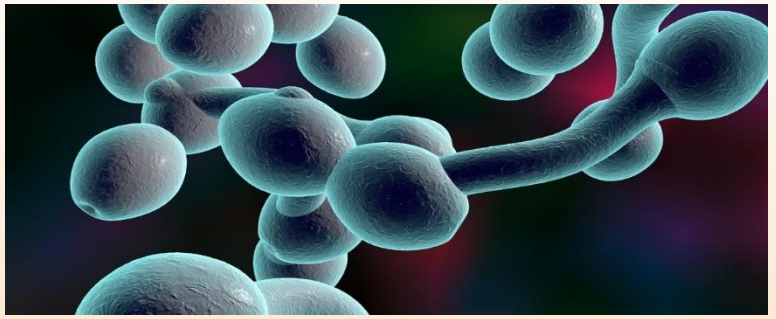

Crisis in the shadows: confronting the escalating Fungal Sepsis Challenge

BY

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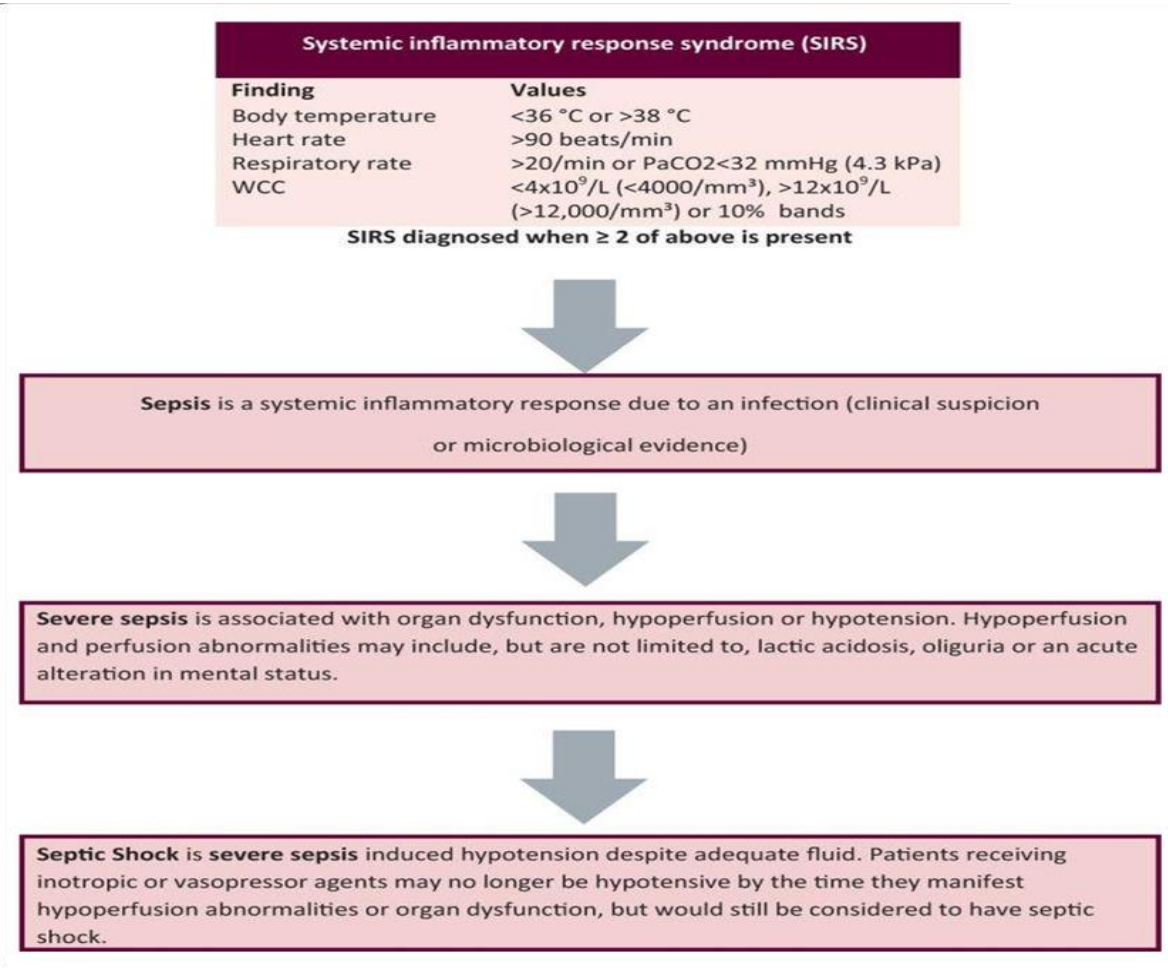
Outlines

- Outline immunopathogenesis of fungal sepsis.
- Identify causes of invasive fungal infections.
- Determine obstacles in laboratory diagnosis of invasive fungal infections.
- Outline preventive measures for invasive fungal infections

Definition of Sepsis:

Sepsis was defined as a systemic inflammatory response secondary to an infection.

