

# Invasive candidiasis

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**Abstract** | Invasive candidiasis is an important health-care-associated fungal infection that can be caused by several *Candida* spp.; the most common species is *Candida albicans*, but the prevalence of these organisms varies considerably depending on geographical location. The spectrum of disease of invasive candidiasis ranges from minimally symptomatic candidaemia to fulminant sepsis with an associated mortality exceeding 70%. *Candida* spp. are common commensal organisms in the skin and gut microbiota, and disruptions in the cutaneous and gastrointestinal barriers (for example, owing to gastrointestinal perforation) promote invasive disease. A deeper understanding of specific *Candida* spp. virulence factors, host immune response and host susceptibility at the genetic level has led to key insights into the development of early intervention strategies and vaccine candidates. The early diagnosis of invasive candidiasis is challenging but key to the effective management, and the development of rapid molecular diagnostics could improve the ability to intervene rapidly and potentially reduce mortality. First-line drugs, including echinocandins and azoles, are effective, but the emergence of antifungal resistance, especially among *Candida glabrata*, is a matter of concern and underscores the need to administer antifungal medications in a judicious manner, avoiding overuse when possible. A newly described pathogen, *Candida auris*, is an emerging multidrug-resistant organism that poses a global threat.

Candidiasis is a broad term that refers to cutaneous, mucosal and deep-seated organ infections caused by fungi of the *Candida* genus, which can occur at any age and usually occur in the setting of easily identifiable risk factors for infection. Invasive candidiasis refers to bloodstream infections with *Candida* spp. (that is, candidaemia) and deep-seated infection — such as intra-abdominal abscess, peritonitis (inflammation of the peritoneum, the tissue that covers the inner wall of the abdomen and abdominal organs) or osteomyelitis (infection of the bones) — with or without candidaemia (FIG. 1).

Invasive candidiasis is an emerging infection closely linked to advances in medical technology and is widely recognized as a major cause of morbidity and mortality in the health-care environment<sup>1–6</sup>. At least 15 distinct *Candida* spp. can cause human disease, but the majority of invasive infections are caused by five pathogens: *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Candida krusei*<sup>2–5</sup>. In certain parts of the world, a previously rare organism, *Candida auris*, has emerged as a major pathogen<sup>7–9</sup> (BOX 1).

*Candida* spp. colonization is regarded as a prerequisite for subsequent infection. *Candida* spp. are commensal yeasts that are part of the normal human skin and gut microbiota, and they are detectable in up to 60% of healthy individuals<sup>2,4</sup>; thus, invasive disease

is usually a consequence of increased or abnormal colonization together with a local or generalized defect in host defences. Invasive candidiasis is not a single clinical entity but rather is a disorder with myriad clinical manifestations that potentially affect any organ, as each *Candida* sp. possesses its own unique characteristics relative to invasive potential, virulence and antifungal susceptibility. Overall, *C. albicans* is the most common pathogen in most clinical settings, but non-*albicans* *Candida* spp. collectively could represent >50% of the bloodstream isolates in certain regions<sup>2–5</sup>.

Key challenges to the management of candidaemia and invasive candidiasis include prevention, early recognition and rapid initiation of appropriate systemic antifungal therapy. Owing to the paucity of rapid diagnostic assays for invasive candidiasis, most clinicians rely on routine fungal cultures (which have low sensitivity) and empirical evidence (for example, unexplained fever or sepsis in a patient in the intensive care unit (ICU) with prior exposure to antibacterial agents, a central venous catheter and recent abdominal surgery) to establish a diagnosis. This approach can lead to the unnecessary use of antifungals in individuals without invasive candidiasis and also leads to delays in intervention with effective antifungal therapy for those who are infected. These delays in diagnosis and intervention could lead to much worse clinical outcomes.

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Antifungal resistance is an emerging problem worldwide, and this further complicates the selection of appropriate antifungal therapy. *Candida* spp. strains that are resistant to first-line antifungals (such as echinocandins and fluconazole) are increasingly being recognized, and their appearance usually correlates with high azole and/or echinocandin background usage in hospitals or specific hospital units<sup>10–14</sup>. The term multidrug-resistant (MDR) *Candida* sp. is used to designate *Candida* spp. strains that are resistant to two antifungal drug classes, whereas the term extensively drug-resistant (XDR) *Candida* sp. can be used to designate *Candida* spp. strains that are resistant to  $\geq 3$  antifungal drug classes.

In this Primer, we discuss invasive candidiasis in adult patients. Candidiasis in neonates and other paediatric populations and cutaneous and mucosal *Candida* spp. infections (such as intertrigo (inflammation of body folds), oropharyngeal candidiasis and candidal vaginitis) are not reviewed here.

## Epidemiology

### The fungi

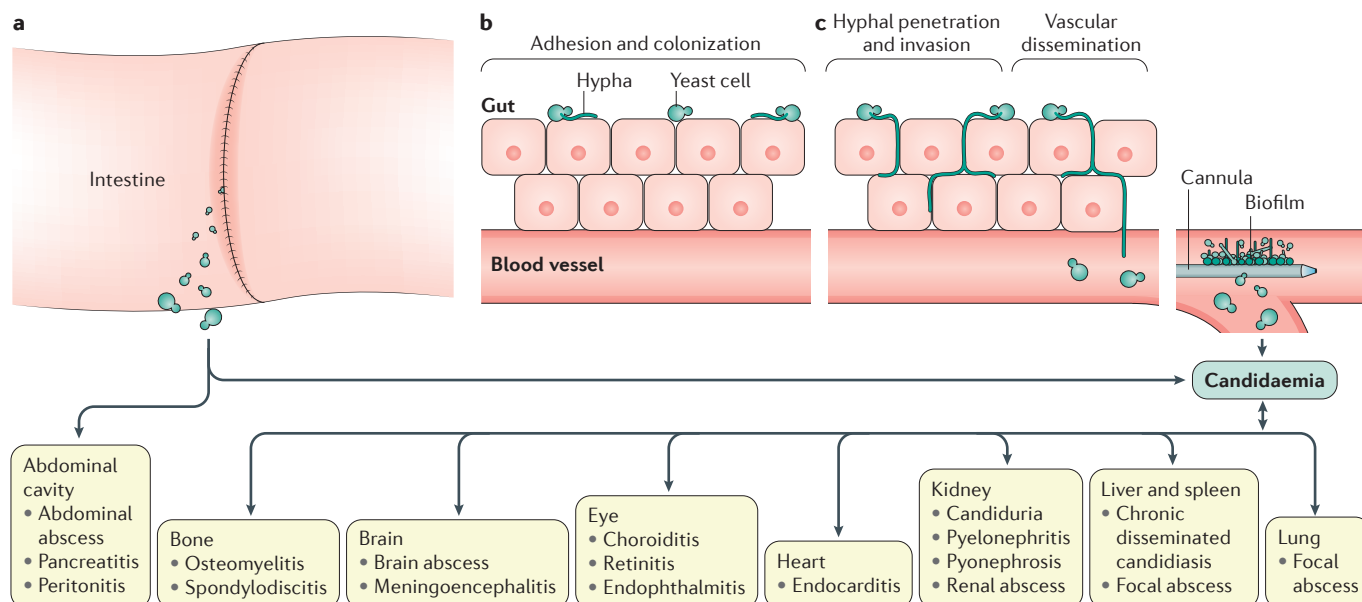
There is substantial geographical, centre-to-centre and unit-to-unit variability in the prevalence of *Candida* spp. (FIG. 2). *C. albicans* continues to be the most prevalent *Candida* sp. causing disease in both adult and paediatric populations, but the past decades have witnessed an increase in diagnoses of non-*albicans* candidaemia in both groups<sup>10,11</sup>. The prevalence of non-*albicans* *Candida* spp. in most regions is usually determined by factors such as background antifungal usage patterns in the region, individual patient risk factors and clonal outbreaks, that is, outbreaks involving a *Candida* spp. molecular strain that is unique in a health-care environment. In the United States and northwestern Europe, the second most common species is generally *C. glabrata* in the non-outbreak setting<sup>10,11</sup>. *C. glabrata* is also generally more-common among individuals of >60 years of age and among recipients of solid organ transplant<sup>2,6</sup>. In Latin America, Southern Europe, India and Pakistan, *C. parapsilosis* and/or *C. tropicalis* are much more frequently encountered than *C. glabrata*. *C. krusei* is the least common of the five

major *Candida* spp., and it is most often found among patients with underlying haematological malignancies who have received antifungal prophylaxis with fluconazole<sup>10,11</sup>. A very worrisome global trend is the emergence of *C. auris*<sup>7–9</sup> (BOX 1).

### The disease

In many developed countries, *Candida* spp. rank in the top three or four pathogens causing health-care-associated bloodstream infections, which also include *Staphylococcus aureus*, coagulase-negative staphylococci and *Enterococcus* spp.<sup>2,4</sup>. In the United States, *Candida* spp. have been reported to cause up to 22% of health-care-associated bloodstream infections based on data from the National Health and Safety Network; of note, this was a highly selected patient population comprising only patients receiving antimicrobials<sup>1</sup>. In a 2009 point prevalence survey of ICUs from around the world, *Candida* spp. accounted for 18% of infections reported<sup>15</sup>. Approximately 50% of episodes of candidaemia occur in the ICU setting, reflecting the complexity of illness usually associated with this infection. There are well-described risk factors associated with invasive candidiasis that apply to all hospitalized persons but especially to those in the ICU<sup>16,17</sup>. Some risk factors are intrinsic to the host or the disease state, whereas others are the result of iatrogenic interventions. The most common individual risk factors include the presence of an indwelling central venous catheter, exposure to broad-spectrum antibacterial agents, long-term ICU stay with or without assisted ventilation, recent major surgery, necrotizing pancreatitis, any type of dialysis, total parenteral nutrition and iatrogenic immunosuppression (BOX 2).

**Disease burden.** The incidence of candidaemia varies with geographical region, local epidemiology, age and other factors. Most large national surveys report an incidence of 3–5 per 100,000 persons in the general population and 1–2% of all medical and surgical ICU admissions<sup>2,6</sup>. Community-acquired candidaemia (that is, acquired in the community rather than in the hospital) is a somewhat new observation in the United States and other developed countries and reflects the increased utilization of long-term intravenous access devices (for example, peripherally inserted central catheters and tunneled intravascular catheters) and parenteral outpatient antimicrobial therapy<sup>6,18</sup>. The attributable mortality among all patients with candidaemia has been reported to be between 10% and 47%, but a more-accurate estimate is probably 10–20%, with the risk of death being closely related to increasing age, higher Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, the infecting *Candida* sp. (for example, *C. parapsilosis* is less virulent than other *Candida* spp. and is generally associated with lower all-cause mortality), the use of immunosuppressive agents, pre-existing renal dysfunction and other comorbidities, venous catheter retention and specific antifungal treatment<sup>2,4,18</sup>. The attributable cost of candidaemia is reported to be ~US\$40,000 per patient<sup>18</sup>.



**Figure 1 | Pathogenesis of invasive candidiasis.** *Candida* spp. can be detected on the mucosal surfaces of ~50–70% of healthy humans. **a** | When breaches in the intestinal barriers occur, for example, after gastrointestinal surgery, *Candida* spp. can disseminate to the abdominal cavity directly and invade the bloodstream (candidaemia). **b** | Under normal conditions, the fungus behaves as a commensal organism without causing disease. **c** | Impairment of immune response, among other factors, can promote fungal overgrowth in the gut and candidaemia, which can lead to deep-seated opportunistic infections in various organs (invasive candidiasis).

### Mechanisms/pathophysiology

As invasive candidiasis is most often caused by *C. albicans*<sup>2,4</sup>, this section will focus on this species, which has been the most extensively studied *Candida* sp. to date. However, the emergence of non-*albicans* *Candida* spp. has led to increasing studies of host–pathogen interactions in some of these species, predominantly *C. glabrata* and *C. parapsilosis*. Although common features of host immunity against these species and *C. albicans* have been reported<sup>19–20</sup>, additional studies are required to define the species-specific differences in cellular and molecular factors of protective immunity in these emerging *Candida* spp.

