

An Overview on Diagnosis of candida infections

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Abstract:

Candida infections represent a significant cause of morbidity and mortality, particularly among immunocompromised and critically ill patients. The diagnosis of candidiasis remains challenging due to the absence of specific clinical manifestations and the limitations of conventional diagnostic methods. Blood culture, although considered the gold standard, has low sensitivity and may yield negative results in a substantial proportion of invasive cases. Additionally, serological tests and antigen detection methods are often limited by rapid antigen clearance or cross-reactivity due to colonization. Recent advances in molecular and non-culture-based diagnostic techniques have improved the sensitivity and specificity of Candida detection; however, their high cost and technical requirements restrict widespread use. Accurate and early identification of Candida species is essential, as different species exhibit variable virulence and antifungal susceptibility patterns, directly impacting clinical management and therapeutic outcomes. Therefore, a combined diagnostic approach integrating microbiological, serological, histopathological, and molecular methods is often required to achieve optimal diagnosis and guide appropriate antifungal therapy.

Keywords: Candida infections, diagnosis, blood culture, antifungal therapy

Introduction:

Clinically the diagnosis of candida infections is complicated due to lack of specific signs and symptoms of disease. Laboratory diagnosis still a complex work because circulating Candida spp. antibodies may occur in normal individuals due to commensal colonization of Candida on mucosal surfaces thereby reducing the importance of antibody detection to diagnose the disease. Additionally, Candida spp. antigens are often rapidly cleared from the circulation making the antigen detection tests mostly lack the desired level of sensitivity. Microbiological confirmation of candida infections is difficult because blood cultures can be negative in up to 50% of autopsy-proven cases of deep-seated candidiasis or may only become positive late in the infection. Positive cultures from urine or mucosal surfaces do not necessarily indicate invasive disease although can occur during systemic infection. (1).

Importantly, the differences between various Candida spp. as regard the virulence and the antifungal drugs susceptibility; make the definite identification to the Candida spp. much important for clinical management. Recent molecular biological tests have generated interest in most clinical laboratory settings and would be validated in the guidelines. Laboratory surveillance of at-risk patients could result in earlier initiation of antifungal therapy if sensitive and specific diagnostic tests, which are also cost effective, become available. (2).

Isolation of the infecting organism from a normally sterile site is required such as blood, peritoneal fluid, or intravenous lines. However, in cases of hepatosplenic candidiasis, blood cultures are frequently negative; indeed, blood cultures were reported to be negative in up to 50% of all autopsy-proven cases of invasive candidiasis in such patients. (1).

Similarly; Vitális et al., (3) reported that; blood cultures from patients with Invasive Candidiasis (IC) may be negative in a significant number, making the diagnosis frequently rests on a combination of clinical and laboratory findings. Clinically helpful clues in a patient at risk include the presence of unexplained fever or sepsis and metastatic skin lesions (in neutropenic hosts only).

In such patients, complete physical examination should be repeated thoroughly; in a search for a potential focus (intraabdominal or deep organs), the presence of skin lesions, myalgia and muscular abscesses and/or endophthalmitis. Laboratory diagnosis of IC depends upon three approaches: microbiologic, histopathologic and immunologic and on close collaboration between clinicians, Laboratory findings (4).

Identification of the specific infecting organism to the spp level has gained increasing importance for several reasons. First, not only the changed distribution of the *Candida* spp., but also the *Candida* differ in their susceptibility to antifungal agents (5). Second, the specific identification of spp. is relevant for epidemiological purposes; e.g. identification of a particular species in a given hospital ward or locale may indicate a point source outbreak. Third, the risk of developing deep organ involvement, and the severity of clinical manifestations, differs depending on the infecting species. (6).

Nosocomial *Candida* infections are often severe, rapidly progressive, difficult to diagnose clinically and refractory to therapy.

Therefore, rapid and accurate laboratory diagnosis is very important to provide appropriate antifungal treatment. The laboratory diagnosis of *Candida* infection remains problematic as no single laboratory test provides satisfactory and rapid results (7).

Although blood culture is considered the gold standard for diagnosis of disseminated candidiasis, it lacks sensitivity. New culture media, lysis centrifugation and automated blood culture monitoring systems have decreased the assay time required for isolation of etiological agent (8).

However, these techniques have moderate sensitivity. Automated blood culture systems like BACTEC 9240 and ALERT 3D have demonstrated appreciable sensitivity and specificity for diagnosis of disseminated candidiasis (9).

