or yeast-like fungi. Identification at our facility is performed using both CHROMagar Candida (Kanto Kagaku, Tokyo, Japan) and the VITEK 2 automated system (bioMérieux, Marcy-l'Étoile, France). In CHROMagar Candida testing, cultivation is carried out at 35°C and evaluation is made after 48 hours. The VITEK 2 automated system uses a VITEK 2 YST card (bioMérieux) and a modified broth microdilution method for identification. Antifungal susceptibility testing is ordered as needed by the attending physician and performed using Kouboyoushinkin FP (Eiken Kagaku, Tokyo, Japan) according to Clinical and Laboratory Standards Institute procedures (CLSI)[2].

Here, in genetic analysis testing, molecular identification by polymerase chain reaction (PCR) amplification and sequencing analysis of the internal transcribed spacer (ITS) region was performed using DNA extracted from the isolates. The universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were used. Analysis was performed using the GenBank Basic Local Alignment Search Tool (BLAST) database. For matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS), a Bruker MALDI Biotyper CA System (Billerica, MA, USA) was used in line with the manufacturer's instructions. The pattern-matching results were expressed as log score values of 0-3 using MALDI Biotyper CA System software.

The clinical background of 22 cases (sex, age, length of hospital stay, risk for fungemia, primary site of infection, Sequential Organ Failure Assessment (SOFA) score, treatment, death within 30 days, presence of persistent fungemia) was determined from the medical records. Patients who died within 30 days were classifed as the death group, whereas those who survived after 30 days were assigned to the survival group and the two groups compared. The results of PCR and MALDI-TOF-MS were used to evaluate whether the initial treatments were appropriate in both groups. The appropriateness of treatment was determined based on the results of susceptibility tests. When only the fungal species was known, the literature was referred to. It was regarded as unknown if the effect of the selected antifungal drug was not clear, even in the literature. The statistical analysis was peformed using IBM SPSS Statistics for Windows, version 26 (IBM Japan, Tokyo, Japan). Means for continuous variables were compared using independent group *t*-tests when the data were normally distributed. Proportions for categorical variables were compared using the χ^2 test, although the Fisher exact test was used when the data were limited. All tests were two-tailed and a *p*-value of ≤ 0.05 were taken to indicate statistical significance. This study was approved by the Ethical Committee of Tokyo Medical University (approval number : SH4059). The need for consent was formally waived by the Ethical Committee of TMU.

Results

Among the 22 patients, the median patient age was 68.4 years (range 34-85 years) ; 73% were male. The median length of hospital stay before fungemia developed was 28 days (range 1-137 days). Most of the patients had risk factors for invasive fungal infection that have been described previously[1]. Use of broad-spectrum antibacterial drugs (82%) and intravenous catheter use (82%) were the most frequently reported predisposing factors. The most frequent diagnosis of infectious disease was catheter-related bloodstream infection (CRBSI ; 64%), followed by peritonitis (14%). The mean SOFA score was 5.09 (range 0-24). The antifungal drugs selected were micafungin (MCFG) in 16 cases (73%), liposomal amphotericin B (L-AMB) in 3 cases (14%), and fluconazole (FLCZ) in 3 cases (14%). Eleven patients died within 30 days, and the remaining 11 survived. Persistent fungemia was observed in 6 cases (27% ; see Table 1).

The distribution of *Candida* species identified via PCR showed that *C. parapsilosis* (8/22) was the most common, followed by *C. krusei* (3/22). *Cryptococcus neoformans*, *Cyberlindnera fabianii*, *Lodderomyces elongisporus*, *Saccharomyces cerevisiae*, and *Trichosporon montevideense*, which are relatively rarely detected in blood, were also identified using PCR. Use of MALDI-TOF MS enabled the testing of 19/22 cases, but 3/22 cases could not be tested because the samples had been discarded. Cases identified as *C. africana* or *C. krusei* by PCR were identified as *C. albicans* and *C. guilliermondii* by MALDI-TOF MS, but all others were consistent with the PCR results (Table 2).