When perturbations of mucosal microbiota and/or weakening of host immunity occur, *Candida* spp. transition from commensalism to opportunism, which is associated with the induction of key virulence factors<sup>4,21–23</sup>. Specifically, there are three major conditions that predispose to human invasive infection. The first is long-term and/or repeated use of broad-spectrum antibiotics, which enable increased *Candida* spp. gut colonization. In fact, antibiotics confer *Candida* spp. a selective advantage over bacteria because commensal gut microbiota species are important in inducing the release of anti-*Candida* spp. protective factors from the mucosa; thus, depletion of these microbiota species by antibiotics removes these protective factors and enables *Candida* spp. overgrowth<sup>2,4,23</sup>. Another predisposing factor is breach of the gastrointestinal and cutaneous barriers by cytotoxic chemotherapy-induced mucositis (inflammation of the mucosa of the gastrointestinal canal), gastrointestinal surgery or perforation and/or central venous catheters, which collectively enable commensal

*Candida* spp. to translocate from mucocutaneous sites into the bloodstream (FIG. 1). The third factor is iatrogenic immunosuppression, such as chemotherapy-induced neutropenia or corticosteroid therapy, which impairs innate immune defences in tissues and thereby facilitates *Candida* spp. invasion from the bloodstream<sup>2,4</sup> into organs such as the liver, spleen, kidneys, heart and brain. In contrast to mucosal candidiasis, in which T lymphocytes of the T helper 17 (T<sub>H</sub>17) cell differentiation programme are crucial for host defence, effective immunity during invasive *Candida* spp. infection relies on myeloid phagocytes (neutrophils and mononuclear phagocytes, that is, monocytes, macrophages and dendritic cells (DCs)), not lymphocytes<sup>22,24–26</sup>.

### The fungus

Several *C. albicans* virulence traits are integral to its ability to cause opportunistic infections and represent promising targets for vaccine and antifungal drug development. First, the ability of *C. albicans* to filament and interchange its morphotypes between unicellular yeast cells and pseudohyphae and hyphae (that is, multicellular, filamentous forms) critically promotes invasive disease, as strains locked in either the yeast or the filamentous form exhibit impaired virulence in mice<sup>27,28</sup>. Of note, this morphogenic transition is not required for virulence in *C. glabrata*, an important emerging cause of human invasive candidiasis despite its intrinsic inability to form hyphae<sup>2,4</sup>. The versatility of *C. albicans* to undergo morphogenic changes in response to various environmental factors also has important implications for immune evasion, because the differential composition and exposure of surface components (including

mannoproteins (glycoprotein-containing mannose), glucans and chitin (a *N*-acetylglucosamine polymer that is a major component of the cell wall)) in different *Candida* spp. morphotypes affect fungal sensing by the host innate immune system<sup>21,22,29–31</sup>. There are clinical strain-specific differences in fungal immune evasion and virulence<sup>30</sup>.

Second, *C. albicans* secretes a variety of factors in the context of invasive infection, including secreted aspartyl proteases and phospholipases that activate the innate immune response but are also important for promoting fungal tissue invasion and organ damage<sup>32,33</sup>. Candidalysin is a *Candida* spp. cytolytic peptide toxin<sup>34</sup> that induces epithelial cell damage and activates danger signals during mucosal infection; however, its role in promoting the invasion of colonizing *Candida* spp. from the mucosa into the bloodstream and subsequent development of deep-seated infection is not well defined.

Third, effective adherence and invasion of *Candida* spp. in endothelial and epithelial cells enable their dissemination into the bloodstream<sup>35–37</sup>. Among the fungal proteins that mediate adherence, agglutinin-like protein 3 (encoded by *als3*), a *C. albicans*-specific adhesin and invasin, was targeted in the development of a fungal vaccine candidate in humans, which has now been successfully tested in a phase II clinical trial<sup>38</sup>. The capacity of *Candida* spp. for effective adherence also facilitates biofilm formation on implanted medical devices such as central venous catheters, which represents a major source of long-term candidaemia<sup>2,4,39</sup> (FIG. 1). Much work has characterized the fungal regulatory circuits crucial for biofilm formation, which include transcriptional regulators and downstream target genes involved in adherence and filamentation<sup>39,40</sup>.

**Drug resistance.** Resistance in *Candida* spp. is either intrinsic (that is, found in all isolates within a species, such as fluconazole resistance in *C. krusei*) or acquired (that is, found in an isolate from a species that is normally susceptible, such as echinocandin resistance in *C. glabrata*). Echinocandin resistance in *Candida* spp. is emerging, particularly in *C. glabrata*. The target for the echinocandins is the  $\beta$ -D-glucan synthase enzyme, which is important for the cell wall synthesis. This enzyme is encoded by *FKS1* in all *Candida* spp.

and is also encoded by *FKS2* in *C. glabrata*. Mutations in two hotspot regions (HS1 and HS2) of these genes have been identified as the underlying mechanism for echinocandin resistance, where the level of resistance is dependent on the position of the mutated codon (and the corresponding amino acid), the specific amino acid alteration (which amino acid replaces the original one) and in which species the mutation occurs<sup>13,41,42</sup>.

Azoles target the enzyme encoded by *ERG11*. Acquired azole resistance is less common, and from a mechanistic point of view, it is often more complex, as several mechanisms including *ERG11* mutations, upregulation of the target protein production and efflux pumps often play in concert<sup>43</sup>. Although multi-drug resistance is a rare phenomenon<sup>44</sup>, mutations in *MSH2*, which encodes a DNA mismatch repair protein, in *C. glabrata* have been found in clinical isolates and have been demonstrated to facilitate selection of resistance to azoles, echinocandins and polyenes *in vitro*<sup>45</sup>. This mechanism may explain in part why echinocandin resistance in *C. glabrata* has been significantly linked to prior echinocandin and also fluconazole exposure or resistance, despite the fact that azoles and echinocandins have completely different targets in the cell (OR 3.6, 95% CI 2.0–6.4)<sup>12,46</sup>.

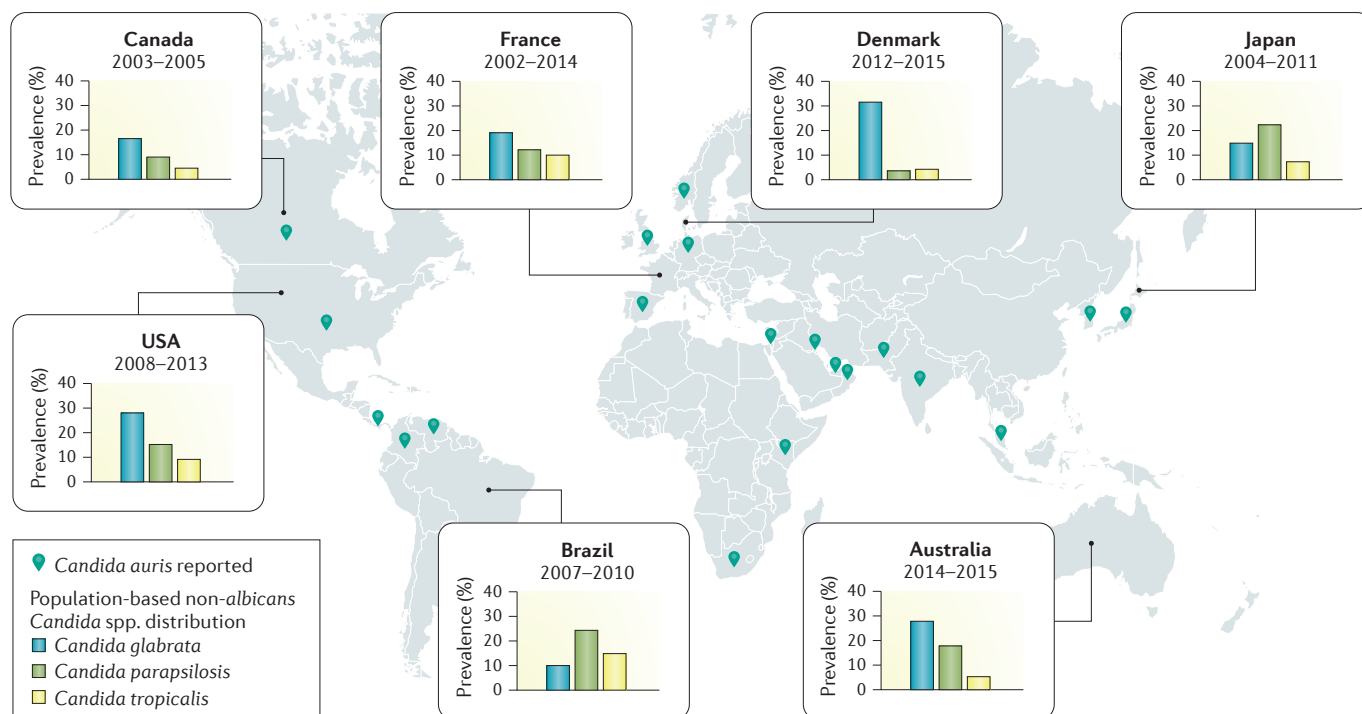
### The host response

***Candida* spp. recognition.** The first step in developing anti-*Candida* spp. immune responses is recognition of extracellular or intracellular pathogen-associated molecular patterns (PAMPs) of invading *Candida* spp. yeast and hyphae by various families of soluble and membrane-bound pattern recognition receptors (PRRs)<sup>21,22,29</sup>. These PRRs are predominantly expressed by myeloid phagocytes and include Toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain (NOD)-like receptors, retinoid-inducible gene 1 protein (RIG1, encoded by *RARRES3*)-like receptors and complement components and receptors<sup>47–53</sup> (FIG. 3). *Candida* spp. also activate the NLRP3 (NACHT, LRR and PYD domains-containing protein 3) and NLRP10 inflammasomes, which are multiprotein intracellular complexes that activate inflammatory responses. NLRP3 and NLRP10 activation results in the production of protective pro-inflammatory cytokines such as IL-1 $\beta$ , IFN $\gamma$  and IL-17 (REFS 54–56). Besides *Candida* spp. sensing by individual PRRs, synergistic interactions between TLR2 and DC-associated C-type lectin 1 (dectin 1, encoded by *CLEC7A*), galectin 3 or complement C5 anaphylatoxin (C5a), between TLR4 and C5a and between dectin 2 (encoded by *CLEC6A*) and dectin 1 or dectin 3 (encoded by *CLEC4D*) also occur, which broaden PAMP recognition and augment or curtail downstream responses<sup>22,29,48,57–60</sup>. Collectively, *Candida* spp. sensing by individual and/or synergistic PRRs initiates complex signalling cascades<sup>47–49,61–64</sup> that mediate the production of pro-inflammatory cytokines and chemokines; promote the recruitment, phagocytosis, generation of reactive oxygen species (ROS) and killing capacity of the phagocytes; and shape the activation of T<sub>H</sub> cell responses<sup>22,65,66</sup>.

#### Box 1 | *Candida auris*

*Candida auris*, a pathogen originally described in Japan in 2009 that has reduced susceptibility to the major antifungal classes, is emerging as a cause of nosocomial outbreaks with rapid spread and high mortality<sup>7–9</sup>. *C. auris* was fluconazole resistant in 93% of patients; in 35% of patients *C. auris* was resistant to amphotericin B, in 7% was resistant to echinocandins, in 41% was multidrug resistant and in 4% was extensively drug resistant<sup>8</sup>. Outbreaks of *C. auris* have been reported in Asia (Japan, India and Pakistan), the United Kingdom, Spain, Colombia, Venezuela, Panama and the United States, and the list of countries continues to expand<sup>7–9</sup>.





