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**Mycology**  
Medical Perspective

*Edited by Felix Bongomin*





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# Mycology - Medical Perspective

*Edited by Felix Bongomin*

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Edited by Felix Bongomin

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# Infectious Diseases

Volume 42

## Aims and Scope of the Series

This series will provide a comprehensive overview of recent research trends in various Infectious Diseases (as per the most recent Baltimore classification). Topics will include general overviews of infections, immunopathology, diagnosis, treatment, epidemiology, etiology, and current clinical recommendations for managing infectious diseases. Ongoing issues, recent advances, and future diagnostic approaches and therapeutic strategies will also be discussed. This book series will focus on various aspects and properties of infectious diseases whose deep understanding is essential for safeguarding the human race from losing resources and economies due to pathogens.



# Meet the Series Editor



Dr. Alfonso J. Rodriguez-Morales received his MD from Universidad Central de Venezuela in Caracas and his MSc in Protozoology/Parasitology from Universidad de Los Andes in Trujillo, Venezuela. He received his Diploma in Tropical Medicine & Hygiene (DT-M&H) from Universidad Peruana Cayetano Heredia, Lima, Peru, and the University of Alabama at Birmingham, Alabama, USA. He also holds a DipEd. Dr. Rodriguez-Morales is a fellow of the Royal Society for Tropical Medicine & Hygiene (FRSTMH), London, United Kingdom; of the Faculty of Travel Medicine (FFTM) of the Royal College of Physicians and Surgeons of Glasgow (RCPSG), Glasgow, Scotland, United Kingdom; of the American College of Epidemiology (FACE), USA; and the International Society for Antimicrobial Chemotherapy (FISAC). He has a HonDSc from Universidad Privada Franz Tamayo (UniFranz), Cochabamba, Bolivia. He is the President of the Latin American Society for Travel Medicine (SLAMVI) (2023–2025) and the Past President of the Colombian Infectious Diseases Association (2021–2023). He is a member of the Council (2020–2026) of the International Society for Infectious Diseases (ISID). He is a senior researcher at Colciencias (2015–2027) and a professor at the Fundación Universitaria Autónoma de las Américas in Pereira, Risaralda, Colombia, as well as at the Universidad Científica del Sur (UCSUR) in Lima, Peru. He is a visiting professor at multiple national and international universities.



# Meet the Volume Editor



Dr. Felix Bongomin is a physician, clinical researcher, and academic specializing in Internal Medicine and Infectious Diseases, with a strong focus on fungal diseases. He holds an MBChB from Gulu University and an MMED in Internal Medicine from Makerere University, MSc in Medical Mycology, and a Ph.D. in Infectious Diseases from the University of Manchester, UK. He is a lecturer at Gulu University School of Medicine. Dr. Bongomin has over a decade of professional experience as a clinician, researcher, and lecturer, with more than five years as a specialist physician in both private and public healthcare facilities in Uganda. His research spans fungal epidemiology, antifungal resistance, emerging fungal pathogens, and the intersection of climate change with fungal diseases. As an editor and author, he is committed to advancing knowledge, training future healthcare providers, and shaping global conversations on fungal infections.



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Perspective Chapter: Zhuxiang Syndrome – A Novel Mechanism Linking  
Chronic Fungal Infections to Systemic Inflammatory Diseases in Aging

*by Xiujuan Yan, Jian An and Charles Xiaoxiang Zhu*

# Preface

Fungal diseases have increasingly emerged as a significant challenge to global health. Once considered relatively rare, invasive and opportunistic mycoses are now recognized as major causes of morbidity and mortality, particularly among immunocompromised populations. Advances in modern medicine, such as transplantation, chemotherapy, and biologic therapies, have improved survival rates for many conditions; however, they have also expanded the pool of individuals at risk of severe fungal infections. At the same time, antifungal resistance is steadily rising, limiting the effectiveness of existing therapies and complicating treatment strategies.

This edited volume, *Mycology – Medical Perspective*, brings together contemporary insights into the epidemiology, diagnosis, clinical management, and therapeutic advances in medical mycology. It highlights the role of conventional and non-culture-based diagnostics, reviews current and novel antifungal therapies, and explores emerging threats such as resistant fungal pathogens. Attention is also given to the impact of environmental and global factors, including climate change, on fungal pathogenicity and patterns of resistance.

By synthesizing contributions from experts in the field, the book aims to provide clinicians, researchers, and public health practitioners with a comprehensive resource that bridges scientific knowledge and clinical application. It underscores the urgent need for awareness, innovation, and multidisciplinary approaches to tackle fungal infections as a growing public health concern.

I hope that this volume not only informs medical practice and research but also inspires continued efforts to strengthen surveillance, improve diagnostics, and expand therapeutic options in the fight against fungal diseases.

**Felix Bongomin**  
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# Introductory Chapter: Mycology – Medical Perspective

*Felix Bongomin*

## 1. Overview of medical mycology

Fungi represent a vast and diverse biological kingdom, distinct from plants, animals, and bacteria. In medical mycology, fungi are studied as both commensal organisms and pathogenic threats, capable of causing diseases known as mycoses. These infections range from superficial skin conditions to life-threatening systemic illnesses, particularly in immunocompromised individuals. The global impact of fungal diseases is staggering, contributing to over 1.5 million deaths annually, a mortality rate comparable to tuberculosis and exceeding malaria [1]. This growing burden is driven by increasing numbers of at-risk patients, including those with HIV/AIDS, uncontrolled diabetes, hematologic malignancies, and organ transplants, as well as the expanding geographic ranges of endemic fungi due to climate change [2].

Fungi exhibit remarkable morphological diversity, which is central to their identification and pathogenicity. Medically important fungi are classified into yeasts, molds, dimorphic fungi, yeast-like fungi, and atypical fungi. Yeasts, such as *Candida albicans* and *Cryptococcus neoformans*, are unicellular and reproduce by budding, often colonizing mucosal surfaces but capable of causing severe infections in susceptible hosts [3]. Molds, including *Aspergillus fumigatus* and Mucorales, grow as multicellular filaments called hyphae, forming intricate networks known as mycelia. These organisms produce spores that facilitate environmental survival and airborne transmission. Dimorphic fungi, such as *Histoplasma capsulatum* and *Coccidioides immitis*, exhibit temperature-dependent morphogenesis, existing as molds in the environment but converting to pathogenic yeast forms within human tissues. Yeast-like fungi, such as *Candida* species, under certain conditions, form pseudohyphae—elongated chains of budding cells that enhance tissue invasion. Finally, atypical fungi, including *Pneumocystis jirovecii* and Microsporidia, lack conventional fungal features but are clinically managed as fungal pathogens due to their susceptibility to antifungals and their impact on immunocompromised patients.

Fungal diseases encompass a wide spectrum of conditions beyond direct infections, including mycotoxicosis, colonization by fungal mycelium, infections (mycoses), and allergies. Mycotoxicosis results from ingestion or exposure to toxic fungal metabolites, such as aflatoxins produced by *Aspergillus flavus*, which contaminate crops and cause liver damage, or ergot alkaloids from *Claviceps purpurea*, leading to vasoconstriction and neurological symptoms. Fungal mycelium, while often benign, can colonize tissues or medical devices, such as airway fungal balls caused by *Aspergillus* in preexisting lung cavities or catheter-associated biofilms formed by *Candida* species. Infections (mycoses) range from superficial conditions like tinea pedis (athlete's foot) caused by dermatophytes to life-threatening invasive

aspergillosis in immunocompromised hosts. Additionally, fungi provoke allergic reactions, such as allergic bronchopulmonary aspergillosis (ABPA), a hypersensitivity response to *Aspergillus* antigens, or fungal sinusitis, where chronic inflammation arises from mold exposure. Together, these examples illustrate the multifaceted impact of fungi on human health, extending far beyond conventional infections.

Clinically, mycoses are categorized based on the depth of tissue involvement and the host's immune status. Superficial mycoses, such as those caused by *Malassezia* species, are confined to the outermost layers of skin and hair, often causing cosmetic concerns rather than serious disease. Cutaneous mycoses, including dermatophytoses (e.g., tinea pedis, tinea capitis) and cutaneous candidiasis, involve deeper keratinized tissues but rarely invade living layers. Subcutaneous mycoses, such as sporotrichosis, mycetoma, and chromoblastomycosis, typically arise from traumatic inoculation of fungi into subcutaneous tissues and are more common in tropical regions. Systemic mycoses are the most severe, divided into endemic dimorphic infections (e.g., histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, blastomycosis) and

| Mycoses      | Key points                                                                                                                                                                                                    | Disease                                         | Causative fungi                                                                                                                                                     |
|--------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Superficial  | <ul style="list-style-type: none"> <li>• superficial infection of the skin or hair shaft</li> <li>• no invasion of living tissue</li> <li>• lack of host cellular response</li> </ul>                         | Tinea nigra                                     | <i>Hortaea werneckii</i>                                                                                                                                            |
|              |                                                                                                                                                                                                               | White piedra                                    | <i>Trichosporon</i> spp.                                                                                                                                            |
|              |                                                                                                                                                                                                               | Black piedra                                    | <i>Piedraia hortae</i>                                                                                                                                              |
| Cutaneous    | <ul style="list-style-type: none"> <li>• superficial infection of skin, nails, or hair</li> <li>• no invasion of living tissue</li> <li>• cellular host response</li> </ul>                                   | Dermatomycosis                                  | Non-dermatophyte molds<br><i>Neoscytalidium dimidiatum</i><br><i>Scopulariopsis brevicaulis</i>                                                                     |
| Subcutaneous | <ul style="list-style-type: none"> <li>• chronic, localized cutaneous and subcutaneous infections</li> <li>• usually following traumatic inoculation of the agent</li> <li>• regional epidemiology</li> </ul> | Sporotrichosis                                  | <i>Sporothrix</i> spp.                                                                                                                                              |
|              |                                                                                                                                                                                                               | Chromoblastomycosis                             | <i>Fonsecaea</i> spp.<br><i>Cladophialophora carrionii</i> .<br><i>Rhinocladiella aquaspersa</i> ,                                                                  |
|              |                                                                                                                                                                                                               | Phaeohyphomycosis                               | <i>Cladophialophora</i> spp.<br><i>Exophiala</i> spp.<br><i>Bipolaris</i> spp.<br><i>Exserohilum</i> spp.<br><i>Phialophora</i> spp.<br><i>Pyrenochaeta romeroi</i> |
|              |                                                                                                                                                                                                               | Eumycotic mycetoma                              | <i>Scedosporium apiospermum</i><br><i>Madurella</i> spp.<br><i>Acremonium</i> spp.<br><i>Exophiala</i> spp.<br><i>Aspergillus nidulans</i>                          |
|              |                                                                                                                                                                                                               | Actinomycotic mycetoma*                         | <i>Nocardia</i> spp.*<br><i>Actinomadurea</i> spp.*<br><i>Streptomyces</i> spp.*                                                                                    |
|              |                                                                                                                                                                                                               | Subcutaneous zygomycosis (Entomophthoromycosis) | <i>Basidiobolus ranarum</i><br><i>Conidiobolus coronatus</i>                                                                                                        |
|              |                                                                                                                                                                                                               | Lobomycosis                                     | <i>Loboa lobi</i>                                                                                                                                                   |
|              |                                                                                                                                                                                                               | Rhinosporidiosis*                               | <i>Rhinosporidium seeberi</i> *                                                                                                                                     |
|              |                                                                                                                                                                                                               | Protothecosis*                                  | <i>Prototheca</i> spp.*                                                                                                                                             |