Addition to organ dysfunction, hypoperfusion or hypotension to “sepsis” turns into “Severe Sepsis”



Definition of Sepsis:

In 2016, new sepsis definitions were proposed by the Society of Critical Care Medicine and the European Society of Intensive Care Medicine , that updated the definition of “sepsis” to be “a life-threatening organ dysfunction caused by a dysregulated host response to infection” ,which corresponds to “severe sepsis” according to the traditional definition.

SOFA (sequential organ failure assessment) and qSOFA (quick sequential organ failure assessment) replaced SIRS criteria.

qSOFA score is preferred at predicting hospital mortality in comparison to SOFA score when applied to severe sepsis and septic shock patients. It has higher sensitivity and specificity

Fungal Sepsis

-Sepsis is defined as host inflammatory response to severe, life-threatening infection with the presence of organ dysfunction.

-Sepsis is the most frequent cause of mortality in most ICUs.

-Severe Inflammatory response (SIRS) can occur in association with bacterial, viral, protozoal or fungal infection.

-The incidence of sepsis is increasing in the past few years due to increased incidence of impaired immunity.

-Fungal sepsis is associated with a higher mortality than bacterial & viral sepsis.

- *Candida* species are by far the predominant cause of fungal sepsis accounting for 10% to 15% of health-care associated infections and 5% of all cases of sepsis .

- Invasive candidiasis has become the fourth leading cause of bloodstream infection, causing up to 40% mortality.

Immuno-pathogenesis of Sepsis

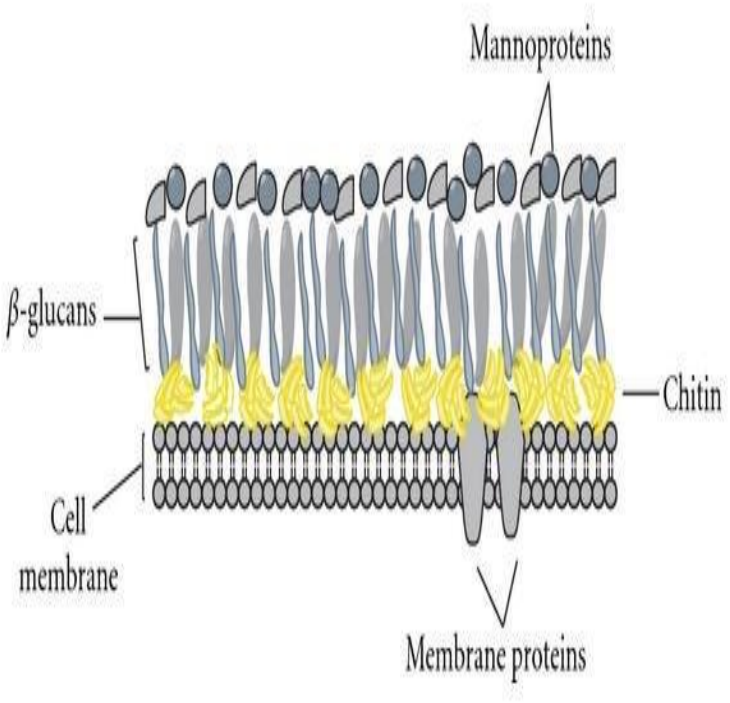
-The pathophysiology of sepsis remains incompletely understood.