Table 1 Demographic characteristics and outcomes of patients with unidentified fungemia.

Variable		Description
Sex, *n* (%)		
	Male	16 (73%)
	Female	6 (27%)
Age (years)		
	Mean	68.4
	Median	70
Length of hospital stay (days)		
	Median	28
Risk factor(s) for fungemia		
	Use of broad-spectrum antibacterial drugs	18 (82%)
	Presence of central venous catheter	18 (82%)
	Cancer	8 (36%)
	Use of immunosuppressive drugs	7 (32%)
	Use of parenteral nutrition	7 (32%)
	Chronic renal failure	4 (18%)
	Diabetes mellitus	3 (14%)
Primary site of infection		
	Catheter-related bloodstream infection	14 (64%)
	Peritonitis	3 (14%)
	Cholangitis	1 (5%)
	Ileus	1 (5%)
	Artificial vascular infection	1 (5%)
	Urinary tract infection	1 (5%)
	Unknown	1 (5%)
SOFA score		
	Mean	5.09
	Median	4
Treatment		
	Micafungin	16 (73%)
	Fluconazole	3 (14%)
	Liposomal amphotericin B	3 (14%)
Outcome		
	Died within 30 days	11 (50%)
	Persistent fungemia	6 (27%)

Micafungin was the most commonly used drug in both the death and survival groups. Based on the species of fungus identified, susceptibility results, and previous literature, treatment in 4 cases may have been ineffective. The combinations of fungal species and antifungal drugs considered ineffective comprised MCFG for *Cryptococcus neoformans*, *Lodderomyces elongisporus*, *Trichosporon montevideense*, and *Saccharomyces cerevisiae.* All 4 of these combinations were found found in the suvival group (Table 3).

Differences between the death and survival groups were also investigated in terms of sex, age, primary site of infection, SOFA socre, PCR results, and treatment (Table 4). The only significant difference was found in SOFA score (death group = 8 vs. survival group = 2.18 ; *p*-value = 0.002).

Discussion

Identification using the CHROMagar Candida and VITEK 2 automated system is highly sensitive, but not 100%[3]. We initially speculated that only rare fungi would prove might unidentifiable. The results showed, however, that *C. parapsilosis*, a common fungal species, was the most often determined to be unidentifiable.

In terms of drug used, MCFG was selected the most frequently. We believe that MCFG was the most commonly selected because the attending physician probably imagined infection due to non-albicans *candida* when they received the report of an unidentified fungus[4].

The results of MALDI-TOF MS were almost identical

Table 2 Results of PCR and MALDI-TOF MS identification.

Species	PCR	MALDI
Candida parapsilosis	8	8
Candida krusei	3	1
Candida glabrata	2	2
Candida africana	1	0
Candida albicans	1	2
Candida guilliermondii	1	2
Candida tropicalis	1	0
Cryptococcus neoformans	1	1
Cyberlindnera fabianii	1	1
Lodderomyces elongisporus	1	1
Saccharomyces cerevisiae	1	0
Trichosporon montevideense	1	1
–	0	3
Total	22	22

– : The case isolate was not stocked.

to those of PCR (Table 2). Use of MALDI-TOF MS offers the advantage of shorter identification time compared to PCR[5]. In the present study, the results of MALDI-TOF MS were not related to the selection of antifungal drug because MALDI-TOF MS was performed using the remaining strains after all treatments were completed. Further studies are needed to determine whether the introduction of MALDI-TOF MS changes the prognosis.

Whether appropriate treatment was given in each patient in the death and survival groups was assessed (Table 3). In the death group, *C. parapsilosis* was the most common strain identified (4/11). Although *C. parapsilosis* has been reported to be resistant to MCFG, no highly resistant strain was observed[6]. In other *C. parapsilosis* cases, FLCZ and L-AMB were also used, suggesting that the treatment for *C. parapsilosis* was appropriate. For other species (such as *C. glabrata* and *C. krusei*), standard treatment was performed for the identified strain. In the survival group, *C. parapsilosis* was the most common species in 4 of 11 cases. Two cases were treated with MCFG, but no highly resistant strain was observed. The remaining *C. parapsilosis* cases were treated appropriately.