| Mycoses                                        | Key points                                                                                                                                                                                                                                               | Disease                                        | Causative fungi                                                                                                                                                                                               |
|------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Dimorphic systemic (incl. The endemic mycoses) | <ul style="list-style-type: none"><li>• Thermally dimorphic fungi that are capable of changing morphology (mycelial saprophytic form at 25°C and yeast-like pathogenic form at 37°C)</li><li>• Mainly restricted to certain geographical areas</li></ul> | Histoplasmosis                                 | <i>Histoplasma capsulatum</i><br><i>Histoplasma dubosii</i>                                                                                                                                                   |
|                                                |                                                                                                                                                                                                                                                          | Blastomycosis                                  | <i>Blastomyces dermatitidis</i>                                                                                                                                                                               |
|                                                |                                                                                                                                                                                                                                                          | Coccidioidomycosis                             | <i>Coccidioides immitis</i><br><i>Coccidioides posadasii</i>                                                                                                                                                  |
|                                                |                                                                                                                                                                                                                                                          | Paracoccidioidomycosis                         | <i>Paracoccidioides brasiliensis</i>                                                                                                                                                                          |
|                                                |                                                                                                                                                                                                                                                          | Talaromyces infection (formerly Penicilliosis) | <i>Talaromyces marneffei</i>                                                                                                                                                                                  |
| Opportunistic systemic                         | <ul style="list-style-type: none"><li>• Generally inherent low virulence</li><li>• Infections are opportunistic, i.e., occur in immunocompromised and immunosuppressed hosts</li></ul>                                                                   | Pseudallescheriasis/<br>Scedosporiosis         | <i>Scedosporium</i> spp.<br><i>Lomentospora prolificans</i>                                                                                                                                                   |
|                                                |                                                                                                                                                                                                                                                          | Hyalohyphomycosis                              | <i>Penicillium</i> spp.<br><i>Paecilomyces</i> spp.<br><i>Beauveria</i> spp.<br><i>Fusarium</i> spp.<br><i>Scopulariopsis</i> spp.<br><i>Trichosporon</i> spp.<br><i>Aspergillus</i> spp.<br><i>Mucor</i> spp |
|                                                |                                                                                                                                                                                                                                                          | Phaeohyphomycosis                              | <i>Cladophialophora</i> spp.<br><i>Exophiala</i> spp.<br><i>Bipolaris</i> spp.<br><i>Exserohilum</i> spp.                                                                                                     |
|                                                |                                                                                                                                                                                                                                                          | Adiaspiromycosis                               | <i>Emmonsia crescens</i><br><i>Emmonsia parva</i>                                                                                                                                                             |
|                                                |                                                                                                                                                                                                                                                          |                                                |                                                                                                                                                                                                               |

*\*non-fungal causative agents to be considered as part of the differential diagnosis (With contribution from Dr. Charlotte Sriruttan and Prof David W. Denning).*

**Table 1.**  
Classification of clinically important fungi and fungal diseases.

opportunistic infections (e.g., invasive candidiasis, pneumocystosis, aspergillosis). Endemic mycoses are geographically restricted and often present as pulmonary diseases, whereas opportunistic infections predominantly affect immunocompromised individuals, leading to high mortality if untreated. Emerging threats, such as *Candida auris* and mucormycosis, highlight the evolving challenges in fungal disease management, particularly with the rise of antifungal resistance and hospital-acquired outbreaks (Table 1).

Diagnosing fungal infections requires a multifaceted approach. Direct microscopy using potassium hydroxide (KOH) or calcofluor white staining allows rapid visualization of fungal elements in clinical specimens. Culture techniques, such as growth on Sabouraud dextrose agar, remain the gold standard for species identification but may be slow, particularly for dimorphic fungi. Histopathological examination with special stains (e.g., Grocott's methenamine silver, periodic acid-Schiff) is crucial for detecting tissue-invasive fungi. Modern molecular methods, including polymerase chain reaction (PCR) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, have revolutionized fungal diagnostics

by enabling rapid and precise identification [4]. Serological and antigen-based tests, such as  $\beta$ -D-glucan assays and cryptococcal antigen testing, provide valuable non-invasive diagnostic tools, particularly for deep-seated infections. Despite these advances, diagnostic delays remain a significant challenge, especially in resource-limited settings where access to advanced testing is restricted [5].

Antifungal therapy targets unique fungal structures, primarily the cell wall and membrane, which are absent in human cells [6]. The five major classes of antifungals include polyenes, azoles, echinocandins, allylamines, and antimetabolites. Polyenes, such as amphotericin B, bind to ergosterol in fungal membranes, causing cell leakage and death. While highly effective, their use is limited by toxicity, particularly nephrotoxicity. Azoles, including fluconazole and voriconazole, inhibit ergosterol biosynthesis by targeting the enzyme lanosterol 14 $\alpha$ -demethylase, offering broad-spectrum activity with oral bioavailability. However, azole resistance is an increasing concern, particularly in *Aspergillus* and *Candida* species. Echinocandins, such as caspofungin, disrupt fungal cell wall synthesis by inhibiting  $\beta$ -1,3-glucan synthase, making them first-line agents for invasive candidiasis. Allylamines, like terbinafine, block squalene epoxidase in the ergosterol pathway and are primarily used for dermatophytoses. Flucytosine, an antimetabolite, interferes with fungal DNA and RNA synthesis but is rarely used alone due to rapid resistance development. Combination therapy is often employed for severe infections, such as cryptococcal meningitis, where amphotericin B and flucytosine are used synergistically.

The epidemiology of fungal diseases is shaped by host immune status, environmental exposure, and geographic factors. HIV/AIDS remains a major risk factor for opportunistic infections, particularly cryptococcal meningitis and *Pneumocystis* pneumonia. Transplant recipients and oncology patients are at high risk for invasive aspergillosis and candidiasis due to prolonged immunosuppression. Climate change is altering the distribution of endemic fungi, with *Coccidioides* expanding into new regions and extreme weather events potentially increasing spore dispersal. Antifungal resistance, driven by both clinical and agricultural antifungal use, poses a growing threat, exemplified by multidrug-resistant *Candida auris*. In low-resource settings, limited diagnostic capacity and antifungal access contribute to high mortality rates, underscoring the need for global health initiatives to improve fungal disease management.

Medical mycology has evolved from a niche discipline to a critical component of modern medicine, driven by the rising prevalence of fungal infections and the challenges posed by antifungal resistance. Future progress hinges on enhanced diagnostics, novel antifungal development, and global collaboration to address disparities in fungal disease burden. This text aims to provide a comprehensive foundation in medical mycology, bridging basic science with clinical practice to equip students and practitioners with the knowledge needed to tackle these complex infections.

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# Invasive Fungal Diseases Caused by Fungi of the Genera *Aspergillus* and *Fusarium* and Agents of the Order Mucorales

*Roseli Freitas-Xavier, Eduarda Santos, Camila Silva  
and Isabel Maciel*

## Abstract

Immunocompromised patients or those undergoing immunosuppressive therapies are more susceptible to fungal infections. Fungi confer persistence in the host through virulence factors and diverse interactions with interference in both innate and adaptive immune response pathways, which can alter both homeostatic and effector function, affecting the immune response of T lymphocytes during infection. Pathogen-associated molecular patterns and their interaction with pattern recognition receptors on host cells may be determining factors in the immune response to these infections. Invasive fungal diseases caused by *Aspergillus*, *Fusarium*, and fungi of the order Mucorales are an emerging global problem caused by the widespread use of aggressive immunosuppressive therapies in patient populations and the increasing use of invasive devices. New immunomodulatory drugs have opened up a new spectrum of patients at risk of invasive fungal diseases. There is a need for further studies on the pathogens responsible for invasive fungal diseases whose detailed molecular mechanisms have yet to be clarified. These studies will provide a better understanding of the epidemiology and pathophysiology of these pathogens, with special attention to diagnosis and treatment in various clinical situations. The scientific community and government agencies need to work together to reduce the burden of these complex diseases that are difficult to diagnose and treat.

**Keywords:** invasive filamentous fungal diseases, *Aspergillus*, *Fusarium*, Mucorales order, host-parasite relationship

## 1. Introduction

Research has highlighted the global problem of invasive filamentous fungal diseases (IFDs), diagnosed primarily by microscopic analysis and associated with abnormal clinical findings. Fungal isolation should be associated with an abnormal

clinical or radiological picture consistent with an infectious disease process, amplification of fungal DNA by polymerase chain reaction (PCR) and DNA sequencing when filamentous fungi are present in formalin-fixed tissue [1].

Although invasive lung disease is likely to be host-related, such as a recent history of neutropenia temporally related to the onset of invasive fungal disease (haematological malignancy), receipt of allogeneic stem cell transplantation, receipt of solid organ transplantation, prolonged use of corticosteroids (excluding patients with allergic bronchopulmonary aspergillosis), treatment with other approved T-cell immunosuppressants in the past 90 days, treatment with approved B-cell immunosuppressants, severe hereditary immunodeficiency, grade III or IV acute graft-versus-host disease involving the gut, lung or liver refractory to first-line steroid therapy [1, 2]. IFDs are an emerging global problem, driven by the widespread use of aggressive immunosuppressive therapy in patient populations and the increasing use of invasive devices. New immunomodulatory drugs have opened up a new spectrum of patients at risk of IFDs [2]. Another risk factor is when the etiological agent is part of the host organism's ecological niche associated with the underlying disease.<sup>1</sup> Environmental factors and trauma also play a role in the epidemiology of fungal infections, such as hospital outbreaks [3]. Environmental fungal biodiversity can lead to co-infections and potentiation of the infectious process, and nonculture-based methods are increasingly used for rapid, accurate diagnosis to improve patient outcomes [4]. The increase in IFDs in immunocompromised and critically ill patients is accompanied by an increase in fungal infections [5, 6]. Giacomazzi et al. [6] report that more than 3.8 million people in Brazil may be suffering from serious fungal infections. Patients undergoing intensive chemotherapy have significant impairment of immunity, whether cellular or humoral, thus leading to a high risk of complications from infectious processes. Suppression of the immune response, whether cellular or humoral, is associated with high morbidity and mortality. Treatment of fungal infection leads to disruption of mucosal membrane surfaces, impairment of intestinal peristaltic activity and microbiological imbalance of the host's intestinal flora [5]. The scientific community and government agencies must work closely together to reduce the burden of these complex diseases that are difficult to diagnose and treat [6]. There is an urgent need for early diagnosis and implementation of antifungal therapy, as late implementation leads to poor clinical outcomes. However, the diagnosis of IFD remains problematic because conventional diagnostic methods used in routine practice are slow, have low sensitivity and specificity, and are unable to distinguish between colonisation and disease [5]. Furthermore, the ability of fungi to colonise and/or infect the human host demonstrates an enormous capacity for specific reprogramming of the adaptive mechanisms developed by the fungus to ensure its survival under various adverse conditions in the struggle for nutrients [7].

## **2. Fungal cell wall**

The fungal cell wall is a dynamic structure that undergoes continuous biochemical modification and remodelling during biosynthesis. The main components are chitins,  $\beta$ -glucans, polysaccharides, glycoproteins, enzymes and lipids [8]. Most of the available information relates to the wall structure of *Saccharomyces cerevisiae*, which is composed of  $\beta$ -D-glucans, chitins and mannoproteins (MPs) [9]. Its structural composition is an excellent target for the action of antifungal drugs and for the recognition of immune system cells. Its components are not found in human, animal or plant cells [8, 10].

The fungal cell wall contained (50–60%) glucans (glucose polymers), in particular ( $\beta$ -1,3), which is the main component of the inner wall; (20–30%) branched glycoproteins ( $\beta$ -1,6), which link the components of the inner and outer walls; mannans (chains of several mannose molecules); and a small amount of chitin. Chitin, a long linear homopolymer formed by residues of (N-acetylglucosamine) ( $\beta$ -1,4), varies between fungal species, with about (1–2%) in yeast and (10–20%) in filamentous fungi [11].