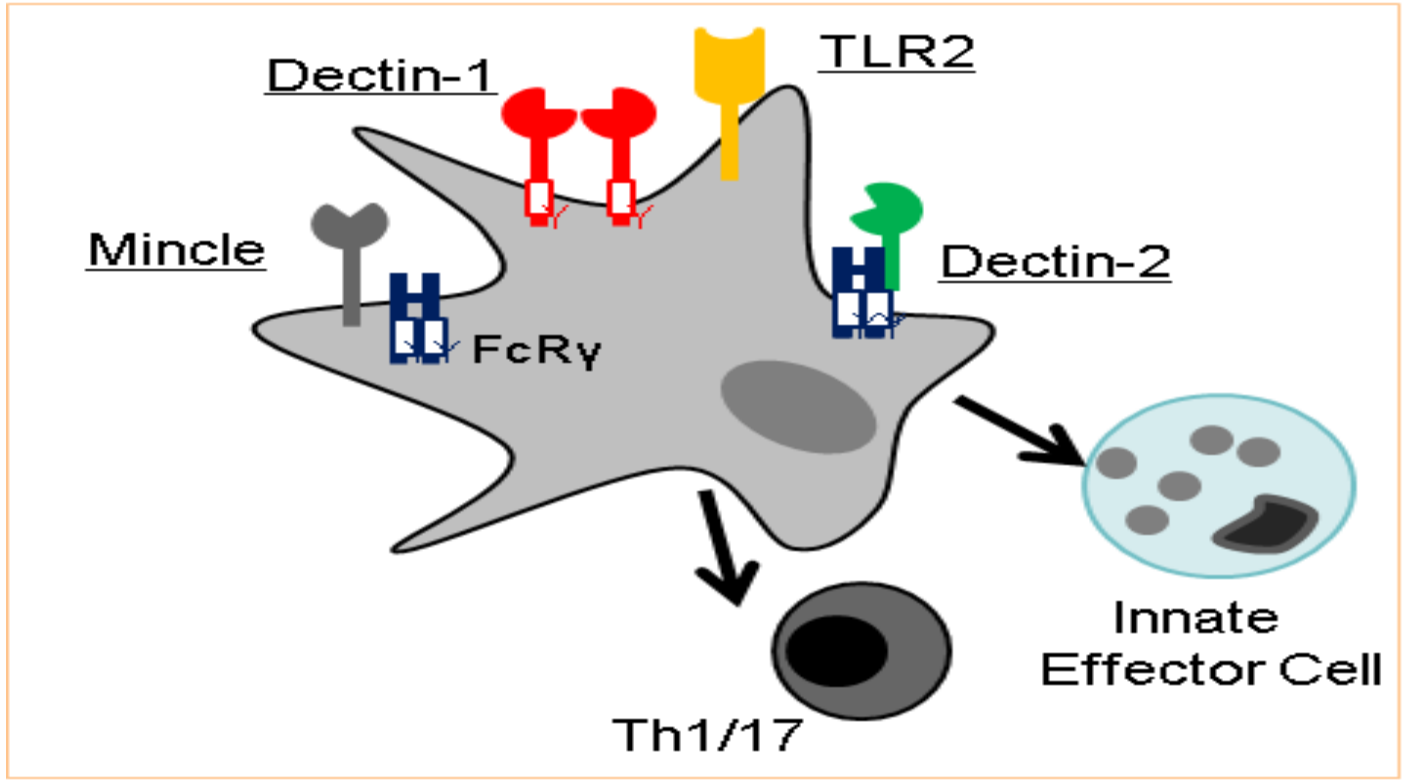
-A multitude of cell types, inflammatory mediators, and coagulation factors are involved and recent research had focused on the contributions of the innate immune system and T cells.

-Previouis studies had shown that patients who die of sepsis have a longer duration and a greater degree of organ injury caused by dysfunction of innate immune-driven inflammation than those who survive.

Immunological responses in fungal sepsis

- The cell wall of fungi predominantly comprises rigid polysaccharide layers. An inner layer consists of a chitin layer and an adjacent layer of β-(1,3) and β-(l,6)-glucans. An outer layer consists of N- and O-linked mannoses, β-glucans occupy 50% of the dry weight of the fungal cell wall.
- Innate responses to fungal pathogens are initiated by fungal component recognition via an array of PRRs including C-type lectin receptors (CLRs), TLRs and complement receptors.
- By recognizing the fugal cell wall components, TLRs and CLRs play important roles in the initiation of innate immune response for the immediate control of fungal propagation, and the differentiation of $CD4^+$ Th1 and Th17 cells for later control and long-term memory of fungal infection.




Dectin-1
TLR2
Mincle
FcRγ
Dectin-2
Innate
Effector Cell
Th1/17

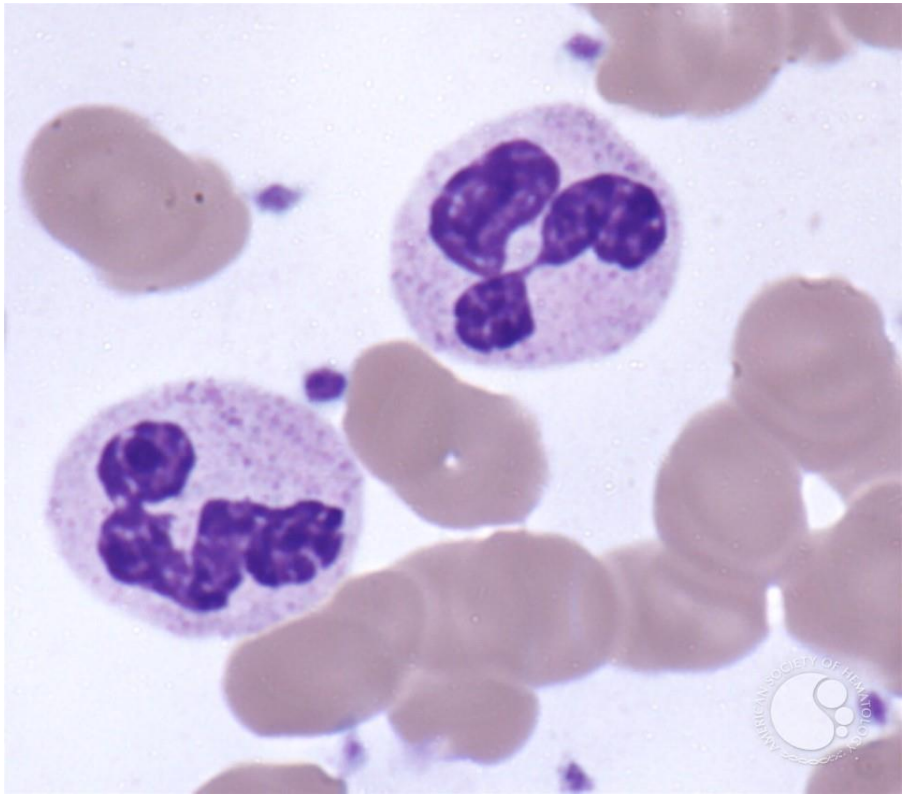
Impact of Sepsis of Immune cells:

