REVIEW

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# Invasive Mould Infections in Intensive Care Units: Epidemiology, Microbiologic Diagnosis and Antifungal Resistance

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## ABSTRACT

**Invasive fungal infections present with a rising incidence and high morbidity and mortality rates. Among these infections, there are those due to moulds which draw particular attention due to the diagnostic difficulties and high mortality rates. While a relative decrease is observed in mortality rates regarding the use of prophylactic, empirical or preemptive antifungal regimens, there is a tendency of increase in antifungal resistance rates. Furthermore, due to an increase in the number of cases with immunosuppression, infections caused by rare and emerging moulds are now more frequently observed. Breakthrough infections, nosocomial infections, and infections due to risk factors other than immunosuppression are also a clinical concern.**

**The diagnosis of invasive mould infections requires a multidisciplinary approach with clinical, radiological, histopathological and microbiological data. However, nonspecificity of clinical signs and radiological findings and difficulties in the differentiation of infection and colonization are major problems in patients with invasive mould infections in intensive care units. Limited availability of routine microbiology laboratories with adequate facilities in mycological diagnostics and problems in specificity and sensitivity of diagnostic tests for intensive care unit patients result in further difficulties in the diagnosis of these infections.**

**In this review article, epidemiological data and microbiological diagnostic methods for invasive mould infections in intensive care units were reviewed per the published reports and the recommendations of current guidelines. Finally, antifungal resistance and clinical impact of resistance were discussed.**

**Keywords:** amphotericin B, azoles, echinocandins, aspergillosis, mucormycosis, invasive mould infection, invasive fungal infection, intensive care unit

## Introduction

Nowadays, invasive fungal infections (IFI) have begun to be observed in different risk groups at an increasing incidence and present with high mortality rates. Although these infections are mostly observed in patients without normal immune function for different reasons, epidemiological changes are also experienced in terms of the risky patient group. One of the special host groups that have gained importance in this concept is patients receiving treatment in intensive care units (ICU). Nevertheless, the intensive clinical use of both old antifungals (e.g., amphotericin B) and newly developed antifungals (e.g., equinocandins) leads to a relative decrease in the isolation rates of common IFI agents and also an increase in rare agents and the reporting of infections caused by fungi that have not been previously reported as infectious agents. On the other hand, the intensive use of environmental (such as pesticide) and clinical antifungals has also made the problem of strains resistant to antifungal drugs a current issue (1-4). In this review article, the current status of invasive mould infections (IMI) observed in patients treated in ICUs was reviewed especially in terms of epidemiology, diagnosis and antifungal resistance.

### Epidemiology: General Information on Risk Factors and Agents

The risk factors that play a role in the development of IFIs are usually a hemato-oncological malignancy, bone marrow or organ transplantation, the presence in ICUs, severe

clinical presentation, uncontrolled diabetes mellitus, previous major surgical intervention or HIV infection. This underlying pathology also directly affects the epidemiology of IFI agents (2,3,5). While especially C*andida* species of yeasts lead to IFI, *Aspergillus* species of moulds most frequently come to the forefront. In nearly 90% of IMIs, *Aspergillus* species stand out as an agent (Invasive Aspergillosis; IA), followed by the members of the Mucorales order (Mucormycosis) and *Fusarium* and *Scedosporium* species (2). *Paecilomyces* and *Phialemonium* species and brown (dematiaceous) fungi can be isolated as IMI agents, although it is very rare (3,5). The incidence of IA reported from various European countries varies between 0.4-23% depending on the characteristics of the patient community, and the geography (2). The infections caused by *A. fumigatus* complex take the lead among all IMIs. Secondly in this species, *A. flavus* complex stands out in areas dominated by the tropical climate. *A. terreus* is also one of the agents reported from Europe. Although mucormycosis cases generally follow aspergillosis cases, fusariosis has been reported at high rates from South American countries such as Brazil (6). The members of the Mucorales order are reported at increasing rates in IMI cases due to rare hyalohyphomycosis agents (*Fusarium* spp., *Scedosporium* spp.) and phaeohyphomycosis agents (*Bipolaris* spp., *Exophiala* spp., *Wangiella* spp.) (3). IFI develops in 17-25% of patients in ICUs (7,8). More than 10% of these infections are IMI (9). While the incidence of IA in ICUs is 0.3-5.8%, mortality is above 80% (10). Although it is observed at higher rates in hematological patients, IA develops in 4% of ICU patients who are not in this group (11). Epidemiological data specific to ICUs for mucormycosis and other agents are very limited. The data from our country are very insufficient, especially for ICUs. In an article recently published in our country, an agent could be isolated in only nearly 60% of infection, sepsis, and septic shock patients in ICUs, only *Aspergillus* spp. was reported from moulds, which was reported to be only 0.3% of microorganisms (12). Attention should be paid to the low rate of agents that can be isolated, the difficulty in diagnosing IMIs, and the lack of specificity of clinical presentations. IFI agents probably cause more infections than current infections in epidemiological data, however, patients are lost before the agent can be isolated and diagnosis is made.