Glucans mainly contain the  $\beta$ -1,3 linkage, and a small proportion, about 10%, of the linkages with branches vary as  $\beta$ -1,6 and  $\beta$ -1,4 [12]. Mannosylation plays a role in fungal growth and virulence. Studies in experimental models show that N- and O-linked mannans are critical for virulence during infections [8]. O-linked mannose residues are linked to serine/threonine and linked to O-glycosylation to promote high fungal colonisation in tissues. At the same time, N-linked mannans recognise innate immune cells and cytokine production by macrophages and dendritic cells [8].

MPs are key antigens that stimulate T-cell responses, resulting in delayed-type hypersensitivity (DTH) reactions and cytokine production [8, 9]. Mannoproteins on the cell wall surface are highly glycosylated polypeptides, i.e., glycoproteins, consisting of approximately 50–95% carbohydrates [8, 9].

They have a functional domain rich in serine and threonine at the C-terminus that forms a fixation anchor for glycosylphosphatidylinositol (GPI), an anchor that binds to cell membrane proteins or to ( $\beta$ -1,6) glucans in the cell wall, which in turn are glycosidically linked to the ( $\beta$ -1,3) glucan-chitin complex that forms the inner wall fibres [8, 9]. The structures of these modules show that these processes take place outside the plasma membrane [8, 9, 12]. The mannosylation that occurs in this serine and threonine region is extremely important because it facilitates recognition by antigen-presenting cells through these exposed mannose receptors, demonstrating a crucial contribution of mannosylation to immunogenicity [13, 14].

The Genome Project has already identified more than 40 serine/threonine (GPI)-rich N-terminal regions, but only a few proteins with these functional domains are known [14]. The mannosylation of glycoconjugates appears to be a conserved pattern in fungi; these glycosylation patterns may be determinants in fungal pathogenesis. Therefore, studies with more approaches targeting these glycosylation pathways are needed to understand the virulence of pathogenic fungi.

### **3. Innate immunity and the integrity of the organism**

Innate and cellular immunity are fundamental to host defence [7, 15]. However, this depends on the infectious agent, the antigenic stimulus, and the microenvironment in which cytokines are produced [7].

In fungal infections, the immune response depends on how these cells are activated [15]. There is a collection of pattern recognition receptors (PRRs) expressed on phagocytic cells that recognise a variety of pathogen-associated molecular patterns (PAMPs)-chitin,  $\beta$ -glucans and mannans [7, 16, 17].

These interactions between host pattern recognition receptors (PRRs), such as those of the dectin and toll-like receptor (TLR) families, and pathogen-associated molecular patterns (PAMPs) determine the course of infection and the host immune response [7]. PRRs eliminate the invading pathogen from the host through phagocytosis, cytokine and chemokine release, and promote the initiation of innate immune defences against infection (NLRs) [16].

### **3.1 Receptors, TLR, NOD, RLR and CLR**

The well-characterised PRRs are the dectin and toll-like receptor (TLR) families, nucleotide-binding oligomerisation domain (NOD-like), Pyrin Domain family (NLR), receptor signalling pathways (RIG-I-like—RLR) and C-type lectin-like (CLR). They differ in their ligand recognition, signalling and cellular localisation. Studies have shown that CLRs are mainly recognition receptors for fungi, while TLRs and NLRs play auxiliary roles [7, 16].

CLRs are expressed in myeloid cells, macrophages, dendritic cells (DCs), lymphocytes, granulocytes, osteoclasts and epithelial cells. Dectin-1 is a CLR that recognises  $\beta$ -1,3 glucans present in the cell walls of several fungal species [7, 16]. Dectin-2 also recognises fungal and mannose structures in a calcium-dependent manner. Transmembrane TLRs recognise PAMPs such as lipopolysaccharide (LPS) and peptidoglycan, whereas TLRs located in intracellular membranes recognise fungal nucleic acids in the infected cell [7, 16].

Family Pyrin Domain (NLRs) are cytoplasmic receptors that recognise PAMPs from internalised pathogens, can form inflammasomes upon activation, and associate with caspase-1 to form the basis of the inflammasome. Studies show that family Pyrin Domain containing 3 NLRP3 is an inflammasome that contributes to immunity during fungal infections [7, 16].

The fungal cell wall is a dynamic structure that can deceive the immune system, preventing the inflammatory process and ensuring its evasion, adaptation and opportunism [18].

### **3.2 Macrophages, neutrophils and dendritic cells**

Macrophages, neutrophils, and dendritic cells have a number of receptors that can recognise and trigger stimulatory, modulatory, or even inhibitory signals for the different cell types of the immune system [7, 18]. Macrophages produce cytokines and induce the proliferation of alpha/beta LT. When exposed to a microenvironment dominated by interferon-gamma (IFN- $\gamma$ ), they differentiate into macrophages activated by the classical pathway, known as macrophages (M1) [19, 20]. When exposed to a microenvironment consisting of interleukins of the type of IL-4, IL-13, and IL-10, they are activated by the alternative pathway (M2), differentiate into anti-inflammatory cells, and have a low capacity for antigen presentation [19, 20].

Neutrophils exert fungicidal activity through oxidative reactions and the production of pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF- $\alpha$ ), IL1- $\beta$ , IL-6, and IL-8 [15]. In addition to increasing the production of these cytokines, TNF- $\alpha$  favours the migration of leukocytes to the site of infection [15].

Dendritic cells DCs play an important role from the outset of the immune response by promoting the interface between the innate and adaptive immune systems during infection [21]. They are phagocytic cells specialised to recognise a wide variety of pathogens and are efficient in processing antigens and activating the adaptive immune system [21].

## **4. History/aetiology, *Aspergillus* sp.**

Plaignaud [22] relates that the first aspergillosis case occurred during the French Revolution in 1789 when a 22-year-old soldier suffered severe facial pain

and protrusion of the right eye due to a fungal mass in the maxillary sinus. After surgery, the mass grew back, causing difficulty swallowing and breathing. He was treated with excision and cauterisation and was discharged 134 days later without recurrence [22].

The species *fumigatus* was described in 1863 [23]. Disseminated *Aspergillus* occurred from 1920 until the beginning of the Second World War and was treated with potassium iodide, followed by nystatin and amphotericin in the 1950s [24, 25].

The genus *Aspergillus* contains over 250 species, divided into 22 distinct sections. Of these 13 sections, including *Candidi*, *Circumdati*, *Flavipides*, *Fumigati*, *Nidulantes*, *Nigri*, *Ornati*, *Resctricti*, *Tanneri*, *Terrei*, *Usti*, *Versicolores* and *Warcupi*, they contain species of clinical relevance [26]. Seyedmousavi and Verweij [27] reported that approximately 80% of isolated cases of invasive aspergillosis (IA) are due to *A. fumigatus*, which belongs to the section *Fumigati* [27]. These complex groups comprise approximately 30 species, which are distinguished only by molecular identification. However, they show very similar phenotypic aspects, such as *A. fumigatus*, *A. lentulus*, *Neosartorya udagawe*, *N. pseudofischeri* and others. However, *A. flavus* (Section *Flavi*), *A. niger* (Section *Nigri*) and *A. terreus* (Section *Terrei*) have been isolated more frequently, strongly suggesting the emergence of other *Aspergillus* species associated with patients with immunodeficiencies [27].

#### 4.1 *Aspergillus* wall cell

In the genus *Aspergillus*, polysaccharides galactosaminogalactans and galactomannans (GM) are a virulence factor. Galactosaminogalactans contain galactosamine, a linear exocellular molecule identified in *A. nidulans*. Galactomannans associated with galactosaminogalactans are the major components of biofilm formation found in aspergillomas and lung lesions [8, 10, 28]. Galactosaminogalactans have immunosuppressive properties, inhibiting the production of IFN- $\gamma$  and neutrophil chemokines, thereby promoting the pathogenesis of infection [8, 10]. Galactomannans contain mannose and are the major antigens detected in serum and bronchoalveolar lavage in invasive aspergillosis caused by *A. fumigatus* [8, 28].

Aspergillosis infection by *A. fumigatus* occurs with detection in three different forms: (1) found in the culture medium as a free polysaccharide, (2) covalently linked to  $\beta$  (1  $\rightarrow$  3) glucans of the cell wall, and (3) anchored to the glycosylphosphatidylinositol (GPI) membrane forming the inner layer of the fungal cell wall [8, 10]. *Aspergillus fumigatus* and *A. flavus* also have functional structures similar to the serine- and threonine-rich regions where glycosylation occurs [8, 14].

Kang et al. [10] assessed the composition of the fungal cell wall of *A. fumigatus* by solid-state (NMR) spectroscopy and found that chitin and  $\alpha$ -1,3-glucan form a hydrophobic structure surrounded by a hydrated matrix of diversely linked  $\beta$ -glucans and covered by a dynamic layer of glycoproteins and  $\alpha$ -1,3-glucan. The distribution of two  $\alpha$ -1,3-glucan domains demonstrates the dual function of this molecule, contributing to cell wall rigidity and fungal virulence on bronchoalveolar lavage [10]. However, the role of  $\alpha$ -glucans is not fully understood; exposure of *A. fumigatus* to  $\alpha$  (1  $\rightarrow$  3) glucans during conidial germination promotes aggregation and development of these germ tubes [8, 10, 28].

The polysaccharides,  $\beta$  (1  $\rightarrow$  3) -glucans, have been characterised in *Aspergillus* spp.  $\beta$ -D-glucans are present in practically all fungi and are recognised as immunomodulators, and large particles of  $\beta$ -glucans induce the production of cytokines and

reactive oxygen species (ROS) that promote phagocytosis by neutrophils and macrophages, while soluble  $\beta$ -glucans act as antagonists of these responses [8, 10].

Chitins and  $\beta$ -glucans are conserved, abundant polysaccharides found in most fungi. The chitin, an insoluble component of *A. fumigatus*, can be pure or associated with chains, as chitin is associated with  $\beta$ -1,3-glucans and GM is associated with the branched chains of  $\beta$ -1,3-glucans. Chitin induces activation of leukocytes and macrophages via the alternative pathway; however, when phagocytosed by macrophages, only chitosans can induce the NLRP3 inflammasome and caspase-1 activation and IL-1 $\beta$  release [8, 10, 28].

## 4.2 Epidemiology and pathophysiology *Aspergillus*

This genus includes ubiquitous and saprophytic fungi found in humid environments, whose ecological niche is the soil, where there is decomposing organic matter present [29, 30]. Many species are used in biotechnology for the production of metabolites, organic acids, drugs, enzymes, and food fermentation [26]. Aspergillosis has a social and economic impact. They spoil food and produce mycotoxins [31]. They are also human and animal pathogens [31]. *Aspergillus* spores are found in air, soil, food, air conditioning systems, and various buildings, including hospitals with poor structural or hydraulic conditions [2]. These environmentally dispersed conidia can be inhaled and reach the pulmonary alveoli and, depending on the immune status of the host, can lead to chronic and non-invasive forms of infection with overlapping features ranging from the development of a fungal ball (aspergilloma) to a chronic inflammatory and fibrotic process currently classified as chronic pulmonary aspergillosis [32]. Currently, invasive aspergillosis (IA) manifests mainly in intensive care unit (ICU) patients as invasive pulmonary aspergillosis (IPA) and other factors such as pre-existing lung disease or comorbidities such as chronic obstructive airway disease, bronchiectasis, decompensated liver disease, chronic heart failure and adult respiratory distress syndrome [33].

Continued exposure to the fungal environment is a convenient source of IFD for recipients and in lung transplantation (SOT) due to inhalation/colonisation of *Aspergillus* through the respiratory tract [34].

Rapid deterioration of respiratory function is possible in ICU patients infected with influenza or coronavirus (SARS-CoV-2) [35].

In 2020, the European Organisation for Research and Treatment of Cancer and Mycoses Study Group Education and Research Consortium consensus definitions of DFI to promote communication and comparison of DFI study results in cancer patients undergoing allogeneic haematopoietic stem cell transplant (HSCT) or solid organ transplantation to evaluate antifungal therapy, diagnostic tests and epidemiological studies [1].

