ISSN: 2754-4516

Journal of Medicine and Healthcare

SCIENTIFIC Research and Community

Research Article

Open Access

Diagnosis, Prognostic Factors and Therapeutic Management of Invasive Fungal Infections. The Results of a Cross-Sectional Study in a Tunisian Hospital

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ABSTRACT

Introduction: Invasive fungal infection (IFI) is associated to a high morbidity, mortality and healthcare costs.

Patients and Methods: Our cross-sectional study focuses on patients who developed an IFI and treated with systemic antifungals, over a period of 14 months in a tunisian hospital. Clinico-biological data, risk factors, and therapeutic management were collected and analyzed.

Results and Discussion: We report 51 cases with IFI, aged between 15 and 85 years. 56.9% were hospitalized in intensive care units and nearly 80% already suffer from other pathologies. Based on the EORTC/MSGERC criteria and clinical, radiological and biological data, IFIs were classified as follows : 49.3% cases of "proven infection", 10.1% cases of "probable infection" and 17.4% cases of "possible infection". Two risk factors revealed to correlate with a poor prognosis: intubation or invasive ventilation and long-term corticosteroid therapy. *C.glabrata* was the most common isolated species, agent responsible for invasive candidiasis. Resistances for fluconazole were not noticed. The anti-fungals prescribed for management of the IFI were: caspofungin, anidulafungin, voriconazole, amphotericin B and fluconzaole (following de-escalation).

Conclusion: A close multidisciplinary collaboration between clinicians, radiologists, mycologists and pharmacists can improve the prognosis of these infections.

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Received: August 13, 2023; **Accepted:** August 18, 2023; **Published:** August 25, 2023

Keywords: Invasive Fungal Infection, Prognostic Factors, Candidiasis, Aspergillosis

Highlights

- Invasive fungal infection (IFI) is associated to a high morbidity, mortality and healthcare costs.
- Diagnosis of invasive candidiasis is based on biological diagnosis tools, whereas diagnosis of aspergillosis is based mainly on radio-clinical presumptions (fever, cough, etc.).
- Based on the EORTC/MSGERC criteria and clinical, radiological and biological data of each patient, IFIs were classified as "proven infection" in nearly 50% of cases.
- Two factors were identified as correlating with a poor prognosis for fungal infections: intubation or invasive ventilation and long-term corticosteroid therapy.
- The anti-fungals prescribed for management of the IFI were: caspofungin, anidulafungin, voriconazole, amphotericin B and fluconzaole (following de-escalation). Management of aspergillosis was based on the use of voriconazole in 53.8% of cases.

Declaration of Interest Statement

All the authors declare that they have no established conflicting financial interests or personal relationships that may have influenced the research presented in this paper.

Introduction

Invasive fungal infection (IFI) represents currently a serious problem in healthcare units, due to their high mortality. Therapeutic management is also associated with elevated costs [1]. In contrast to bacterial infections, the mycological diagnosis of an IFI remains difficult, despite technical progress and automation [2]. According to the consensus ot the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC), IFIs are classified into three sub-categories: "proven," "probable," or "possible," based on host factors, clinic, and mycologic evidence. The "proven" category applies to all patients regardless of immune status and requires documented evidence (microscopic examination, culture result from a sterile site, blood culture, or presence of DNA by PCR-sequencing). The "probable" category applies only to the

Citation: Ghedira Donia, Samaali Eya, Sayadi Mouna, Sameh Belgacem, Maha Mastouri, et al. (2023) Diagnosis, Prognostic Factors and Therapeutic Management of Invasive Fungal Infections. The Results of a Cross-Sectional Study in a Tunisian Hospital. Journal of Medicine and Healthcare. SRC/JMHC-290.
DOI: doi.org/10.47363/JMHC/2023(5)240

immunocompromised patients and requires an identified risk factor in the host with clinical symptoms and mycological evidence. « Possible » infection requires an identified risk factor in the host with the presence of clinical symptom(s). Several arguments for infection should therefore be sought rapidly in order to initiate treatment early and avoid aggravation [3].

On the other hand, multi-resistant strains (such as *Candida auris*) and strains with reduced sensitivity to fluconazole (such as *Candida glabrata*) are increasingly isolated [4]. Available anti-fungal treatments are limited, even with the recent introduction of echinocandins.