-Neutrophilis:

-Neutrophils are essential for the early control of invading pathogens.

-Animal models of sepsis and experimental studies in patients revealed disrupted neutrophil functions including impaired clearance of infectious agent reduced production of reactive oxygen species and decreased recruitment to infected tissues.

-Loss of chemotactic activity is probably the most frequently documented dysfunction of circulating neutrophils during sepsis.



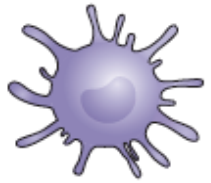
-Several studies have reported that impaired neutrophil function precedes the development of invasive fungal infections.

-Reduced neutrophil function had also been reported in a mouse model of polymicrobial sepsis in which the mice had an increased susceptibility to secondary infections.

a Effects of protracted sepsis on the innate immune system

Follicular dendritic cell

- ↑ Apoptosis
- ↓ Antigen presentation to B cells



Dendritic cell

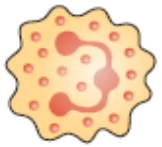
- ↑ Apoptosis
- ↓ Antigen presentation to T cells
- ↓ Cytokine secretion

Macrophage

- ↑ Anti-inflammatory cytokine secretion
- ↓ HLA-DR expression
- ↓ Pro-inflammatory cytokine secretion
- ↓ Pathogen killing

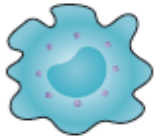
NK cell

- ↑ Apoptosis
- ↓ Cytotoxic function
- ↓ Cytokine secretion



Neutrophil

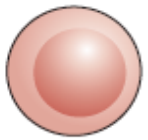
- ↑ Release of immature neutrophils
- ↑ IL-10 secretion
- ↓ Apoptosis
- ↓ Reactive oxygen species release
- ↓ Nitric oxide release
- ↓ Expression of adhesion markers



MDSC

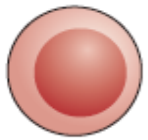
- ↑ Apoptosis
- ↓ Cytotoxic function
- ↓ Cytokine secretion

b Effects of protracted sepsis on the adaptive immune system



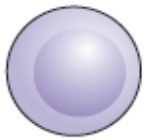
$CD4^+$ T cell

- • Cell exhaustion
- ↑ Apoptosis
- ↑ T_H2 cell polarization
- ↓ Adhesion molecule expression
- ↓ CD28 expression
- ↓ TCR diversity



$CD8^+$ T cell

- • Cell exhaustion
- ↑ Apoptosis
- ↓ Cytotoxic function
- ↓ Cytokine secretion
- ↓ TCR diversity



T_{Reg} cell

- • Resistance to apoptosis
- ↑ Suppressive activities

B cell

- ↑ Apoptosis
- ↓ Antigen-specific antibody production

Risk factors for Sepsis:

1-Extrems of age (infants and old patients).

2-Cancer patients.

3-Immunocompromised patients.

4-Chronic illness.