An increase has also been observed in the incidence of IFI in the neonatal group, especially with the progress of neonatal care and with higher survival of the community of patients who are premature and/or have a problematic immune system. Nevertheless, mould infections are rarely observed in premature newborns. Epidemiologically, although there are limited data, the distribution of infection-causing moulds is similar to that of adults. Infections originating from *Aspergillus* and Mucorales order are mostly observed. They are usually cutaneous infections due to risk factors such as skin trauma and the presence of a catheter, however, they also have a risk of dissemination (13). In neonatal units, wooden tongue depressors and contaminated closure covers were reported as the sources of Mucorales (14).

The IMI agent and the incidence of the agent vary according to the primary pathology of the patient. When IMI cases caused by *Aspergillus* species are examined, it is remarkable that the underlying factors in most of the cases are chronic obstructive pulmonary disease (COPD), asthma, solid organ transplantation, and solid organ tumors, especially hematologic malignancies and hematopoietic stem cell transplantation. In these cases, the risk factors for *Aspergillus*-induced IFI were identified as neutropenia, broad-spectrum and long-term antibiotic therapy, the use of corticosteroids and other drugs effective on the immune system, graft-versus-host disease (GVHD) and cystic fibrosis (CF) (*Aspergillus* colonization). The presence of cytomegalovirus (CMV), influenza, *Pneumocystis jirovecii* infection and decompensated cirrhosis in the patient is also considered to be among the risk factors (even a prognostic factor). In cases of mucormycosis, uncontrolled diabetes mellitus, deferoxamine treatment, deterioration of skin integrity (burns) and severe traumas, surgical wounds and voriconazole prophylaxis are added to the same risk factors. For other IMI agents, risk factors are also similar to those mentioned (2,3,6).

## Infections Developing Under Treatment

Depending on the use of antifungal drugs and their spectrum of action, breakthrough infections have also been added to IFIs. While the options of using prophylaxis, empirical and preemptive antifungals in cases with IMI risk are recommended by international guidelines, there can be reduction especially in IA cases and generally positive effects on IA prognosis. On the other hand, breakthrough invasive infections caused by moulds, especially mucormycosis, in which the effectiveness of the administered antifungal is relatively low, have begun to be observed. As it is understood, factors that cause these infections also vary according to the antifungal drug administered, the factors of the patient, the control of the underlying disease and the local epidemiology. In terms of antifungal drugs, the spectrum of action and accordingly the presence of secondary resistance acquired for fungal species or infectious agent strains in which primary resistance is observed play a determining role in breakthrough infectious agents.

In terms of primary resistance, mucormycosis developing under voriconazole treatment is the most typical example, as it has been previously mentioned (3,15-18).

## Hospital-Acquired Outbreaks

Hospital-acquired outbreaks of fungal infection can also be observed in patients in the risk group and in ICUs. In IFI cases originating from hospital/patient-care caused by various species of moulds, the sources are usually hospital ventilation, renovations and constructions carried out in hospitals, contaminated medical solutions or medical instruments and devices (1). Such infections seem to be associated with the socioeconomic conditions of countries, which are more frequently reported from low and middle-income countries. This is probably due to inadequate infection control measures (19). Therefore, it is essential to keep these patient groups under the control of hospital infection control committees, to eliminate the condition which would pose a risk of mould infection in the hospital environment, if available, or to protect patients from exposure if it cannot be eliminated (1).