In Tunisia, the incidence of IFI has increased significantly in recent years. According to a Tunisian multicenter study conducted over five years (2011-2015), four invasive candidiasis occur per day. Another tunisian study reports that 80% of IFIs affect patients with one or more risk factors [5,6]. Identifing the risk factors, as well as factors correlating with a poor prognosis, is of great interest, as it allows to recognize the category of patients at risk of fungal infections.

Our study aims to describe the profile of patients who contracted IFI in a Tunisian university hospital. The clinico-biological diagnosis of the fungal infection, the risk factors as well as factors correlating with a poor prognosis, and finally the therapeutic management were described and analyzed.

Patients and Methods

We report herein a descriptive and cross-sectional study, conducted in the pharmacy department of a tunisian hospital (Fattouma Bourguiba of Monastir), over a period of 14 months (January 1, 2019-February 28, 2020). All patients who contracted IFI and received a systemic antifungal drug (except fluconazole). Patients treated only with fluconazole, those who had already started treatment before the begining of the study, and patients who died after less than 48 h of treatment were excluded. We justify the exclusion of fluconazole by the high frequency of its prescription in the hospital and its low cost compared to other molecules.

Clinico-biological data, risk factors, and therapeutic management were collected from the request of the anti-fungal drug received by the pharmacy department.
The results were statistically processed using SPSS Statistics version 22 software. A p-value of less than 0.05 was considered statistically significant.

Results

Diagnosis of fungal infection

We identified 51 patients distributed as follows: 32 (62.7%) patients with invasive candidiasis, 6 patients (19.6%) with invasive pulmonary aspergillosis (n=10), 6 patients (11.8%) with secondarily disseminated infections (n=6), and 3 patients (5.9%) with candidiasis and aspergillosis co-infection (n=3).
Patients were aged between 15 and 85 years, with a mean age of 53.22±16.5 years, and a sex ratio of 1.125.
Based on the EORTC/MSGERC criteria and clinical, radiological and biological data of each patient, IFIs were classified as follows : 49.3% cases of "proven infection" (n=34), 10.1% cases of "probable infection" (n=7) and 17.4% cases of "possible infection" (n=12) (Figure 1).

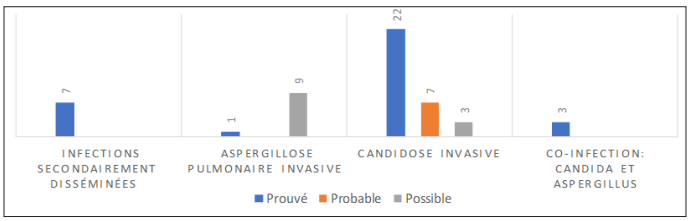

Figure 1: Distribution of invasive fungal infections (in number)

Investigation of invasive candidiasis was achieved using diverse diagnostic tools. Mapping was performed for 22 patients hospitalized on the intensive care units, revealing a positive colonization index (CI) (superior to 0.5) for 18 patients, among whom one had "proven" invasive candidiasis. The candida score was calculated for 17 patients and was superior to 3 in all acses. An antifungal test was performed in 59.4% (n=41) of cases, corresponding to positive culture for yeasts. *Candida glabrata* was the most frequently isolated species (36.5%), followed by *Candida albicans* (32.7%), then *Candida tropicalis* (15.4%) (Figure 2, table 1).

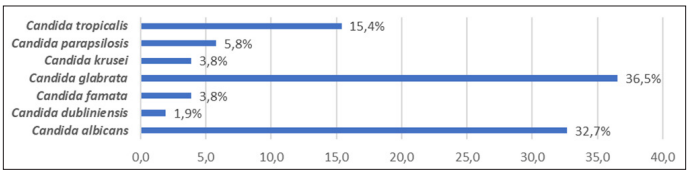

Figure 2: Distributiuon of Candida isolated species (in percentage)

Table 1: Results of the *in vitro* susceptiblity testing of *C.albicans* and *C.glabrata*

	Sensitive*		Intermediate		Resistant	
	Fluconazole	Amphothericin B	Fluconazole	Amphothericin B	fluconazole	Amphothericin B
*C.albicans**	80%	100%	20%	-	-	-
*C.glabrata***	18.2%	77.8%	54.5%	-	27.3%	22.2%

* *C.albicans* is considered susceptible to fluconazole if MIC ≤0,5 mg/L, and to amphothericin B if MIC≤1 mg/L (EUCAST 2022)
* *C.glabrata* is considered susceptible to fluconazole if MIC ≤16 mg/L, and to amphothericin B if MIC ≤1 mg/L (EUCAST 2022)

Candidiasis serology was performed for 26% of patients with invasive candidiasis and revealed positive in 23% of cases.