**Figure 2 | Geographical variations in the distribution of *Candida* species.** Globally, *Candida albicans* is the most prevalent species associated with invasive candidiasis; however, the distribution of non-*albicans* *Candida* spp. varies greatly, as exemplified in the representative countries shown. Of note, the species distribution may have changed since the data were collected. An increasing number of countries have reported cases of *Candida auris* infection. Data presented are from Australia, Brazil, Canada, Denmark, France, Japan and the United States<sup>6,200–205</sup>. Data on *C. auris* are from the [Centers for Disease Control and Prevention](#) (last accessed 27 March 2018).

Inborn errors of immunity (also known as primary immunodeficiencies) in various PRRs and downstream adaptor genes have shed light on the differential contributions of these pathways in host defence against human invasive candidiasis; undoubtedly, the most crucial PRR signalling cascade for human anti-*Candida* spp. immunity is that downstream of CLRs. Defects in the TLR and complement signalling pathways do not severely compromise the host's response to invading *Candida* spp. Indeed, patients with *MYD88* (which encodes myeloid differentiation primary response protein MYD88) mutations, who lack TLR signalling and IL-1 $\beta$  receptor signalling, which is also dependent on MYD88, develop pyogenic bacterial infections but no invasive candidiasis<sup>67</sup>. Similarly, patients with genetic or acquired deficiencies in early complement components are primarily susceptible to invasive bacterial disease<sup>68</sup>. Conversely, patients with an autosomal recessive deficiency in the CLR pathway adaptor protein caspase recruitment domain-containing protein 9 (CARD9) have selectively heightened susceptibility to fungal infections (specifically, invasive candidiasis, chronic mucocutaneous candidiasis, extrapulmonary aspergillosis and phaeohyphomycosis) but not to bacterial or viral disease, which is consistent with the CLR–CARD9 pathway being activated by *Candida* spp. and other fungal PAMPs<sup>65,66,69</sup>.

Strikingly, in individuals with CARD9 deficiency, invasive candidiasis typically affects the central nervous

system (CNS) (although little is known on the mechanisms that enable *Candida* spp. to cross the blood–brain barrier) but not the kidney, liver or spleen<sup>65,66,69,70</sup>. The aetiology for the CNS tropism of invasive candidiasis in CARD9 deficiency can be explained by the fact that resident glial cells and myeloid cells recruited to the *Candida* spp.-infected CNS require CARD9 to generate the chemoattractants that mediate neutrophil recruitment<sup>70</sup>. Of note, patients with null mutations in *CLEC7A* develop mucocutaneous but not invasive candidiasis, indicating that other CLRs upstream of CARD9 compensate for the lack of dectin 1 signalling in humans<sup>71</sup>.

**Neutrophils.** Neutrophils are essential for effective immunity during invasive candidiasis in mice, and neutropenia is a well-established risk factor for development of the infection and mortality in humans<sup>22,24,72,73</sup>. Neutrophil recruitment to the site of infection is of paramount importance for *Candida* spp. clearance, particularly early in the course of the infection, as in a mouse model, neutrophil accumulation is especially protective within the first 24–48 hours after infection<sup>72</sup>. In mouse tissues, such as the liver and spleen, that recruit large numbers of neutrophils within the first 24 hours post-infection, *Candida* spp. growth can be successfully controlled, whereas the delayed trafficking of neutrophils into the *Candida* spp.-infected kidney is associated with ineffective renal host defence<sup>74</sup>.

## Box 2 | Risk factors for invasive candidiasis

## General risk factors

- Intrinsic: colonization with *Candida* spp., diabetes mellitus, gastrointestinal perforation, increased age, pancreatitis, sepsis and severity of illness.
- Iatrogenic: any type of dialysis (especially haemodialysis), broad-spectrum antibiotics, central venous catheter, corticosteroids and other immunosuppressants, gastrointestinal surgery or other major surgery, left ventricular assist device, long-term stay in hospital or intensive care unit, mechanical ventilation and total parenteral nutrition.

## Additional risk factors in patients with compromised immune system

- Intrinsic: graft-versus-host disease, mucositis and profound neutropenia (absolute neutrophil count <500 cells/mm<sup>3</sup>).
- Iatrogenic: solid organ transplant and stem cell transplant.

Assembly of the NADPH oxidase complex at the phagosomal membrane and activation of myeloperoxidase result in ROS generation, which promotes oxidative *Candida* spp. killing<sup>75–77</sup> (FIG. 4). Dectin 1-dependent activation of both the calcineurin pathway and of the exchange factors for RHO-family GTPases proto-oncogene vav (encoded by *Vav1*) and guanine nucleotide exchange factor VAV3 (encoded by *Vav3*) also contributes to ROS formation in mouse neutrophils, indicating that patients treated with calcineurin inhibitors could have impaired anti-*Candida* spp. defences<sup>78,79</sup>. Indeed, NADPH oxidase-deficient and myeloperoxidase-deficient neutrophils from mice and humans exhibit impaired *Candida* spp. killing capacity, and a proportion of patients (<5%) with chronic granulomatous disease or complete myeloperoxidase deficiency (two types of primary immunodeficiency) develop invasive candidiasis<sup>76,77,80</sup>. However, the majority of individuals with deficiency in the oxidative killing machinery never develop invasive candidiasis<sup>25,76,77</sup>, indicating that lack of ROS in human neutrophils can be largely compensated by non-oxidative killing mechanisms, whose importance is emerging<sup>47,81,82</sup>. Studies of patients with primary immunodeficiencies have shed light on the different phagolysosomal mechanisms that are employed by neutrophils for anti-*Candida* spp. killing when *Candida* spp. are opsonized compared with when the fungi are unopsonized<sup>183,84</sup> (FIG. 4).

Although neutrophils are crucial for host defence during invasive candidiasis, their functions that are aimed to control *Candida* spp. infection could come at the cost of immunopathology. In fact, excessive neutrophil accumulation in tissue late in the course of the infection is deleterious in mice, and certain pathways have been identified that may drive neutrophil-mediated immunopathology<sup>72</sup>. Indeed, pathogenetic neutrophil effects can be observed in patients with invasive candidiasis with renal involvement and in a subset of patients with neutropenia who develop invasive candidiasis with hepatosplenic involvement during the time of their neutrophil recovery<sup>85,86</sup>, and administration of corticosteroids has been employed in such situations to ameliorate the immune response-mediated tissue damage. Thus, pharmacological inhibition of these neutrophil-dependent immunopathology pathways

may represent targeted therapeutic strategies in selected patients with invasive candidiasis.

**Mononuclear phagocytes.** In addition to neutrophils, other cell types including monocytes, macrophages and DCs are also important phagocytes for protection during invasive candidiasis<sup>87–89</sup>. Monocytes and tissue-resident macrophages exhibit marked efficiency in internalizing and ensnaring *Candida* spp. yeasts and filamentous forms *in vivo* within the first hours after infection and in producing pro-inflammatory cytokines and chemokines, which recruit and activate neutrophils in infected tissue<sup>87–89</sup> (FIG. 4). Although less potent than neutrophils, monocytes and macrophages possess substantial anti-*Candida* spp. killing capacity<sup>87</sup>, which is accomplished at least in part via peroxynitrite production<sup>47</sup>.

Monocytes and macrophages also confer lymphocyte-independent protection following a subsequent occurrence of invasive candidiasis via epigenetic reprogramming, which results in ‘innate memory’ or ‘trained immunity’, that is, the ability of the innate immune system to mount resistance to reinfection<sup>90</sup> (FIG. 3).

Moreover, CD11b<sup>+</sup>, but not CD103<sup>+</sup>, DCs are indispensable for host defence during invasive candidiasis via the production of key inflammatory mediators, direct fungal killing — which is inferior to that of monocytes and macrophages — and priming of the candidacidal activity of neutrophils through an IL-23–granulocyte-macrophage colony-stimulating factor (GM-CSF) pathway that involves natural killer cells<sup>91–95</sup> (FIG. 4). Indeed, a randomized clinical trial demonstrated that recombinant GM-CSF therapy resulted in decreased mortality from invasive candidiasis in recipients of allogeneic haematopoietic stem cell transplant<sup>96</sup>.

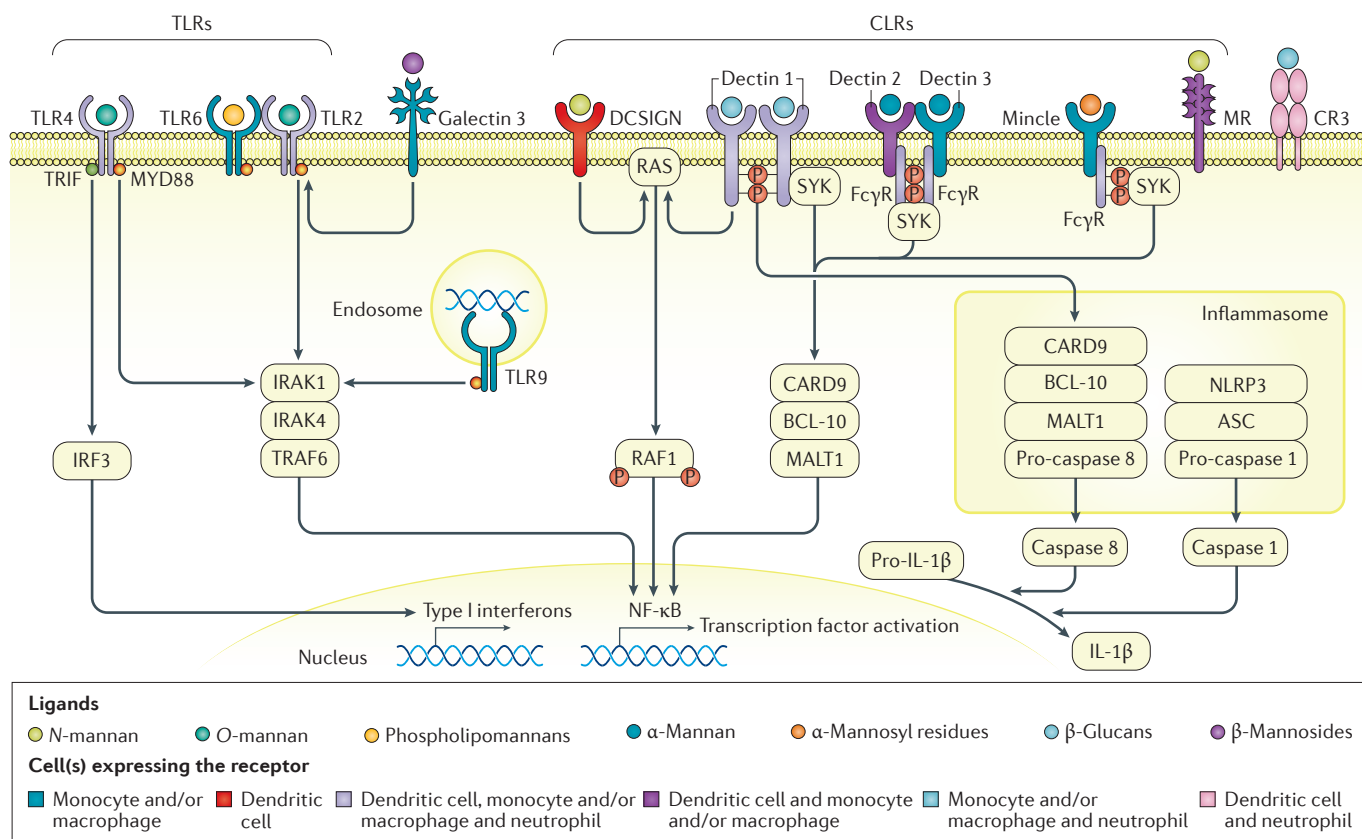
**Immunogenetics for precision medicine**

The majority of patients in the ICU do not develop invasive candidiasis despite sharing similar risk factors, and the clinical outcome of the infection varies substantially among patients with similar clinical and microbiological risk factors. Thus, it is possible that individual genetic variations in key immune-related genes (singularly or in combination) confer heightened susceptibility to or protection from infection<sup>2,4,87,97–101</sup>. Indeed, population studies have demonstrated increases in susceptibility to invasive candidiasis in humans with selected genetic variants involving *IL10*, *IL12B*, *TNF*, *CXCR1*, *CX3CR1*, *STAT1*, *PSMB8*, *SP110*, *CCL8*, *TLR1*, *CD58*, *TAGAP* and *LCE4A-C1orf68* (REFS 81, 87, 97–102).