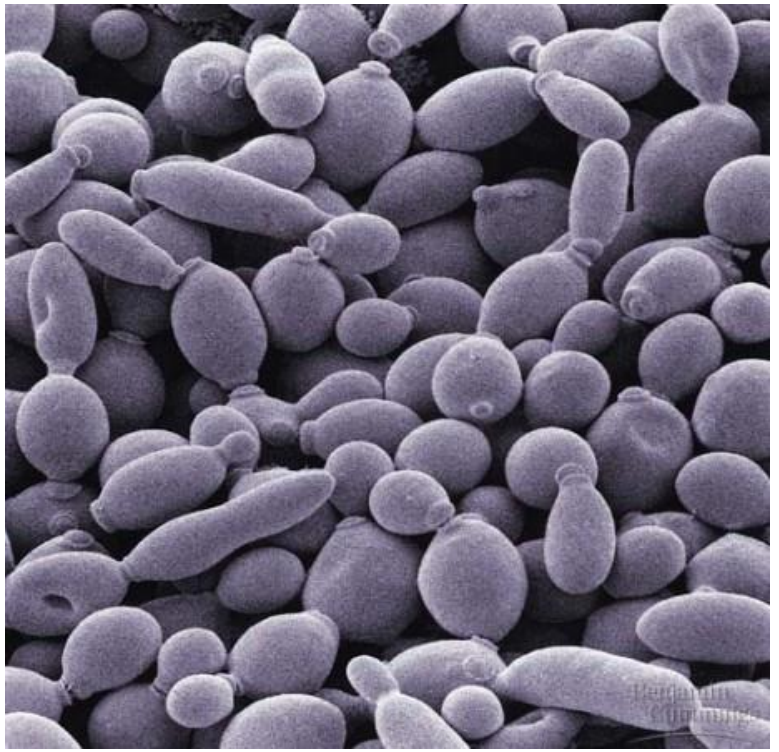
Epidemiology of Invasive fungal infections

- It is estimated that 1.9 million patients get an acute invasive fungal infection (IFI) each year, while an estimated 3 million people globally suffer from chronic severe fungal infections.
- Many of these are life-threatening infections, with an estimated greater than 1.6 million deaths per year attributed to all fungal diseases.
- Nearly 70% of all IFIs in the world are caused by invasive candidiasis (IC)

Causative agents of invasive fungal infections:

- -Invasive fungal infections are a growing problem in critically ill patients and are associated with increased morbidity and mortality.

- Most of them are due to *Candida* species, especially *Candida albicans*.



Predisposing Factors for invasive fungal infections:

-Predisposing risk factors for invasive fungal infections as candidiasis and aspergillosis is immunocompromised hosts due to neutropenia, cell-mediated immune dysfunction, and disruption of mucosal integrity.

- The prevalence of invasive devices, especially intravascular central lines, has resulted in an increase in catheter-related bloodstream infections (CRBSIs), candidemia and disseminated candidiasis.

Incidence of invasive Candidasis

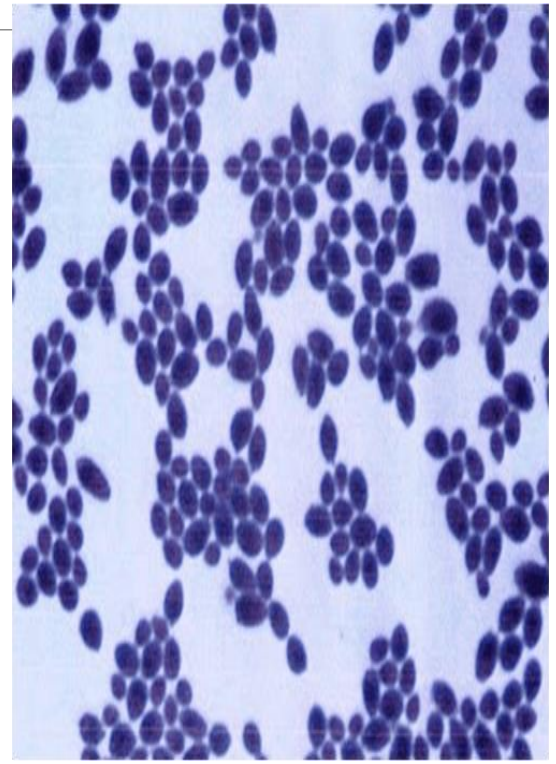
- Candida bloodstream infections (BSIs) constitute the vast majority of HAIs fungal infections. In a large surveillance study in United States *Candida spp* were ranked fourth among the hospital-acquired BSIs.
- The majority of infections (51%) were noticed in ICU.
- The true incidence of HAI candidemia is likely to be higher because of the relatively poor diagnostic tools that only yield approximately 50% of positive blood culture results in patients with disseminated candidiasis.
- **Candida** is the most frequent cause of fungal severe sepsis or septic shock in ICU patients.
- Overall crude mortality among patients with invasive candidiasis or candidemia is in the range of 40% to 60%.

Candaida spp

-*Candida* species constitute part of the normal human flora. Only a small percentage of the identified species cause disease in humans. *Candida* spp. is responsible for an extremely large spectrum of diseases.

Candida spp are the most common fungal pathogens causing serious HAIs especially in patients admitted to ICU.