The diagnosis of aspergillosis was based essentially on radio-clinical presumptions (fever, cough, etc.). Mycelia were observed on direct examination of bronchial specimens in 2 cases, corresponding to invasive pulmonary aspergillosis. Identification *Aspergillus fumigatus* and *Aspergillus flavus*.

Clinico-Biological Profile of the Population

The distribution of prescriptions of anti-fungal drugs according to the hospitalization department was as follows: intensive care units (56.9%, n=29), infectious diseases (17.6%, n=9) and clinical hematology (13.7%, n=7).

80.4% (n=41) of the population had a pathological history such as: pneumopathy (34.6%, n=18), cancer (17.3%, n=9), chronic disease (hypertension, diabetes, 11.5%, n=6) and renal failure (11.5%, n=6).

Patients received antibiotics simultanously in 89.9% of cases. Broad-spectrum beta-lactams (carbapenems, ureidopenicillins and 3rd generation cephalosporins) were co-prescribed in 46.15% of cases (n=24), followed by colistin (18.4%), fluoroquinolones (13.4%) and aminoglycosides (12.8%).

All risk factors for fungal infection identified in the study population are summarized and statistically analyzed in Table 2.

Table 2: Summary of Identified Risk and Prognostic Factors

Risk factor	N	%	khi2 value	p-value	Odds Ratio* OR	Confidence interval 95%
Stay duration at hospital > 72h	47	18,5%	4,25	0,062	1,91	[1,45 ; 2,52]
Diabetes	17	6,7%	0,16	0,769	1,143	[0,6 ; 2,18]
Intubation or invasive ventilation	31	12,2%	4,413	0,046	2,194	[0,96 ; 4,9]
Broad spectrum antibiotics	47	18,5%	0,083	1	0,851	[0,3 ; 2,39]
Prophylaxis using azole agents	6	2,4%	0,131	1	1,184	[0,49 ; 2,83]
Central catheter	30	11,8%	3,088	0,094	1,867	[0,878 ; 3,97]
Neoplasia, anti-cancer chemotherapy	12	4,7%	0,615	0,518	0,722	[0,30 ; 1,72]
Long duration corticosteroids	19	7,5%	4,948	0,041	2,021	[1,09 ; 3,75]
Immunosuppression/ neutropenia	23	9,1%	0,375	0,581	1,217	[0,65 ; 2,28]
MDR Acinetobacter *baumanii infection***	6	2,4%	0,266	0,688	0,75	[0,23 ; 2,44]
Parenteral nutrition exclusively	5	2,0%	0,022	1	0,92	[0,3 ; 2,83]
Recent surgery	11	4,3%	1,439	0,312	0,572	[0,21 ; 1,59]

The evolution of patients was favorable in 29 cases (56.9%) and unfavorable (concluded by death) in the 22 other patients (43.1%). Among these 22 patients, 76.47% were hospitalized in intensive care units. Statistical analysis using the chi-2 test allowed us to identify 2 factors correlating with a poor prognosis for fungal infections: intubation or invasive ventilation (p=0.046, OR=2.194) and long-term corticosteroid therapy (p=0.041, OR=2.021) (table 2).
OR : *Odds-ratio*/ MDR : *multi-drung resistant*

Therapeutic Management

The anti-fungals prescribed for management of the IFI were: caspofungin, anidulafungin, voriconazole, amphotericin B and fluconzaole (following de-escalation).

Invasive candidiasis was treated by caspofungin in 58.6% of cases and anidulafungin in 10.3% of cases. Patients treated with echinocandins were hospitalized in the intensive care units in 78.37% of cases. According to EORTC/MSGERC criteria, echinocandin treatment corresponded to “proven” IFI in 71.42% of cases and “probable” IFI in 20% of cases.

Management of aspergillosis was based on the use of voriconazole in 53.8% of cases. The remaining cases received caspofungin or amphotericin B. According to the EORTC/MSGERC, 40% of indications were “proven” and 60% were “possible”.

Discussion

Diagnosis of Candida Infection

In the case of candidiasis, the sensitivity of classical mycological diagnosis (microscopic examination ± culture) is about 70% and depends ont the pathology [7]. Furthermore, identification of the fungus is only possible after culture, which takes few days to two weeks. The culture allows the biologist to conclude in only 50% of cases for candidemia, and even less in the case of molds [8]. In our study, 62.3% of the IFIs were proven by a mycological argument, i.e. the identification of yeasts or molds by a direct examination, a culture, a PCR in a sterile specimen or biopsy. In 22 cases of invasive candidiasis, the infection was classified as proven, compared to only one case of invasive aspergillosis.

