Infectious Diseases

A Threat Emerging in Patients with Hematological Malignancy: Invasive *Magnusiomyces capitatus* and *Magnusiomyces clavatus* Infections

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ABSTRACT

Background: *Magnusiomyces capitatus* (*M. capitatus*) and *Magnusiomyces clavatus (M. clavatus)* are rare cause of fungemia leading to high mortality rates, particularly in neutropenic patients with hematological malignancies. This research set out to explore the clinical characteristics of patients with hematological malignancies with *M. capitatus* and *M. clavatus* fungemia.

Methods: Eight patients from whom *Magnusiomyces* spp. were isolated, from among patients hospitalized at the Atatürk University Hospital between October 2017 and November 2022, were enrolled in this retrospective observational study. The 8 patients' medical data were subjected to analysis.

Results: *Magnusiomyces capitatus* emerged as the pathogen in 5 cases and *M. clavatus* in 3. The patients' median age was 35.5 years. The most common underlying hematological malignancy was acute leukemia. Neutrophil values of 500 cells/mm^3 were detected in all patients during *Magnusiomyces* spp. isolation, with severe neutropenia at less than 100 cells/mm^3 in 5. The mean duration of neutropenia prior to *Magnusiomyces* spp. isolation was 29 days. Breakthrough fungemia developed in 7 patients using echinocandins, fluconazole, and posaconazole. Liposomal amphotericin B and voriconazole were used for initial treatment. The general mortality rate was 37%. All isolates were resistant to echinocandins. Voriconazole possessed the lowest minimum inhibitory concentration value against all isolates. The survival rate was higher among young patients. Mortality was higher among patients followed up in the intensive care unit.

Conclusion: Life-threatening *Magnusiomyces* spp. can spread among patients with long-term neutropenia under treatment for hematological malignancies. Awareness and prompt initiation of treatment can reduce the risk of mortality in invasive infections caused by *Magnusiomyces* spp.

Keywords: Hematological malignancy, *Magnusiomyces capitatus*, *Magnusiomyces clavatus*, neutropenia, opportunistic infection

Introduction

Invasive fungal infections pose a severe threat to patients with hematological malignancies, particularly in immunosuppressed individuals. Although *Candida* spp. and *Aspergillus* spp. are still the most commonly identified causes of invasive fungal infections in immunosuppressed patients, there is a growing increase in non-Candida yeast fungi, such as *Magnusiomyces* spp., as the spectrum of fungal pathogens changes. *Magnusiomyces capitatus* (*M. capitatus*) (or *Saprochaete capitata*, formerly known as *Blastoschiomyces capitatus* or *Geotrichum capitatum*) and *Magnusiomyces clavatus* (*M. clavatus*) (formerly known as *Saprochaete clavata* or *Geotrichum clavatum*) are arthroconidial yeast-like filamentous fungi, the taxonomy of which has been subject to several revisions in recent years.[1] *Magnusiomyces* spp. are rare opportunistic pathogens that cause severe invasive infections in patients with hematological malignancies, particularly those developing neutropenia following chemotherapy.[1-3] *Magnusiomyces* spp., which can be isolated from soil and water and can also be present in the normal flora, are non-fermentative and urease-negative fungi.[4-6] *Magnusiomyces* spp. infections are difficult to diagnose, generally relying on clinical suspicion and isolation from blood, tissue specimens, or sterile body fluids.[4] Due to the limited data concerning the antifungal drugs that should be used in the treatment of *Magnusiomyces* spp. infections, the durations of treatment, and their antifungal susceptibility patterns, treatment is difficult, and the optimal form is still unclear.[7] The current therapeutic recommendations rely

on expert opinions and data elicited from small case series.[7,8] Infections linked to *Magnusiomyces* spp. are therefore associated with high mortality.[2,9] The high mortality rate, which comes with the difficulty of diagnosis and treatment, can be attributed to the lack of awareness among clinicians and microbiologists about infections caused by this rare yeast. The present study focuses on the epidemiological characteristics and outcomes of 8 cases of infection caused by *M. capitatus* and *M. clavatus* detected in patients with hematological malignancies over a 5-year period. In addition, it aims to contribute to the limited data in the literature about this rare but potentially mortal yeast by reviewing the risk factors, diagnosis, treatment, and outcomes of the infection.

