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RESEARCH AGENDA

Intensive care medicine research agenda on invasive fungal infection in critically ill patients

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Abstract

Purpose: To describe concisely the current standards of care, major recent advances, common beliefs that have been contradicted by recent trials, areas of uncertainty, and clinical studies that need to be performed over the next decade and their expected outcomes with regard to *Candida* and *Aspergillus* infections in non-neutropenic patients in the ICU setting.

Methods: A systematic review of the medical literature taking account of national and international guidelines and expert opinion.

Results: Severe invasive fungal infections (IFIs) are becoming increasingly frequent in critically ill patients. Approximately 80% of IFIs are due to *Candida* spp. and 0.3–19% to *Aspergillus* spp. Recent observations emphasize the necessity of building a worldwide sentinel network to monitor the emergence of new fungal species and changes in susceptibility. Robust data on the attributable mortality are essential for the design of clinical studies with mortality endpoints. Although early antifungal therapy for *Candida* has been recommended in patients with risk factors, sepsis of unknown cause, and positive *Candida* serum biomarkers [β-1 → 3-D-glucan (BDG) and *Candida albicans* germ tube antibody (CAGTA)], its usefulness and influence on outcome need to be confirmed. Future studies may specifically address the optimal diagnostic and therapeutic strategies for patients with abdominal candidiasis. Better knowledge of the pharmacokinetics of antifungal molecules and tissue penetration is a key issue for intensivists. Regarding invasive aspergillosis, further investigation is needed to determine its incidence in the ICU, its relationship with influenza outbreaks, the clinical impact of rapid diagnosis, and the significance of combination treatment.

Conclusions: Fundamental questions regarding IFI have to be addressed over the next decade. The clinical studies described in this research agenda should provide a template and set priorities for the clinical investigations that need to be performed.

Keywords: *Candida*, *Aspergillus*, Antifungals, Echinocandins, Fluconazole, Beta-D-glucan

Introduction

Invasive fungal infections (IFI) in critically ill patients are associated with considerable morbidity and mortality. This research agenda on IFI focuses on the more frequent diseases, namely invasive candidiasis and invasive aspergillosis in non-neutropenic patients, but not on the so-called ultra-orphan fungal infections that may be as rare as less than 1 in 1 million population, e.g., mucormycosis or trichosporonosis [1]. This article aims to highlight open questions and gaps in our knowledge and

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understanding of epidemiology, risk factors, use of diagnostic tools, and antifungal compounds.

The critically ill patients treated today are a very heterogeneous group. This is why the authors of this article represent microbiology, intensive care, and clinical infectious disease experts with views from Asia, Europe, and North America.

It was a major step forward that an international consensus was established on the definitions of what constitutes invasive fungal disease [2]. A common set of definitions allows comparison of results between studies to a certain extent, but even the definitions continue to evolve [3].

Treatment delays in critically ill patients negatively impact on outcomes. Invasive fungal infections are no exceptions to this rule. Thus the field moves towards earlier diagnosis followed by earlier treatment. The early strategic option obviously is prophylaxis, which has been proven successful in other clinical settings [4]. To avoid overtreatment in a large group of critically ill patients, any prophylactic approach needs a population at sufficiently high risk, but currently it is unclear how to select such appropriate target groups.

Whether superiority of a certain treatment or strategy can be proven at all may depend on the clinical endpoint chosen. While echinocandins appear superior to azoles in terms of treatment success [5], it is much more challenging to improve survival rates by applying a single diagnostic tool or treatment decision. The diverse and complex problems that the individual critically ill patient at risk for IFI may face could easily mean several "competing" life-threatening conditions at the same time. Thus, single interventions proven to save lives in a randomized comparison are rare findings.

In critically ill patients we do not expect the next advance to come from a single pivotal drug trial, but rather from strategic trials integrating genetics profile of host and pathogen, diagnostic tools, antifungal treatment, and therapeutic drug monitoring (TDM). Nothing less than individualized treatment may advance the field. Unfortunately, no such clinical trial has succeeded yet.

Methods

A systematic review for this project was conducted with a timeline from 2006 through 2016. Two authors of each of the two sections (Candida and Aspergillus) searched PubMed using the terms *Candida*, *Aspergillus*, antifungal therapy, and critically ill patients. Searches were completed in February 2017. One author (MB) also manually screened reference lists of articles selected for inclusion to identify additional studies. The selection of articles and topics for inclusion in this research agenda from the systematic review were based on their likely importance for yielding clinically important practice changes over the next decade as determined by the writing committee.

What is the current standard of care for delivering the best possible critical care in the field?

Invasive candidiasis

Invasive fungal infections (IFIs) are an increasingly frequent cause of severe infections with between 30% and 40% of IFIs episodes occurring in critically ill patients [6]. Approximately 80% of IFIs are due to *Candida* species. In a worldwide ICU prevalence study, *Candida* was the third most frequently isolated microorganism accounting for 17% of all infections [7, 8]. The rank order of *Candida* in bloodstream infections varies by country and is influenced by the type of patients studied. *Candida* is number three or four in mostly ICU-based US studies and number six to ten in population-based European studies [9]. The incidence of candidemia is age-related with higher frequencies at both ends of the spectrum. Globally, invasive candidiasis ranges from 1 to 10 per 1000 ICU admissions. While it is responsible for no more than 5% of the total number of sepsis or septic shock, candidemia is associated with sepsis or septic shock in 10–40% of cases [10].

Conventional culture-based microbiological tests are suboptimal for the diagnosis of candidemia and deep-seated candidiasis. Blood cultures are insensitive and take several days to obtain *Candida* species and antifungal susceptibilities [11]. Although fluorescence in situ hybridization with peptide nucleic acid probes (PNA-FISH) allows the detection of yeasts within 30–90 min of blood culture positivity, the commercially available test (PNA-FISH Yeast Traffic Light assay) does not distinguish between *C. albicans* and *C. parapsilosis* or *C. glabrata* and *C. krusei*.