The colonization index (CI) was positive in 86.66% of patients with proven invasive candidiasis. This percentage is very close to that reported by Egimann P. and coll. (92.9%) [9]. The CI is therefore very significant in invasive candidiasis and has a positive predictive value. It is the most commonly used tool to assess the risk of developing an invasive fungal infection. According to the same [9], the CI allows a better detection of fungal colonization, especially with *Candida spp*., and to distinguish low-risk patients from high-risk ones, who should therefore benefit early from empirical antifungal treatment.

Concerning the Candida score, it was positive in only 17 cases. It consists in a simple and interesting diagnostic tool used to better manage candidemia [10]. Given its reduced sensitivity (77.94%) and specificity (66.67%), as recently determined by Kazancioglu and coll. in 2022 [11], this score makes it possible to pre-detect candidemia.

Among the 41 antifungigrams, *Candida glabrata* was found to be the most isolated species, which is in contrast with other national and international studies (Table 3).

Table 3: Isolated Species in Literature

Author	*C. glabrata*	*C. albicans*	*C. tropicalis*	*C. parapsilosis*
Trifi and coll. (8)	23%	54 %	-	-
Fuller and coll. (29)	20.8%	49.6%	5.2%	12%
Our study	36.5%	32.7 %	15.4 %	5.8%

A global epidemiological study published by Quindos and coll. [12] in 2018, revealed that *C.glabrata* occupies the 1st or 2nd position in several countries: China, Japan, Latin America, Mediterranean countries of Africa, Asia and Europe. The change in the epidemiology from *C.albicans* to other *Candida* species is mainly due to the selection pressure of antifungal drugs used excessively, especially in hospitals. Te results of the in vitro susceptibility testing revealed that the isolated *C.glabrata* strains tended to be diminished towards fluconazole, whereas the *C.albicans* strains were all susceptible to fluconazole. The emergence of *C.glabrata* with decreased susceptibility to fluconazole has been described worldwide. Fuller and coll found that 8-10% of *C.glabrata* strains are resistant to fluconazole. Among these resistant isolates, 9-10% are cross-resistant to echinocandins [13]. One mechanism of resistance to fluconazole is the over-expression of the CgCDR1 and CgCDR2 genes encoding for efflux proteins of the antifungal agent [14, 15]. Other mechanisms contribute to this resistance, the best known being mutation of the FKS gene, with production of a mutated 1,3 β-D-Glucan synthase. This results in resistance to echninocandins, which are generally the first line of treatment for invasive candidiasis [16, 17]. Resistance among C.glabrata and C.auris has been reported, but remains uncommon among *C.albicans* [18]. In our study, C. albicans still remains susceptible to fluconazole. Fuller and coll. estimated that the percentage of resistance is less than 1% for this species and that MICs have a normal distribution [13].

As for amphotericin B, Candida resistance is not frequent. In contrast, *C. auris* shows resistance to amphotericin B in up to 30% of cases [18].

Diagnosis of Aspergillosis Infection

During IA, treatment was initiated based on radiological and clinical evidence in patients at risk. The diagnosis of invasive aspergillosis is established by the meeting of a cluster of arguments: host factors, clinic and suggestive images on chest CT scan. Our study found that the radiological exam was always in favor of pulmonary aspergillosis. Chest CT scan is the key exam for the early diagnosis of invasive pulmonaty aspergillosis, the suggestive aspects being: the halo sign and the gas crescent sign. The direct examination of a specimen reveals mycelial filaments, which are very characteristic of *Aspergillus*. Their presence in a sterile respiratory specimen confirms the infection. The strains isolated were: *Aspergillus fumigatus* and Aspergillus flavus, corresponding to the strains most incriminated in IPA [19].

Due to the small number of cases, we cannot draw a conclusion regarding the sensitivity profile of Aspergillus strains in our hospital. The IDSA does not recommend to carry an antifungigram for initial infection, but recommends it in case of non-response, suspicion of an azole-resistant strain or for epidemiological purposes [20]. Recent studies have described the emergence of resistance to azoles antifungal agents (voriconazole, itraconazole, posaconazole), which represent the first line of treatment for IPA according to the IDSA guidelines. Rensdiz-Sharpe and coll found a prevalence of 20.2% of voriconazole-resistant A [21]. *fumigatus* in several European hospitals among patients hospitalized in clinical hematology. Several mechanisms of resistance are involved, the most common being over-expression of the Cyp51 protein and ATP-binding casette efflux pumps [22].