In terms of pathogen, *C. parapsilosis* was identified the most often and MCFG used the most frequently in both groups. The efficacy of MCFG against highly resistant strains of *C. parapsilosis* is not clear, however, and it may be necessary to be cautious in its use, considering that the fungus determined to be unidentified by CHROMagar Candida and VITEK 2 automated system was *C. parapsilosis*[6].

Four patients in the survival group received antifungal

Table 3 Initial treatments and outcomes.

Reported species	PCR result	Sensitivity result	Initial treatment	Appropriate treatment ?	Outcome
Candida sp.	*Candida africana*	No data	MCFG	YES	Death
Candida sp.	*Candida glabrata*	MCFG < 0.01	MCFG	YES	Death
Candida sp.	*Candida glabrata*	MCFG < 0.01	MCFG	YES	Death
Candida sp.	*Candida krusei*	No data	L-AMB	YES	Death
Candida sp.	*Candida krusei*	No data	MCFG	YES	Death
Candida sp.	*Candida parapsilosis*	MCFG =1	MCFG	YES	Death
Candida sp.	*Candida parapsilosis*	MCFG =1	MCFG	YES	Death
Candida sp.	*Candida parapsilosis*	No data	L-AMB	YES	Death
Candida sp.	*Candida parapsilosis*	FLCZ = 0.25	FLCZ	YES	Death
Candida sp.	*Candida tropicalis*	No data	MCFG	YES	Death
Candida sp.	*Cyberlindnera fabianii*	No data	FLCZ	YES	Death
Candida sp.	*Candida albicans*	No data	MCFG	YES	Survival
Yeast-like fungi	*Candida guilliermondii*	MCFG < 0.01	MCFG	YES	Survival
Yeast-like fungi	*Candida krusei*	MCFG = 0.12	MCFG	YES	Survival
Candida sp.	*Candida parapsilosis*	No data	FLCZ	YES	Survival
Candida sp.	*Candida parapsilosis*	MCFG = 1	MCFG	YES	Survival
Candida sp.	*Candida parapsilosis*	L-AMB = 0.5	L-AMB	YES	Survival
Candida sp.	*Candida parapsilosis*	MCFG = 1	MCFG	YES	Survival
Yeast-like fungi	*Cryptococcus neoformans*	MCFG > 16	MCFG	NO	Survival
Yeast-like fungi	*Lodderomyces elongisporus*	No data	MCFG	Unknown	Survival
Yeast-like fungi	*Trichosporon montevideense*	MCFG > 16	MCFG	NO	Survival
Yeast-like fungi	*Saccharomyces cerevisiae*	No data	MCFG	Unknown	Survival

L-AMB : liposomal amphotericin B ; MCFG : micafungin ; FLCZ : fluconazole.
Death : Death within 30 days.

Table 4 Differences between death group and survival group.

Variable		Death group		Survival group
Sex (*n*)	Male	6		10
	Female	5		1
Age (years)	Mean	71.6		65.2
	Median	74		70
Primary site of infection	CRBSI	7	CRBSI	7
	Peritonitis	2	Peritonitis	1
	Cholangitis	1	Urinary tract infection	1
	Fungemia	1	Ileus	1
			Artificial blood vessel infection	1
SOFA score	Mean	8		2.18
	Median	6		2
PCR result	*Candida parapsilosis*	4	*Candida parapsilosis*	4
	Candida glabrata	2	*Candida albicans*	1
	Candida krusei	2	*Candida guilliermondii*	1
	Candida africana	1	*Candida krusei*	1
	Candida tropicalis	1	*Cryptococcus neoformans*	1
	Cyberlindnera fabianii	1	*Lodderomyces elongisporus*	1
			Saccharomyces cerevisiae	1
			Trichosporon montevideense	1
Treatment	MCFG	7	MCFG	9
	L-AMB	2	L-AMB	1
	FLCZ	2	FLCZ	1