Serological tests to detect antigen or antibody in serum or other body fluids are useful for presumptive diagnosis and initiation of antifungal therapy but not for identification of species. Common antigens detected are galactomannan and β -D-glucan. Other antigens that can be seen circulating in the blood are D-arabinitol, enolase and SAP (10).

Due to low levels of circulating antibodies, in immunosuppressed individuals, immunoglobulin detection test lacks sensitivity. Molecular diagnostic techniques are gaining importance in recent years because they are highly accurate, sensitive and specific for identification of *Candida* spp. However, these are expensive and require expertise technical support (11).

A combination of serological, microbiological, histological, and molecular biological tests may be required for the best possible diagnosis. (2).

1. Fungal culture:

Multiple blood cultures on successive days, especially after removal of or in the absence of central lines, are compelling evidence for invasive disease. Advances in blood culturing techniques have been developed which appear to have improved the sensitivity and/or reduced the time required to obtain a positive blood culture. These include the development of lysis centrifugation tubes and automated monitoring of blood culture bottles. (2).

CHROM agar is a selective and differential medium for the identification of *Candida* species and can be used to identify *C. albicans*,

C. parapsilosis, *C. dubliniensis*, *C. tropicalis* and *C. krusei*. It is widely used in mycology and it is found to be an effective primary identification test, where each species gives different colours of the colony forming units when species-specific enzymes split the chromogenic substrates. (12).

The low sensitivity of blood cultures for detecting candidemia has been significantly improved with newer technologies such as the continuous monitoring automated blood culture systems (particularly the BactT/Alert (BioMerieux) and the Bactec® (Becton-Dickinson) systems) and the lysis-centrifugation method (Isolator, Wampole) (13).

Isolation of *Candida* from blood requires preliminary broth culture to amplify the usually low numbers of yeasts, which can then be plated on other media for isolation and identification. Biphasic blood culture media and vented culture bottles are optimal for detection of *Candida* yeasts and pre-treating blood samples by cell lysis and centrifugation greatly enhances the yield (14).

Combination of lysis-centrifugation and the automated Bactec® identification system offers an average time of 3 to 4 days for detection of *C. albicans*, *C. parapsilosis* and *C. tropicalis*, whereas *C. krusei* and

C. glabrata often take longer to grow. Therefore, blood cultures obtained to rule out hematogenous candidiasis should be evaluated up to 2 weeks. (15).

Sampling various anatomic sites (throat, sputum, stools, urine, others) for candidal colonization is useful to

identify risk categories for IC (low if persistently negative cultures versus high if multifocal colonization and/or *C. tropicalis* colonization in immunocompromised patients) and those in whom antifungal therapy with agents other than fluconazole should be considered (colonization with *C. glabrata* or *C. krusei*). (16).

Candida spp. grow well on standard mycologic media at 35°C, such as Sabouraud agar pH 5.6 with chloramphenicol and gentamicin, and on many bacterial media, including blood agar, brain-heart infusion agar and tryptose agar. Cycloheximide (Actidione) inhibits candidal growth and hence should not be incorporated in the laboratory work-up for *Candida*. (17).

The time needed for species identification can be shortened by using media that can differentiate *Candida* spp. by colony color such as CHROMagar *Candida*®, which inhibits bacterial growth and provides presumptive identification of *C. albicans*, *C. tropicalis*, *C. dubliniensis*, and *C. krusei*. (18).

Fongiscreen 4H® also allows similar detection. Other culture media that allow rapid identification of *C. albicans* include CandiSelect®, Sanofi Diagnostic Pasteur, France; Fluoroplate® *Candida*, Merck, Germany; Murex *Candida albicans*®, Murex Diagnostic, USA; and *Albicans ID*®, BioMérieux, France (19).

The most common strategy for identification of yeasts is to start with rapid, simple, and specific tests to identify *C. albicans* since this single species accounts for the majority of yeasts grown from clinical samples. This identification is easily done on any of the isolation media described above. Alternatively, identification of *C. albicans* could rely on this species' ability to produce germ tubes in serum after 3 hours at 37°C (>90% of *C. albicans* isolates produce germ tubes) (20).

However, germ tube production is also seen with *C. dubliniensis* and *C. stellatoidea*. *C. dubliniensis* closely resembles *C. albicans* in screening tests. Only routine testing of presumed *C. albicans* isolates for absence of β -glucosidase activity can confirm that the isolate is *C. albicans* without need for DNA fingerprinting (4).