## IMI Developing in Cases without Demonstrable Risk Factors

Although risk factors pave the way for the development of IMIs, the inability to observe the typical clinical presentation of the

disease in such cases causes physicians not to suspect IMI and makes diagnosis difficult. For example, in aspergillosis cases of neutropenic and non-neutropenic patients, the clinical presentation may be different and also the sensitivity and specificity of the diagnostic tests vary (3,6,20). On the other hand, retrospective autopsy studies show that there are considerable numbers of cases in which IMI was not/could not be diagnosed despite the tests performed but the agent could be understood as a result of the autopsy, even if the patient had a risk factor (21).

## IMI and Mortality

The mortality rates of mould infections are generally higher compared to yeasts. Although it is very difficult to distinguish mortality caused by the mould infection itself from other accompanying pathologies of the patient while calculating mortality rates, it was demonstrated in the studies that a significant decrease was achieved in mortality rates both by the prevention of mould infection with prophylactic and empirical antifungal applications and by early diagnosis and correct treatment for mould infection (3,5,6). In IA cases, mortality ranges from 38% to 100% although it varies according to primary pathology. It is above 60% in Mucorales infections while it is between 50-90% in other moulds (2,3,6). The immune status of the patient (such as the presence of neutropenia, bone marrow transplantation), the presence of hematologic malignancy, the presence of antifungal resistance and hospitalization in ICUs are the independent factors that mostly increase mortality (2,5,22).

## IMI especially in Intensive Care Units (ICUs)

In autopsy studies carried out, IFIs constitute an important group for undiagnosed infections. Here, the difficulties in distinguishing between colonization and infection, and non-specificity of radiological and clinical symptoms are the biggest problems.

Furthermore, the fact that especially some serological diagnostic tests reveal sensitivity and specificity problems for non-hematologic ICU patients leads to the questioning of the reliability of tests (20,23).

Although the patient is in the ICU, risk factors are similar to the general epidemiology of IMI according to the agent (3). However, IMI (especially IA) cases are increasingly reported in ICU patients although there is no defined immunosuppression condition. It is stated that ICU ventilation systems along with contaminated water and medical fluids may also have an effect on the development of infection. On the other hand, further exposure of ICU patients to invasive procedures such as catheter and mechanical ventilation, long-term and intensive use of antibiotics, corticosteroid treatments used in sepsis and similar cases, and organ failures are also thought to be effective (20,23). Here, it is necessary to focus especially on COPD, which is a risk factor for IMI by itself. In the world, the prevalence of COPD varies between 8% and 20%, and 25% of these patients are admitted to ICUs and require mechanical ventilation. Systemic and inhaled corticosteroids play a role in the treatment of all COPD patients whose admission to ICU is/is not considered necessary. In other words, a COPD patient has many risk factors simultaneously for IMI (24,25).

In intensive care units, invasive yeast infections are 7 times more common than mould infections. However, IMI cases lead to higher mortality (35-80%; above 60% in general). IA is the most common mould infection among ICU infections (0,3-6,9%) and its mortality is above 80% (9,23,26). Although there is an increase in cases of mucormycosis reported by years, there are no epidemiological data specific to ICU. Although the cases of mucormycosis are observed at the rates of 0.43-1.2/1.000.000, 37% of the cases of mucormycosis are diagnosed in ICUs (23).

## Diagnosis

The diagnosis of IMI is made by a multidisciplinary approach with the components of clinical, radiological, histopathological and microbiological findings. The main issue here is that the combined use of these diagnostic approaches is essential. In this review article, only microbiological diagnosis was discussed.

As a microbiological diagnostic method, the culture is important and is the gold standard in terms of the finalization of the diagnosis and especially performing an antifungal susceptibility test. However, the sensitivity of the culture is low and it is possible to come across problems such as contamination in the culture, and sometimes difficult/contraindicated sampling from these patients. The first procedure is direct microscopy while sampling. In IA cases, positivity can only be detected by 50% on average in the direct microscopic examination (20). It is understood that negative microscopy does not eliminate the infection. The use of a fluorescent dye (calcofluor-white, etc.) significantly increases sensitivity and also shows a high positive predictive value (if the patient is not IA, 89-100% negative fluorescence microscopy result is achieved) (27). On the other hand, even if microscopy is positive, it is not possible to determine species by microscopy (28). Therefore, the culture is essential. Culture positivity was detected in only 4 of sputum cultures and in bronchoalveolar lavage (BAL) cultures in pulmonary aspergillosis cases in transplant patients (29).