In the first large prospective cohort of patients with candidaemia, in which patients in the ICU with the same underlying disease but without candidaemia were selected as matched controls, three *TLR1* single-nucleotide polymorphisms (SNPs) were associated with susceptibility to candidaemia<sup>99</sup>. In another study, in patients in the ICU who developed candidaemia the presence of SNPs leading to increased circulating IL-10 and decreased IL-12b was associated with progression of disease and persistent candidaemia despite antifungal therapy<sup>97</sup>. The importance of the balance between

pro-inflammatory and anti-inflammatory cytokines for the ability to clear the infection in humans was further underlined by a systems biology approach that identified the type I interferon pathway as the most prominent transcriptional response specifically activated by *Candida* spp.<sup>100</sup>. In the first genome-wide association study assessing genetic susceptibility to candidaemia, ~119,000 SNPs across 186 loci known to be associated with immune-mediated diseases were analysed in 217 patients with candidaemia, which is the largest cohort to date, and ~12,000 control individuals<sup>98</sup>. This approach identified three novel candidaemia genetic risk factors, mapping to *CD58*, the *LCE4A-C1orf68* locus and *TAGAP*. Carrying two or more risk alleles (variants that

are positively associated with candidaemia) of these loci increases the risk of candidaemia by 19-fold<sup>98</sup>. Significant findings were confirmed in a clinical cohort of patients matched for disease. This 19-fold increased risk of developing candidaemia in individuals carrying specific SNPs suggests that it is feasible to develop screening strategies to identify patients in the ICU who are at risk and could benefit from antifungal prophylaxis. Further delineation of such genetic factors holds promise for devising immunogenetics-based risk stratification and prognostication strategies for identifying patients in whom targeted vaccination, antifungal prophylaxis and/or intensified diagnostic and therapeutic interventions could be beneficial.



**Figure 3 | Recognition of *Candida* spp. by pattern recognition receptors of myeloid phagocytes.** Surface and intracellular pattern recognition receptors, the different myeloid phagocytes that express them and the corresponding *Candida* spp. pathogen-associated molecular patterns (ligands) that they recognize are shown. Cell-surface Toll-like receptor 4 (TLR4), intracellular TLR9 and cell-surface TLR2, which forms heterodimers with cell-surface TLR6, signal through the adaptor protein myeloid differentiation primary response protein MYD88. The C-type lectin receptor (CLR) family of receptors recognize fungal carbohydrates and result in activation of the spleen tyrosine kinase (SYK)–caspase recruitment domain-containing protein 9 (CARD9) axis, formation of the CARD9–B cell lymphoma/leukaemia 10 (BCL-10)–mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) complex and nuclear factor-κB (NF-κB) activation. Among CLRs, dendritic cell-associated C-type lectin 2 (dectin 2) forms heterodimers with dectin 3, and dectin 2 and macrophage-inducible C-type lectin (mincle, encoded by *CLEC4E*) require immunoglobulin-γ Fc region receptor (FcγR) cooperation for signalling. The nucleotide-binding oligomerization domain (NOD)-like

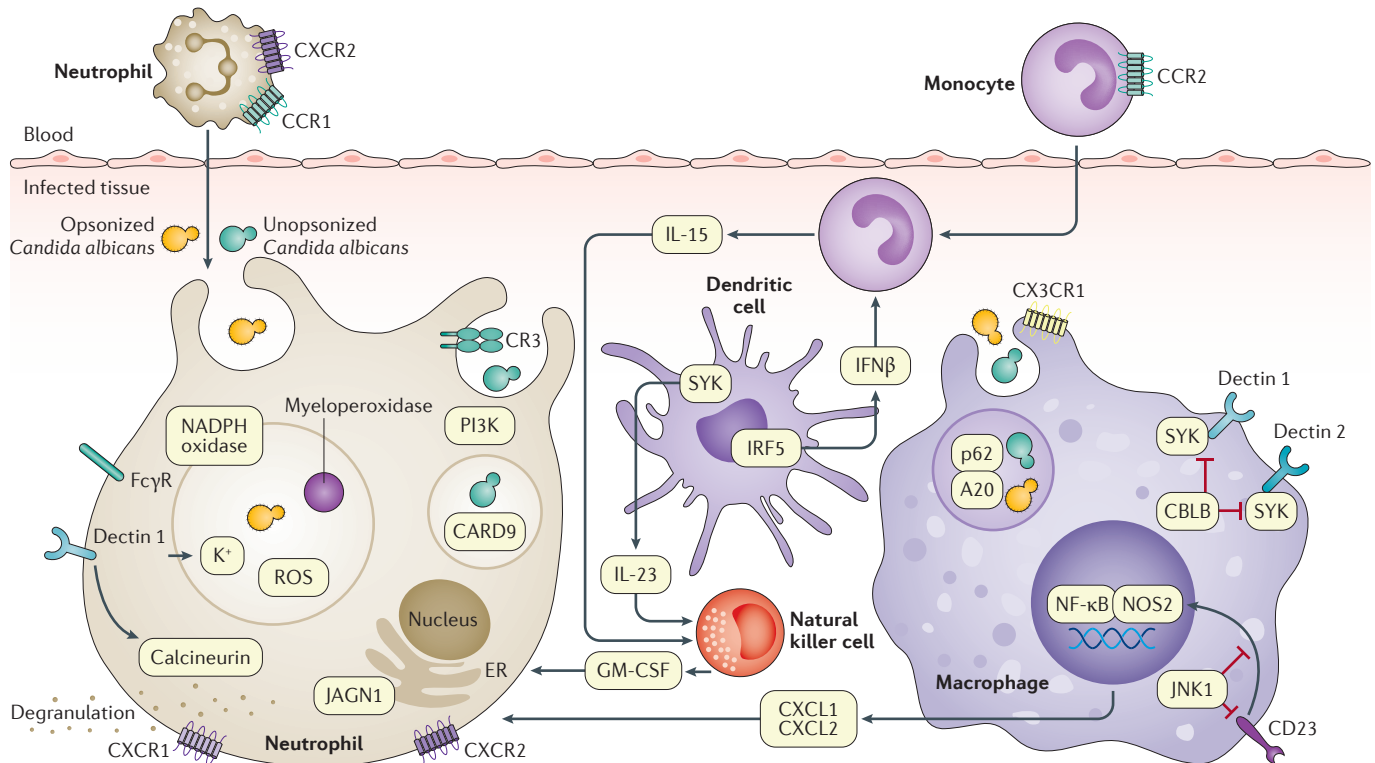
receptor NLRP3 (NACHT, LRR and PYD domains-containing protein 3) forms an inflammasome complex with apoptosis-associated speck-like protein containing a CARD (ASC) and caspase 1 that results in production of IL-1β. In addition, dectin 1 signalling engages the CARD9–BCL-10–MALT1 complex to promote non-canonical inflammasome activation that results in IL-1β production via caspase 8. *Candida* spp. β-glucans induce trained immunity in monocytes and macrophages via the dectin 1–RAF proto-oncogene serine/threonine-protein kinase (RAF1) signalling cascade through a pathway that involves RAC serine/threonine-protein kinase (AKT), hypoxia-inducible factor 1α (HIF1α) and serine/threonine-protein kinase mTOR and through a switch of cell glucose metabolism from oxidative phosphorylation to aerobic glycolysis (not depicted)<sup>206</sup>. CR3, complement receptor 3; DCSIGN, dendritic cell-specific ICAM3-grabbing non-integrin 1 (also known as CD209 antigen); IRAK, IL-1 receptor-associated kinase; IRF3, interferon regulatory factor 3; MR, mannose receptor; RAS, GTPase RAS; TRAF6, tumour necrosis factor receptor-associated factor 6; TRIF, TIR domain-containing adaptor molecule 1 (also known as TICAM1). Adapted from REF. 24.

### Diagnosis, screening and prevention

No clinical signs or symptoms are specific for invasive candidiasis. Thus, invasive candidiasis should be suspected in patients with known risk factors who have an unexplained fever that is unresponsive to antibacterial treatment. Timely diagnosis of invasive candidiasis is key to ensure a favourable outcome<sup>103,104</sup>. In fact, a 1–2-day delay in initiation of effective antifungal therapy has been associated with a doubling of mortality<sup>103,104</sup>. However, diagnosing invasive candidiasis can be challenging. Although the classical microbiological methods (light microscopy and culture) in general enable

detection of *Candida* spp., concomitant presence of bacteria in clinical specimens can outcompete fungal growth. Thus, if invasive candidiasis is suspected, the diagnostic laboratory should be notified to use selective media (containing inhibitors of bacterial growth) and additional specific diagnostic options, such as antigen detection and PCR tests. Combinations of different diagnostic options could facilitate earlier and more-sensitive diagnosis (TABLE 1).

Distant sites of infection are quite uncommon among non-neutropenic patients with candidaemia. An estimated 15% of patients with candidaemia have



**Figure 4 | Effector mechanisms of myeloid phagocytes for control of invading *Candida* spp. in infected tissue.** Neutrophils, which are recruited into tissue via CXC-chemokine receptor 2 (CXCR2) (early phase) and CC-chemokine receptor 1 (CCR1) (late phase), are the myeloid cells with the most potent candidacidal activity and depend on both oxidative and non-oxidative mechanisms to kill *Candida* spp. The NADPH oxidase complex and dendritic cell-associated C-type lectin 1 (dectin 1)–calcineurin activation promote oxidative fungal killing. Differential signalling pathways are engaged during killing of opsonized (NADPH oxidase complex, immunoglobulin-γ Fc region receptors (FcγRs) and protein kinase C) and unopsonized (complement receptor 3 (CR3), caspase recruitment domain-containing protein 9 (CARD9) and phosphoinositide 3-kinase (PI3K)) fungal organisms. Neutrophil extracellular traps are released by neutrophils and contain antimicrobial proteins such as elastase and calprotectin, and they are thought to contribute to extracellular destruction of *Candida* spp. filamentous forms (not depicted). Extracellular damage to large *Candida* spp. filamentous forms is also mediated by neutrophil degranulation, which depends on jagunal homologue 1 (JAGN1) and the chemokine receptor CXCR1. Inflammatory monocytes are recruited to the *Candida* spp.-infected tissue in a CCR2-dependent manner, produce pro-inflammatory cytokines, kill *Candida* spp. and differentiate into inflammatory dendritic cells. CD11b<sup>+</sup> dendritic cells produce type I interferons (including IFNβ) and also secrete IL-23 via spleen tyrosine kinase (SYK) activation; IL-23 acts on natural killer

cells to produce granulocyte–macrophage colony-stimulating factor (GM-CSF), which potentiates the candidacidal activity of neutrophils in the *Candida* spp.-infected tissue. The IL-15 produced by CCR2-expressing inflammatory monocytes also stimulates natural killer cells to release GM-CSF for neutrophil activation. Tissue-resident macrophages effectively internalize *Candida* spp. yeast forms, encircle *Candida* spp. filamentous forms, exert candidacidal activity and produce a variety of pro-inflammatory cytokines and CXC-chemokines via the autophagic adaptor ubiquitin-binding protein p62 (also known as sequestosome 1), which sequesters zinc-finger protein A20 (also known as tumour necrosis factor α-induced protein 3) in the autophagosome. Resident macrophage survival, accumulation in tissue and contact with *Candida* spp. *in vivo* depend on the CX<sub>3</sub>C-chemokine ligand 1 (CX<sub>3</sub>CL1)–CX<sub>3</sub>C-chemokine receptor 1 (CX<sub>3</sub>CR1) axis in both mice and humans<sup>87,207–209</sup>. Inhibition of SYK-dependent dectin 1 and dectin 2 signalling in macrophages by the E3 ubiquitin-protein ligase CBLB impairs macrophage effector functions. Similarly, inhibition of the C-type lectin receptor CD23 signalling by JUN N-terminal kinase 1 (JNK1; also known as mitogen-activated protein kinase 8) results in impaired macrophage production of nitric oxide and defective fungal killing. Thus, CBLB and JNK1 could represent potential therapeutic targets<sup>207,210</sup>. CXCL, CXC-chemokine ligand; ER, endoplasmic reticulum; IRF5, interferon regulatory factor 5; NF-κB, nuclear factor-κB; NOS2, nitric oxide synthase, inducible; ROS, reactive oxygen species.