Materials and Methods

This retrospective and observational research was carried out in the 1400-bed Atatürk University Hospital, Türkiye, between October 2017 and November 2022. Eight patients followed up in the hematology and pediatric hematology departments and with *Magnusiomyces* spp. isolated in blood cultures were enrolled.

The study data were retrieved from the Atatürk University Hospital microbiology laboratory and automated computer database. A review of the microbiology laboratory records over the last 5 years identified a total of 8 patients with *Magnusiomyces* spp. isolation from blood and other samples despite all efforts. The 8 patients were visited daily when the microbiological culture results were issued. Epidemiological (age, sex, underlying disease, and risk factors), chemotherapeutic regimen, clinical manifestation, imaging, antimicrobial therapy, antifungal therapy, microbiological data, and clinical outcomes were collected and analyzed. Due to the limited case series and insufficient literature concerning this rare infection, the statistical power could not be calculated exactly.

Approval for the study was granted by the Atatürk University Medical Faculty clinical research ethical committee (Decision no: B.30.2.ATA.0.01.00/276; Date: March 3, 2023). Informed consent was obtained from patients.

Microbiological Examination

Blood specimens were collected concomitantly from both arms at the patient's bedside. These were then inoculated in line with the relevant standards into 2 blood culture sets containing aerobic and non-aerobic bottles (bioMerieux, Marcy l'Etoile, France) in line with the manufacturer's instructions and sent to the microbiology laboratory. A BacT/Alert (bioMerieux, Marcy l'Etoile, France) automated blood culture system was employed for the incubation of the blood culture bottles. The cultures were first incubated in the automated system for a maximum of 7 days. Blood culture bottles exhibiting signs of positive growth were inoculated on sheep blood agar, chocolate agar, and eosin methylene blue agar and evaluated for growth following incubation. Gram staining was also performed on bottles yielding signs of positive growth for direct microscopic examination. Since yeast cells were observed in the gram-stained preparations, these were inoculated on sabouraud dextrose agar (SDA) (Oxoid, UK) and on chromogenic agar (HiCrome, India). Soft, rough, and wrinkled cream-colored colonies were detected following an incubation period of 24-48 h (Figure 1). A VITEK® 2 (bioMerieux, Marcy l'Étoile, France) automated identification and susceptibility system was employed to identify these microorganisms isolated from the blood cultures. *M. capitatus* or *M. clavatus* were identified as the agents on the VITEK® 2 device.

The microdilution method was applied to determine antifungal susceptibility to amphotericin B, micafungin, anidulafungin, caspofungin, posaconazole, voriconazole, fluconazole, itraconazole, and flucytosine with the Sensititre YeastOne Y10 panel (Thermo Fisher Inc.). The study was carried out according to the procedure recommended by the manufacturer included in the kit. Antifungal sensitivity test results were given by evaluating color changes visually. Evaluations were carried out on the 24th and 48th hours of incubation. If the color of the positive control well of the microplate changed from blue to pink at the end of the incubation, the microplate was evaluated. The first well in which there was no growth or partial growth was observed was considered the minimum inhibitory concentration (MIC).

Serum galactomannan levels were determined using the Dynamiker Aspergillus Galactomannan Assay (Tianjin, China) in line with the manufacturer's instructions. A test serum index <0.5 was considered negative for galactomannan, while values ≥0.5 were regarded as positive.