The distinction between colonization and infection is also another problem even if the culture is positive in ICU patients (20). Histopathological examination is useful in this case if it can be performed.

Although the conventional culture is diagnostic along with clinical data in the microbiological diagnosis of IMI cases, to wait for the result of the culture in these cases in whom rapid diagnosis and initiation of emergency treatment are required does not allow for early treatment. There is also a limited number of routine microbiology laboratories with experienced expert staff working at international standards in the field of mycology. 1,3-β-D-glucan (serum), galactomannan (BAL, serum), *Aspergillus* immunochromatographic test ("Lateral flow device"-LFD) (BAL) and molecular tests (PCR) (BAL) have been developed to contribute to the rapid diagnosis. The most important advantage of these tests over the culture is that they provide quick results, however, their sensitivity and specificity can be significantly affected by many factors such as the patient's comorbidities and drug use (3,6,20,29). Furthermore, the data on ICU patients are more limited since these tests have been developed especially for hemato-oncological patient groups (20). Another issue is that

these tests have been mostly developed for IA and that there are no specific markers for mould infections other than *Aspergillus* (6). Therefore, diagnosis is made by the evaluation of other parameters in non-*Aspergillus* IMI cases. The areas of usage of the tests, the recommendations and levels of evidence of guidelines, and important issues about the tests are presented in Table 1.

## Antifungal Resistance

Antifungal resistance in moulds is a frequently discussed issue nowadays. Antifungal resistance may be in the form of primary resistance, in other words, without encountering the antifungal drug and natural resistance and also in the form of resistance due to drug exposure of the secondary (gained, acquired), in other words, the infected strain. The active mould should be produced from a clinical sample and the antifungal susceptibility test should be performed for the determination of antifungal resistance. There are two reference methods that can be used for this: The Clinical & Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) reference methods. In these two methods, epidemiological cut-off values (ECOFF) were determined for different antifungals in

**Table 1.** Serological and Molecular Tests in the Diagnosis of IMI, and their Properties. (adapted from sources number 3,6,13,16,20,23,26,29, 38,39,41.)

| Test | Clinical Example | ESCMID-ECMM-ERS *Aspergillus* Guideline Recommendation and Level of Evidence | ESCMID-ECMM Mucormycosis Guideline Recommendation and Level of Evidence | ESCMID-ECMM Other Hyalohyphomycosis Agents Guideline Recommendation and Level of Evidence[b] | (if any) Notes on ICU | Notes and Exceptions |
|---|---|---|---|---|---|---|
| 1,3-β-D-glucan (Panfungal test; for *Cryptococcus* and non-Mucorales fungi) | Serum | C II | D III | B III | Sensitivity and specificity are low for ICU patients. | It also gives a positive result in fungi such as *Pneumocystis jirovecii* and *Candida* species. IV albumin or immunoglobulin administration, hemodialysis, wound dressings, bacterial infections, abdominal surgery, and cirrhosis are the reasons for false positivity that can increase β-glucan levels. It is valuable in excluding the diagnosis since its NPV is high. This test cannot be used in the diagnosis of mucormycosis. |
| Galactomannan (*Aspergillus* Antigen test) | Serum | A I (A II in patients with neutropenia, B II in ICU, D II in those with antifungal prophylaxis) | B III | B III | There is a sensitivity problem in patients in the ICU and without neutropenia | It is useful in the diagnosis of IA, repeated tests are recommended when it is applied as a screening test. It may also give a positive result in patients with another mycosis (*Penicillium*, *Fusarium*, *Paecilomyces*, *Saprochaete capitata*, *Histoplasma capsulatum*), intestinal mucositis or GVHD. Due to the use of β-lactam antibiotics and *Bifidobacterium* spp. found in the flora in neonatal cases, false positive results can be obtained. It is not recommended in those receiving antifungal prophylaxis with a mould effect. The suspicion of IMI in radiological findings associated with Galactomannan negativity should suggest mucormycosis. |
| | BAL | A II | B III | B III | The recommended type of sample for galactomannan analysis in ICU and non-neutropenic patients. | The suspicion of IMI in radiological findings associated with Galactomannan negativity should suggest mucormycosis. |
| "Lateral-Flow Device" LFD-Aspergillus | BAL | B II | - | - | - | It is useful in the diagnosis of IA and is recommended to be used together with galactomannan antigen. Sensitivity is 100% and specificity is 81% in patients with hematologic malignancies and solid organ transplantation. |
| PCR | BAL, CSF | B II | B II [a] | C III | - | It is useful in the diagnosis of IA. CSF PCR sensitivity is 100% and specificity is 93% in patients with hematologic malignancies. The combined use of PCR and galactomannan antigen test is recommended (Especially in hematologic patients A II). |
| | Blood, Serum, Tissue | B II | B II [a] | C III | - | It is useful in the diagnosis of IA. In particular, its specificity is high and between 96-100% in tissue samples. |