Aspergillosis is a neglected disease despite its high mortality rate, with approximately 3,000,000 cases of chronic pulmonary aspergillosis and 250,000 cases of IA. In Brazil, there were 1854 cases of IA, representing (4.5%/100,000) people, of which 13.4% were in patients with acute myeloid leukaemia, 2.3% in HSCT recipients, 0.5% in kidney transplant recipients and 13.3% in lung transplant recipients (no data on chronic obstructive pulmonary disease) [36].

A multicentre study involving 30 ICUs from eight countries found *A. fumigatus* to be the most common species (92%) in IA13. Case fatality rates for *A. flavus*, *A. terreus* and *A. niger* range from 65 to 90%, with missed diagnoses often occurring at autopsy of ICU patients [37].

### 4.3 Diagnosis *Aspergillus*

There is no gold standard test for the diagnosis of aspergillosis, but a combination of microbiological and histopathological findings, host factors and clinical and radiological evidence is required [38].

It remains a challenging problem in clinical mycology, delaying the initiation of antifungal therapy [38].

Isolation and identification of *Aspergillus* spp. from clinical specimens is the standard approach to establishing a proven case. Cultures obtained from respiratory specimens may not reflect the severity of the disease, as there may be a low fungal load or airway or environmental contamination, in contrast to a biopsy. It is important to emphasise that the visualisation of hyphae suggestive of *Aspergillus*, both in direct and histological examination, does not allow differentiation from other agents of hyalohyphomycosis and only after isolation is a result available for evaluation [39].

Cocio et al. [40] report the first case of pulmonary aspergillosis associated with COVID-19 (CAPA) caused by *A. welwitschiae* in America, specifically in the rural area in the northwestern region of the state of São Paulo, Brazil. *Aspergillus welwitschiae* was detected in bronchoalveolar lavage (BAL) and identified using morphological methods, with significant morphological changes, as shown in **Figure 1** [40].

In view of these difficulties, immunodiagnosics become important in this group of patients with a high morbidity rate. The main aim of serological tests is to detect

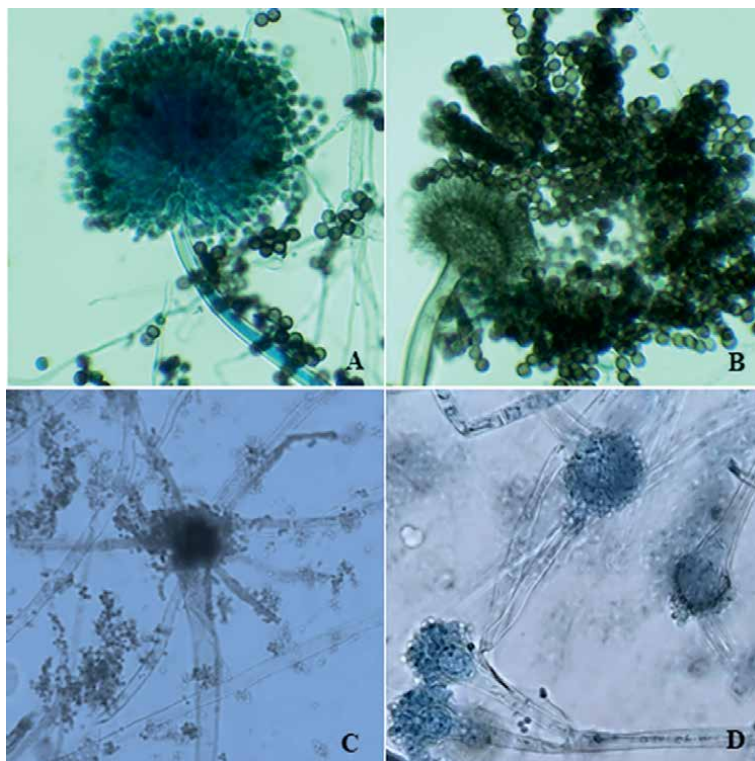


**Figure 1.** Mycological diagnosis of this black *Aspergillus*, section *Nigri* was evaluated using *A. tubingensis* (CBS 122719), as a control. Micromorphology was studied on potato dextrose agar. In Figure A and B, *Aspergillus tubingensis*; C and D *Aspergillus welwitschiae* [37].

circulating antibodies to the infecting fungus in the host serum, since the detection of circulating antigens, particularly GM, is important. Among the serological techniques used in the diagnosis of aspergillosis, the following stand out: double immunodiffusion in agarose gel, counterimmunoelectrophoresis (CIE), enzyme-linked immunosorbent assays (ELISA), which allow the detection of both antibodies and circulating antigens and immunoblotting (IB) [38].

It is important to emphasise that GM testing is a screening test in clinical practice. However, it has limitations, including high rates of false-positive results and reproducibility, which require caution in interpreting results [41].

The detection of anti-*Aspergillus* antibodies is necessary for the diagnosis of aspergillosis. However, there is no international consensus on the serological tests used and no fully standardised test is available [1].

The French nomenclature of biological tests established in 1995 distinguishes between screening and confirmatory tests for *Aspergillus* serology in two phases: (1) a screening phase with electrosyneresis, haemagglutination, enzyme immunoassay or double agar gel immunodiffusion tests and (2) a confirmatory phase with positive immunoelectrophoresis or Western blot tests [42]. According to the French consensus, the main serological screening tests were used in 20 tertiary care centres. ELISA was used for screening in 81% of centres, while immunoelectrophoresis was used as a confirmatory test in 70% of centres. Standardisation of immunoelectrophoresis and the advent of commercial Western blot kits are still needed but are promising alternatives for confirmation and diagnosis [42].

In a review study, Fang et al. [43] reported overall sensitivities and specificities of 67–100% and 86–100% for the fungal cell wall components GM and 1,3b-d-glucan (BDG) biomarkers to identify patients with IA; Bio-Rad's Platelia *Aspergillus* enzyme immunoassay (EIA) is the only approved GM test [40]. Sulahian et al. [44] compared the Fungitell b-glucan assay with the GM test in a case-control study of 105 IA patients and 147 controls. The BDG assay was found to be more sensitive (97% versus 82%,  $P = 0.0001$ ) but less specific (81% versus 49%,  $P < 0.0001$ ) than the GM assay for the diagnosis of IA [44].

Lu et al. [45] evaluated the performance of the Dynamiker enzyme-linked immunosorbent assay kit, used for (IgG) detection of *A. fumigatus* strains, in diagnosing non-neutropenic patients with IPA [42]. The authors found that the combined positive diagnosis of immunoglobulin (IgG) and bronchoalveolar lavage GM resulted in higher sensitivity compared to serum GM. Given the convenience of blood sampling, this is a good screening and diagnostic method for non-neutropenic patients with IPA, especially those who cannot tolerate invasive procedures. Rozaliyani et al. [46] analysed the efficacy of Western blot (WB) and rapid immunochromatography (ICT) tests in detecting *Aspergillus* in 88 patients divided into chronic pulmonary aspergillosis (CPA) and non-CPA groups [46]. The results showed that 39% of the patients had positive WB results, with a sensitivity of 80% in the CPA group, compared to only 26% in the non-CPA group. The 16 and 18–20 kDa bands were the most prevalent in the CPA group, and the total intensity of the bands was significantly higher compared to the non-CPA group. The ICT also showed high sensitivity (85%) in the CPA group. The agreement between the two methods was substantial, with 86% of results in agreement, although 14% showed disagreement. The results suggest that both tests detect *Aspergillus*, but WB is superior in detecting CPA. However, combining the tests may improve diagnostic accuracy [46].

A polymerase chain reaction (PCR) test is readily available in reference laboratories. Many in-house PCR tests or commercial assays are used for molecular detection

of *Aspergillus* spp., especially in immunocompromised patients with underlying diseases and without antifungal prophylaxis. The main concern for PCR is the standardisation of blood PCR, which remains complicated [39]. Therefore, the routine molecular diagnosis of aspergillosis is not yet available in many medical centres, despite the advances that allow the detection of *Aspergillus* DNA directly from clinical samples and the standardisation of PCR assays, although variable sensitivity and specificity results with higher values than classical methods [4, 47]. Detection of fungi in clinical samples using DNA amplification coupled with DNA sequencing for fungal identification is a new technology that is emerging to support routine diagnosis (e.g., isothermal and PNA) fish methods, platforms that allow high throughput analysis (e.g., DNA microarrays and Luminex® xMAP™) and/or commercial PCR assays [4].

In a review study, Terrero et al. [48] compared and addressed independent tests and the combination of GM tests and *Aspergillus* PCR diagnostics that use a variety of genetic targets, such as mitochondrial DNA, 28S rRNA, 18S rRNA, and internal transcribed spacer (ITS) regions of ribosomal DNA, to screen for IA in high-risk patient populations and that the combination of these tests to define cases reaches a sensitivity of 99% in some approaches to guide the treatment of these high-risk patients [48].

AsperGenius is a multiplex PCR that identifies the presence of *Aspergillus* species and detects four resistance-associated mutations (RAMs, TR34/L98H/T289A/Y121F) in the CYP51A gene responsible for azole resistance. It showed a sensitivity and specificity of 84% and 80%, respectively, in the diagnosis of IA and BAL in patients suspected of having an underlying haematological malignancy. The performance of AsperGenius in serum samples yielded a sensitivity and specificity of 78.6% and 100%, respectively [49].

Zvezdanova et al. [50], using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) for *A. fumigatus* isolates from 842 identified isolates, showed an identification rate of 97%. The majority (86%) of isolates had high confidence scores ( $\geq 2.0$ ) [50]. Mutational analysis of the CYP51A gene in azole-resistant isolates identified three groups reflecting different mutations. MALDI-TOF accurately identifies *A. fumigatus* and is promising for species identification, characterisation and resistance. Américo et al. [51] investigated the VITEK MS platform for the identification of 106 species of *Aspergillus*. The identification accuracy was 91.6% at the genus and section level and 84.7% at the species level, including the detection of azole-resistant strains such as *A. lentulus* and *A. calidoustus* [51].

#### 4.4 Treatment *Aspergillus*

The wide availability of modern molecular techniques has allowed the characterisation of cryptic *Aspergillus* species, some of which have shown reduced susceptibility to several antifungal drugs, and optimal treatment remains a challenge, with therapy modified according to in vitro susceptibility testing. The authors highlight that the exponential increase of cryptic species in different sections of *Aspergillus* is emerging with recent descriptions, with scarce data on antifungal treatment [52].

The secure trial evaluated the efficacy and safety of isavuconazole compared with voriconazole in patients with IFD. The phase 3 trial was a double-blind, global, multicentre comparison group study. The 527 adult patients with suspected IFD were randomised in a 1:1 ratio, stratified by geographical region, allogeneic haematopoietic stem cell transplantation and active malignancy at baseline, to receive isavuconazole or voriconazole. The authors conclude that isavuconazole was non-inferior to

voriconazole for the primary treatment of suspected invasive IFD. Isavuconazole is well tolerated compared with voriconazole, with fewer adverse events [53].

A specific prophylactic antifungal regimen influences the onset of this IFD in SOT patients [34]. In immunosuppressed patients, the indiscriminate use of fluconazole as prophylaxis for invasive candidiasis increases the incidence of AI, as this antifungal agent has no fungicidal/fungistatic activity against this genus [54]. Resistance to treatment of aspergillosis with azole drugs has been associated with treatment failure or indiscriminate use of azole antifungals in agriculture, leading to resistance in environmental fungi [27]. A retrospective study of the treatment of immunocompromised cancer patients diagnosed with proven or probable IA divided into two groups: voriconazole and non-voriconazole (90% amphotericin B). The authors demonstrated that primary therapy with voriconazole was associated with improved response and survival compared with non-voriconazole [55].