Various studies have shown that delays in initiating adequate antifungal therapy are associated with increased mortality [12, 13], therefore driving the field to explore advanced strategies such as prophylaxis, marker-based pre-emptive therapy, and risk-based empirical therapy. Numerous risk factors for invasive *Candida* infections have been identified, including higher Acute Physiology and Chronic Health Evaluation II scores, diabetes mellitus, renal insufficiency, surgery (especially abdominal surgery), pancreatitis, the use of broad-spectrum antibiotics, parenteral nutrition, hemodialysis, mechanical ventilation, the presence of central vascular catheters, and therapy with immunosuppressive agents [9]. The development of invasive *Candida* infections is often preceded by extensive colonization of the skin or of the mucus membranes of the gastrointestinal and urogenital tracts, and the degree of colonization, assessed using the colonization index, has been shown to be an

independent risk factor for development of candidiasis [9]. Fluconazole and caspofungin decrease the incidence of invasive candidiasis when given prophylactically in selected patients [14, 15]. Biomarker-guided pre-emptive therapy with echinocandins triggered by elevated serum β-1 → 3-D-glucan (BDG) has also been shown to reduce the incidence of proven disease and to target antifungals for patients that would be most likely to benefit from them [15]. While fluconazole has not been shown to be effective for empirical therapy [16], a recent study has shown that empirical therapy with micafungin in high-risk hosts also decreased the incidence of proven disease [17]. None of these studies have shown a survival benefit as none of them have been powered to do so; however, the standard of care at this time is utilizing a clinical prediction rule such as the *Candida* score [18] or the MSG-01 rule [15] to identify high-risk hosts and then monitor serum biomarkers such as BDG or PCR for biomarker-based pre-emptive therapy or the clinical prediction rules themselves as triggers for empirical therapy. A recent study has also shown that the negative predictive value of BDG can be used to stop empirical therapy as part of an antifungal stewardship intervention [19].

Polyenes, azoles, and echinocandins are the antifungal drug classes available for the treatment of invasive candidiasis (Table 1). All new antifungal drugs have been compared to a standard regimen in one or more randomized trials. In the 1990s, fluconazole became the standard treatment regimen for invasive candidiasis, as it was shown to be as effective as conventional amphotericin B deoxycholate (dAmB), but associated with significantly lower toxicity [20]. Since those trials, dAmB is no longer considered a treatment option for invasive candidiasis [21]. Also voriconazole and caspofungin were shown to be as effective as dAmB but less toxic [22, 23]. Subsequently, in two randomized trials, micafungin was shown to be as effective as caspofungin and as liposomal amphotericin B (LAmB) [24, 25].

Despite the limited but significant advent of *Candida* strains with reduced susceptibility to fluconazole (especially *C. glabrata*), the drug has remained the mainstay of anti-*Candida* treatment for more than two decades.

Invasive aspergillosis

The incidence of invasive pulmonary aspergillosis (IPA) in the ICU is unclear, ranging from 0.3% to 19% because of the difficulties of diagnosis (diagnostic tests vary in their sensitivity and specificity) and related to biopsy or autopsy difficulties in the ICU setting (coagulation abnormalities, difficult oxygenation, next of kin consent for autopsy, etc.) [26]. The "classical" risk factors for IPA are classified in high, intermediate, and low risk categories (Table 2). New risk factors, however, have been

Table 1 Data on different antifungal treatment in randomized controlled studies

Study drug	Comparator drug	Number of patients, total (candidemia/localized)	Patients in ICU (%)	Standardized success rate[a]	Mortality	References
Fluconazole 400 mg/day	Amphotericin B 0.5–0.6 mg/kg/day	206 (206/0)	Unknown	Fluconazole 70%, amphotericin B 79%, $P = 0.22$	Fluconazole 40%, amphotericin B 33%, $P = 0.20$	[20]
Caspofungin 50 mg/day	Amphotericin B C 0.6–0.7 mg/kg/day	224 (181/43)	Unknown	Caspofungin 73%, amphotericin B 62%, $P = 0.09$	Caspofungin 34%, amphotericin B 30%, $P = 0.23$	[23]
Voriconazole 3 mg/kg b.i.d.	Amphotericin B 0.7–1.0 mg/kg/day followed by fluconazole 400 mg/day	370 (370/0)	49	Voriconazole 65%, amphotericin B → fluconazole 71%, $P = 0.25$	Voriconazole 36%, amphotericin B → fluconazole 24%, $P = 0.23$	[22]
Anidulafungin 100 mg/day	Fluconazole 400 mg/day	245 (219/26)	Unknown	Anidulafungin 76%, fluconazole 60%, $P = 0.01$	Anidulafungin 23%, fluconazole 31%, $P = 0.13$	[5]
Micafungin 100 mg/day	Liposomal amphotericin B 3 mg/kg/day	392 (333/59)	56	Micafungin 74%, L-AmB 70%, $P = 0.27$	Micafungin 40%, L-AmB 40%, $P = 0.94$	[24]
Micafungin 100 or 150 mg/day	Caspofungin 50 mg/day	576 (492/84)	Unknown	Micafungin 100 mg/day, 76%, micafungin 150 mg/day 71%, caspofungin 72%, $P = 0.36$	Micafungin 100 mg/day 29%, micafungin 150 mg/day 33%, caspofungin 26%, $P = 0.19$	[25]
Isavuconazole 200 mg/day	Caspofungin 50 mg/day	400 (333/67)	Unknown	Isavuconazole 60%, caspofungin 71%	Isavuconazole 15%, caspofungin 12%, $P =$ n.s.	[80]

[a] Standardized success rate (modified) intent to treat population; end of iv therapy, or at last available study visit

identified during the last decade including COPD, liver failure, cirrhosis, and post-H1N1 influenza [27, 28]. The diversity of patients and risk factors complicates diagnostic and therapeutic procedures while the available data in critically ill patients are extremely limited.