-Source of *Candida* infection can be endogenous or exogenous even leading to local outbreaks

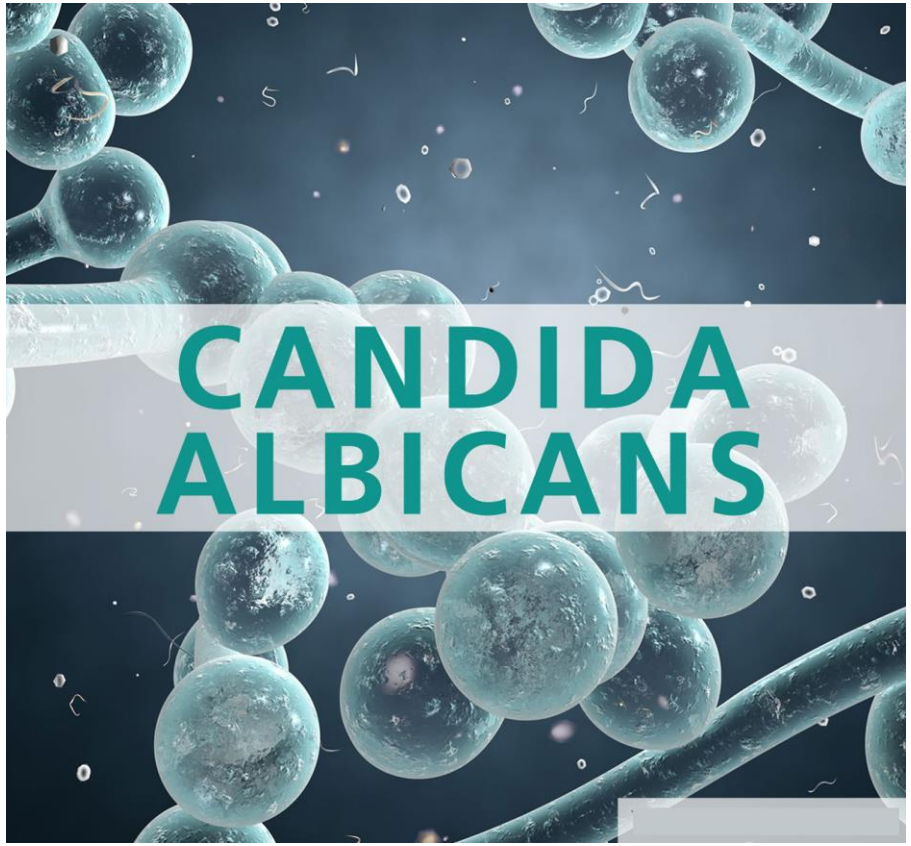


The Changing Epidemiology of *Candida spp*.

-*Candida albicans* is the most frequently recognized followed by *C. glabrata , C. tropicalis & C. krusei.*

-During the last years a shift to non-*albicans* species had been noted.

- Several risk factors contributed to presence of *non albicans species* as presence of a central venous catheter, prophylactic antifungal administration & mean number of antibiotics per day were associated with an increased risk of BSIs due to *non albicans* compared to *C. albicans.*



The Changing Epidemiology of Candida spp cont.

-The increased proportion of *non-albicans* species has been also observed in critically ill patients as hematological malignancies.

-The shift to *non-albicans* spp. is important as most of these species **are resistant to fluconazole or other antifungal agents**.

- Rapid species identification and antifungal susceptibility testing are required for the proper and timely treatment of these infections.

Candida non albicans spp

-***C. glabrata:***

-Most common in elderly patients.

-Most common in malignancies.

-***C. krusei***

-Haematological malignancies

-Neutropenia

Predisposing Factors for Invasive Candidiasis:

1- Prolonged ICU stay.

2-Central venous catheter.

3-Prolonged antimicrobial use.

4-Immunosuppressive agents

5-Chemotherapy.

6-Neutropenia

Risk factors for invasive *Candida* infections in the critically ill

Age
Colonization of body sites with *Candida*
Length of ICU stay
Administration of broad-spectrum antibiotics
Intravascular access devices
Diabetes mellitus
Parenteral nutrition
Mechanical ventilation
Renal insufficiency
Hemodialysis, hemofiltration
Antifungal prophylaxis
Surgery
Acute necrotizing pancreatitis
Treatment with corticosteroids

Pathophysiology of invasive candidiasis

- Weakening of host immunity occur, Candida spp. transition from **commensalism to opportunism**, which is associated with the induction of key virulence factors.
- The first is long-term and/or **repeated use of broad-spectrum antibiotics**, which enable increased **Candida spp, gut colonization** thus, 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.
- Another approach is **breach of the gastrointestinal and cutaneous barriers** by cytotoxic chemotherapy-induced mucositis , venous catheters, which collectively enable commensal
- Candida spp. to translocate from mucocutaneous sites into the bloodstream.