**BAL:** Bronchoalveolar lavage; **BOS:** Cerebrospinal Fluid; **NPV:** Negative Predictive Value; **PCR:** Polymerase Chain Reaction; **GVHD:** Graft-Versus-Host Disease.
[a]No commercial kit available. [b]*Fusarium*, *Scedosporium*, and other species
**Suggestion Levels:** A) Strong Suggestion; B) Moderate Suggestion; C) Low Suggestion; D) Not suggested
**Levels of Evidence:** I) At least one randomized controlled trial; II) At least one well-designed non-randomized trial; III) Opinions of known authorities, case studies, expert commission reports
According to the ESCMID-ECMM feohypomycosis guideline, there are no data on the tests given in the table in the diagnosis of invasive infections caused by feohypomycosis agents. Diagnosis is based on clinical data and the isolation of the agent from the clinical sample.

certain moulds as a result of extensive epidemiological studies. When a mould is tested for antifungal susceptibility, if a minimum inhibitory concentration (MIC) value below ECOFF values is determined, this strain is regarded as wild type and it is anticipated that the strain does not contain any resistance mechanism to that antifungal. The determination of a MIC value above ECOFF indicates an acquired resistance mechanism. However, since the ECOFF value does not contain clinical data, no comment can be made on the susceptibility of the relevant mould to the antifungal tested (in other words, the treatability of the patient with that drug). Clinical threshold values (CBP) were determined for this purpose. The EUCAST method includes CBPs determined for some *Aspergillus* species and antifungal drugs. MIC results on CBP can be reported as dose-dependent susceptible (SDD), moderately susceptible (I) or resistant (R) according to the microorganism and antifungal, however, those below are reported as susceptible (S). The CLSI and EUCAST documents do not contain ECOFF and/or CBP data against each antifungal for every mould. This is due to the fact that sufficient studies have not been carried out yet. Therefore, the laboratory can report susceptibility in those with CBP data, but it can only report wild type or not in those without it according to the ECOFF data.

Since a microorganism has the potential of drug resistance when a microorganism is not wild type, it is generally recommended that the drug is not preferred in primary treatment. In strains and antifungals without any CBP or ECOFF value, the laboratory only reports the MIC levels (30,31).

*Aspergillus*: Some species in *A.ustus* and *A.niger* complex of the genus *Aspergillus* show resistance or decreased sensitivity to azoles, *A.terreus*, *A.flavus* complex and *A.nidulans* show resistance or decreased sensitivity to amphotericin B, and *A.lentulus* (a member of *A.fumigatus* complex) shows resistance or decreased sensitivity to azoles and amphotericin B (16,22,32).