Isolates that cannot be recognized as *C. albicans* or *C. dubliniensis* should be examined for the morphology of their blastoconidia and for their ability to produce pseudo hyphae and chlamydoconidia on suitable semi-starvation media such as corn meal or cream of rice-Tween agars (3).

2. Automation:

Vitek YBC system: The Vitek YBC system (bioMérieux Vitek, Inc., Hazelwood, MO, USA) is an auto-microbial system that is widely applied in research centres and laboratories. (21).

VITEK® 2 ID-YST system: VITEKR 2 Yeast identification (YST) is a developmental system. This is a high-speed, rapid, and simple system that could simultaneously conduct multiple reactions. This system can perform 47 carbohydrate assimilation-based fluorescent biochemical reactions including deamination and oxidation reactions with various arylamidases and oxidases of *Candida* species. This system is more suitable for differentiating *C. dubliniensis* and *C. glabrata* in addition to the identification of yeast microorganisms. (22).

3. Direct microscopy:

Microscopic examination can be done either directly from fresh samples or from fungal cultures. However, direct microscopy is non-specific, as different species can show the same morphological patterns and it is not possible to identify the *Candida* species causing the Candidiasis. (23).

Direct microscopic examination of clinical material with wet mounts, Gram, Giemsa, periodic acid-Schiff (PAS) or Calcofluor stains can rapidly identify the characteristic combination of budding yeast cells and pseudohyphae seen with *Candida* although *Trichosporon* and *Geotrichum* may produce similar findings (13).

In patients with suspected high-grade candidemia, an early diagnosis can be made by examination of blood smears, particularly if the microscopist is specifically asked to look for yeasts (Wright-Giemsa stain, 100 high-power field). Cytologic and histologic stains, including the hematoxylin and eosin (H&E), GMS and PAS stains, can detect *Candida* spp. in various fluids and tissues (24).

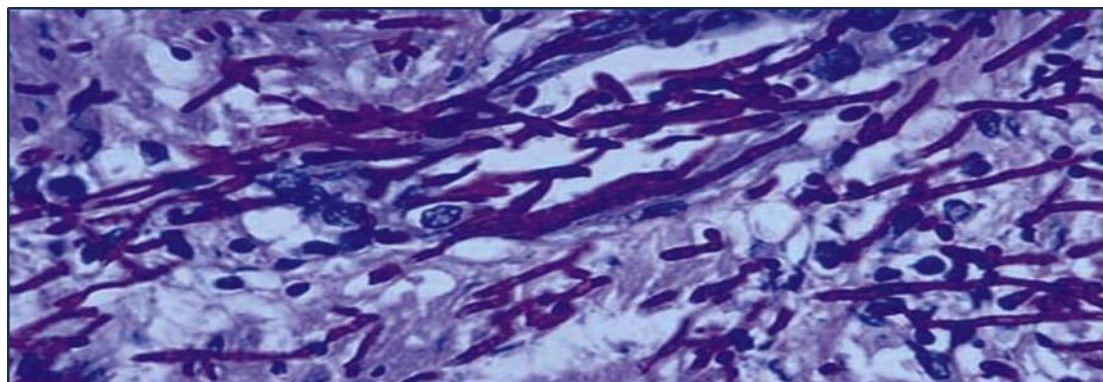


Figure (1): Direct smear of urine from a patient with candidiasis of the kidney showing *C. albicans* in mycelial or tissue phase with blastoconidia budding from the pseudo hyphae (25).

However, the H&E stain may be inadequate when there are few fungal elements. In such settings, GMS and PAS stains can detect smaller numbers of fungi and better delineate their morphology. When needed, *Candida*-specific immune fluorescent stains can confirm a presumptive histologic identification (26).

4. Non-culture methods (Serological methods):

Extensive work has been done on non-culture methods for diagnosis of hematogenous candidiasis, with somewhat disappointing results. The only promising approaches appear to be serial testing of the combination of the Platelia *Candida* antigen test (detects mannan) with the Platelia antibody test (detects antimannan antibodies) and the β 1-3- glucan (BG) test (Fungitell® BG assay, Associates of Cape Cod Inc, Falmouth, MA) (24).

BG is a component of the cell wall of most fungal pathogens including *Candida* and can be detected and quantitated in the bloodstream of patients with fungal infections and has a sensitivity and specificity >90% in patients with hematogenous candidiasis (15).