## 5. History/aetiology, *Fusarium* sp.

The review by Summerell et al. [56] reports that link 1809 designated the genus *Fusarium* and named it *Fusisporium*, but it was not until 1821 that Fries validated it according to the International Botanical Code. Nevertheless, Wollenweber and Reinking 1935 consolidated this act with descriptions of 135 taxa, which remain the basis for all subsequent taxonomic systems [56]. The genus *Fusarium* has about 200 species, which are grouped into species complexes with little or no morphological differences between them.

They are cosmopolitan organisms and comprise a diverse group of species based on morphological, physiological and phylogenetic characteristics. Genetic and molecular techniques have defined many new *Fusarium* species that have been described and grouped into complexes according to their clinical relevance, including the *F. solani* complex, *F. oxysporum* complex, *F. fujikuroi* complex, *F. incarnatum-equiseti* complex, *F. dimerum* complex, and others [48, 56].

### 5.1 *Fusarium* wall cell

Virulence factors in *Fusarium* spp. are proteases, protein kinases, multifunctional proteins, collagenases and mycotoxins [57]. Cellular proteins and metabolites mediate fungal-host interactions and promote hyphal adhesion, invasion and penetration into tissues. Biofilm formation is the main virulence factor of *Fusarium* and can contribute to the virulence of other human pathogens by conferring protection with the fungal biofilm and generating drug resistance. The biofilm can promote angiogenesis, adhesion, infiltration and immunomodulation [58]. *Fusarium* secretes enzymes such as carboxypeptidases, aminopeptidases and toxic mycotoxins [17].

### 5.2 Epidemiology and pathophysiology *Fusarium*

This genus includes ubiquitous and saprophytic fungi, or plant pathogens, commonly found in soil, decaying vegetables, plants and other organic substrates [59]. It is known to contain phytopathogenic species with economic impact, affecting agriculture and horticulture worldwide [56].

Schmale et al. [60] report that the dispersal processes of *Fusarium* species can be simple or combined, mainly by airborne dispersal, soil dispersal and dispersal with

infected host plants [59]. Airborne species can reach continental distances by air transport. *Fusarium* species have been isolated from hospital water supply systems and are pathogenic to patients, with fungal propagules probably being transported in these environments and infecting these hosts by inhalation during the use of taps and showers, in addition to the respiratory tract, skin and tissues, and possibly membranes and mucous membranes [59–62].

*Fusarium* species cause a wide spectrum of infections in humans, ranging from superficial to locally invasive and disseminated infections [63]. However, the clinical form of the disease, fusariosis, is related to the portal of entry of the infection and the immunity of the host [63]. In immunocompetent hosts, keratitis and nail lesions are the most common infections, but in immunosuppressed patients, the outcome is fatal in most cases. Invasive infection is a severe IFD and mainly affects patients with acute leukaemia and allogeneic bone marrow transplant recipients due to the greater immunosuppression associated with intense and prolonged neutropenia [63]. Mortality is approximately 75% in patients with disseminated disease compared to 36% in those without this clinical presentation; this association remains statistically significant even after controlling for neutropenia, haematopoietic stem cell transplantation, comorbidities and organ involvement [63]. The prognosis depends on the immune status of the host, with poor survival observed in people with haematological malignancies [64]. Many cases with clinical features of disseminated fusariosis may resemble AI and require differential diagnosis [59].

### 5.3 Diagnosis and treatment *Fusarium*

For the diagnosis of fusariosis, an anemophilous fungus, there are criteria for obtaining biological material, as it is part of the environmental microbiota. After adequate collection, a direct mycological examination revealed the presence of septate hyaline hyphae, sometimes irregular and with branches forming ramifications that can be confused with other genera. Therefore, only the isolation of the aetiological agent allows a definitive diagnosis, although the histopathology helps in the diagnosis of fusariosis; the morphology of the hyphae is not always characteristic and may resemble other hyalohyphomycetes [63, 65].

Immunohistochemistry is not standardised, and non-specific results occur in a high percentage of cases [65]. In addition, the commercial availability of antibodies for filamentous fungi is limited [65]. Probes developed for *Fusarium* sequences for in situ hybridisation assays target sequences in the ribosomal DNA region and have low specificity [65]. The study showed that despite exponential amplification of the target DNA, which makes it possible to detect even small amounts of fungi, the molecular tool has a decreasing specificity. In addition, what is commercially available depends on internal validation [65]. The PCR technique, where available, can confirm infection [65]. Felix [66] evaluated *Fusarium* genomic material in fresh and paraffin-embedded tissues using real-time PCR. BALB/c mice were used to develop models of invasive infection by *Fusarium solani*. Organs were harvested for culture, semi-nested PCR and real-time PCR. Histopathological analyses were performed using haematoxylin-eosin and Gomori-Grocott staining, which showed the presence of fungal structures and changes in tissue morphology, confirming the spread of infection. In molecular diagnostics, the different *Fusarium* primers showed cross-reactivity, mainly with *Aspergillus* and *Rhizopus* DNA samples, and low specificity shows the culture and histopathological analysis of *Fusarium solani* as shown in **Figure 2** [66].

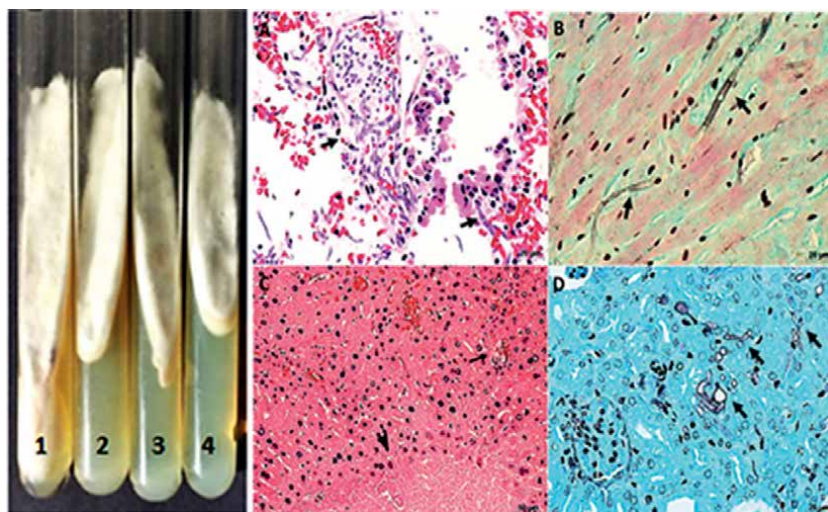


**Figure 2.** Morphology of *Fusarium solani* cultures obtained from mouse tissues: heart (1), lung (2), liver (3) and kidney (4); and histopathological analysis: A. Lung (HE stain, X40); B. Heart (Gomori-Grocott stain, X40); C. Liver (HE stain, X20); D. Kidney (Gomori-Grocott stain, X40) [64].

Detection of GM antigen in the serum of patients with invasive fusariosis has a sensitivity and specificity of 83% and 67%, respectively, but there is a cross-reaction with *Aspergillus*, probably due to the similarity of the cell wall [67, 68]. Ledoux et al. [68] compared the relationship between the biomarkers GM and beta-D-glucan assay in cases of fusariosis and found significant results. Of the 29 cases of IFD caused by *Fusarium*, only two were positive for GM, suggesting that the biomarker is ineffective in detecting *Fusarium* infections. On the other hand, the beta-D-glucan assay was positive in 50% of the cases evaluated, including *F. verticillioides* infections, demonstrating that the biomarker is effective in detecting *Fusarium* infections [68].

Zeller et al. [69] demonstrate the standardisation of a pan-fungal real-time PCR assay for the broad detection of fungal DNA (fungi assay) in various clinical specimens [69]. This internal HybProbe real-time PCR assay targets the ITS2 region of fungal DNA and assesses clinical specimens with suspected fungal infection. The authors noted that a limitation of the assay is that a blast search or comparison to the CBS database using the ITS2 region alone does not allow identification of the species level of all fungal pathogens detected, and for the genus *Fusarium* only members of the *F. oxysporum* and *F. solani* complex can be identified as such [69].

Electronic Nose (e-Nose) technology offers an innovative method of detecting fungi such as *Fusarium* species by identifying their emitted volatile compounds. This non-invasive device uses gas sensors and pattern recognition to monitor odour resistance [70]. Research has demonstrated its ability to discriminate between the odours of different fungal species, including *F. oxysporum* and *Rhizoctonia solani*. By integrating e-Nose with a machine learning model, researchers have developed a low-cost solution using metal oxide sensors and logistic regression for classification. This combination represents a promising approach for rapid detection of *Fusarium* and other fungi, improving diagnosis and control of invasive fungal diseases [70].

Barião et al. [71] assess the MALDI-TOF technique's effectiveness in determining the minimum profile change concentration (MPCC) in clinical isolates of *Fusarium*

spp., comparing it to the inhibitory concentration minimum (MIC) from the M38-A2 protocol. Results show that 93.3% of strains had (MIC) values of 1–4 µg/mL, except for clinical strain (LMC 7108.01), which exhibited resistance. The MPCC assessment indicated that 80% of strains had values between 1 and 8 µg/mL, while the resistant strain had an MPCC over 32 µg/mL. A significant correlation was found between MPCC and MIC with 86.7% agreement. MALDI-TOF MS spectra were essential for strain identification and understanding phenotypic changes due to amphotericin B treatment, highlighting the role of the technique in fungal resistance research [71].

Guarro [72] reviewed the susceptibility of *Fusarium* spp. Overall, they found low in vitro activity, with amphotericin B showing low MICs [72]. Posaconazole and terbinafine have some in vitro activity, while caspofungin has high MICs alone but synergistic activity with other antifungals [72]. This limited in vitro activity is consistent with poor efficacy in animal models, leading to high mortality despite new antifungal agents [72]. Clinical trials show modest results with posaconazole, and voriconazole is less effective in neutropenic patients, where reversal of neutropenia is critical to improve survival. Information on combination therapies such as amphotericin B and voriconazole is limited [72].

## 6. History/aetiology, order Mucorales

The first description of a case of mucormycosis was in 1876 by Fürbinger et al. [73] in Germany, who described a patient who died of cancer with a haemorrhagic infarct in the right lung with fungal hyphae and some sporangia [71]. In 1885, the pathologist Arnold Paltauf [74] published a case of disseminated mucormycosis, which he called “mycosis mucorina” [74].

O'Donnell et al. [75] and Voigt and Wöstemeyer [76] discuss the phylogeny and evolutionary relationships of mucormycetes, currently classified in the families Cunninghamellaceae, Lichtheimiaceae, Mucoraceae, Saksenaceae and Syncephalastraceae, with the vast majority of human infections caused by members of Mucoraceae and Lichtheimiaceae [75, 76]. Molecular phylogenetic analyses have placed the genus *Rhizomucor* outside the Mucoraceae, placing the genus in the Lichtheimiaceae. However, Hoffmann et al. [77] reclassified the Mucorales on the basis of a phylogenetic analysis of 201 isolates and 103 species [77]. The data analysed included the extant families Lichtheimiaceae and Cunninghamellaceae, as well as the genera *Backusella* and *Lentamyces*, which have recently received family status, and Rhizopodaceae, which includes *Rhizopus*, *Sporodiniella* and *Syzygites* [77]. Analysis of compensatory base shifts in the Lichtheimiaceae confirmed the lower-level placement of *Lichtheimia* and *Rhizomucor*, while genera such as *Circinella* or *Syncephalastrum* still lack compensatory base shifts [77].

The order Mucorales consists of abundant, rapidly growing mycelium, which usually forms large amounts of anamorphic structures. The mycelium is often inseparable, sessile or irregularly septate [78]. Anamorphic sporangiospores are produced in multispore-bearing sporangia or in poorly sporulated sporangioles or merosporangia [78]. Most species rarely produce chlamydospores, arthrospores or yeast cells. A key feature of sporangia is the presence of a columella, which is a synapomorphy for Mucorales [78]. Conjugation in homothallic species or compatible heterothallic types leads to the formation of zygospores, which may display various exospore ornaments and protective appendages on the supporting cells [78]. Some species of Mucorales show dimorphism, alternating between filamentous and yeast-like states [78].