The use of echinocandins to treat azole-resistant Aspergillus infections was approved by the Food and Drug Administration in 2002. Nowadays, an emergence of resistance of *Aspergillus* strains to this therapeutic class has been observed. Satish and coll. in 2019 showed that this resistance is due to different mechanisms : genomic, involving FSK, and non-genomic, disrupting the interaction with the target in *A.fumigatus* [23].

Clinico-Biological Profile and Risk Factors for IFI

The demographic characteristics (mean age and sex-ratio) of the population were similar to those reported in other studies, such as Ouachi and coll. [24] (mean age : 48 years with an extreme age of 85 years, sex-ratio=1.5). It should be noted that we did

not collect cases of fungal infections in the pediatric population in our study period, as they would probably be under-diagnosed.

Determination of risk factors allows us to identify the category of patients at risk for fungal infections. In addition to the host risk factors cited in the EORTC/MSGERC criteria (3), the list is expanded from the review of literature to cover all patients at risk (Table 2).

More than half of the study population was hospitalized in intensive care units (56.8%). Currently, it is well established that a stay in intensive care units is associated with a higher incidence of contraction of fungal infections, especially invasive ones. Several data in the litterature support this finding, including the four-year retrospective study by Touhami K and coll. (Casablanca, Morocco) and the study published by Denis and coll. (Strasbourg, France) [25, 26]. The authors reported a stay in an intensive care unit in 29 and 37.5% of cases, respectively. Also, we found that a length of stay superior to 72H and the use of broad-spectrum antibiotic therapy are the most common risk factors in the population (18.5%). The suspicion of a fungal infection arises when apyrexia is not obtained 72 hours after broad-spectrum antibiotic treatment. In the face of non-specific clinical signs of IFI, bacterial infection is often primarily suspected, thus delaying the diagnosis and management of fungemia [27]. Antibiotic therapy was identified in our studty as the first risk factor in candidiasis and the second risk factor in aspergillosis. Indeed, exposure to antibiotics disturbs the commensal bacterial flora, particularly in the digestive tract, resulting in an imbalance that favors the multiplication of fungi that colonize the mucous membranes [28]. The bacteria of the commensal flora activate the innate immune system through a transcription factor (HIF-1α) and an antimicrobial peptide (LL-37), resulting in resistance to candidal colonization [29]. In the same context, a study published in 2016 on the epidemiology of candidiasis in France [30]. Estimated the prevalence of candidal infection to be between 10 and 20% in a group of patients previously exposed to broad-spectrum antibiotics and with multifocal colonization. Hebert C and coll. [31] suggest through their study publised in 2010 that exposure to piperacillin/tazobactam is associated with subsequent infection with fluconazole resistant yeasts.

A percentage of 12.2% of the study population was ventilated under the "controlled assisted ventilation" regimen. This factor therefore comes in second place among all risk factors. A meta-analysis achieved by Chakraborti and coll. in 2020 [32]. Concluded that airway colonization by *Candida spp*. in ventilated patients is associated with a high risk of mortality. Immunosuppression, whether as a result of prolonged corticosteroid therapy, chemotherapy or related to autoimmune disease, accounted for 21.3% of all factors.

Several studies propose immunosuppression (defined as neutropenia (PNN<500/mm3), corticosteroid therapy, chemotherapy, HIV infection, transplant patient) as a risk factor strongly associated with fungal infections [33].

A suprising finding of our study is that fungal infections are associated with multidrug-resistant *A.baumanii* infection in only 2.4% of candidiasis cases. This result is not consistent with the results of other studies. Tan X and coll. found that *Candida* colonization of the airways facilitates the occurrence of *A.baumanii* pneumonia in both animal and human models. The small size of our sample may explain this discrepancy with previously published results [34].

Only 2.4% of patients received fluconazole for fungal prophylaxis, as proposed by the IDSA for high-risk patients in intensive care units [17]. Indeed, the massive use of antifungal agents leads to the emergence of resistant or even multi-resistant isolates. A study carried out at the University Hospital of Rennes showed that in patients with chronic aspergillosis, the selection pressure from the intensive use of azole drugs furnish an explanation for the appearance of resistant *A. fumigatus* [35]. The acquisition of *de novo* mutations is the most likely mechanism.