L-AMB : Liposomal amphotericin B ; MCFG : micafungin ; FLCZ : fluconazole.

drugs which were ineffective or potentially ineffective : MCFG for *C. neoformans* was considered invalid based on the literature[7] ; the efficacy of MCFG against *Lodderomyces elongisporus* was considered unknown as the CLSI has not set the breakpoint of MCFG for *Lodderomyces elongisporus*[8] ; MCFG for *Trichosporon montevideense* was considered invalid based on the literature[9] ; and the efficacy of MCFG against *Saccharomyces cerevisiae* is unknown[10]. The present evaluation suggests that appropriate treatment was performed in the death group, with all 4 cases in which treatment was considered ineffective being found in the survival group. Investigation into why these 4 patients survived despite ineffective treatment revealed that the infectious disease specialist had suggested catheter removal for CRBSI patients and proposed changing the antifungal drug depending on the patient's condition. The biggest difference between the death and survival groups was the SOFA score. Scores in the death group (mean SOFA score, 8) tended to be higher than those in the survival group (mean SOFA score, 2.1 ; p-value = 0.002), which suggest the death group performed worsen condition at the onset. Catheter removal was performed and antifungal drugs changed in the death group as much as possible. This suggests that the difference between patient death and survival was due to differences in their general condition.

In conclusion, identification of even common fungi may be difficult using conventional automated microbiology systems. When strains were judged as unidentified, MCFG was used in most cases. With molecular identification, however, *C. parapsilosis* was the most commonly observed pathogen. Unfortunately, this species may be resistant to MCFG, rendering its use invalid in such cases. When treating patients determined to have fungemia due to unidentified species according to the results of analysis using *Candida* medium and an automated identification system, physicians should pay attention to the condition of the individual patient and whether they are responding to initial treatment.

Conflict of interest

None to declare.

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一般的方法で同定出来なかった真菌菌血症の予後の検討

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【要旨】 本研究は東京医科大学病院において 2013 年 6 月 1 日から 2017 年 11 月 30 日までの期間の後方視的研究である。一般的な方法では同定できなかった真菌を PCR と MALDI-TOF MS を用いて同定し、適切な治療が行われていたのか及び患者の予後を調査した。当院では CHROMagar Candida、VITEK 2 automated system によって真菌の菌種同定が行われているが、126 症例のうち 22 症例で菌種を同定できなかった。これらの菌種を PCR、MALDI-TOF MS を用いて同定を行った。同定できなかった菌種の内訳では、*Candida parapsilosis* が全体の 36% と最も多かった。他の菌種では、*Cryptococcus neoformans*、*Cyberlindnera fabianii*、*Lodderomyces elongisporus*、*Saccharomyces cerevisiae*、*Trichosporon montevideense* など比較的稀な菌種も含まれていた。初期治療では Micafungin（MCFG）が同定できなかった症例の 73% に投与されていた。30 日以内に死亡した症例は 11 例（50%）あり、死亡群と生存群にわけて検討を行った。効果がない可能性があると考えられた真菌と抗真菌薬の組み合わせは 4 例あったが、それらは全て生存群の患者であり、死亡群の治療は適切に行われたと考えられた。効果がない可能性のある 4 例は 4 例とも治療に MCFG が用いられていたが、適切な介入と抗真菌薬の変更が提案されたため生存できたと考えられた。適切な治療が行われたにも関わらず死亡群の患者が死亡した原因は、SOFA スコアから診断時の全身状態が生存群に比べて悪かったためと考えられた。以上より一般的方法で同定できなかった真菌には MCFG が選択される傾向があるが、主治医は MCFG を選択した際に患者の全身状態を注意深く観察し、治療に反応しているかを確認する必要があると結論づけた。

〈キーワード〉 同定できなかった真菌菌血症、PCR、MALDI-TOF MS