Table 1 | Specimen types and tests for the diagnosis of invasive candidiasis

Diagnostic test	Specimen(s)	Advantages	Disadvantages
Fungal culture	Blood	<ul style="list-style-type: none"> <li>Enables species identification and subsequent susceptibility testing</li> </ul>	<ul style="list-style-type: none"> <li>Slow (median detection time 2–3 days)</li> <li>Sensitivity suboptimal, particularly if high volume (<math>\geq 60</math> ml) and a fungal blood culture bottle are not employed</li> </ul>
	Tissue and sterile body fluids	<ul style="list-style-type: none"> <li>Enables species identification and subsequent susceptibility testing</li> </ul>	<ul style="list-style-type: none"> <li>Selective media, proper spreading of the sample and 3 days of incubation required for optimal performance</li> </ul>
Microscopy	Cerebrospinal fluid, tissue and sterile body fluids	<ul style="list-style-type: none"> <li>Highly sensitive, particularly if using fluorescent brightener staining</li> </ul>	<ul style="list-style-type: none"> <li>No species identification</li> <li>Lower sensitivity in absence of fluorescent brightener staining</li> </ul>
Histopathology	Tissue and sterile body fluids	<ul style="list-style-type: none"> <li>Enables evaluation of tissue invasion and inflammation</li> </ul>	<ul style="list-style-type: none"> <li>No species identification</li> <li>Lower sensitivity in absence of fluorescent brightener staining</li> </ul>
Mannan antigen and antimannan antibody detection	Serum or plasma (EDTA) or cerebrospinal fluid	<ul style="list-style-type: none"> <li>Increased diagnostic sensitivity when combined antigen and antibody testing is performed (although in neonates (in any sample) and in cerebrospinal fluid, antigen testing suffices)</li> </ul>	<ul style="list-style-type: none"> <li>Heavy colonization (many non-sterile body sites culture positive for <i>Candida</i> spp. and/or with heavy growth in semi-quantitative culture) could cause positivity for blood testing</li> </ul>
$\beta$ -D-glucan detection	Serum or plasma (EDTA)	<ul style="list-style-type: none"> <li>Pan-fungal marker</li> </ul>	<ul style="list-style-type: none"> <li>No separation between <i>Candida</i> spp. and other fungi</li> <li>Many sources for false positivity</li> </ul>
PCR	Blood (EDTA)	<ul style="list-style-type: none"> <li>Rapid tests</li> <li>Some commercial tests are FDA approved</li> </ul>	<ul style="list-style-type: none"> <li>Commercial tests are expensive</li> <li>May not detect all species</li> </ul>

some manifestation of ocular involvement, and 1–2% will develop severe, sight-threatening endophthalmitis (inflammation of the retina, choroid and vitreous humour)<sup>105</sup>. For all patients with candidaemia, a dilated funduscopy examination performed by an experienced clinician is indicated within the first week after initiation of specific antifungal therapy<sup>106,107</sup>. Among patients with candidaemia in the setting of neutropenia, the funduscopy examination should be delayed until neutrophil recovery because the characteristic inflammatory retinal and choroidal changes might not become clinically evident during periods of profound neutropenia<sup>106</sup>. Endocarditis is an uncommon complication of candidaemia, and the routine performance of an echocardiography among patients with candidaemia is not generally recommended unless there are risk factors present for endocarditis such as intravenous drug abuse, pre-existing valvular disease or the presence of a prosthetic cardiac valve.

### Classical diagnostic techniques

Blood culture remains the gold standard for diagnosis of invasive candidiasis, but it is an insensitive tool, as it is positive in 21–71% of patients with autopsy-proven invasive candidiasis depending on sampling frequency and volume of blood drawn<sup>108</sup>. Nonetheless, it is an essential diagnostic test and enables species identification and susceptibility testing<sup>106,108–110</sup>. During an episode of candidaemia, the number of circulating *Candida* spp. cells is usually  $<1$  colony forming unit (CFU) per ml (REF. 111). *Candida* spp. multiply more slowly than bacteria, a fact that partly explains the suboptimal sensitivity and prolonged time to positivity (median of 2 days) for blood cultures<sup>111,112</sup>. Frequent sampling (once daily or more frequently if the patient has a fever), larger blood culture volumes (optimally  $\geq 40$ –60 ml daily), the use of specialized fungal blood culture bottles with selective medium and sampling before initiation of

antifungal therapy increase sensitivity<sup>110,112–116</sup>. For non-candidaemic invasive candidiasis, diagnosis is firmly established based on positive culture for *Candida* spp. and/or positive histopathology from normally sterile sites, such as intra-abdominal and intra-thoracic sites. Tissue and/or cytology specimens from normally sterile sites or newly placed drains (placed within the past 24 h) should undergo microscopy, preferably using staining with a fluorescent brightener, and also be sent for histopathological examination using special staining for fungi (Grocott–Gomori methenamine silver stain, which recognizes carbohydrates in the fungal cell wall, and periodic acid–Schiff, which detects polysaccharides and glycoproteins).

As early as in the mid-1980s, a matched case–control study showed that isolation of *Candida* spp. from sites other than blood was associated with candidaemia (OR 10.37)<sup>117</sup>. Subsequent genotyping studies have confirmed the strong association between the colonizing and invasive *Candida* sp. isolates obtained from the same individual, supporting the concept that invasive infection usually arises from the colonizing microbiota organisms owing to a barrier breach<sup>118,119</sup>. Consequently, cultures from non-sterile body sites yielding the presence of colonizing *Candida* spp. can be informative regarding likelihood of invasive candidiasis<sup>120</sup> and regarding which species is most likely involved; such information can be used to select antifungal therapy in patients in whom the diagnosis is suspected but not confirmed by culture from a normally sterile site. In an attempt to optimize the predictive value of such surveillance cultures from non-sterile sites, studies were undertaken to determine whether the number of body sites and degree of colonization (light, moderate or heavy *Candida* spp. growth) at these body sites were crucial to the development of candidaemia. A colonization index  $\geq 0.5$  (the ratio of the number of *Candida* spp. positive specimens to the number of samples taken) and a corrected colonization

index  $\geq 0.4$  (the colonization index multiplied by the proportion of sites with heavy growth (quantified as +++ from semi-quantitative culture) to the total number of body sites with *Candida* spp.) were developed and associated with invasive candidiasis in some<sup>16,121</sup> but not all studies<sup>122</sup>. However, regular routine surveillance cultures for patients in the ICU who are at risk of invasive candidiasis are labour intensive and not broadly used. A 2015 study examining cultures obtained 72 hours after ICU admission and then twice weekly showed that culture of throat and perineum samples within the first two time points after ICU admission captures 84% (61/73 patients) of patients with subsequent invasive candidiasis, with negative predictive values of 99%<sup>120</sup>, but a randomized empirical treatment study failed to show a correlation between empirical antifungal therapy based on positive colonization cultures at study inclusion (from mouth, throat, upper respiratory tract, skin, urine and digestive tract (rectal swab or faeces) and, if appropriate, drains and catheters) and patient survival in non-neutropenic patients with sepsis and organ failure in the ICU<sup>122</sup>.

### Antigen detection

**Mannan antigen and antimannan antibody.** *Candida* spp. mannan antigen and antimannan antibody detection can be useful for the detection of invasive candidiasis<sup>123–127</sup>, including in paediatric patients and in those with CNS infection<sup>123,128–130</sup>. However, in a 2016 study including patients in the ICU with severe abdominal conditions, the performance of combined mannan antigen and antimannan antibody detection was disappointing (sensitivity 55% and specificity 60%)<sup>131</sup>. Antibodies are often present in patients with compromised immune system with prior candidaemia or heavy colonization; thus, the positive predictive value of detection of antibodies alone in the absence of rising concentration is low<sup>123,126</sup>. This observation and the unexplained variable performance across various studies are important caveats that could lead to inappropriate antifungal treatment of patients who are unlikely to have candidiasis unless the clinicians understand the strengths and limitations of the tests. *Candida* spp. mannan and antimannan antibody detection is not US FDA approved as a diagnostic tool in the United States and is mainly available in Europe.

**$\beta$ -D-glucan.**  $\beta$ -D-glucan is a pan-fungal marker of invasive fungal infection. The antigen can be detected in blood during *Candida* spp., *Aspergillus* spp., other mould (not Mucorales) and *Pneumocystis jirovecii* infections<sup>132,133</sup>. Hence, the test cannot distinguish between candidiasis and infections caused by the other fungi. The sensitivity for invasive candidiasis was generally high (76.7–100.0%) in seven studies from 2011 to 2018, with half of the studies reporting sensitivity above 85.0%. However, the specificity was more-variable (40.0–91.8%)<sup>131,134–139</sup>. Multiple sources for false positivity have been reported, including heavy *Candida* spp. colonization without infection<sup>131</sup>, haemodialysis with cellulose membranes, human blood products (immunoglobulins or albumin), antibiotics (amoxicillin-clavulanate or piperacillin-tazobactam),

serious bacterial infections, surgical sponges and gauzes containing glucan and severe mucositis, which is particularly challenging in the ICU and haematological setting, and these sources often lead to lower specificities<sup>131,134,140</sup>. However, many of these sources are often present in the same patient, making it difficult to dissect which are more-important and whether the risk of contamination is limited to certain types or batches of each of these sources. Nevertheless, the specificity was below 60% in half of the studies, illustrating that false positivity is a recurrent challenge. The negative predictive value has been high in most studies, reflecting a high sensitivity and suggesting that invasive candidiasis, including candidaemia, is unlikely when the  $\beta$ -D-glucan test result is negative.

***C. albicans* germ tube antigen.** Another *Candida* spp.-specific antibody test is the *C. albicans* germ tube antigen (CAGTA) test; the antigen was originally found in *C. albicans*, but the test also detects candidiasis with other *Candida* spp., although it detects some non-*albicans Candida* spp., including *C. parapsilosis*, with lower sensitivity<sup>131,138,141,142</sup>. The experience with this test is more limited than that with *Candida* spp. mannan antigen and antimannan antibody and  $\beta$ -D-glucan tests. The sensitivity ranges from 53.3% to 74.1%, and specificity ranges from 56.5% to 92.0%<sup>131,138,141,142</sup>.