Traditionally, mucormycosis pathogens have been identified on the basis of their morphology [78]. **Figure 3** is an illustration of the morphology of *Mucor indicus* [79].

### 6.1 Mucorales cell wall

Virulence factors recently identified in *Rhizopus* spp. include the cell wall, spore germination, enzymes and proteins related to iron sequestration, as well as the fungal protein CotH with upregulation of the cellular receptor protein that acts as a chaperone within the endoplasmic reticulum GRP78, which is associated with immune evasion and survival in phagocytes, among other things [80]. Mucorales use free iron to take advantage of available sources when host defences are altered [80]. Iron is obtained by two mechanisms: using siderophores (iron chelators) or permeases consisting of ferric reductase (Fre), ferroxidase (Fet3), and high-affinity iron permease (FTR1) [80, 81]. The high-affinity free iron uptake system is considered an important virulence factor of the fungus [78, 81]. However, lack of iron availability can reduce virulence, affect fungal growth and even induce apoptosis [80, 81].

Fungal siderophores are small molecules that sequester iron by intrinsic or extrinsic means. Rhizoferrin is the major siderophore produced by several species of Mucorales. Fungi can synthesise several endogenous siderophores and misappropriate exogenous siderophores (xenosiderophores) synthesised for other microorganisms [80, 81].

GRP78 is a receptor required for Mucorales invasion, as its specific fungal ligands are CotH. CotH is a protein kinase belonging to the spore coat protein family. In many fungal species, such as *Rhizopus oryzae*, the protein is required for spore germination and formation. Spore germination is a pathogenic mechanism

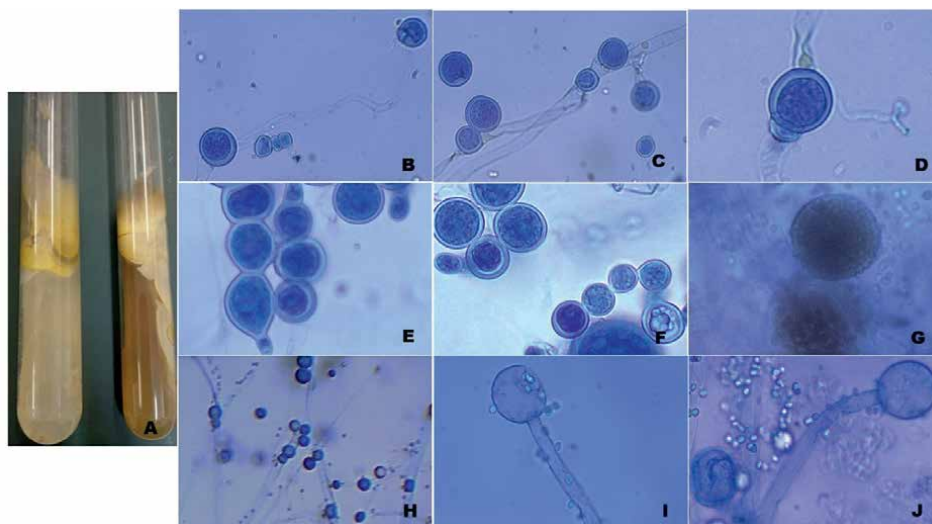


**Figure 3.** Macromorphology: A. Culture on potato agar or Sabouraud-dextrose agar: colonies rapidly growing, yellowish to greenish, often several centimetres high, usually forming a thick mat due to abundant production of erect sporangiophores. Micromorphology: B–D. Primary isolation on Sabouraud dextrose agar: numerous zygospores, between unequal suspensors; E–G. Re-culturing from primary isolation on Sabouraud dextrose agar: chlamydospores, zygospores and sporangiophores and sporangia; and H–J. Recultivation from primary isolation on potato agar: yeast-like and filamentous forms and sporangiophores with spherical to subspherical sporangia [77].

in angioinvasion, thrombosis and tissue necrosis [80, 81]. There is evidence that the *CotH2* and *CotH3* genes are expressed in *R. oryzae*, with *CotH3* being the most highly expressed. However, both genes, which are linked to *GRP78*, can adhere to and invade nasal endothelial and epithelial cells and lead to fungal dissemination [80, 81]. Immunosuppression and susceptibility predispose to *Rhizopus* spp. infection, such as poor glycaemic control with persistent hyperglycaemia, available free iron, and an acidic microenvironment that may favour the expression of *CotH* genes on the fungal cell surface, resulting in the *GRP78/CotH3* interaction [16, 80]. The human body needs iron for vital processes such as cell growth and development. It must therefore be bound to host transport proteins (transferrin, ferritin and lactoferrin) to avoid the toxic effects of free iron [80, 81].

## 6.2 Epidemiology and pathophysiology Mucorales

Fungi of the order Mucorales are found in the soil, decaying matter, and contaminated food [82]. Mucormycosis is rare but can be fatal, especially in diabetic patients, with mortality rates as high as 46% [82]. Liang et al. [83] reported that the global prevalence of mucormycosis is still unclear [83]. In India, the incidence of mucormycosis is about 70 times higher than the global average. The COVID-19 pandemic has significantly increased the number of associated cases. Increases in annual infection rates have also been observed in China, Pakistan, Iran, and several high-income countries, including France, Belgium, Switzerland and Spain [82, 83].

Hyperglycaemia leads to glycosylation of transferrin and ferritin, resulting in high levels of free iron, which promotes Mucorales growth [84]. In addition, elevated cytokines such as interleukin-6 in COVID-19 patients increase iron availability, increasing the risk of infection [84]. Acidic and glucose-rich environments, common in ketoacidosis, promote the growth of Mucorales, which can reduce ketones [84]. This highlights the increased risk of mucormycosis in diabetes mellitus, particularly during periods of immunosuppression, such as during the COVID-19 pandemic [84]. Diabetes also causes endothelial complications such as endothelitis and thrombosis, which reduce CD4+ and CD8+ cell levels and increase susceptibility to mucormycosis [85]. Studies show that severe mucormycosis is often associated with high glycated haemoglobin (HbA1c > 10%) and COVID-19 positivity, highlighting a significant association between these conditions [85]. Diabetic ketoacidosis promotes the germination of Mucorales spores. Corticosteroids, often used for COVID-19 treatment, can reduce the phagocytic activity of leukocytes in diabetic patients, increasing their risk of infection and upregulating proteins that aid angioinvasion, such as spore-binding *CotH* and glucose-regulating *GRP-78* [80]. In adults, mortality rates range depending on the clinical condition of the patient, the site of infection and treatment [80].

## 6.3 Diagnosis Mucorales

Early recognition of mucormycosis is essential for timely treatment and improved outcomes. Diagnosis requires direct microscopy or histopathological examination to identify broad, non-septate hyphae invading tissue, together with a culture of samples from affected sites. Identification of the pathogen and determination of antifungal susceptibilities are essential for the selection of appropriate therapy [86]. However, samples are often not cultured, or organisms may fail to grow due to cell death after maceration [86].

Radiographic findings alone are non-specific for diagnosis. Pulmonary infections can have a variety of non-specific appearances, such as aspergillosis pneumonia. CT findings of pleural effusion, pulmonary nodules and sinusitis may suggest mucormycosis, especially in immunocompromised patients. The “reversed halo” sign is more commonly associated with pulmonary mucormycosis than aspergillosis [87].

Non-culture-based serological tests such as BDG and GM, which are derived from fungal cell wall components, are not present in Mucorales, meaning that a positive result on these tests can “rule out” mucormycosis [88]. Burnha-Maruish et al. [89] evaluated a monoclonal antibody (2DA6) in a sandwich ELISA which showed strong reactivity with fucomannan from *Mucor* spp. [89]. They developed a more convenient lateral flow immunoassay (LFIA) for the detection of fucomannan in BAL, serum, urine and tissue which effectively identified *R. delemar*, *L. corymbifera*, *M. circinelloides* and *C. bertholletiae* [89].

Molecular methods such as PCR are increasingly being used to improve tissue detection, allowing species-level identification via targets such as ITS or 18S rRNA [90].

Kasai et al. [91] developed a qPCR specific for 28S rDNA, while Bernal-Martinez et al. [92] developed a multiplex real-time qPCR targeting ITS1/ITS2 specific for *R. oryzae*, *R. microsporus* and *Mucor* spp. [91, 92]. In addition, Springer et al. [93] developed two Mucorales-specific real-time qPCR assays targeting the ribosomal operon regions (18S and 28S) to detect different clinically relevant Mucorales species [93]. Tests for invasive mucormycosis use multiple probes and primers, making standardisation and clinical implementation difficult. The commercial pan-Mucorales qPCR kit offers rapid and sensitive detection directly from blood samples and can predict the final diagnosis [91–93].

Guegan et al. [94] validated its efficacy against four in-house qPCR assays targeting 18S rDNA in lung samples, detecting *Mucor-Rhizopus*, *Lichtheimia*, *Rhizomucor* and *Cunninghamella* [94]. This commercial test is easy to use and outperforms conventional methods for detecting fungal DNA. However, it may miss infections with low fungal burden [92]. Springer et al. [95] reported early detection of Mucorales in haematological patients using a rapid real-time PCR test that provides specific results in approximately three hours, allowing earlier diagnosis of mucormycosis compared to conventional methods (8 days), and imaging tests (3 days) demonstrated efficacy for screening and monitoring high-risk patients and contributed to higher survival rates [95, 96]. Millon et al. [97] demonstrated that three qPCRs could identify *L. corymbifera*, *Mucor/Rhizopus* and *Rhizomucor* with high sensitivity, detecting infections 3 to 68 days earlier than conventional methods validated by RESSIF (Pasteur Institute, Paris) [97]. In addition, Legrand et al. [98] showed that the detection of circulating Mucorales DNA allows earlier diagnosis of invasive mucormycosis in patients with severe burns [98].

Koshy et al. [99] analysed volatile breath metabolites in a mouse model of invasive mucormycosis using three Mucorales species: *Rhizopus arrhizus* var. *arrhizus*, *R. arrhizus* var. *delemar*, and *R. microsporus*, and *A. fumigatus* as controls [99]. They also studied five patients with mucormycosis caused by *R. microsporus*. The results showed distinct volatile profiles for the Mucorales species, facilitating real-time identification of mucormycosis and differentiation from aspergillosis. A non-invasive method has diagnostic potential for fungal infections, although further evaluation is required.

Shao et al. [100] validated the efficacy of MALDI-TOF MS for the identification of filamentous fungi of the order Mucorales using 111 isolates from six genera [100]. The basic Bruker Filamentous Fungi Library identified 49.5% of isolates at the species level and 66.7% at the genus level [98]. However, combining the Bruker library

with the BMU database improved identification rates to 81.1% for species and 100% for genus [98]. MALDI-TOF MS, only the Bruker library accurately identified *M. irregularis* after incorporating relevant profiles [100]. The extended Bruker library improved the accuracy of the identification of pathogenic Mucorales [100].

## 6.4 Treatment Mucorales

Liang et al. [81] reviewed a comprehensive approach to the management of mucormycosis, emphasising radical surgical debridement as the primary treatment, with systemic antifungal therapy and management of underlying conditions such as strict glycaemic control [81]. While surgery can improve survival rates, its effectiveness is reduced in predisposing conditions, particularly in CNS cases [81]. Both open and minimally invasive procedures benefit pulmonary mucormycosis, and cutaneous forms have a better prognosis with faster recovery when combined with antifungal therapy [81]. Amphotericin B is the most effective drug for Mucorales but has significant side effects [81]. New lipid nanocrystal formulations of oral amphotericin B show promise with reduced toxicity [81]. Posaconazole (POS), effective against Mucorales, achieved a success rate of 54% and is useful in salvage therapy scenarios [81]. Isavuconazole shows better tolerability and efficacy than amphotericin B, with the potential for prophylaxis in high-risk patients [81]. Echinocandins target cell wall synthesis but are less effective against Mucorales [81]. However, they may enhance the efficacy of polyenes when used in combination, showing potential as part of combination therapies [81]. Further research is needed to understand the treatment of mucormycosis [81].