The long-term and frequent use of prophylaxis, empirical or therapeutic antifungals may affect the development of antifungal resistance. On the other hand, especially for *Aspergillus* species, antifungal resistance increases in clinical strains due to the intense use of azole group pesticides as pesticides (22,33). Studies on this subject have been mostly concentrated on *A. fumigatus* complex, and the resistance to azoles, which are the main drugs used against this agent (considering resistance to all azoles) ranges from 1% to 20% (34). However, the important issue here is the clinical impact of antifungal resistance, the observation of triazole resistance in IMI cases caused by *A. fumigatus* complex was found to be directly associated with mortality (in resistant cases; 88%-100) (33-35). Furthermore, for IA, azole-resistant Aspergillus strains are mostly observed in ICU patients and in patients with high risk factors (33). On the other hand, there are also studies that cannot establish a relationship between resistance and mortality. This indicates the effects of other factors in the patient as well as antifungal resistance on mortality (such as ongoing neutropenia, comorbidities) (32). Here, it is necessary to remind the result "91% clinical success is achieved if the fungal agent is susceptible to the antifungal drug while 48% clinical success is achieved if it is resistant" determined in a very comprehensive meta-analysis study (36). Especially with respect to ICUs, the resistance-mortality relationship is not significant and the issue needs to be studied more comprehensively (35).

Nowadays, only the determination of mould species is no longer sufficient for effective treatment. Species-level identification and screening for antifungal resistance when needed (e.g., an agar screening test for azole resistance in *Aspergillus fumigatus* strains) are the recommended techniques in routine microbiology nowadays (37). Antifungal susceptibility profiles may vary considerably even within the same species complex due to cryptic species found in a species complex that cannot be separated morphologically (16). Moreover, the prevalence data about cryptic species are very limited and high MIC values against antifungals can be directly associated with treatment failure (22).

*Non-Aspergillus fungi:* The identification of strains such as *Fusarium* spp., *Scedosporium* spp. and *Lomentospora prolificans* (previously *Scedosporium prolificans*) may directly change the treatment protocols due to primary antifungal resistance data available for this genus/species (38). *Fusarium* species are generally resistant to azoles and amphotericin B or are not sufficiently susceptible while the Mucorales order is naturally resistant to voriconazole. On the other hand, *L. prolificans* is a very serious problem with its resistance to all drugs (22,38).

Since MIC values and resistance vary according to the active strain, when any IMI is suspected and/or a patient with risk factors is encountered, it is important to examine the current resistance epidemiology data and current treatment guidelines in diagnosis, prophylaxis, empirical approach, preemptive and treatment approaches (16, 38-40).

## Current Status and Results

Both diagnostic difficulties, and clinical awareness of IMI and the limited number of laboratories and experts competent in mycology restrict the isolation and identification of IMI agents.

As it can be envisaged from the aforementioned autopsy studies, the prevalence and incidence of IMI are expected to be higher than those determined by studies (12,14,27).

Studies have revealed a dramatic conclusion that there is a significant lack of information on the proper use of antifungal drugs and the pros and cons of diagnostic tests (6). The guidelines published in recent years have been guiding for the elimination of these situations (16,38-41).

Early diagnosis of the patient, prophylaxis, and the correct application of preemptive or agent-specific therapeutic antifungals are directly associated with antifungal resistance and patient mortality (3,22). In the studies published from ICUs from different regions, for example, significant incidence and mortality rates were reported for IA (6). IMI-induced mortality rates in ICUs are higher than in general. The main reason for this is that patients have many comorbidities and patients do not show the typical clinical presentation (23,29). This actually reveals the difficulty of diagnosis and treatment of IMIs. Therefore, the multidisciplinary approach of the Infectious Diseases and Clinical

Microbiology Specialist, the Medical Microbiology Laboratory, the Infection Control Committee together with the branch physician who undertakes primary treatment of the patient, and the close follow-up of patients using clinical, radiological and conventional microbiological tests and fungal biomarkers are of critical importance for patients in the risk group, even if the development of IMIs is not suspected.

Here, the determination of the risk factors of patients, early diagnosis of IMI and accordingly the initiation of early treatment, and the algorithmic realization of all of them led by guidelines are important for reducing mortality and morbidity.

Nowadays, studies are also going on for the development of scores that can be used in the clinic by complying with the current guidelines published by expert groups from international and various branches (e.g., EQUAL "Aspergillosis Score 2018" and "Mucormycosis Score") (42,43). Furthermore, studies on establishing diagnostic criteria specific to ICUs are also going on to overcome the difficulty of diagnosis as much as possible (FUNDICU project) (44).

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