**Combination tests.** As none of the above-mentioned antigen and antibody tests are perfect, several studies have investigated the performance of various combinations of these tests or of the combination of one of these and DNA-based techniques. For example, positive CAGTA antibody and  $\beta$ -D-glucan tests in a single blood sample or  $\beta$ -D-glucan positivity in two consecutive blood samples enabled the discrimination of invasive candidiasis from *Candida* spp. colonization in critically ill patients with severe abdominal conditions<sup>131</sup>. The combination of CAGTA and  $\beta$ -D-glucan or CAGTA and *Candida* spp. mannan antigen had a very high negative predictive value and could be used for discontinuing unnecessary empirical therapy in patients with suspected candidaemia<sup>138</sup>. The drawback of such approaches is increased cost, and if two consecutive positive tests are required, the diagnosis can be delayed, unless the tests are offered on a daily basis. As procalcitonin testing is available on a daily basis in most institutions, the combination of  $\beta$ -D-glucan and procalcitonin testing has been evaluated: the combination of a positive  $\beta$ -D-glucan test with low procalcitonin ( $<2$  ng/ml) had a sensitivity and specificity of 66% and 98% for invasive candidiasis, respectively, whereas if only one of these two criteria was met, the performance parameters were 96% and 60%<sup>139</sup>.

### DNA-based techniques

The development and implementation of PCR-based tests for the detection of fungal infections in daily routine lag behind those for viral and bacterial infections. The main challenges have been efficacious DNA extraction from the fungal cells, low numbers of fungal cells in blood, similarity between fungal and human DNA and

the ubiquity of many fungal pathogens or their DNA in the environment, on skin, in buffers and on utensils leading to false positive results. Thus, the performances reported for locally developed PCR assays vary substantially, and PCR-based tests have not yet been incorporated into official guidelines or classification criteria for *Candida* spp. disease<sup>106,143</sup>.

*Candida* spp. can be detected by various commercially available PCR assays targeting bacterial and fungal pathogens; these assays can be used in patients with sepsis and include the LightCycler SeptiFast Test MGRADE (Roche Molecular Diagnostics, Pleasanton, CA, USA), Sepsitest (Molzym Molecular Diagnostics, Bremen, Germany), the IRIDICA BAC BSI assay (Abbott Diagnostics, Lake Forest, IL, USA), which uses PCR and electrospray ionization mass spectrometry technology, and a test targeting *Candida* spp. and *Aspergillus* spp. using the RenDx Fungiplex assay (Renishaw Diagnostics, Glasgow, United Kingdom)<sup>144–148</sup>. SeptiFast is the most thoroughly evaluated system. Although the numbers of patients with candidaemia are low in all studies, SeptiFast detected more *Candida* spp.-positive specimens than blood culture, especially from patients receiving antifungal therapy, and produced a *Candida* spp. distribution mirroring that found in blood cultures<sup>149–152</sup>. Drawbacks are that the test is labour intensive, that only the five most common *Candida* spp. are included and that the sensitivity for detecting the haploid species *C. glabrata* is lower (100 CFU/ml are required for 100% sensitivity). Nonetheless, these studies support that DNA-based assays for *Candida* spp. can potentially improve timeliness and sensitivity of the diagnosis. In contrast to these broad-spectrum sepsis tests, the T2Candida Panel test (T2 Biosystems, Lexington, MA, USA) is an FDA-approved test for diagnosis of candidaemia specifically. It detects the five most common *Candida* spp. from whole blood and additionally detects *Saccharomyces cerevisiae*, *Candida bracarensis*, *Candida metapsilosis* and *Candida orthopsilosis*. However, the test cannot identify a specific species, and the results fall into one of the three following groups: *C. albicans* or *C. tropicalis*; *C. glabrata*, *C. krusei*, *S. cerevisiae* or *C. bracarensis*; or *C. parapsilosis*, *C. metapsilosis* or *C. orthopsilosis*. The pooled sensitivity and specificity were 91.1% and 99.4%, respectively, in a study combining data from approximately 1,500 clinical samples and 300 artificial positive control samples created with various concentrations of the five most common species<sup>153</sup>. In practice, the sensitivity might be somewhat lower depending on the prevalence of candidaemia caused by species not covered by the T2Candida Panel test. Such species are responsible for ~5% of candidaemia infections according to a nationwide fungemia programme in Denmark but can exceed 20% in selected settings<sup>154–156</sup>; of note, the test does not detect the emerging MDR species *C. auris*. The test seems promising and cost effective in recent studies<sup>156,157</sup>.

### Drug susceptibility testing

Once the diagnosis of invasive candidiasis is confirmed, it is important to establish the susceptibility profile of the identified species. Intrinsic resistance is diagnosed by a

correct species identification, whereas identification of acquired resistance of the specific isolate requires correct susceptibility testing and application of clinical breakpoints. Clinical breakpoints are used for categorizing the susceptibility test result as susceptible, intermediate or resistant and for everyday use in the clinical laboratory to advise on patient therapy.

The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) have developed reference standards for antifungal susceptibility testing with associated clinical breakpoints for most common species–compound combinations<sup>158–161</sup>. Both tests are microbroth dilution tests, where the ability of the *Candida* sp. isolate to grow in serial dilutions of the antifungal agent is investigated. The lowest concentration that causes prominent or at least 50% growth inhibition compared with an uninhibited control is the test result, the MIC (minimum inhibitory concentration). These are excellent methodologies for identifying resistant isolates, but they are not user friendly in the context of the routine clinical microbiology laboratory. A range of other commercial susceptibility tests is available, and none is superior across all organism–antifungal combinations, emphasizing that local validation and experience are important<sup>162–165</sup> (TABLE 2). The most common are agar diffusion tests (disk and gradient strips), commercial microbroth dilution tests or the semi-automated system VITEK (bioMérieux, Marcy l'Etoile, France).

### Prevention

**Antifungal prophylaxis and pre-emptive therapy.** Most published data suggest that antifungal prophylaxis with fluconazole reduces the incidence of invasive candidiasis in patients in the ICU who are at high risk of developing the infection, but reduction of mortality has not been convincingly reported<sup>166,167</sup>. Of note, antifungal prophylaxis could theoretically favour the emergence of acquired resistance. Data supporting the use of targeted prophylaxis among patients with certain risk factors of invasive candidiasis are conceptually promising, but the results of clinical trials have been unconvincing<sup>168</sup>. Stronger data support the use of targeted antifungal prophylaxis in selected high-risk recipients of liver, pancreas, small bowel or haematopoietic stem cell transplant<sup>106</sup>. Much less clear is the value of pre-emptive therapy (that is, based on positive biomarkers, such as  $\beta$ -D-glucan, during patient monitoring at frequent intervals before blood or tissue culture yield positive results) and of empiric antifungal therapy among patients at high risk of invasive candidiasis with unexplained symptoms but without clear mycological evidence of disease. Several studies have attempted to define a clear role for antifungals in this setting but have failed to do so convincingly<sup>121,122,169–171</sup>.

### Infection prevention and hospital epidemiology.

Practical measures to prevent infection with *Candida* spp. include judicious use of antibacterials and mitigation of some of the preventable risk factors mentioned previously<sup>16–18,172–175</sup>. *Candida* spp. (and other yeasts) can

Table 2 | Overview of various available antifungal susceptibility tests

Test (method)	End point reading	Resistance detection			Advantages	Disadvantages
		Echinocandins	Azoles	Amphotericin B		
Reference tests						
EUCAST*¶ (microbroth dilution)	Automated	Easy	Easy	Easy	<ul style="list-style-type: none"><li>• Pattern for the most resistant organism reported if mixed cultures are tested</li><li>• Growth inhibition can be quantified in per cent of growth control by a spectrophotometer</li><li>• Objective end point reading</li></ul>	<ul style="list-style-type: none"><li>• Not all antifungal compounds are commercially available</li><li>• Most laboratories are not familiar with plate production and test principle</li><li>• Requires an ELISA reader</li><li>• The amphotericin B MICs tend to cluster in a narrow concentration range (which may lead to difficulties in correctly separating susceptible from resistant strains)</li></ul>
CLSI†¶ (microbroth dilution)	Visual (50% or 100% growth inhibition)	Easy	Difficult for isolates displaying trailing growth, which is defined as only partial growth inhibition over a wide concentration range	Easy	<ul style="list-style-type: none"><li>• Pattern for the most resistant organism reported if mixed cultures are tested</li><li>• Growth inhibition can be quantified in per cent of growth control by a spectrophotometer</li></ul>	<ul style="list-style-type: none"><li>• Not all antifungal compounds are commercially available</li><li>• Most laboratories are not familiar with plate production and test principle</li><li>• Subjectivity in end point reading is inevitable</li><li>• The amphotericin B MICs tend to cluster in a narrow concentration range</li></ul>
Other available tests						
Etest‡# (gradient strip agar diffusion)	Visual (growth)	Easy, but recommended CLSI breakpoint for caspofungin bisects <i>Candida glabrata</i> and <i>Candida krusei</i> (that is, it leads to a random classification of the pathogens as susceptible, intermediate and resistant)	Difficult, potentially leading to misclassifications of resistant isolates	Easy, but recommended breakpoint bisects <i>Candida krusei</i>	<ul style="list-style-type: none"><li>• Potentially the best test for the detection of amphotericin B resistance</li><li>• Most laboratories are familiar with the principle</li></ul>	<ul style="list-style-type: none"><li>• MIC ranges do not mirror those of the reference methods for all species and compounds, which leads to misclassifications when reference breakpoints are adopted</li><li>• Pattern for the most susceptible organism reported if mixed cultures are tested</li><li>• Subjectivity in end point reading is inevitable</li></ul>
Disk and/or tablet diffusion§**	Visual (growth)	Easy, but laboratory-to-laboratory variation prohibited establishment of meaningful CLSI disk breakpoints	Difficult, potentially leading to misclassifications of resistant isolates	Difficult, small inhibition zones	<ul style="list-style-type: none"><li>• Most laboratories are familiar with the principle</li></ul>	<ul style="list-style-type: none"><li>• Pattern for the most susceptible organism reported if mixed cultures are tested</li><li>• Substantial interlaboratory variation, potentially leading to misclassifications</li><li>• Subjectivity in end point reading is inevitable</li></ul>
VITEK  ‡‡ (based upon broth dilution using a limited number of concentrations)	Automated	Easy	Easy	Easy	<ul style="list-style-type: none"><li>• Semi-automated and electronic output improves objectivity in end point reading</li><li>• Many laboratories are familiar with the principle</li></ul>	<ul style="list-style-type: none"><li>• The test MIC range for caspofungin is too high (for example, sensitive and intermediate categories for <i>Candida glabrata</i> cannot be differentiated)</li><li>• Approximately 20% VMEs in a study for caspofungin<sup>211</sup></li><li>• Test MIC range for voriconazole is too high to allow detection of any minor MIC drift in normally susceptible species</li><li>• The amphotericin B MICs tend to cluster in a narrow concentration range</li></ul>



Table 2 (cont.) | Overview of various available antifungal susceptibility tests

Test (method)	End point reading	Resistance detection			Advantages	Disadvantages
		Echinocandins	Azoles	Amphotericin B		
Other available tests (cont.)						
SensiTitre YeastOne <sup>®</sup> (microbroth dilution test using alamarBlue)	Visual (colour)	Easy, but recommended CLSI breakpoint for caspofungin bisects <i>Candida glabrata</i> and <i>Candida krusei</i>	Difficult for isolates displaying trailing growth, leading to a gradual colour change of blue to pink	Easy, narrow concentration range limits discriminatory potential	<ul style="list-style-type: none"><li>• Ready-to-use panels with antifungals</li><li>• Growth inhibition is relative to the inherent growth control</li></ul>	<ul style="list-style-type: none"><li>• SensiTitre-specific ECVs have been published for echinocandins, the adoption of which reduced VMEs to 6.2–11.1% depending on which echinocandin was used<sup>164</sup></li><li>• Subjectivity in end point reading is inevitable, particularly for azoles</li><li>• The amphotericin B MICs tend to cluster in a narrow concentration range</li></ul>

CLSI, Clinical and Laboratory Standards Institute; ECV, epidemiological cut off value (that is, the MIC value that describes the upper MIC value for isolates without acquired resistance mechanisms); ELISA, enzyme-linked immunosorbent assay; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimum inhibitory concentration; VME, very major error. \*Low sensitivity to inoculum variation. †Medium sensitivity to inoculum variation. ‡High sensitivity to inoculum variation. ‡Sensitivity to inoculum variation not tested. ‡High reproducibility. ‡Medium reproducibility. ‡Low reproducibility. ‡Reproducibility not tested.

also spread through nosocomial transmission: they can be transferred to patients via hands of health-care workers or contaminated material (for example, intravenous saline for central venous catheter flushing shared among patients), which can cause outbreaks<sup>176–180</sup>. *C. parapsilosis* is most commonly associated with nosocomial transmission, targeting neonates, patients with immunosuppression and patients with indwelling catheters. Some observations suggest person-to-person transmission between hospitalized patients may occur more frequently than previously estimated. Data from molecular typing studies suggest that minor clusters of infections involving isogenic isolates occur regularly<sup>181,182</sup>. Moreover, data from the Atlanta–Baltimore Centers for Disease Control and Prevention (CDC) surveillance programme describe an exceptionally high proportion of echinocandin-resistant *C. glabrata* with FKS mutations among echinocandin naive patients, suggesting nosocomial transmission<sup>12</sup>. Thus, strict adherence to routine infection prevention procedures including hand hygiene and central venous catheter management are crucially important. In this regard, the adoption of ‘catheter care bundles’ (a standard combination of interventions to prevent catheter-associated bloodstream infections) as an approach to central venous catheter management has been important in reducing catheter-associated bloodstream infections.