## 7. Conclusion

Although *Aspergillus* genera are one of the main aetiological agents of IFD in immunosuppressed patients, other “unusual” filamentous fungi have emerged, especially species belonging to the genus *Fusarium* and the order Mucorales. In these patients, the clinical manifestation of the disease deviates from the conventional pattern, making the diagnosis of the infectious process difficult and challenging and delaying the initiation of antifungal therapy. In view of the above, methods should be sought in this group of patients to allow early diagnosis of infectious processes caused by emerging and/or re-emerging opportunistic fungi, which will contribute to the implementation of antifungal prophylaxis with a consequent increase in survival rates. The diagnosis of IFDs remains problematic because conventional diagnostics have low sensitivity and specificity and cannot distinguish between colonisation and disease. Better treatment and diagnosis for patients susceptible to fungal infections must be developed, based on rigorous meta-analyses of well-described case reports, with appropriate identification, treatment and reporting of outcomes. The scientific community and government agencies need to work closely together to reduce the burden of these complex diseases that are difficult to diagnose and treat.

## Conflict of interest

The authors declare no conflict of interest.

## **Nomenclature**

|               |                                                                   |
|---------------|-------------------------------------------------------------------|
| IFDs          | invasive filamentous fungal diseases                              |
| HSCT          | allogeneic haematopoietic stem cell transplant                    |
| MPs           | mannoproteins                                                     |
| DTH           | delayed-type hypersensitivity                                     |
| GPI           | glycosylphosphatidylinositol                                      |
| PRRs          | pattern recognition receptors                                     |
| PAMPs         | pathogen-associated molecular patterns                            |
| TLRs          | dectin and toll-like receptor families                            |
| NOD-like      | nucleotide-binding oligomerization domain                         |
| NLRs          | family Pyrin Domain                                               |
| RIG-I-like    | receptors signalling pathways                                     |
| CLR           | C-type lectin-like                                                |
| LPS           | lipopolysaccharide                                                |
| NLRP3         | family Pyrin Domain containing 3                                  |
| LT            | lymphocytes T                                                     |
| IL            | interleukins                                                      |
| IFN- $\gamma$ | interferon-gamma                                                  |
| TNF- $\alpha$ | tumour necrosis factor-alpha                                      |
| DCs           | dendritic cells                                                   |
| ROS           | reactive oxygen species                                           |
| IA            | invasive aspergillosis                                            |
| ICU           | intensive care unit                                               |
| IPA           | invasive pulmonary aspergillosis                                  |
| SOT           | recipients in lung transplantation                                |
| SARS-CoV-2    | viruses from the coronavirus family                               |
| CIE           | counterimmunoelectrophoresis                                      |
| ELISA         | enzyme-linked immunosorbent assays                                |
| IB            | immunoblotting                                                    |
| GM            | galactomannan                                                     |
| BDG           | galactomannan 1,3b-d-glucan                                       |
| EIA           | enzyme immunoassay                                                |
| IgG           | immunoglobulin                                                    |
| WB            | Western blot                                                      |
| ICT           | immunochromatography                                              |
| CPA           | chronic pulmonary aspergillosis                                   |
| PCR           | polymerase chain reaction                                         |
| ITS           | internal transcribed spacer regions                               |
| RAMs          | resistance-associated mutations                                   |
| MALDI-TOF     | Matrix-Assisted Laser Desorption/Ionization Time-of-Flight        |
| MPCC          | minimum profile change concentration                              |
| CIM           | inhibitory concentration minimum                                  |
| CotH          | spore coat proteins constitute a kinase family                    |
| GRP78         | protein that acts as a chaperone within the endoplasmic reticulum |
| LFIA          | lateral flow immunoassay                                          |
| CNS           | central nervous system                                            |
| POS           | posaconazole                                                      |

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# Cryptococcosis in Non-HIV Immunocompromised Hosts: Epidemiology, Pathogenesis, and Management

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## Abstract

*Cryptococcus* sp. is a globally distributed yeast responsible for significant morbidity and mortality, particularly in immunocompromised populations. While cryptococcosis has been well described in people living with HIV, the incidence of this fungal infection has been rising in non-HIV immunosuppressed hosts. Mortality has been reported as high as 27%. In non-HIV patients, cryptococcosis usually presents in a non-specific manner, making the diagnosis challenging. It can manifest as pneumonia, meningitis, disseminated disease, or infection in unusual sites such as eyes, skin, bones, prostate, and the gastrointestinal tract. This difficulty in diagnosis and treatment relates to the low clinical suspicion and the high relapse rates. The need for further research and the development of tailored treatment strategies for these diverse populations is emphasized.

**Keywords:** *cryptococcus*, immunosuppression, pathogenesis, epidemiology, antifungal treatment

## 1. Introduction

*Cryptococcus* spp. is a yeast with a global distribution. It causes high morbidity and mortality among immunocompromised hosts. *Cryptococcus neoformans* and *Cryptococcus gattii* are the main species responsible for human infection.

Although cryptococcal meningitis death reports have an increased impact on people living with HIV, countries have reported an increasing impact of the disease among non-HIV immunocompromised people. It is the most prevalent systemic mycosis in immunosuppressed hosts. Some risk factors are solid organ transplantation (SOT), malignancy, systemic lupus erythematosus (SLE),

cirrhosis, use of corticosteroids, intravenous drug use, diabetes mellitus type 2, neutralizing autoantibodies against granulocyte-macrophage colony-stimulating factor, and sarcoidosis [1–6].

## 2. Epidemiology

*Cryptococcus neoformans* is usually found in soil and bird feces distributed in the environment, and it was listed as a top-priority fungal pathogen by the WHO in 2022. *Cryptococcus gattii* is related to exposure to eucalyptus, which grows in tropical and subtropical regions. The major focus of this last species was Australia, although recent reports have isolated this species in the USA and parts of Europe. Small studies suggest that *C. gattii* usually affects immunocompetent people and its presentation is usually pulmonary; it tends to form cryptococcomas. This species is more frequently found in chronic pulmonary disease than *C. neoformans*.

*C. neoformans* presentation is usually meningeal; IRIS is less frequent, and 12-month mortality is higher. There are studies that present these different epidemiologies and clinical presentations, highlighting the importance of species-specific identification for treatment and follow-up [6–8].

## 3. Pathogenesis

This fungal infection occurs when infectious particles, called basidiospores, are inhaled and deposited in the lung; other ways are through inoculation or ingestion, but they are less frequent [9]. One of the virulence mechanisms of this fungus is the presence of the polysaccharide capsule, composed of glucuronoxylomannan (GXM), which confers resistance to the host immunologic response and enhances its pathogenesis and invasive action. Other important virulence factors are thermotolerance and the use of melanin. Most hosts may clear the infection, but macrophages will encounter *Cryptococcus* in different circumstances and form persistent asymptomatic pulmonary granulomas. This latency form is more frequent in *C. gattii*, although central nervous system (CNS) infections, the most lethal manifestation, are usually seen with *C. neoformans*. CNS infection is developed through the “trojan horse” mechanism, where macrophages that phagocytize the yeast travel through the hematocerebral barrier. Another site that can act as a reservoir is the prostate [6, 9, 10].

## 4. Cryptococcal infections in non-HIV immunosuppressed hosts

### 4.1 Cryptococcosis in organ transplant recipients

*Cryptococcus* spp. is the third most frequent fungal infection in solid organ transplant (SOT) patients. It accounts for 7% of invasive fungal infections in this group, just after *Aspergillus* spp. and *Candida* spp. This infection usually appears during the 1 year after liver and lung transplants, while it presents after 16 months in kidney transplants. People with cirrhosis have an increased risk of unrecognized infection, which usually manifests as peritonitis and has a high mortality rate. Cryptococcosis in this population is believed to be caused by the reactivation of the pathogen, but primary infections have also been identified. Transmission from donors has also been

reported, and this should be considered when the disease is diagnosed during the first 30 days of transplant, if cryptococcosis is diagnosed in more than one recipient, or if it is isolated from the surgical site or graft. Antithymocyte globulin and monoclonal antibodies such as alemtuzumab and glucocorticoids have been described as risk factors for this fungal infection. On the other hand, using calcineurin inhibitors is associated with lower rates of disseminated disease; this can be explained by the fact that these drugs target the fungal homologs of calcineurin in these microorganisms.

Clinical presentation in this group of people is non-specific. It can present as pneumonia, meningitis, or disseminated disease. Infections in the skin, eyes, gastrointestinal tract, prostate, bones, and joints, head and neck, cardiovascular system, genitourinary system, thyroid, or adrenal glands have been described. Pulmonary involvement manifests as fever, cough, dyspnea, malaise, night sweats, and weight loss. This presentation is not always severe; it may present as colonization or mild infection. The image findings are usually nodules that are not specific to this fungal infection and can also present as pleural effusion, consolidation, or cavitated nodules (**Figures 1–3**). Meningitis can manifest with fever, malaise, prolonged headache, and altered mental status; nuchal rigidity is rare. Around 75% of SOT patients are present with disseminated disease. Overall mortality has been reported to be up to 20% [11, 12].

#### **4.2 Cryptococcosis in other immunosuppressed people (non-HIV, non-transplant)**

Individuals with other immunosuppressive conditions or receiving immunosuppressant drugs and people putatively immunocompetent are also affected by cryptococcosis [2, 10, 13, 14]. Risk factors in non-HIV, non-transplant (NHNT) patients include glucocorticoid treatment, idiopathic CD4 lymphopenia, and case reports and small case series have implicated immunosuppressive therapy, including

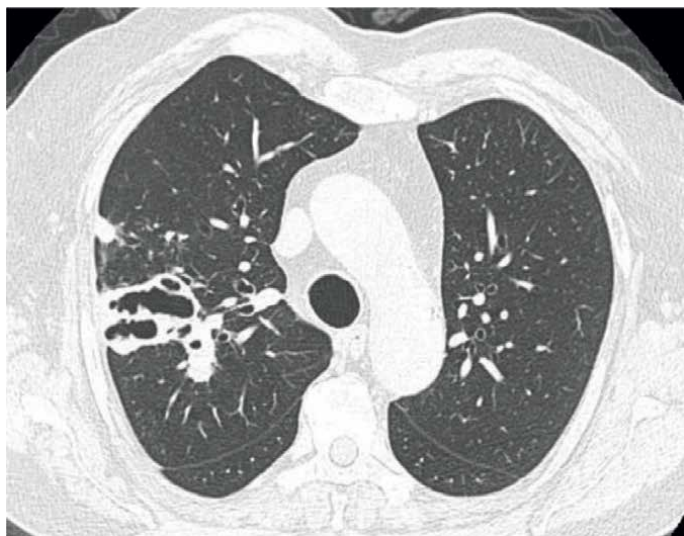


**Figure 1.**  
*Thorax CT scan with areas of alveolar occupation at the expense of cavitated consolidations, with thick and irregular walls, ground glass, and associated peripheral micronodules in the right upper lobe due to C. neoformans in a 78-year-old patient with prostate adenocarcinoma.*