Virulence factors of Candida

- 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,
- Also, C. albicans secretes a variety of factors in the context of invasive infection, including secreted **aspartyl proteases** and **phospholipases** that are also important for promoting fungal tissue invasion and organ damage.
- Third, effective adherence and invasion of Candida spp. in endothelial and epithelial cells enable their dissemination into the bloodstream.
- Among the fungal proteins that mediate adherence, **agglutinin-like protein 3** , C. albicans-specific adhesin and invasin, was targeted in the development of a fungal vaccine .

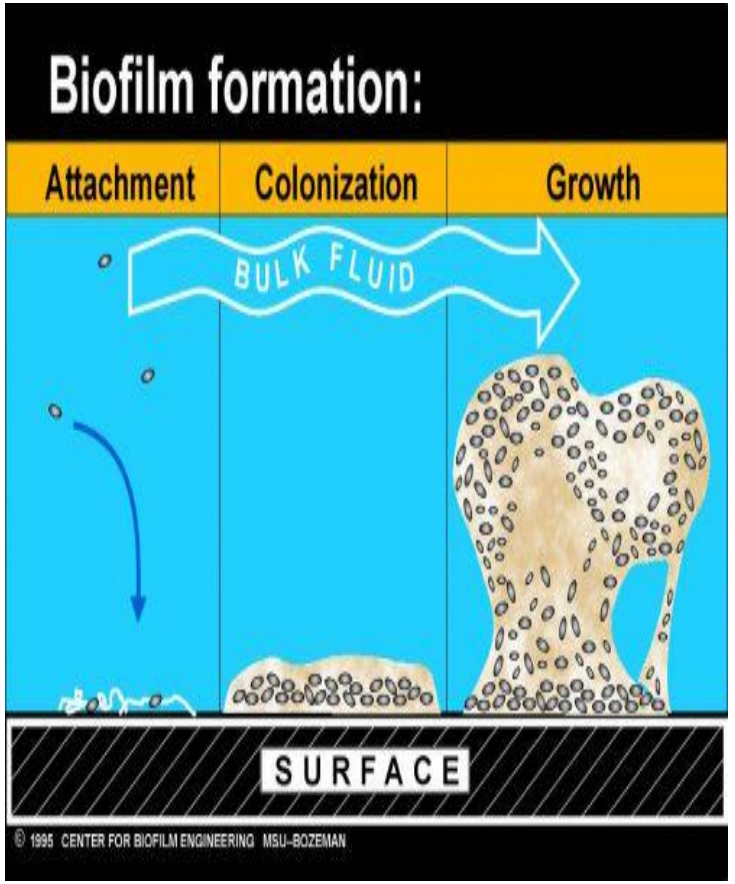
Other Virulence factors of Candida

-Biofilm formation:

It is an important virulence factor that enables attachment of *Candida* to surfaces , aggregation of extracellular matrix. This matrix is capable to sequester antifungal agents and makes it resistant to antifungal drugs.

Hydrolase secretion:

It secrets hydrolase after attachment to host surface which facilitates their penetration into cells.



Clinical Manifestations

- -Candidemia is the most common clinical presentation of all forms of invasive candidiasis.
- -The clinical presentation of fungal sepsis is not different from that of bacterial sepsis.
- In a prospective, randomized, double blind, multiple center study comparing the clinical manifestations of septic shock caused by bacteria or Candida spp., higher levels of lactate dehydrogenase in patients with bacterial septic shock and a **higher incidence of renal and hepatic failure in patients with candidal** septic shock were the only striking differences.
- Fungal sepsis does not present with specific clinical manifestations or laboratory abnormalities and thus remains a real challenge for physicians.

Diagnosis

- -Invasive candidiasis should be suspected in patients with known risk factors who have an unexplained fever that is unresponsive to antibacterial treatment.
- Diagnosis of invasive candidiasis Is key to ensure a favorable outcome. Delay in initiation of effective antifungal therapy has been associated with a doubling of mortality.
- However, diagnosing invasive candidiasis can be challenging as presence of bacteria in clinical specimens can outcompete fungal growth.
- 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.

I-Classical diagnostic techniques

-Blood culture remains the gold standard for diagnosis of invasive candidiasis. it is an essential diagnostic test and enables species identification and susceptibility testing.

-However, It is positive in 21–71% of patients with autopsy-proven invasive candidiasis depending on sampling frequency and volume of blood drawn.

-Frequent sampling (once daily or more frequently if the patient has a fever), larger

blood culture volumes, **the use of specialized fungal blood culture bottles** with selective medium and sampling before initiation of antifungal therapy increase sensitivity.

- In case of invasive candidiasis, blood culturing is considered a gold standard, but the long turnaround time (in the case of yeasts, up to five days; and moulds, up to four weeks) may put the patient at increased risk due to delay in delineating the necessary treatment plan.

- Many cryptic fungal species are unable to be isolated and grown on fungal culture media, thus escaping conventional detection. For example, (C. albicans, C. glabrata, C. parapsilosis, and C. haemulonii) which they referred to **as cryptic**, as they were **nonculturable on** conventional fungal media and were identified based on molecular methods.