What have been the major recent advances in the field?

Invasive candidiasis

Geographical and environmental factors, the patient's age, and exposure to antimicrobial agents impact on the distribution of *Candida* species isolated from patients with invasive candidiasis (IC). Historically, *C. albicans* was responsible for about two-thirds of IC, but non-*albicans Candida* species (i.e., *C. glabrata, C. krusei, C. tropicalis*, and *C. parapsilosis*) now account for about half of all cases of IC in hospital [10]. The proportion of IC due to *C. glabrata* has increased in Western countries (i.e., Europe and North America) and tends to be higher in older patients [9, 10]. *C. parapsilosis* is more predominant in Southern Europe, Southeast Asia, and Latin America [10]. The occurrence of infections caused by *C. krusei* or by *C. glabrata* had been linked to previous exposure to antifungal agents, especially azoles. *C. dubliniensis*, *C. guilliermondii*, *C. kefyr*, and *C. lusitaniae* species are a less frequent cause of IC [9] *C. auris* is an emerging, multidrug-resistant yeast causing invasive healthcare-associated infections [29].

Table 2 Risk factors for IPA in ICU patients

1. High risk
Neutropenia (500/mm^3)
Hematological malignancy
Allogeneic HSCT
2. Intermediate risk
Prolonged treatment with corticosteroids before admission to the ICU
Autologous HSCT
COPD
Liver cirrhosis
Solid organ cancer
HIV infection
Lung transplantation
Systemic immunosuppressive therapy
3. Low risk
Severe burns
Solid organ transplant
Steroid treatment for >7 days
Prolonged stay in the ICU (>21 days)
Malnutrition
Post cardiac surgery
Near drowning

COPD chronic obstructive pulmonary disease, *HIV* human immunodeficiency virus, *HSCT* hematopoietic stem cell transplantation, *ICU* intensive care unit, *IPA* invasive pulmonary aspergillosis

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has overcome several of the shortcomings of conventional microbiology for the detection and identification of yeasts [30–32]. Turnaround times are 10–15 min after the growth of a fungal colony or a positive blood culture, and diagnostic accuracy is over 95%. Additional work is required to make MALDI-TOF MS suitable for the detection of antifungal resistance [33].

Over the last few decades, non-culture-based diagnostic methods have been developed to detect fungal metabolites, cell wall antigens (mannan and BDG), nucleic acids, and antibodies directed against fungal products in blood and other body fluids. A meta-analysis showed that the detection of mannan antigen and anti-mannan antibodies had low sensitivities (around 60%) but high specificities (90–95%) for the diagnosis of IC [34]. Combining antigen and antibody assays increased the sensitivity to 83% but not the specificity. Sensitivities are higher for infections caused by *C. albicans* than by other *Candida* species. Commercially available BDG assays had sensitivities of 73–75% and specificities of 97% for the diagnosis of probable or proven *Candida* infections [35].

Up to two-thirds of adult ICU patients receive empirical systemic antifungal treatment (ESAT) [21, 36]. Although early ESAT has been recommended in patients with risk factors for invasive candidiasis (IC), sepsis of unknown cause, and positive *Candida* serum biomarkers BDG and *Candida albicans* germ tube antibody (CAGTA) [37], its usefulness and influence on outcome need to be confirmed.

In the challenging scenario of early diagnosis of IC, recent studies have demonstrated the diagnostic value of BDG and CAGTA [19, 38–42]. Although the use of BDG measurement in daily practice for establishing the indication or the withdrawal of ESAT should be defined, recent observational studies [41, 42] showed that negativity of the BDG assay may be used as a strategy to discontinue ESAT because of the high sensitivity and negative predictive value of the test. Until new proteomic (MALDI-TOF) or genomic (PCR) techniques could be extrapolated to IC for improving its diagnosis, a therapeutic algorithm based on stratification of the clinical condition, *Candida* score, and results of *Candida* serum biomarkers may be useful to improve the management of ICU patients at high risk of invasive fungal infection (Fig. 1).

In a pivotal randomized trial, anidulafungin was compared to fluconazole for the treatment of candidemia and invasive candidiasis in non-neutropenic patients [5]. In that study, the overall response was significantly better with anidulafungin (76%) than with fluconazole (60%; $P < 0.01$). The inferior outcomes with fluconazole were retained in post hoc multivariate analyses,