Molecular techniques are based on either Polymerase Chain Reaction (PCR), or non-PCR based techniques. Various PCR based techniques include conventional, semi-nested and nested-PCR, PCR-enzyme immunoassay, real time PCR and multiplex PCR. However, the non-PCR based techniques include The Yeast Traffic Light peptide nucleic acid fluorescence in situ hybridization assay (PNA-FISH) and pyrolysis and matrix- assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOFMS) (27).

Limitations of the BG test include false-negative results (if hyperbilirubinemia or hypertriglyceridemia presents) and false-positive results. False positives can result from excess sample manipulation and exposure to gauze or other materials that contain glucans, or in presence of Gram-positive bacteremia, hemolysis, hemodialysis with cellulose membranes and therapy with IV immunoglobulins and albumin (26).

Minimal sample manipulation and two sequential positive results improve test performance. Use of the polymerase chain reaction for the diagnosis of IC remains limited by lack of standardization of testing protocols and limited clinical validation (19).

A trehalose test can identify *C. glabrata*, as can the differential growth on eosin methylene blue agar (rapid growth, versus no growth or slow growth on blood agar). Most laboratories continue to rely on a battery of biochemical tests offered in the API-20C strip for yeast identification or a more extended kit ID 32C, API *Candida* kit (19).

Recently, a molecular method has been developed that uses a fluorescein-labelled, peptid nucleic acid probe that targets *C. albicans* 26S rRNA for identification of yeasts directly from blood culture bottles (*C. albicans* PNA FISHTM test, AdvanDx Inc, Woburn, MA). The method allows for very rapid identification (within 2–3 hours) of *C. albicans* versus non-*albicans* species provided the blood culture bottle tests positive and yeasts are visible on Gram stain (13).

The test is highly sensitive and specific regardless of the blood culturing system or broth formulation used. Applying this method results in identification 24–48 hours earlier than with conventional methods and may offer substantial savings as a result of more directed antifungal therapy (16).

Anti-fungal drugs

Fungi are eukaryotic, like their human hosts, limiting the number of molecular targets that can be selectively exploited for drug development without the risk of cross-target toxicity. (28).

Classically four classes of FDA-approved antifungal agents clinically used in the treatment of invasive fungal infections, namely the polyenes, flucytosine, the azoles, and the echinocandins. (29).

The efficacy of these agents is compromised by host toxicity, fungi static activity, or the emergence of drug resistance in pathogen populations; polyenes, azoles, and echinocandins. (28).

Principal classes of antifungals available are:

1. **Polyenes:** amphotericin B deoxycholate, liposomal amphotericin B, amphotericin B lipid complex, nystatin. (30).
2. **Pyrimidines:** Flucytosine (5FC).
3. **Azoles:** triazoles (flucanazole, itraconazole, posaconazole) and imidazole (ketoconazole, miconazole and clotrimazole).
4. **Echinocandins:** anidulafungin, caspofungin, micafungin, rezafungin. (31).

Table (1): The four main classes of clinically-used antifungal agents for the treatment of invasive fungal infections.

Antifungal Class	Mechanism of Action	Biological Effect	Spectrum of Action
Polyenes	Target ergosterol and extract sterols from fungal cell membranes	Fungicidal	Broad spectrum antifungal in treatment of invasive fungal infections; resistance is rare
Flucytosine	Inhibits DNA and RNA Synthesis	Fungicidal against <i>Cryptococcus</i> spp.	cryptococcal meningitis, but resistance is extremely common so never used in monotherapy
Azoles	Inhibit 14- α -lanosterol demethylase thereby inhibiting ergosterol synthesis	Mostly fungistatic	As a class they display broad spectrum against yeasts and filamentous fungi, although some species display intrinsic resistance to commonly used derivatives; secondary resistance can often develop during treatment
Echinocandins	Target 1,3- β -d-glucan synthase thus preventing production of cell wall 1,3--d-glucan	Fungicidal against <i>Candida</i> spp., but fungistatic against <i>Aspergillus</i> spp.	First line of defense for candidiasis and used in aspergillosis when refractory to other treatments; resistance is emerging

Polyenes, are broad spectrum antifungal agents discovered in the 1950s, include Amphotericin B (AmB), Nystatin and Natamycin. AmB, available in conventional (deoxycholate, AmB-d) and lipid formulations (ABLC lipid complex, liposomal L-AmB), acts by binding to ergosterol, an essential component of the fungal cell membrane, causing it to destabilize and form pores, and finally causes leakage of cellular contents and cell death. Amphotericin B is the only polyene that can be administered systemically (32).