Results

M. capitatus was identified as the pathogen in 5 patients (62%) hospitalized in the hematology clinic over the 5-year study period and *M. clavatus* in 3 (38%). The patients' median age was 35.5 years (9-50), and 4 were men (50%). The most common underlying hematological malignancy was acute leukemia, with acute myeloid leukemia (AML) being observed in 4 patients (50%) and acute lymphoblastic leukemia (ALL) in 4 (50%). *M. capitatus* and *M. clavatus* infections were diagnosed in all patients by means of blood culture (Table 1). All patients had received chemotherapy containing cytarabine prior to the development of fungemia. All patients had been administered granulocyte colony-stimulating factor. Broad-spectrum antibiotics with known efficacy against both gram-positive and gram-negative bacteria had also been given. Fever was present in all patients before the

Main Points

- Magnusiomyces spp. are a rare cause of fungemia leading to high mortality rates in patients with hematological malignancies, particularly those developing neutropenia following chemotherapy.
- The survival rate was significantly higher among younger patients.
- A significantly higher mortality rate was thus determined among the patients admitted to the intensive care unit.

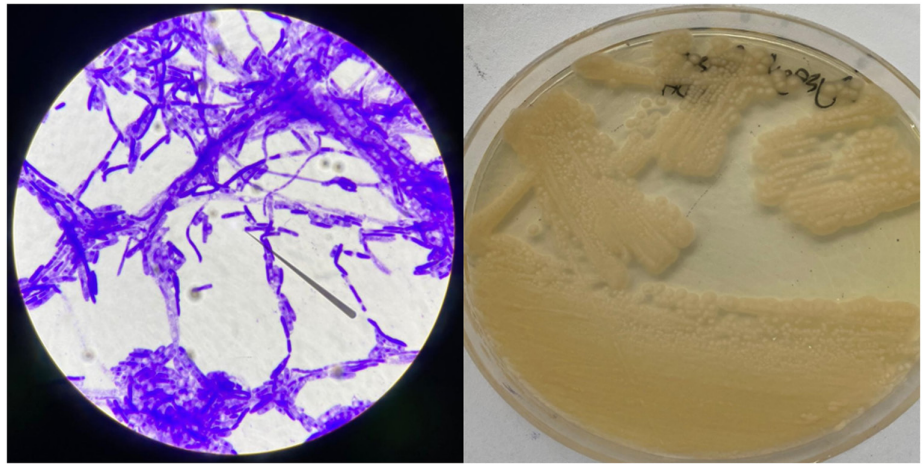

Figure 1. Microscopic Image of *Magnusiomyces* species (100x magnification). Appearance of yeast cells at gram staining and the SDA.

Table 1. The Epidemiological, Microbiological, and Clinical Characteristics of the Cases with Magnusiomyces spp. Fungemia

Patient	Sex-Age	Underlying Hematological Malignancy	Clinical Symptoms	Number of Positive Blood Cultures	Infection Sites
Magnusiomyces capitatus (n = 5)					
1	Female-19	B-ALL	Fever, cough, shortness of breath, abdominal pain, diarrhea	3	Blood, lung, liver, spleen, kidney, pancreas
2	Female-43	Recurrence AML	Fever	7	Blood
3	Male-34	T-ALL	Fever, abdominal pain,	2	Blood, liver, spleen
4	Female-37	Recurrence AML	Fever, shortness of breath, abdominal pain	1	Blood, spleen
5	Male-50	AML	Fever	2	Blood, lung, spleen
Total	2 Male, 3 Female	2 ALL, 3 AML			
Magnusiomyces clavatus (n = 3)					
6	Male-24	B-ALL	Fever	3	Blood, liver
7	Female-42	B-ALL	Fever, shortness of breat	6	Blood, lung
8	Male-9	AML	Fever, shortness of breat	1	Blood, lung
Total	2 Male, 1 Female	2 ALL, 1 AML			

ALL: Acute lymphoblastic leukemia; AML, Acute myeloid leukemia.

diagnosis of fungemia. Respiratory symptoms were observed in 2 (40%, 2/5) of the patients with *M. capitatus* infection and in 2 (66%, 2/3) of those with *M. clavatus* infection (such as cough, dyspnea, and chest pain). Gastrointestinal symptoms (such as abdominal pain and diarrhea) were present in 3 (60%, 3/5) of the patients with *M. capitatus* infection. Visceral spread, including the lungs, spleen, kidneys, liver, and pancreas documented via radiological examinations, are shown in Figure 2. The epidemiological, microbiological, and clinical characteristics of the patients with *Magnusiomyces* spp. infection are shown in Table 1.