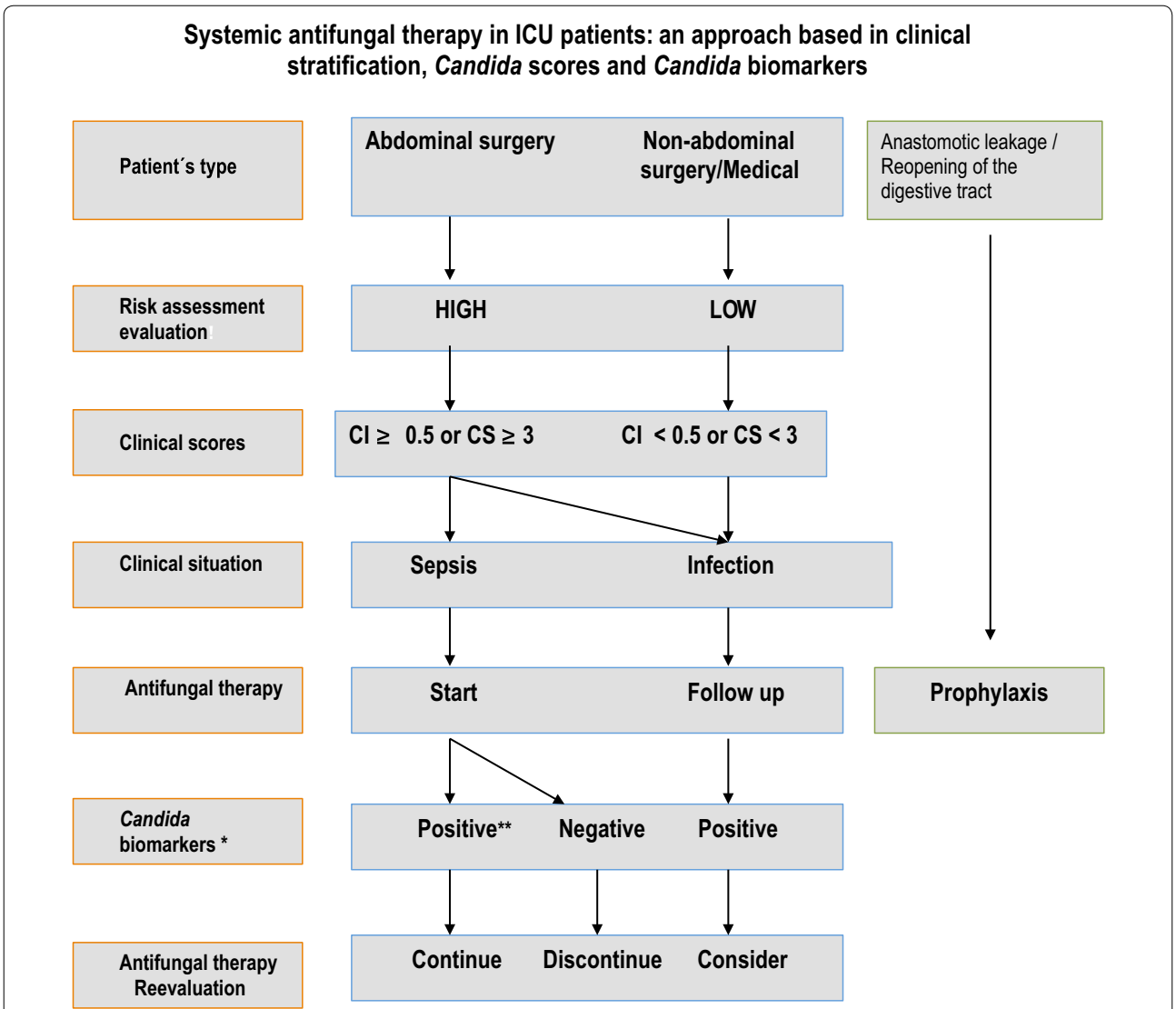

Fig. 1 Systemic antifungal therapy in ICU patients: an approach based on clinical stratification, *Candida* scores and *Candida* biomarkers. *CI* Colonization index (range, 0–1) indicates the number of positive sites colonized with *Candida* divided by the number of sites sampled, *CS* Candida score (range, 0–5) items are surgical admission (1 point), severe sepsis (2 points), multiple sites positive with Candida species (1 point), and parenteral nutrition (1 point), * *Candida* biomarkers: (1→3)-ß-D-glucan (ßDG) and *Candida* albicans germ tube antibody (CAGTA), ** Positive: ßDG ≥cutoff in two consecutive samples or ßDG and CAGTA ≥cutoff in one determination

and the difference was consistent over a broad range of APACHE II scores [43]. Interestingly, while the infecting *C. albicans* strains were uniformly susceptible to fluconazole, the inferiority of fluconazole was most prominent in patients infected by *C. albicans* (success rate, fluconazole 62% vs. anidulafungin 81%; $P < 0.02$) [5]. In addition, while the trial was not powered for mortality differences, the mortality in the fluconazole group tended to be higher than in the anidulafungin group (31% vs. 23%; $P = 0.13$).

On the other hand, the influence of severe pathophysiological changes that are present in the critically ill patient may also cause pharmacokinetic (PK) alterations of the different antifungals [44, 45]. Recent studies showed that echinocandins exposure in ICU patients was low compared with healthy volunteers and other (non-)critically ill patients, most likely as a result of a larger volume of distribution, suggesting that a weight-based dose regimen should probably be more suitable for patients with substantially altered drug distribution [45–47].

Invasive aspergillosis

Early diagnosis of IPA is a challenge based on the integration of microbiological, radiological, and clinical data. The laboratory diagnosis is based on galactomannan

(GM) detection in bronchoalveolar lavage (BAL) specimens (GM cutoff 0.5–1.0) and not in serum (BAL GM sensitivity 66.7%, serum GM sensitivity 53.3%). There is a limited role for BDG testing alone, although the combination with GM or PCR improves specific detection [48]. The *Aspergillus* lateral flow device (LFD) assay in serum and BAL samples—a rapid test for the detection of *Aspergillus*—is not yet commercially available, whilst the SeptiFast assay method in blood samples is associated with a sensitivity of 66%, specificity 98%, PPV 93%, and NPV 88% [49]. CT scanning of the lungs is associated with greater than 90% specificity and poor sensitivity (30–40%). In critically ill COPD patients multiple nodules distributed along with bronchovascular bundles are common, whilst a deteriorating chest X-ray combined with laboratory tests could support the diagnosis of probable IPA. The clinical signs and symptoms are non-specific. Because of all these diagnostic uncertainties and difficulties, an algorithm by AspICU investigators has been suggested in order to facilitate the diagnosis of probable IPA in the ICU setting (Table 3) [50].

What are the common beliefs that have been contradicted by recent trials and what are remaining areas of uncertainty?