II-Antigen detection

-**Mannan antigen and antimannan antibody:**Candida spp. mannan antigen and anti-mannan antibody detection can be useful for the detection of invasive candidiasis , including in pediatric patients and in those with CNS infections.

-β-D-glucan. β-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. The sensitivity for invasive candidiasis was generally high (76.7–100.0%) in seven studies. The only FDA-approved BDG assay is the **Fungitell Assay**. It has been shown to be useful for diagnosing intra-abdominal candidiasis **and blood culture negative** cases of pneumophila pneumonia

-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 nonalbicans Candida spp., including C. parapsilosis, with lower sensitivity.

- The advantage of performing serology based tests is **the rapid results** obtained, unlike culture methods.
- Serology test may give a positive result even if the culture test is negative or the fungal species is nonculturable.
- One major limitation of antibody-based testing **is seen in immunocompromised** or immunosuppressive patients that are **unable to elicit adequate levels of antibodies** and may show false negative results. However, fungal antigen detection in such patients offers the solution.
- **Polysaccharides or proteins (fungal antigens)** secreted during fungus growth may end up in different bodily fluids, making them ideal for detection in both immunocompetent and immunocompromised people as possible disease markers.

III-DNA-based techniques

The development and implementation of PCR-based tests for the detection of fungal 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 fungal cell wall interferes with cell lysis and the release of DNA resulting in false-negative signals.

Combination tests:

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 β-D-glucan tests** in a single blood sample or β-D-glucan positivity in two consecutive blood samples enabled the discrimination of invasive candidiasis from Candida spp. colonization in critically ill patients.

The combination of **β-D-glucan and procalcitonin** testing has been evaluated: their combination showed higher sensitivity & specificity.

Artificial intelligence and machine learning: a new era in fungal diagnostics

- Artificial intelligence (**AI)** is the simulation of human intelligence processes by machines, especially computer systems, and this simulation includes learning, reasoning, and self-correction.

- Machine learning (**ML**) is a branch of AI and computer science that focuses on the use of data and algorithms, allowing the software application to become more accurate at predicting outcomes just like humans do.

-Many AI and ML-based models have been explored to assist at many levels in fungal diagnostics.

(a) assistance in **microscopic image analysis for precise detection**;

(b) assistance in histopathological slide analysis

(c) assistance with medical imaging scans (X-rays, MRI & CT).

-Assistance via automated microscopic image analysis Initial diagnosis of fungal infections still relies on microscopic examination of fungi or fungal structures.

Obstacles in laboratory Diagnosis :

-The major problem with laboratory diagnosis of invasive fungal infections is due to the difficulty of providing **rapid and easy tools of diagnosis**.

-One difficulty in diagnosing invasive aspergillosis is defining the precise criteria required to establish the diagnosis.

-The gold standard for diagnosis remains identification of the organism by histopathology and/or growth in culture from tissue biopsy or aspirate from a sterile site.

-However, culture of the organism from non-sterile sites (such as sputum or bronchoalveolar lavage) from an immunocompromised host who has clinical evidence of infection can be utilized to support a **probable** diagnosis of invasive aspergillosis.

Targeted Antifungal Therapies

- -Several studies have shown that inappropriate empirical therapy or delays in the introduction of appropriate antifungals are associated with an increased mortality in patients with candidemia or septic shock due to Candida.

- Consider empirical antifungal therapy in critically ill patients with risk factors for invasive candidiasis and no other known cause of fever.

Prevention of invasive fungal infections

-Implementation of infection prevention and control strategies can prevent HAIs/invasive fungal infections.

- Application of standard precautions is the best way to decrease morbidity and mortality of fungal infections.

-Adherence to **hand hygiene** is a single best way to reduce the risk of HAIs fungal infections.

-Strict adherence for CDC guidelines to prevent CRBSI.

-Education of all healthcare personnel, including environmental services staff and ensure adequate supplies are available to implement infection control precautions.

Prevention cont

-Physicians should be aware of fungal infections.

-New diagnostic tools to help quick and accurate diagnosis are needed to identify infecting pathogens and assess antimicrobial susceptibility.

-Increase awareness about the rise in **antifungal drug resistance**.

-Conducting **multi-center candidemia surveillance** and performing species confirmation and antifungal susceptibility testing.

Conclusions:

- Fungal sepsis is associated with a higher mortality than bacterial & viral sepsis.
- Clinical manifestations of fungal infections are non specific, diagnosis & management are still a challenge.
- The increase in invasive fungal infections and the emergence of other than C. albicans species with reduced susceptibilities or intrinsic resistances to antifungal drugs highlight the absolute need of developing new diagnostic tools that could help identify among critically ill patients with invasive candidiasis.


Thank You!