At the time of detection of fungemia, neutrophil counts were below 500 cells/mm^3 in all patients, and lower than 100 cells/mm^3 in 5 (62%). The mean duration of neutropenia prior to *Magnusiomyces* spp. isolation was 29 days for *M. capitatus* and 31 days for *M. clavatus*. Seventy-five percent of the serum galactomannan antibody results were negative. *Magnusiomyces* spp. infection developed during antifungal prophylaxis in 7 patients (87%). The majority of patients were using echinocandin or azole prophylaxis before *Magnusiomyces* spp. isolation (Table 2). *Magnusiomyces* spp. were isolated during voriconazole therapy in 5 patients (62%). The majority of patients (87%) received targeted treatment with liposomal amphotericin B (LAMB 5 mg/kg/day) and voriconazole (2 × 6 mg/kg loading, 2 × 4 mg/kg maintenance). A combination of LAMB and posaconazole (2 × 300 mg loading, 1 × 300 mg maintenance) was used in 1 patient (13%) due to the development voriconazole allergy. Voriconazole continued to be administered for 6 weeks, while LAMB was discontinued after 3 weeks in patients receiving a LAMB and voriconazole combination. However, mortality occurred in 3 patients (37%) a mean of 8 days following isolation of *Magnusiomyces* spp. The general mortality rate was 37%, with rates of 40% (2/5) in patients with *M. capitatus* infection and 33% (2/3) in those with *M. clavatus* infection. The cases' laboratory and clinical characteristics are summarized in Table 2.

Susceptibility testing of 4 *Magnusiomyces* spp. isolates yielded MIC values for all analyzed strains. According to the study findings, voriconazole (MIC range 0.12-0.25 µg/mL) exhibited the lowest MIC values against all *Magnusiomyces* strains. Echinocandins exhibited high MIC values against *M. capitatus* and *M. clavatus*. The *M. capitatus* isolate from an exitus patient exhibited a high MIC value against amphotericin B (32 µg/mL), while the MIC values in the others were low. The antifungal susceptibility results of the *Magnusiomyces* spp. isolates are shown in Table 3.

In this study, the mean age of patients who survived was 24 years (37-9), while the mean age of patients who developed mortality was 43 years (50-49). The survival rate was higher among young patients. All 3 patients who died in this study were followed up in the intensive care unit. (ICU), while none of the 5 survivors were followed up in the ICU. Mortality was higher among patients followed up in the ICU.

Discussion

Magnusiomyces spp., one of the opportunistic fungal infections in patients with hematological malignancies, results in mild infections such as onychomycosis in immunocompetent patients but can lead to severe systemic infections characterized by mortality rates as high as 60% in patients with severe neutropenia.[3,7] Survival in the present study was greater in young patients with *Magnusiomyces* spp. infection, while the mortality rate was higher in patients followed up in the ICU.

The presence of hematological malignancy, prolonged and severe neutropenia, admission to intensive care, wide-spectrum antibiotic use, cytotoxic chemotherapies, central venous catheter use, and impairment of skin/mucosal integrity are all factors predisposing individuals to invasive *Magnusiomyces* spp. infection.[2,3,7,10] The

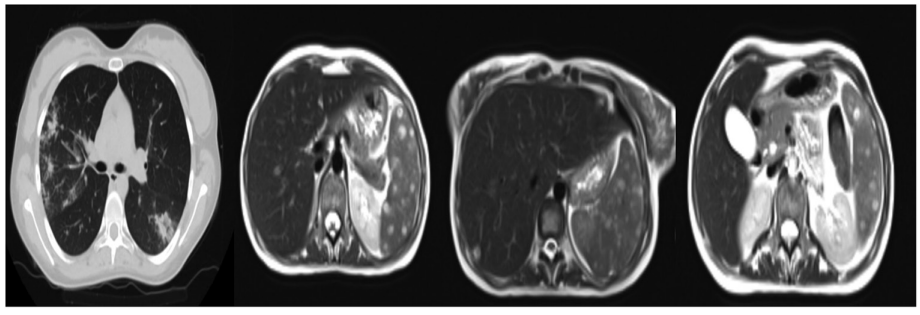

Figure 2. Multiple areas of consolidation and nodular lesions caused by *M. capitatus* in the lung, liver, spleen, kidney, and pancreas at pulmonary tomography and abdominal magnetic resonance imaging.