Invasive candidiasis

IC is associated with an overall crude mortality of 40–60%, which is strongly affected by the underlying conditions and by the presence of sepsis or septic shock [10]. Assessing the attributable mortality of IC is difficult, with studies estimating ranges from 5% to 70% [51]. However, it is most likely in the range of 10–15% [9]. Species-specific survival analyses have yielded ambiguous results in large patient cohorts.

Although use of preemptive therapy is gaining interest, more studies are needed to better define which patients may benefit from this approach and whether more widespread use of antifungal agents may negatively influence fungal ecology. A recent double-blind placebo-controlled trial has also contradicted the widespread belief that critically ill patients with ICU-acquired sepsis, *Candida* colonization, and multiple organ failure should receive antifungal therapy. In this clinical trial, empirical treatment with micafungin, compared with placebo, did not increase fungal infection-free survival at day 28 (primary endpoint). However, this trial was not powered to detect changes in mortality [17].

Despite some studies showing the superiority of echinocandins over fluconazole, a propensity score-derived analysis of a population-based, multicenter prospective cohort has demonstrated that, in patients with candidemia, therapy with fluconazole did not show a significant association with mortality either in the empirical or targeted therapy [52]. These results were similar among patients with severe sepsis and septic shock. In patients with septic shock attributable to *Candida* spp., after

Table 3 A clinical algorithm to diagnose IPA in critically ill patients

1. *Aspergillus* (+) LRT specimen culture (entry criterion)
2. Compatible signs and symptoms (one of the following)
Fever refractory to at least 3 days of appropriate antibiotic therapy
Recrudescent fever after a period of defervescence of at least 48 h while still on antibiotics and without other apparent cause
Pleuritic chest pain
Pleuritic rub
Dyspnea
Hemoptysis
Worsening respiratory insufficiency in spite of appropriate antibiotic therapy and ventilatory support
3. Abnormal medical imaging by portable chest X-ray or CT scan of the lungs
4. Either
4a. Host risk factors (one of the following conditions)
Neutropenia (absolute neutrophil count less than 500/mm^3) preceding or at the time of ICU admission
Underlying hematological or oncological malignancy treated with cytotoxic agents
Glucocorticoid treatment (prednisone or equivalent, >20 mg/day)
Congenital or acquired immunodeficiency
OR
4b. Semiquantitative *Aspergillus*-positive
Culture of BAL fluid (+ or ++) without bacterial growth together with a positive cytological smear showing branching hyphae
a) Probable invasive pulmonary aspergillosis when 1 + 2 + 3 + either 4a or 4b
b) When ≥1 criterion is not met, the case is classified as *Aspergillus* colonization

adjustment for severity of illness and source control, therapy with echinocandins was not associated with a higher survival rate [53]. Two other recent observational studies have confirmed that antifungal choice (fluconazole vs. echinocandin/liposomal-amphotericin B) does not influence mortality in *C. glabrata* bloodstream infection [53, 54]. More data are needed in this area.

Pathophysiological changes associated with critical illness can change drug concentrations so that they are significantly different from those observed in non-critically ill patients. In these circumstances, if standard dosing is used, then suboptimal concentrations (either too low or unnecessarily high) may result, putting the patient at risk of clinical failure or drug toxicity. Antifungal agents tend not to be as markedly affected by altered PK in critical illness. The drugs are mostly lipophilic, hepatically metabolized, and with high protein binding. Drugs such as fluconazole are an exception to these characteristics. Use of renal replacement therapy (RRT) can influence the clearance of some antifungal drugs, particularly those that are predominantly eliminated by renal mechanisms, are not highly protein bound, and have low molecular weights, notably the azoles and 5-flucytosine. Moreover, different RRT characteristics, including the specific mode, membrane characteristics, flow rates, and duration of treatment, may influence the effects of RRT on drug PK and should be taken into consideration when evaluating dosing [6, 9].

Source control, i.e., catheter removal during candidemia or abscess drainage in invasive candidiasis, has proven an independent predictor of reduced mortality in multiple studies [12, 55]. The optimal total duration of therapy for candidemia and invasive candidiasis has not been studied. However, step-down from echinocandins to intravenous or oral azoles appears to be feasible, once the patient has been stabilized, and provided that the isolate is azole-susceptible. While most trials allowed step-down to azoles after at least 10 days of echinocandin therapy, a recent non-comparative trial applied a step-down to an oral azole as early as 5 days after start of iv treatment [56]. In a prospective cohort study of ICU patients, neither discontinuation of empirical antifungal therapy nor de-escalation to fluconazole after 5 days in the case of documented invasive candidiasis was associated with a survival difference [57]. Although these studies were not randomized to compare early step-down therapy to prolonged echinocandins, the efficacy and survival results in patients with early step-down were similar.

Invasive aspergillosis

For the treatment of IPA voriconazole and isavuconazole are recommended, whilst LAmB or AmB lipid complex is recommended for species with intrinsic high azole minimum inhibitory concentrations (MICs). Voriconazole is recommended for IPA due to *A. fumigatus* if the isolate is voriconazole-susceptible (susceptible if MIC $\leq$1 mg/L) whilst in the case of resistance (MIC >2 mg/L) LAmB therapy is preferred. Where the voriconazole MIC equals 2 mg/L (intermediate) the response to voriconazole monotherapy is unknown [58]. A growing problem in IPA treatment is the intrinsic resistance to polyenes and azoles or the acquired resistance to azoles during azole therapy mainly in patients with chronic pulmonary aspergillosis (CPA) [59]. TDM is used to avoid treatment failure, resistance, or toxicity. The optimal plasma trough levels (with HPLC/MS) are 1–4 mg/L for itraconazole, 2–6 mg/L for voriconazole, and greater than 1 mg/L for posaconazole suspension [60–62]. The newer tablet or intravenous formulation of posacoanzole is more likely to achieve target plasma levels but there is limited evidence for routine TDM. Data supporting routine TDM for isavuconazole are also limited, although from phase II/III clinical studies trough levels of 2–3 mg/L are suggested.