### Management

In addition to early diagnosis, two clinical interventions are essential to the successful management of invasive candidiasis: source control and early initiation of treatment with early effective systemic antifungal therapy, usually before the diagnosis of invasive candidiasis has been confirmed. Source control refers to the elimination of the suspected nidus (focus) of infection, such as removal of contaminated intravascular catheters and effective drainage of collections of infected material, for example, peritoneal fluid, pleural fluid and/or abscess material. Removal of these presumably

infected prosthetic devices, such as cardiac pacemaker leads, prosthetic joints or other devices, when feasible, is important to a successful outcome. In addition to source control, early effective antifungal therapy is crucial in the successful treatment of patients with invasive candidiasis, as data indicate significantly higher mortality in patients with candidaemia when antifungal therapy was delayed or inadequate (for example, the drugs were not chosen on the basis of susceptibility data or the dosage was insufficient) and/or when source control was not quickly achieved<sup>183–185</sup>. There are several published guidelines outlining expert recommendations for the management of invasive candidiasis and candidaemia, with detailed recommendations for specific clinical circumstances<sup>106,107</sup>.

### Initial therapy

The selection of an antifungal drug for initial treatment should be based on the patient’s prior exposure or intolerance to an antifungal agent, severity of illness, relevant comorbidities and involvement of the brain, cardiac valves and/or visceral organs. The working knowledge of the main *Candida* spp. and susceptibility data in a particular clinical unit should also be considered.

**Echinocandins.** Most clinicians choose an echinocandin (anidulafungin, caspofungin or micafungin) as first-line therapy for adult patients (FIG. 5). Echinocandins inhibit  $\beta$ -D-glucan synthase, an enzyme required for the formation of the cell wall, and have excellent fungicidal activity against most *Candida* spp. Echinocandins are effective, safe and have very limited drug–drug interactions; however, they require intravenous administration. Each agent in this class was effective in ~70–75% of patients in randomized, comparative clinical trials<sup>186–190</sup>.

An analysis of seven large-scale randomized clinical trials that compared treatment for invasive candidiasis in almost 2,000 patients overall showed that an echinocandin as the first-line agent for initial therapy was associated with significantly lower 30-day mortality

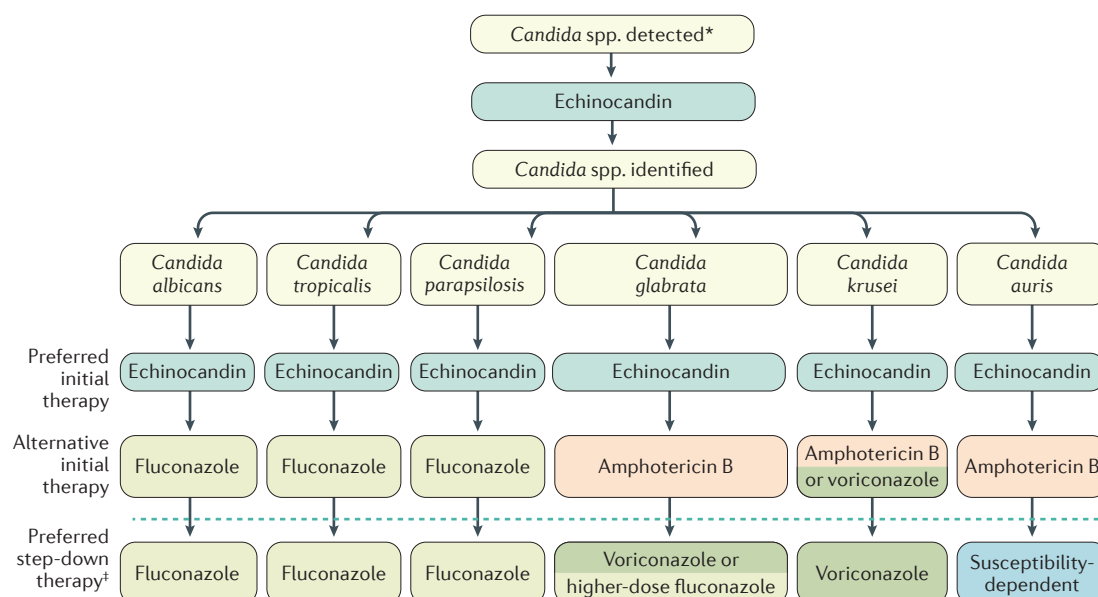


Figure 5 | **Algorithm for the management of invasive candidiasis.** For candidaemia, the total duration of therapy is 14 days from the first negative blood culture. \*As yet unknown species. †Step-down therapy to fluconazole is usually based on documented susceptible minimum inhibitory concentrations to fluconazole (<2 µg/ml for *C. albicans*, *C. parapsilosis* and *C. tropicalis* and <32 µg/ml for *C. glabrata*) and clinical stabilization of the patient. Higher-dose fluconazole consists of 12 mg/kg per day. The information in the presented figure is based in part on Infectious Diseases Society of America (IDSA) and European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines<sup>106,107</sup>.

compared with an azole or amphotericin B (AmB)<sup>185</sup>. Higher APACHE II score, older age, intravascular catheter retention and infection with *C. tropicalis* were associated with worse outcomes and higher mortality, irrespective of antifungal regimen<sup>185</sup>. To date, two randomized studies have directly compared an echinocandin with an azole for initial therapy of candidaemia. A 2007 randomized trial demonstrated that overall response rates were numerically lower with fluconazole (60%) than with anidulafungin (76%)<sup>186</sup>, and a 2016 larger study yielded highly similar outcomes for isavuconazole (60%) compared with caspofungin (71%)<sup>190</sup>.

*C. parapsilosis* is a low-virulence organism that consistently demonstrates higher MICs for echinocandins than most other *Candida* spp. However, in contrast to patients with candidaemia caused by other *Candida* spp. with acquired resistance and high MICs, observational data from Spain and the United States and combined data from several clinical trials suggest no difference in outcome among patients with candidaemia due to *C. parapsilosis* between those who received initial treatment with an echinocandin and those who received other regimens<sup>191–193</sup>. Thus, initial therapy with an echinocandin is appropriate for most patients with *C. parapsilosis* infection.

**Azoles.** The azoles are a group of antifungal compounds with broad activity against most *Candida* spp. They are generally well tolerated, have predictable drug–drug interactions and can be administered as systemic agents either intravenously or orally. Azole antifungals inhibit lanosterol 14 $\alpha$ -demethylase, a key enzyme in the formation of ergosterol, which is a major component

of the fungal cell membrane. Fluconazole was one of the drugs of choice for candidaemia until the echinocandins became generally available in 2003, and now is a second-line agent for initial therapy in most circumstances. Of note, azole therapy is preferred in specific clinical situations, such as endophthalmitis, meningitis and urinary tract candidiasis, in which echinocandins do not reach sufficient concentrations, as echinocandins do not sufficiently pass the blood–brain barrier and are not excreted into the urine. Voriconazole is an effective alternative for invasive candidiasis but offers little substantial advantage over therapy with fluconazole in most instances<sup>187</sup>.

In parts of the world with limited resources and low prevalence of azole resistance, fluconazole remains the standard initial treatment for patients with invasive candidiasis. However, fluconazole should be considered as first-line therapy with caution in light of recent data pertaining to increasing fluconazole resistance, particularly among regions where *C. auris* is prevalent<sup>7</sup>. Haemodynamically stable patients who have had no previous exposure to azoles and who do not belong to a group at high risk of *C. glabrata* infection (such as elderly patients or patients with diabetes mellitus and/or cancer), in settings where access to echinocandins is limited and/or parenteral therapy is impractical, are reasonable candidates for oral fluconazole first-line therapy<sup>106</sup>.

### Step-down therapy

After the patient's conditions are stabilized, step-down therapy is commonly prescribed and supported by clinical data<sup>188</sup>. This transition is generally made within 3–7 days from initial therapy, but the timing

varies substantially on the basis of both the patient's response and the clinician's preference. An oral azole (usually fluconazole) is the first-line agent for step-down therapy, although there must be no evidence of azole resistance based on species determination or antifungal susceptibility data. Non-comparative trials have assessed outcomes among patients with candidaemia caused by azole-susceptible *Candida* spp. who received an echinocandin for at least 5 days and, once their conditions had stabilized and blood cultures were negative, switched to step-down therapy with an oral azole (fluconazole or voriconazole) or continued the echinocandin regimen at the same dosage<sup>188,194</sup>. No differences in 30-day survival or mycological cure rate were observed between patients who continued to receive echinocandin as step-down therapy and those who received an oral azole<sup>188,194</sup>.

Voriconazole is an acceptable step-down agent for patients who are ready for transition from initial therapy to step-down oral therapy and who have fluconazole-resistant isolates such as *C. krusei* and *Candida guilliermondii*, and potentially *C. glabrata*. Posaconazole and isavuconazole also have excellent *in vitro* activity against *C. krusei*, *C. guilliermondii* and *C. glabrata*, but there are few clinical data to support their routine use.

### Duration of treatment

The duration of antifungal treatment is generally determined by the individual clinical and mycological response to therapy. Among patients with candidaemia, follow-up blood cultures at least every other day until documented clearance of *Candida* spp. from the bloodstream are essential to establish the appropriate duration of treatment. In the absence of organ involvement, the duration of systemic (intravenous or oral) antifungal therapy should be 14 days after clearance of *Candida* spp. from the bloodstream (if the blood culture was initially positive) and resolution of all signs of infection, such as fever and hypotension<sup>106,107</sup>.

### Deep-seated infections

For patients with less common manifestations of deep-seated invasive candidiasis, treatment decisions are based more on experience and/or anecdote rather than prospective data. For osteoarticular involvement, chronic hepatosplenic candidiasis and intra-abdominal candidiasis, there are no prospective treatment data, and most clinicians take a modified approach to therapy, such as initial therapy with echinocandins until there is a favourable clinical and/or radiographic response, and then transition to an oral azole (such as fluconazole) for several months<sup>106,107</sup>. The duration of treatment in these situations depends on the site of involvement and ranges from several weeks to 6–12 months. Occasionally, patients require chronic, long-term antifungal suppressive therapy for refractory infections such as prosthetic valve endocarditis, chronic prosthetic joint infection or infected intravascular devices that cannot be removed or replaced (for example, left ventricular assist devices and intracardiac pacemakers or defibrillators)<sup>106,107</sup>.