Lipid formulations of AmB have been developed to mitigate its toxicity, particularly the nephrotoxicity associated with the deoxycholate formulation. A meta-analysis showed that lipid formulations significantly reduced nephrotoxicity compared with conventional formulation. (33).

Among the polyene antimycotics, amphotericin B is the most potent drug currently available for the treatment of systemic fungal infections, as it has fungicidal and fungistatic activities against many fungal pathogens responsible for systemic fungal infections, such as *C. albicans*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Aspergillus*. It is commonly used to treat recurrent fungal infections in patients with compromised defense mechanisms (e.g., patients on immunosuppressive therapy, chemotherapy, and HIV patients). (34).

Recently, two lipid-based formulations developed for oral administration, named amB lipid complex (ABLC) and liposomal amphotericin (L-AmB), have been authorized, characterized by greater selective toxicity towards sensitive fungi, and increased tolerability especially in terms of nephrotoxicity. (35).

Flucytocine (5FC), is a pyrimidine analogue affect fungal pyrimidine metabolism by inhibiting DNA and RNA synthesis and used in combination with Amphotericin B in treating life threatening candida infections like endocarditis, meningitis and hepatosplenic disease. (36).

Allylamines, such as terbinafine, inhibit ergosterol synthesis by acting on the enzyme squalene epoxidase, leading to squalene accumulation and ergosterol depletion, altering fungal cell membrane function. Their use is primarily confined to the treatment of dermatophytoses, with a limited role in invasive fungal infections. (37).

Azoles, introduced since the 1960s, are subdivided into two classes Imidazoles (e.g. Miconazole, Ketoconazole and Clotrimazole), used mainly topically or with limited systemic use, and Triazoles (e.g., Fluconazole, Itraconazole, Voriconazole, Posaconazole, Isavuconazole) widely used systemically. They inhibit ergosterol synthesis by blocking the cytochrome P450-dependent enzyme lanosterol 14- α -demethylase, leading to the accumulation of toxic methylated sterols and depletion of ergosterol, altering membrane function. Azoles are generally fungistatic against yeasts and fungicidal against *Aspergillus* spp. but have a high risk of drug interactions and potential hepatotoxicity. (38).

Fluconazole is extensively used for prophylaxis and treatment of superficial and disseminated candidiasis (27).

Echinocandins introduced in the early 2000s, include Caspofungin, Micafungin and Anidulafungin approved by FDA, these antifungal agents, act by target the cell wall and inhibit 1, 3- β -D- and 1, 6- β -D-glucan synthesis. (40).

All these three compounds have fungicidal (action both in vivo and in vitro) against most *Candida* species irrespective of their resistance or susceptibility to azole or Amphotericin B. Recently echinocandins are used increasingly. Echinocandins (especially caspofungin) are now considered as first line drugs for treatment of disseminated candidiasis or azole resistant candida infection (8).

Candida infections are treated with antifungal medications such as nystatin, clotrimazole, amphotericin B, miconazole. Mild or moderate genital *Candida* infections can have treatment with antifungal vaginal cream. The antifungal creams come in 1, 3, or 7-day treatment. Econazole or fluconazole 150 mg orally one-time dose can also be prescribed (13).

Systemic candidiasis requires treatment with oral or intravenous antifungal medications, include-ing caspofungin, fluconazole, and amphotericin B (14).

In cases of denture stomatitis, the patient should refrain from using their denture for at least two weeks along with the topical application of antifungal medication. Angular cheilitis occurs due to loss of vertical dimension. Thus, after the infection subsides fabrication of new denture prostheses with proper vertical dimensions is essential. Oral application of probiotics can serve as an adjuvant in treating oral candidiasis (16).

The antifungal agents for treating disseminated candidiasis differ in mode of action, pharmacokinetics and pharmacodynamics, route of administration, indication, cost and safety. These differences are to be considered while treating a particular patient for a particular purpose. The purpose for initiation of antifungal treatment may be prophylaxis, initial therapy (empiric, pre-emptive or targeted) or salvage therapy (41).