The use of combination antifungal therapy for IA is debated, although theoretically this can achieve (a) potential synergistic effects, (b) broader antifungal spectrum, and (c) potentially a reduction of acquired resistance [63]. In vitro and animal studies demonstrated synergistic or additive effects of a mold-active triazole (itraconazole, voriconazole, or posaconazole) or amphotericin B with an echinocandin but few human studies support this practice. The combination of voriconazole with anidulafungin failed to demonstrate an overall significant difference over voriconazole alone in primary outcome in IPA cases, although in a post hoc subgroup analysis among galactomannan-positive patients a significant advantage favoring combination treatment was recorded [64]. The use of combination therapy is considered as therapeutic solution (a) in cases of resistance (e.g., Cyp51a mutations), (b) in central nervous system aspergillosis due to azole-resistant *Aspergillus* (favoring flucytosine), (c) as broad initial coverage pending pathogen identification, (d) for salvage therapy in refractory disease [65]. In regions with environmental resistance rates of at least 10%, a voriconazole–echinocandin combination could be used as initial therapy [66]. The duration of IPA treatment ranges between 3 and 50 weeks. Physicians should consider iv to oral switch in stable patients and, before treatment discontinuation, is essential the closed monitoring with imaging and microbiological evaluation.

Chronic pulmonary aspergillosis (CPA) is a rare disease in non-immunocompromised patients with prior or current lung disease including tuberculosis, chronic cavitary pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA), *Aspergillus* nodules

and single aspergilloma, which in moderately immunocompromised patients present as subacute invasive pulmonary aspergillosis (formerly called chronic necrotising pulmonary aspergillosis) leading to a more rapidly progressive infection (<3 months) which should be managed as invasive aspergillosis.

The diagnosis of CPA requires the combination of thoracic imaging findings, direct evidence of *Aspergillus* infection (microscopy or culture from biopsy) or immunological response to *Aspergillus* spp., and exclusion of alternative diagnoses, all present for at least 3 months. Long-term oral antifungal therapy is recommended, whilst surgical excision of simple aspergilloma is suggested if technically possible. Hemoptysis may be controlled with tranexamic acid and bronchial artery embolization [67].

What are remaining areas of uncertainty?

Invasive candidiasis

Given the ubiquitous presence of glucan in the environment, false-positive BDG results have been noted in a number of conditions frequently encountered in critically ill patients such as severe mucositis, bacterial sepsis, hemodialysis, treatment with antibiotics, albumin, or immunoglobulins, and transfusion of human blood products. BDG was superior to the *Candida* score or the colonization index for the diagnosis of intra-abdominal candidiasis or candidemia in high-risk surgical and ICU patients [68, 69]. However, undetectable serum BDG does not rule out an early IFI when the clinical suspicion is high [70].

The detection of fungal DNA by PCR is a technical challenge because of a low DNA yield after cell lysis and the risk of false positivity due to contamination by fungal saprophytes. A broad range of in-house PCR methods have been developed but there still is no commercially available PCR assay. In a systematic review and meta-analysis, PCR tended to be positive at an earlier time point than conventional microbiology with pooled sensitivity and specificity of PCR for candidemia of 95% and 92%, respectively [71]. In a recent study, plasma or serum PCR was more sensitive than BDG in patients with IC or deep-seated candidiasis (80% versus 56% and 88% versus 62%, respectively) but not in those with candidemia [72]. Sensitivities reached 98% when blood culture was combined with PCR. A whole blood assay combining PCR technology and nanoparticle-based hybridization (T2 magnetic resonance) was recently shown to rapidly (<5 h), accurately, and reproducibly detect 1–3 CFU of *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. krusei*, and *C. parapsilosis* per milliliter of spiked blood or in patients with a low incidence of IC [73]. Lately, a PCR/electrospray ionization–mass spectrometry (PCR/ESI) technology was developed to detect more than 800 bloodstream infection-relevant bacterial and *Candida* species within 6 h of sample acquisition. Findings from the RADICAL study showed 81% sensitivity, 69% specificity, and 97% negative predictive value with PCR/ESI when compared with conventional cultures [74]. In a set of prospectively collected blood samples in sepsis patients, PCR/ESI and standard microbiology were concordant in 86% of culture-positive samples [75]. Of note, the PCR/ESI assay is not capable of determining antimicrobial resistance phenotypes, and the inclusive detection of resistance-associated genotypes is, to date, limited to only few broad-spectrum antibiotic resistance markers.

Many questions about the proper management of fungal infections still exist without definitive answers. The absence of definite diagnostic criteria in patients with suspicion of non-candidemic invasive candidiasis makes it clearly difficult to determine the efficacy of different therapeutic approaches. In fact, the negative results of ESAT in the recent clinical trial can be explained by the fact that many of these patients did not truly have an invasive candidiasis despite the fact that all had sepsis, multiple organ failure, and multifocal *Candida* colonization [17].

Although several diagnostic algorithms based on the combination of clinical scores and microbiological tests have been proposed, this approach did not discriminate infected from non-infected patients. New diagnostic tools with higher sensitivity and specificity are clearly needed. For the rapid diagnosis of *Candida* bloodstream infections, technologies such as MALDI-TOF allow the direct identification of bacteria from positive blood cultures whereas yeasts are poorly identified using this technique [76, 77]. New technologies (i.e., fluorescence in situ hybridization) [78] capable of identifying *Candida* spp. in blood cultures within a few hours are highly attractive, but clinical studies are needed to establish the clinical benefits of these diagnostic tools and how to introduce them into the diagnostic work-up. T2 diagnostic technology is an exciting development in the field, but more real-world experience is needed [73, 79].