Table 2. The Laboratory and Clinical Characteristics of the Eight Cases with *Magnusiomyces* spp. Infection

Patient	Neutropenia	Duration of Neutropenia Prior to *Magnusiomyces* Isolation (days)	Duration of Neutropenia (days)	Time Elapsed Between Presentation and First Positive Culture (days)	Galactomannan (serum) (mcg/L)	Antifungal Prophylaxis	Empiric Antifungal Therapy	Targeted Antifungal Therapy	Outcome
Magnusiomyces capitatus (n = 5)									
1	Yes	9	12	44	Negative	None	VRC	VRC + LAMB	Cure
2	Yes	10	14	65	Positive (1,68)	FLC	MFG	VRC + LAMB	Mortal
3	Yes	33	38	33	Negative	MFG	MFG	VRC + LAMB	Cure
4	Yes	73	78	37	Negative	POS	LAMB	POS + LAMB	Cure
5	Yes	11	30	48	Negative	POS	MFG	VRC + LAMB	Mortal
Total	5 Yes				4 Negative, 1 Positive	1 FLC, 2 POS, 1 MFG	1 LAMB, 3 MFG, 1 VRC	5 LAMB, 4 VRC, 1 POS	3 Cure, 2 Mortal
Magnusiomyces clavatus (n = 3)									
6	Yes	22	22	39	Negative	CAS	VRC	VRC + LAMB	Cure
7	Yes	41	45	55	Positive (1,36)	MFG	VRC	VRC + LAMB	Mortal
8	Yes	30	67	66	Negative	MFG	VRC	VRC + LAMB	Cure
Total	3 Yes				2 Negative, 1 Positive	1 CAS, 2 MFG	3 VRC	3 LAMB, 3 VRC	2 Cure, 1 Mortal

CAS, caspofungin; FLC, fluconazole; LAMB, liposomal amphotericin B; MFG, micafungin; POS, posaconazole; VRC, voriconazole.

majority of patients with invasive *Magnusiomyces* spp. infection exhibit impaired immunity due to fungemia as a result of profound neutropenia developing secondary to chemotherapy in the event of hematological malignancy. El Zein et al[4] reported the possibility of a close association between cytarabine use and acute leukemia. Studies have shown the spread of a new and more resistant yeast-like pathogen due to the use of caspofungin in patients with prolonged neutropenia.[10] All the cases consisted of patients with neutropenic AML and ALL who received cytarabine and intensive chemotherapy. In addition, all the patients had used wide-spectrum antibiotics due to neutropenic fever, although the fever persisted despite wide-spectrum antibiotics and antifungal therapy. The presence of hematological malignancy, neutropenia, wide-spectrum antibiotic use, and cytotoxic therapies increased the risk of *Magnusiomyces* spp. infection in the cases and exhibited similar characteristics to the data from previous studies. It was thought that prolonged and profound neutropenia might be the principal risk factor, but no statistically significant association was observed. This may be due to the low case numbers, one of the limitations of this study. This study now needs to be expanded with more comprehensive patient groups.

Studies have shown that the yeast in question was isolated from blood culture in 75% of cases.[3] Similarly to previous research, the agent was isolated from blood cultures from all patients in the present study. As shown in previous studies, *Magnusiomyces* spp. infections frequently affect the lung, liver, and spleen, and more rarely the kidney, bone marrow, brain, and heart. The clinical manifestation mimics invasive candidiasis, and fungemia is widespread, although *Magnusiomyces* spp. affect the lungs and deep organs more commonly than *Candida* spp.[4] However, bloodstream infections are the most common clinical form.[9,11,12] Fever is the most common clinical finding, as in the present cases.[9] Respiratory and gastrointestinal complaints represent the second and third most frequent symptoms, respectively, in both fungi. Similarly to El Zeid et al[4], involvement of the liver, spleen, and lung were most common in the patients with *M. capitatus* fungemia and involvement of the liver and spleen in *M. clavatus*.