Statistical analysis of all data allowed us to identify the two following risk factors as life-threatening for patients with fungal infections: intubation or invasive ventilation (OR=2.194) and long-term corticosteroid therapy (OR=2.021). The study of Massou [36] found a correlation between immunosuppression/neutropenia and IFI. Intubation/invasive ventilation was the incriminated risk factor by Poissy and coll. [37]. This difference may be due to differences in practices and treatment protocols in the intensive care units (Table 4).

Table 4: Reported P-Value of Risk Factors in Other Studies

Risk factor	Our study	*Massou and coll.* (2013, Morroco)*	*Durga and coll.* (2018, India)	*Poissy and coll.* (2020, Switzerland)
Stay duration at hospital > 72h	0,062	-	-	-
Diabetes	0,769	-	1	0,2
Intubation or invasive ventilation	0,046	-	0,88	< 0,001
Broad spectrum antibiotics	1	-	-	0,2
Prophylaxis using azole agents	1	-	-	0,5
Central catheter	0,094	-	0,37	0,6
Neoplasia, anti-cancer chemotherapy	0,518	0,628	-	0,3
Long duration corticosteroids	0,041	0,137	-	-
Immunosuppression/ neutropenia	0,581	0,002	0,003	0,2/0,6
MDR Acinetobacter *baumanii infection***	0,688	-	-	-
Parenteral nutrition exclusively	1	-	-	0,4
Recent surgery	0,312	-	-	0,3

* P-Value calculated in multivariate analysis of factors associated with proven systemic candidiasis

Therapeutic Management of IFI

It should be noted first, that posaconazole, 5-flucytosine and micafungin are not marketed in Tunisia. Caspofungin, an echinocandin, was the most prescribed antifungal agent. This fact is in line with the IDSA guidelines updated in 2016, which recommends the use of an echinocandin as first-line treatment in patients with severity factors, or who have already received an azole as prophylaxis (six cases in our study) [17]. According to the IDSA, a re-assessment should be made to judge the benefit-risk of treatment. Treatment can be continued 14 days after the last positive blood culture. Prior to this update, fluconazole was the first-line drug. The emergence of resistance with *C. glabrata* and the frequent adverse effects of azoles antifungal agents (hepatotoxicity and drug interactions with drugs highly metabolized by the cytochrome P450 system) have positioned echinocandins as the first line agents used for the management of candidiasis [17, 20].

De-escalation from echinocandins to fluconazole is recommended in patients who have remained clinically stable for 5 to 7 days with repeated negative blood cultures, if the isolated strain is sensitive to fluconazole. We noted four occasions when de-escalation from caspofungin to fluconazole was possible but not performed. An evaluation in the intensive care unit at the Bordeaux University Hospital revealed the same finding [38]. Pharmacoeconomic studies can be carried out in order to raise awareness among clinicians. In Tunisia, the high price of echinocandins agents (anidulafungin and caspofungin) leads to a very significant increase in the cost of managing a fungal infection. At the National Bone Marrow Transplant Center in Tunis, a study evaluating the cost of fungal infections management with caspofungin showed that the treatment cost is near to 921.29 tunisian dinars for 19 patients over a period of five months [39]. The rationalization of the prescription of systemic anti-fungals and the implementation of anti-fungal stewardship in hospitals is then necessary.

Therapeutic manaement of the patient with cutaneous and pulmonary aspergillosis with invasive candidiasis was based on the use of the combination of two azoles (voriconazole and fluconazole). This association is not supported by the IDSA guidelines. Gellen and coll. [40], reported the different possible combinations of antifungal therapy, and does not support this combination, mainly because it increases ocular adverse effects, photosensitivity and, above all, the risk of drug interactions through inhibition of cytochrome P450 iso-enzymes.

Voriconazole is the most commonly used antifungal agent (53.8%) for invasive aspergillosis, which is well in line with IDSA recommendations [20]. Amphotericin B (lipid form), previously the molecule of choice in IA, remains an alternative (fungicidal against *A. fumigatus* and *A. flavus* but inactive against *A. terreus*).

Conclusion

In conclusion, this study has contributed to the understanding of invasive fungal infections: prevalence of risk factors, prognostic factors, species involved, and diagnostic procedures. We have underlined the importance of a correct and optimized therapy according to the recommendations. Finally, only a close multidisciplinary collaboration between clinicians, radiologists, mycologists and pharmacists will improve the prognosis of these infections.

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