Recent studies have also cast doubt about the optimal dosing of antifungals in critically ill patients, especially in more severe condition [80]. There are also emerging data linking suboptimal dosing to the emergence of antifungal resistance and a poor outcome. Dosing of antifungal agents in patients with extracorporeal devices needs more studies to define the therapeutic schemes that avoid subtherapeutic levels. In this sense, the impact on outcome of TDM warrants further investigations.

Despite the publication of the randomized trial suggesting the superiority of anidulafungin compared to fluconazole [5], physicians have continued to prescribe

fluconazole with candidemia or invasive candidiasis, supported by some of the guidelines. Many physicians have long favored fluconazole over the new echinocandins, driven by the vast clinical experience with fluconazole, its favorable pharmacokinetics and tissue penetration, and its low price compared to echinocandins.

Recently, a second, large randomized trial comparing azoles to echinocandins as initial therapy for candidemia or invasive candidiasis demonstrated a similar difference. In that study, the success rate in the isavuconazole group was 60.3% vs. 71.1% in the caspofungin group [81]. A pooled analysis was performed of patient-level data from seven randomized antifungal treatment trials, using 30-day mortality as the primary endpoint [55]. In this analysis, randomization to an echinocandin was associated with significantly greater clinical success and better survival than treatment with amphotericin B or azoles. The advantage of echinocandin therapy over azoles was demonstrated in patients with low and moderately high APACHE II scores, suggesting that the survival benefit from echinocandin treatment is not limited to severely ill patients [55]. Multiple cohort studies have identified treatment with an echinocandin to be independently associated with better survival in multivariate analyses, e.g., in severely ill patients with candidemia and septic shock [82], and in patients infected by *C. glabrata* [83].

Despite the lack of randomized trials, antifungal therapy for patients with severe community-acquired or healthcare-associated intra-abdominal infection is recommended if *Candida* is grown from cultures [9, 84].

As patients with candidemia are easier to recognize and to enroll in clinical trials than patients with deep-seated candidiasis, trial results and guidelines have mainly focused on patients with candidemia [9]. Specific studies will need to identify the optimal diagnosis and treatment of deep-seated abdominal candidiasis.

In a large clinical study, ICU patients with polymorphisms in their TLR/interferon-α host defense pathway were more likely to develop candidemia than underlying disease-matched ICU controls [85]. A genome-wide analysis has identified several additional immune polymorphisms that render patients up to 19 times more likely to acquire candidemia than other ICU patients [86]. These findings may lead to screening strategies to identify patients at risk and requiring antifungal prophylaxis, early empiric therapy, or adjunctive immunotherapy when undergoing surgery or ICU admission [87].

Invasive aspergillosis

The survival of patients with IPA has increased in recent years owing to advances in diagnosis and the release of newer antifungal drugs, but the overall outcome still remains suboptimal (attributable mortality is considered 42–64%) especially in certain populations (e.g., allogeneic HSCT) or in cases of extrapulmonary involvement [63]. According to Taccone et al., the mortality predictors for critically ill with IPA include age, SOFA score, mechanical ventilation, and renal replacement therapy at diagnosis [26, 88].

What do the international group of experts recommend as the top 10 studies/trials to be done in the next 10 years and what are expected outcomes/results of these trials?

Invasive candidiasis

Recent observations emphasize the necessity to build a worldwide sentinel network to collect epidemiological data in real time in order to monitor the emergence of new fungal species causing IC and changes in susceptibility of the most common isolates. Obtaining robust data on the attributable mortality of IC is critical for the design of clinical studies with mortality endpoints.

Despite enormous progress made in mycological diagnostics, molecular methods relying on culture amplification remain inadequate to fill the temporal gaps between the release of a laboratory result and the identity of causative fungal pathogens and susceptibility patterns. Upcoming molecular methods applied directly to clinical specimens may greatly accelerate diagnosis and significantly impact on early therapy of IFIs. Trade-offs in terms of increased sensitivity include the risk of false-positive results due to environmental fungal DNA contamination. Yet, fully automated molecular tests may help reduce the risk of contamination, workload, and error rates and thus offer the promise of short turnaround time which is essential for early diagnosis and prompt initiation of appropriate therapy for IFIs.

As the discussion has highlighted, the recent years have seen the development of various new biomarkers for the diagnosis and management of candidemia and invasive candidiasis, along with a better appreciation and validation of various clinical stratification tools. Hence, we suggest three potential studies that should help to elucidate our understanding of these severe infections. First, it remains unclear how the use of biomarkers can be differentially integrated into pre-emptive therapy strategies. A randomized controlled trial linking change in biomarkers and a clinical risk score as part of a pre-emptive treatment strategy as compared to standard therapy would provide substantial insight. Second, with expanding utilization of procalcitonin (PCT) to guide anti-infective treatment initiation and duration, there remains a paucity of knowledge about if and how this test can be applied in cases of either suspected or confirmed candidemia or invasive candidiasis. Simple observational studies to appreciate the natural history of changes in PCT in candidemia are necessary as are studies to define the sensitivity and