A number of case reports have described *M. capitatus* and *M. clavatus* infections during antifungal prophylaxis. The majority of *Magnusiomyces* spp. are inherently resistant or exhibit reduced susceptibility to various antifungals compared to *Candida* spp..[4,13-15] However, dysbiosis caused by antibiotic or antifungal prophylaxis has been

Table 3. The Antifungal Susceptibility Results of the *Magnusiomyces* spp. Isolates

Antifungal Agent	Species	Total	MIC as µg/mL									
			0.06	0.12	0.25	0.5	1	2	4	8	16	32
AMB	*Magnusiomyces capitatus*	3	0	0	0	1	0	1	0	0	0	1
	Magnusiomyces clavatus	1	0	0	0	0	0	1	0	0	0	0
FC	*Magnusiomyces capitatus*	3	0	0	0	0	0	0	0	1	1	0
	Magnusiomyces clavatus	1	0	0	0	1	0	0	0	0	0	0
FLC	*Magnusiomyces capitatus*	3	1	0	0	0	0	0	0	1	1	0
	Magnusiomyces clavatus	1	0	0	0	0	0	0	0	1	0	0
ITC	*Magnusiomyces capitatus*	3	0	0	1	0	0	0	0	0	0	0
	Magnusiomyces clavatus	1	0	0	0	1	0	0	0	0	0	0
VRC	*Magnusiomyces capitatus*	3	0	1	1	0	0	0	0	0	0	0
	Magnusiomyces clavatus	1	0	0	1	0	0	0	0	0	0	0
POS	*Magnusiomyces capitatus*	3	0	0	0	0	2	0	0	0	0	0
	Magnusiomyces clavatus	1	0	0	0	0	1	0	0	0	0	0
CAS	*Magnusiomyces capitatus*	3	0	0	0	0	0	0	0	2	0	1
	Magnusiomyces clavatus	1	0	0	0	0	0	0	0	1	0	0
MFG	*Magnusiomyces capitatus*	3	0	0	0	0	0	0	0	1	0	0
	Magnusiomyces clavatus	1	0	0	0	0	0	0	0	1	0	0
AFG	*Magnusiomyces capitatus*	3	0	0	0	0	0	0	1	0	0	1
	Magnusiomyces clavatus	1	0	0	0	0	0	0	1	0	0	0

AFG, anidulafungin; AMB, amphotericin B; CAS, caspofungin; FC, flucytosine; FLC, fluconazole; ITC, itraconazole; MFG, micafungin; MIC, minimum inhibitory concentration; POS, posaconazole; VRC, voriconazole.

reported to be capable of facilitating the entry into the lungs or intestine of *M. capitatus* and *M. clavatus*.[16] *Magnusiomyces* spp. infection developed in 87% of the cases under antifungal prophylaxis and in all those under empiric antifungal therapy.