### Drug resistance

Echinocandin-resistant isolates, especially *C. glabrata* isolates, are frequently also fluconazole resistant, and resistance to both classes of antifungals seems to be associated with worse clinical outcomes<sup>14</sup>. The recent emergence of MDR *C. auris* poses especially challenging questions relating to effective therapy. As there are currently no prospective data to guide treatment decisions in these situations, most clinicians favour a lipid formulation of AmB for treatment of patients with invasive candidiasis due to proven or suspected MDR *Candida* spp. strains until more data become available.

AmB is an antifungal from the polyene class that binds to ergosterol on the fungal membrane and has broad activity against all *Candida* spp. but not against *Candida lusitanae*, which is frequently resistant. AmB has historically been associated with severe adverse effects, particularly nephrotoxicity. Lipid formulations of AmB are preferred owing to their efficacy<sup>195</sup>, lower incidence of infusion-related adverse effects and reduced risk of nephrotoxicity compared with AmB deoxycholate. An AmB formulation should be taken into account in patients with a history of intolerance to echinocandins and/or azoles, when the infection is refractory to other therapy, in isolates (such as *C. auris*) with suspected or proven resistance to other agents, in some deep-seated infections such as valvular endocarditis and meningoencephalitis and for direct intraocular administration for endophthalmitis.

### Quality of life

There have been no quality of life (QOL) studies among survivors of invasive candidiasis. The most objective QOL measure associated with invasive candidiasis is survival at 30 days. Most studies suggest that this figure should be >60% in large groups of patients<sup>185</sup>. The majority of survivors of uncomplicated candidaemia have no residual effects from the infection, with the exception of patients with ocular involvement associated with candidaemia, which is a major morbidity that can lead to permanent visual impairment including blindness if not recognized early and treated aggressively<sup>192</sup>. Most individuals resume prior activities without effect on QOL, and the risk of recurrent disease is quite uncommon (<10%)<sup>186–190</sup>. When relapse does occur, it is most often associated with persistence of an endovascular focus, such as a retained implantable central venous catheter, prosthetic heart valve or implantable defibrillator. Less commonly, an infected retained prosthetic device such as orthopaedic hardware may be the cause of persistent or recurring disease. Rarely, a persistent intra-abdominal or intra-thoracic source, such as an incompletely drained abscess, can be a source of persistent infection. In each of these instances, QOL can be substantially affected by the morbidity associated with a chronic persistent infection, including the need for additional surgical procedures, such as cardiac valve excision and replacement, removal and replacement of a prosthetic orthopaedic joint, intraocular debridement or intra-abdominal surgical drainage and/or debridement.

Administering systemic antifungal therapy to patients on a long-term basis is often challenging. Long-term therapy is expensive and inconvenient and usually requires at least once-daily administration; additionally, it is also associated with potential drug-related intolerance (for example, nausea and vomiting), drug–drug interactions (for example, voriconazole and fluconazole interaction with the antibiotic rifampin and immune suppressants cyclosporine and tacrolimus) and substantial direct drug-associated toxicity (for example, renal impairment, liver function abnormalities and rash). Most of these drug effects, for example, elevated liver function tests associated with use of fluconazole or voriconazole, are reversible upon discontinuation of the compound. Others, such as AmB-related renal impairment due to long-term treatment, could be irreversible.

### Outlook

Invasive candidiasis is the most common fungal disease among hospitalized patients and has continued to be a major cause of mortality. Although diagnostic tools and management strategies have improved, the changing epidemiology towards non-*albicans* *Candida* spp. and the emerging resistance to antifungal drugs are of increasing concern. The prevalence of non-*albicans* *Candida* spp. has been steadily increasing in many parts of the world. The selective pressure of antifungal use has further driven the emergence of species that are typically less drug sensitive than *C. albicans*, such as *C. glabrata* (in settings with heavy fluconazole use) and *C. parapsilosis* (in settings with heavy use of echinocandins)<sup>12,196</sup>. In addition, acquired echinocandin resistance has been reported with increasing frequency, particularly in *C. glabrata*<sup>12</sup>. The recent emergence of MDR *C. auris* as a cause of nosocomial outbreaks with rapid spread is worrisome<sup>7–9</sup>. Owing to its capability to persist in hospital environments and cause lengthy outbreaks, its propensity to acquire resistance mechanisms and its potential misidentification by current diagnostic systems, *C. auris* is a potential global threat. Outstanding questions are whether *C. auris* outbreaks will be contained to settings with suboptimal *Candida* spp. identification and infection control, or whether this and other MDR species will spread and mark the beginning of a new era. In addition, changes in species distribution will drive changes in future treatment recommendations, as the differential susceptibilities of the various *Candida* spp. to azoles and echinocandins influence treatment. At this time, there is a crucial need for novel antifungal agents with new mechanisms of action to ensure successful treatment of *Candida* spp. that are resistant to azoles and echinocandins.

### Diagnostics

Non-culture-based, PCR-based diagnostic techniques have been rapidly evolving<sup>144,153</sup> and will markedly affect the diagnostic work-up of patients within the next 5 years. By contrast, biomarkers, including *Candida* spp. mannan and  $\beta$ -D-glucan and antimannan antibodies, have had a variable and somewhat disappointing performance. Their specificity for candidaemia or deep-seated candidiasis usually is limited, with many patients

with *Candida* spp. colonization but without infection having positive test results<sup>131</sup>, whereas their sensitivity, and hence their negative predictive value, can be inadequate in selected populations at very high risk of candidaemia<sup>4,131</sup>. Unless new biomarkers are identified, diagnostic strategies will likely shift towards direct, DNA-based *Candida* spp. detection.

At a time when most attention has been focused on detecting candidaemia, the diagnosis and management of deep-seated (for example, intra-abdominal) non-candidaemic infections require further clinical studies. Data suggesting an important role for plasma PCR in detecting deep-seated invasive candidiasis are promising<sup>197</sup>.

### Treatment

On the basis of a growing body of evidence, the management of patients with invasive candidiasis has changed markedly since the 2000s. Source control and early initiation of appropriate antifungal therapy have been consistently reported as major determinants of survival<sup>183,184</sup>, and an emerging body of evidence now supports the superiority of echinocandins for primary treatment of invasive candidiasis<sup>185,186,190</sup>.

Few studies are available to support recommendations on the optimal step-down therapy from echinocandins to azoles or the total duration of therapy. Whereas early initial therapy with echinocandins is associated with reduced mortality, step-down therapy to an azole once the patient is clinically stable and *Candida* spp. have been cleared from the bloodstream seems to be effective as early as 3–7 days into initial therapy<sup>188</sup>. Strategy studies should prospectively identify optimal step-down protocols as well as explore further limitations of the total duration of antifungal treatment in selected patient groups.

Studies in patients with culture-proven invasive candidiasis have identified early appropriate treatment as a major driver of outcomes. Thus, it is plausible that early, presumptive treatment of patients with possible invasive candidiasis is also beneficial. However, such strategies have not been successfully validated by prospective studies. Trials on empirical or presumptive treatment of high-risk patients in the ICU have been disappointing. Even studies in which treatment decisions were refined by taking into account parameters such as *Candida* spp. colonization and serum biomarkers have failed to demonstrate a reduction of mortality or length of stay<sup>131,167</sup>. The failure to reliably identify individuals with yet-undetected invasive candidiasis is partly explained by the low prevalence of invasive candidiasis, even in the ICU, and by the very low positive predictive value of prediction rules applying clinical risk factors and biomarkers. Thus, such rules might at best be an additional component in the evaluation of a patient's risk of developing candidiasis, rather than a decision-making tool<sup>4</sup>.

### Prevention

The challenges in *Candida* spp. vaccine development are highly specific to the diverse manifestations of the disease and to the host defence pathways involved, which demand a cellular vaccine target rather than protective



antibodies, as neutrophil, monocyte and/or macrophage functions are the major components of the anti-*Candida* spp. host defence. Recurrent vulvovaginal candidiasis, the most evident target for vaccine development in view of its chronic and recurrent nature, would require a vaccine aimed at improving T<sub>H</sub>17 cell pathway-driven mucosal immunity and at protecting against mucosal invasion of *Candida* spp. rather than colonization. The first *Candida* spp. vaccine candidate in humans, which targeted agglutinin-like protein 3, was recently tested safely in a phase I clinical trial and was efficacious in reducing episodes of vaginitis in women with recurrent vulvovaginal candidiasis in a phase II clinical trial<sup>36–38</sup>. Mechanistically, vaccine protection during invasive candidiasis is mediated by enhanced neutrophil recruitment in infected tissue<sup>198</sup>. For patients at risk of candidaemia and invasive candidiasis, a vaccine should preferably target T<sub>H</sub>1 cell pathways. Vaccination will likely not be aimed at the general, healthy population but at specific patients at immediate risk, such as candidates for surgery or for stem cell or solid organ transplant. This poses an additional challenge for vaccine efficacy in patients with underlying diseases and immune defects.

Antifungal prophylaxis for selected patients at high risk in the ICU seems appropriate, but studies have shown that such treatment did not improve patient survival<sup>122,167,168</sup>. Future studies should aim to further identify risk factors to select individual patients who will benefit most from prophylaxis. As sets of clinical risk factors have

been explored exhaustively with limited success<sup>168</sup>, the identification of individual immunogenetic traits rendering individuals prone to acquiring invasive candidiasis will open new avenues for individualized, targeted prophylaxis and treatment.

### Determinants for personalized therapy

Specific cytokine pathways and gene polymorphisms have now been identified that are associated with a 10-fold to 20-fold increase in susceptibility to invasive candidiasis among patients in the ICU<sup>199</sup>. Within the next 5–10 years, the rapid development of fungal immunogenetics will likely generate clinical tools to select the highest-risk patients for *Candida* spp. vaccination or prophylactic antifungal treatment upon admission to the ICU or transplant unit. Similarly, immunogenetic traits have been identified that are associated with persistent candidaemia and unfavourable outcomes in patients in the ICU once they have acquired invasive candidiasis<sup>97,199</sup>. Such knowledge will likely steer the use of adjunctive immunotherapy but also long-term antifungal treatment or intensified monitoring.

In conclusion, new antifungal treatment regimens have led to lower mortality among patients with invasive candidiasis. Further studies should focus on earlier diagnosis by non-culture-based molecular tests and on early intervention strategies based on combined clinical prediction rules, molecular tests and personalized, immunogenetics-based risk profiles.

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#### Author contributions

Introduction (P.G.P.); Epidemiology (M.C.A. and L.O.-Z.); Mechanisms/pathophysiology (M.S.L. and M.C.A.); Diagnosis, screening and prevention (M.C.A.); Management (P.G.P. and B.J.K.); Quality of life (P.G.P.); Outlook (B.J.K.); Overview of Primer (P.G.P.).

#### Competing interests

P.G.P. has received research grants from Merck, Gilead, Astellas, Cidara, IMMY Diagnostics, T2 Biosystems and Mayne Pharma and is a scientific adviser for Cidara, IMMY, Matinas, Amplex and Mayne Pharma. He is not affiliated with any speakers' bureau and owns no equities in pharmaceutical companies. M.C.A. has received research grants, payment for contract work on minimum inhibitory concentrations and/or lecture honoraria from the following companies: Merck, Astellas, Basilea, Pfizer, Gilead, Cidara, Amplex, F2G, Novabiotics and T2Biosystems. She is not affiliated with any speakers' bureau and owns no equities in pharmaceutical companies. L.O.-Z. has received research grants, consulting honoraria and/or lecture honoraria from the following companies: Merck, Astellas, Pfizer, Gilead, Scynexis, Cidara, Amplex and Vical. B.J.K. is a scientific adviser for Amplex, Astellas, Basilea, Cidara and Scynexis. He is not affiliated with any speakers' bureau and owns no equities in pharmaceutical companies. M.S.L. declares no competing interests.

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