Table 4 Top 10 studies/trials to be done in the next 10 years

Studies/trials	Expected outcome/results
1. Worldwide sentinel network to collect epidemiological data in real time	Monitor emergence of new fungal species and changes in susceptibility of the most common isolates
2. A randomized controlled trial linking change in biomarkers and a clinical risk score as part of a pre-emptive treatment strategy as compared to standard therapy	Provide substantial insight to better define pre-emptive strategy
3. Utilization of PCT to guide treatment initiation and duration	Provide knowledge about if and how this test can be applied in cases of either suspected or confirmed candidemia or invasive candidiasis
4. Specific study on the treatment of abdominal candidiasis (drug, dose, duration)	Better knowledge about optimal management
5. Study on PK/PD and TDM of antifungal molecules in critically ill patients	Better knowledge about tissue penetration of echinocandins, optimal dosing schedule for echinocandins, when and how to do TDM
6. Identification of immunogenetics-based risk profiles for identifying patients at highest risk for invasive candidiasis	Personalized antifungal prophylaxis or treatment
7. Phase 3 Studies on new antifungals (CD101, SCY078, and APX001)	These new options may contribute to optimal management and lower mortality rates among patients with candidemia or invasive candidiasis
8. The clinical impact of the *Aspergillus* LFD and SeptiFast assay in the rapid diagnosis of invasive aspergillosis	Easier and more rapid diagnosis of invasive aspergillosis
9. The significance of combination treatment patients' outcome and impact of PK/PD profile of combined antifungal molecules	Better knowledge about combination therapy in patients with invasive aspergillosis
10. Study on incidence of extrapulmonary manifestation of aspergillosis	Better knowledge about the epidemiology and the management of mediastinitis after cardiac surgery and pericarditis/endocarditis

specificity of this test for non-bacterial processes. Third, with the advent of new biomarkers and expanding data to explain the value and limitations of older ones, we suggest a randomized controlled study employing multiple biomarkers and changes in them to guide initiation and duration of therapy. We envision moving to a scenario much akin to venous thromboembolism, where a risk score stratifies pretest probability formally and then biomarkers with high sensitivity are measured and followed by an assessment of biomarkers with enhanced specificity—so as to decrease the number of patients unnecessarily exposed to antifungal therapy.

For the management of patients with invasive candidiasis, future studies may specifically address the optimal diagnostic and therapeutic strategies for patients with deep-seated abdominal candidiasis or *Candida* peritonitis. These patients are currently often underdiagnosed; while it is assumed that echinocandins constitute the optimal treatment regimen, specific studies on treatment of abdominal candidiasis are lacking. Second, better knowledge of PK of antifungal molecules and tissue penetration is a key issue for intensivists. The optimal dosing schedule for echinocandins in ICU patients in not known. Given the inter-individual variability observed in ICU patients, the role of TDM should be studied further. Third, identification of immunogenetics-based risk profiles may identify patients at highest risk for invasive candidiasis, leading to personalized antifungal prophylaxis or treatment. Finally, new antifungal drugs are currently under development. At least three compounds are under development: a long-acting echinocandin (CD101, Cidara) and two entirely new antifungal classes (SCY078, Scynexis, and APX001, Amplyx) will enter phase 2/3 candidemia/invasive candidiasis trials shortly. These new drugs and new personalized antifungal strategies may contribute to optimal management and lower mortality rates among patients with candidemia or invasive candidiasis.

Invasive aspergillosis

During the last decade significant progress regarding epidemiology, diagnosis, and treatment of IPA has been recorded. However, further investigation is needed for topics including (a) the incidence of IPA in the ICU, (b) the relationship between IPA and influenza outbreaks, (c) the clinical impact of the *Aspergillus* LFD and SeptiFast assay in the rapid diagnosis, (d) the significance of combination treatment (patients' outcome, impact of PK/PD profile of combined antifungal molecules), and (e) the extrapulmonary manifestation of aspergillosis (mediastinitis after cardiac surgery, pericarditis/endocarditis).

Conclusions

As a result of epidemiologic changes, the continuing evolution of pathogens and new patient groups at risk, infectious diseases scientists and physicians can never be fully satisfied with the tools at hand. However, during recent years, the scientific community made significant progress in epidemiology, diagnosis, and treatment of IFI. This is

an excellent foundation on which to build the next steps of improvement. Further investigation aims to reduce the still high morbidity and mortality of IFI, and to raise awareness of IFI among clinicians.

Combining clinical and microbiological expertise, we recommend the following array of research topics for the agenda of the immediate decade (Table 4).

A worldwide sentinel network needs to collect data on emerging pathogens, changing susceptibility patterns, and IFI-attributable mortality in real time. Studies on accelerated diagnosis would allow earlier targeted treatment and should emphasize molecular characterization of cultured pathogens and ideally the direct application of molecular methods to clinical specimens. The randomized comparison of experimental treatment strategy integrating clinical stratification and old and new biomarkers with current treatment standard would provide valuable strategic insights. At the same time studies on biomarkers should guide clinical decision-making on the fundamental issues of when to start and when to stop antifungal treatment. A less well-studied clinical issue is the various forms of deep-organ IFI, ranging from bone and joint to intra-abdominal infection. In critically ill patients, studies on antifungal PK, tissue penetration, and optimal dosing schedules would elucidate the role of TDM. Finally, studies on immunogenetic risk profiles could eventually lead to personalized antifungal strategies.

Researching the above topics will advance our knowledge of IFI and will allow decisive development against fungal morbidity and mortality in critically ill patients. Current industrial drug development plans may address some of the agenda items, but the vast majority goes beyond the interest and capability of the pharmaceutical industry and requires action of public funding bodies.

Abbreviations

BDG: β-1 → 3-D-glucan; CAGTA: *Candida albicans* germ tube antibody; CCPA: Chronic cavitary pulmonary aspergillosis; CFPA: Chronic fibrosing pulmonary aspergillosis; CPA: Chronic pulmonary aspergillosis; ESAT: Empirical systemic antifungal treatment; GM: Galactomannan; HSCT: Halogenic stem cell transplant; IC: Invasive candidiasis; IFI: Invasive fungal infections; IPA: Invasive pulmonary aspergillosis; LAmb: Liposomal amphotericin B; LFD: Lateral flow device; MALDI-TOF MS: Matrix-assisted laser desorption ionization–time of flight mass spectrometry; PCT: Procalcitonin; TDM: Therapeutic drug monitoring.

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Compliance with ethical standards

Conflicts of interests

No non-financial conflicts of interest exist for any of the authors.

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