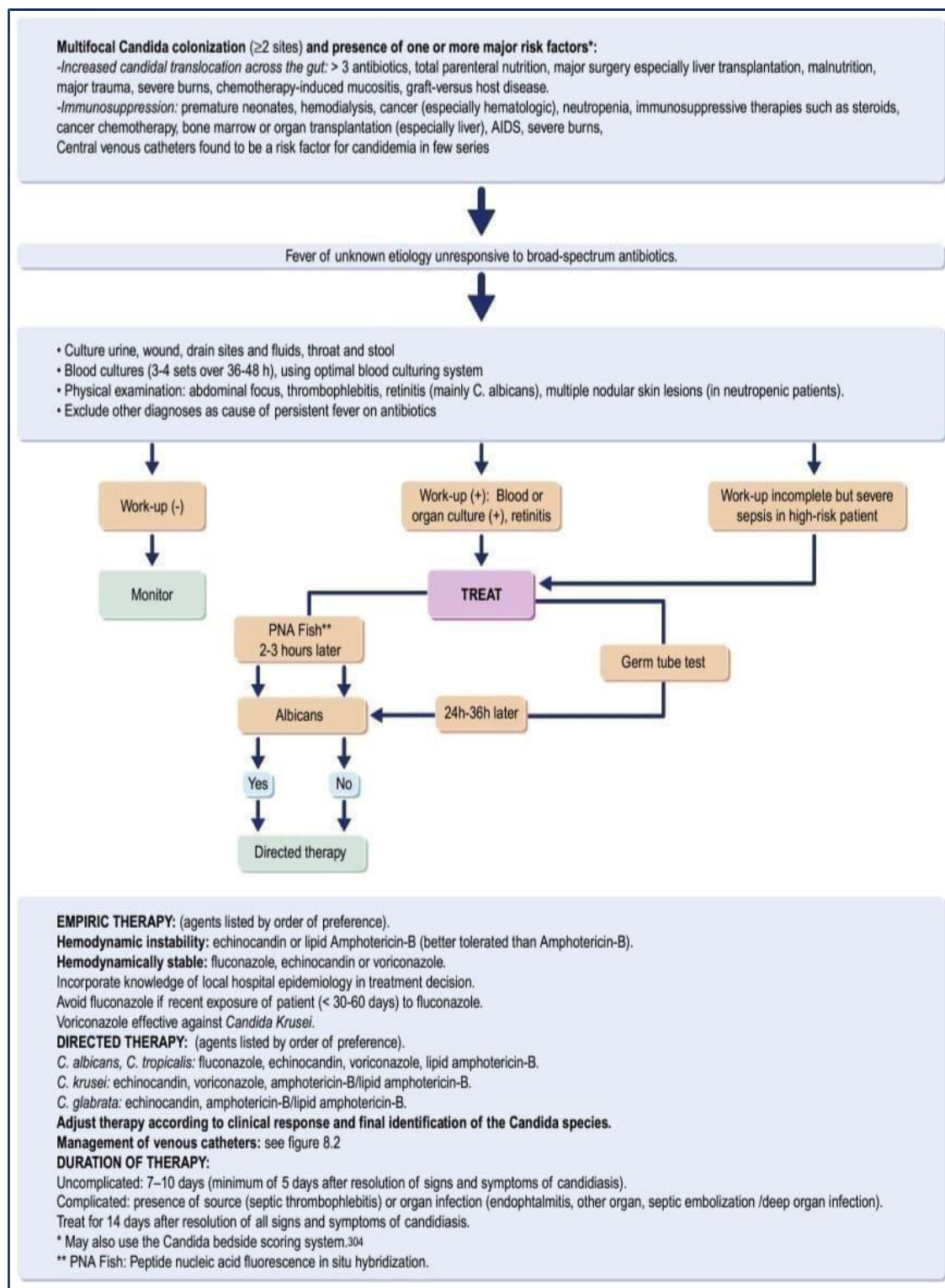


Figure (2): Management summary of patients at risk of invasive candidiasis (25).

Prophylactic therapy is given to avoid infections by *Candida* spp. in high-risk immunosuppressed individuals. But this involves risk of overuse and development of resistance. The pre-emptive therapy is started to avoid disseminated candidiasis in patients. The empiric therapy is started on the basis of presenting clinical signs and symptoms and local epidemiology (8).

However, the role of empirical therapy remains controversial because it leads to overuse and drug resistance. For prophylaxis and treatment of candidiasis.

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