Due to the scarcity of data concerning the experience of treating *Magnusiomyces* spp. infections in patients with hematological malignancies and their susceptibility patterns, insufficient clinical information is available regarding optimal treatment. Although experience is limited, the guidelines for invasive infections recommend amphotericin B and voriconazole as promising agents.[3,7,17] Echinocandins are not recommended for treatment since *Magnusiomyces* spp. are regarded as intrinsically resistant to them, and it has been reported that *Magnusiomyces* infection can develop in patients receiving caspofungin therapy.[7,12,18] Koç et al[19] isolated *M. capitatus* from 20 patients and observed caspofungin resistance in all isolates. Another recommended therapeutic regimen is the combined use of amphotericin B and voriconazole, shown to exhibit good in vitro activity.[7,8] Girmenia et al[2] described in vitro voriconazole as 100% effective. Disseminated infection under empiric micafungin therapy developed in 3 of the cases. This indicates that echinocandins are agents that should not be selected in the treatment of *Magnusiomyces* spp. infections. It should also be borne in mind that *Magnusiomyces* spp. may be the agent in neutropenic patients under echinocandin therapy. LAMB was the most frequently used antifungal agent in all poor cases as an option in the antifungal combination. Following 21-day combination treatment with LAMB and either voriconazole or posaconazole, treatment continued with oral voriconazole in 7 patients as a sequential antifungal option and with oral posaconazole therapy in 1. The MIC values for amphotericin B and voriconazole in the cases' *M. capitatus* isolates were 0.5 µg/mL and 0.12 µg/mL, respectively. In the second case, amphotericin B was measured at >32 µg/mL, while in the third case the values were 2 µg/mL for amphotericin B and 0.25 µg/mL for voriconazole. Although the blood culture results for the first and third cases in which the combined use of these agents was maintained were negative in terms of *M. capitatus, M. capitatus* continued to be isolated in blood cultures taken from the second case on the seventh day of treatment, and the patient was lost on the eighth day of treatment. The MIC values for amphotericin B and voriconazole for the *M. clavatus* strain isolated in one of the cases were 2 µg/mL and 0.25 µg/mL, respectively. *M. clavatus* clearance was achieved with the combined use of these agents.

Prognosis is poor in the majority of patients despite the receipt of adequate antifungal therapy, with rapid progression to multiple organ failure, shock, and, according to previous studies, mortality rates of 60%-80%.[3,4,9] Thirty-day mortality rates in previous case series were between 60% and 100%, although a 39% mortality rate was reported in a 6-year surveillance study involving 47 patients published in 2015.[2,10] Thirty-seven percent of the patients with hematological malignancy and *Magnusiomyces* spp. infection in the present study died within a mean of 8 days following the detection of the infection. A study of fungemia, including *Magnusiomyces spp.,* reported a significant association between septic shock and mortality, while central venous catheter removal exhibited a protective effect on survival.[20] Another study focusing on breakthrough fungemia in patients with hematological malignancies described age ≥60, septic shock, and steroid use as associated with mortality.[21] However, these studies were not focused on *Magnusiomyces* spp., with these representing only 1 component. In the present study, which did focus on *Magnusiomyces* spp., survival was higher among younger patients, while the mortality rate was higher among patients followed-up in the ICU. The older age of the deceased patients in this study and the development of septic shock or fungal sepsis

in ICU follow-up may have played a role in mortality.

There are a number of limitations to this study. In particular, a limited number of cases from a single center were included in this retrospective study. It is believed that the study can be extended by including more comprehensive sample groups. Since it is a rare microorganism, larger multicenter studies are needed.

In conclusion, diagnosis and treatment should be reviewed in neutropenic patients with hematological malignancies with resistant fever that fails to improve during wide-spectrum antibiotic, echinocandin, and/or fluconazole use, bearing in mind that *M. capitatus* and *M. clavatus* may be potential agents of infection with a high risk of mortality. The incidence of these infections may increase even further as new chemotherapeutic and immunosuppressive therapies are developed. Prophylactic and empiric antifungal protocols should therefore be regularly reviewed in order to reflect the evolving local epidemiology of invasive fungal infections.

Further research is needed to elucidate the epidemiology and risk factors of nosocomial invasive fungal diseases. The most effective treatment for Magnusiomyces infections has not been established, and further studies on new antifungal agents and/or combinations are needed. The results of these future studies may increase the awareness of clinicians and microbiologists, preventing mortality with early diagnosis and treatment.

Data Availability Statement: The data that support the findings of this study are available upon request from the corresponding author.

Ethics Committee Approval: Ethics approval for this study was obtained from Atatürk University Faculty of Medicine Scientific Research Ethics Committee (Decision no: B.30.2.ATA.0.01.00/276, Date: March 30, 2023).

Informed Consent: Written informed consent was obtained from all patients who participated in this study.

Peer-review: Externally peer-reviewed.

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