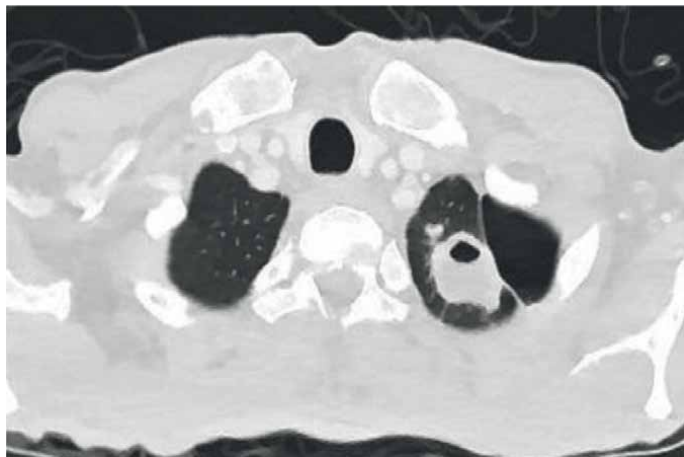


**Figure 2.**  
*Thoracic CT scan of a 49-year-old person living with diabetes mellitus demonstrates a cryptococcal nodular cavitation in the left apicoposterior segment.*

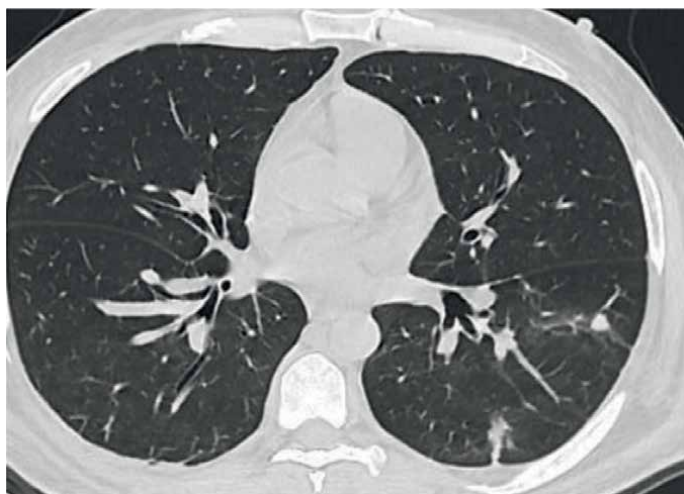


**Figure 3.**  
*Thorax CT scan with a solid nodule in the posterior segment of the left lower lobe of 11 mm due to C. neoformans in a 55-year-old patient with multiple myeloma.*

anti-tumor necrosis factor- $\alpha$  (infliximab), anti-CD52 (alemtuzumab), anti-Bruton's tyrosine kinase inhibitors (ibrutinib), and agents that alter purine metabolism such as azathioprine [4, 13–15]. In rare cases, cryptococcal disease is caused by inborn errors of immunity, including mutations in the GATA2 zinc finger transcription factor and hyper IgM or hyper IgE syndromes [16–18]. Diabetes was found to be the most common underlying condition associated with cryptococcal infection in non-HIV patients in the Atlanta and Houston metropolitan areas between 1992 and 2000 [19], while others found hematologic malignancies to be the most common underlying conditions in the highly active cancer therapy era [13].

As in SOT, clinical presentation in this group of people is non-specific, and the time of diagnosis among these patients is significantly longer when compared to

HIV-positive. Compared to HIV-positive patients, NHNT were less likely to have CNS involvement and cryptococcemia, also like SOT patients [20]. Given these varied host groups, it is difficult to conclude about epidemiology, clinical presentation, prognosis, and outcomes, so no single therapeutic regimen or duration meets all patients' needs. For this reason, cryptococcosis relapses have been reported; the rate of relapse is between 15 and 25% among non-HIV patients, and most relapses have been attributed to inadequate dosing or duration of therapy [5]. Future studies of cryptococcosis focused on NHNT are needed to better understand the disease attributes in these important patient populations.

#### *4.2.1 Hematologic malignancies*

Reports of cryptococcosis in people with multiple myeloma (MM) have been on the rise. Late stages of MM, use of new treatments such as pomalidomide, use of concomitant corticosteroids, and relapsed disease have been described as risk factors in this population. High clinical suspicion is crucial for investigating and treating this infection [21]. Its presentation may be localized (granulomatous sinusitis) [22] or disseminated; the cutaneous disease usually presents in the context of disseminated infection (**Figure 4**), so clinicians must always rule out other sites of infection, such as CNS [23].

In a study in the State of Amazonas, the risk factors most identified were hypertension, diabetes mellitus, and intestinal tuberculosis. Meningitis was registered in 100% of the individuals, with a mortality rate of 57% [24].

Cryptococcal infection is sporadic in people with solid malignant tumors. Patients with hematologic malignancies can be at risk of this fungal infection due to several treatments, immunosuppression, and corticosteroids. About 3% of these patients will develop an invasive fungal infection (IFI). In some reports, hematologic patients were



**Figure 4.**  
*Cutaneous cryptococcosis in a 65-year-old renal transplant recipient.*

more likely to present with disseminated disease or CNS infection. T-cell depression is associated with the risk of acquiring this fungal infection; other malignancies, such as lymphoma and acute myeloid leukemia, have this predisposition. About 50% of the cryptococcal infections in hematologic malignancies have been described in lymphomas. Chronic lymphocytic leukemia without treatment has been associated with this fungal infection as well. Treatment with ruxolitinib for myelofibrosis has also been associated with hematologic diseases. After an allogeneic hematopoietic stem cell transplant (HSCT), the recovery of lymphocytes is essential for avoiding infections and improving survival. In addition to lymphopenia, low levels of immunoglobulin B increase the risk for cryptococcal infection. Some reports suggest that auto-HSCT is at higher risk because of a predominant Th2 response. Although rare because of antifungal infection, cryptococcosis has been reported in this population [25–27].

#### *4.2.2 Cirrhosis*

Cryptococcus is the third cause of IFI in decompensated liver disease. Delayed diagnosis and poor prognosis are described in these patients, so we suggest an early suspicion of this infection in this population. Up to 21–36% of cryptococcosis in non-HIV patients are people with cirrhosis. The cryptococcal infection rate in these patients is 6–21%, with peritonitis as the principal clinical presentation, followed by lung and SNC infection. Physiopathology is thought to be related to the entry of the fungi through the gastrointestinal tract [28].

Cryptococemia is present in 70% of these clinical cases. The Child-Pugh score is a good predictive tool for 90-day mortality in cirrhotic patients with cryptococcal infection [29, 30]. A study suggests that diagnosis and adequate treatment of this fungal infection before liver transplant may be safe and does not exclude the patient from receiving an organ [31].

#### *4.2.3 Diabetes mellitus*

A review integrates 28 articles to study the clinical characteristics and outcomes of cryptococcosis in people with diabetes mellitus (PLDM). They reported that 68% of people living with diabetes mellitus presented with cryptococcal meningitis, followed by 6.4% of disseminated infection. Most of them were diagnosed with culture or microscopy with an all-cause mortality of 38%; cryptococcal meningitis had 36% mortality [32].

A retrospective cohort of 26 patients with diabetes mellitus type 2 and cryptococcal meningitis found that they more often presented visual disorders and higher ICP [33]. Other studies suggest fever, altered mental status, and headache are the most frequent symptoms; one adds shortness of breath [34, 35].

Diabetes has been associated with worse outcomes in cryptococcal meningitis, although it has not been established as an independent risk factor. Some clinical reports describing cryptococcosis in people living with diabetes expose the impaired cell-mediated immunity secondary to hyperglycemia but also explain the chronically activated inflammatory cytokines that explain the intense response to this fungal infection [36].

#### *4.2.4 Intravenous drug users*

Cases of disseminated cryptococcosis have been described in non-HIV people who had a history of intravenous drug use (IVDU) with HIV-negative tests and positive

hepatitis C (HCV) in all the reported cases. These case series report symptoms like headache and ataxia from 1 week to up to 8 months. There are reported cases where cryptococcal meningitis simulated septic emboli. Methamphetamine, specifically, may facilitate the progress of infection by acting as an immunosuppressive agent. This drug stimulates the excessive release of GXM and biofilm, which facilitates entry into the CNS. A molecular study found that this drug interferes with the phagosomal maturation of phagocytic cells, resulting in impaired fungal control. HCV causes T-cell dysfunction, so a relationship between IVDU and this viral infection may be an important risk factor for cryptococcosis. This new possible risk factor must be further investigated [37–39].

## 5. Diagnosis

The importance of a good physical examination and interrogation leads us to look for the places affected by cryptococcus. All patients should be evaluated for extrapulmonary cryptococcal infection. Starting with a serum antigen and its titles, 83% of SOT patients with pulmonary involvement will have a positive serum antigen. The role of serum-positive cryptococcal antigen in predicting CNS infection is still being evaluated. A retrospective study on kidney transplant patients found positive serum cryptococcal antigen (CrAg) correlated with CNS infection [40]. Other studies described lung transplant patients and nodules as less likely to have a positive serum CrAg, but a positive one usually reflected disseminated infection [41]. In a multicenter study where immunocompromised people without HIV were studied, lumbar puncture was performed despite serum CrAg to screen for asymptomatic meningitis, with a positive rate of 13.2%, where fungemia or a high antigen titer could predict 95%. One-year mortality was reported at 21.7% [42]. Negative serum CrAg may not rule out cryptococcosis; if there is a high suspicion, a lumbar puncture should be done. Lateral flow assay is a point-of-care test with higher sensitivity to all cryptococcal serotypes. False-negative results can result from a postzone effect, and adequate dilution should be done in specimens of patients with high suspicion of this fungal infection. The latex agglutination test is less sensitive in *C. gattii* infections. The multiplex PCR panel in cerebrospinal fluid (CSF) may be helpful as it results in less than 3 hours.

Blood cultures are positive up to 45% in SOT patients with cryptococcal meningitis. Pulmonary involvement should be investigated with bronchoalveolar lavage and, if possible, a pulmonary biopsy to rule out other agents. Lumbar puncture should be done with opening pressure measurement and sent to gram stain, fungal culture, cryptococcal antigen, cell count, glucose, and proteins. Indian ink stain (**Figure 5**) is no longer recommended because of the cost and lack of sensibility and specificity, but we know it is sometimes the only option in some countries. Culture is helpful for species identification. Biopsies taken from the lung or other sites can be stained with periodic acid-Schiff or Gomori's methenamine silver. Mucicarmine staining dyes the cryptococcal capsule, distinguishing it from other narrow-based budding fungi.

A thoracic computed tomography (CT) scan is suggested for diagnosing cryptococcal pulmonary disease because it is more sensitive in immunosuppressed patients. Cranial magnetic resonance is the best imaging modality for identifying cryptococcosis in the CNS; up to 33% of patients will have a positive image. Cryptococcomas are more frequent in *C. gattii* infections [12].

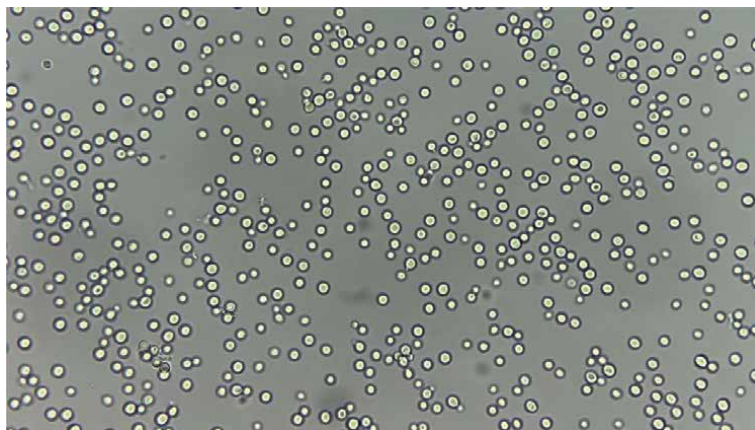


**Figure 5.**

*Indian ink stain of CSF. Indian ink stain shows Cryptococcus as a round yeast with a large halo around it. The halo is the capsule of the Cryptococcus, which is made of a thick polysaccharide. The carbon grains in the Indian ink cannot penetrate the capsule, which creates a clear halo.*

## **6. Treatment**

There are no randomized controlled trials of antifungal treatment for cryptococcosis in SOT patients. The recommendations are based on the extrapolation of the trials done on cryptococcal infections in people living with HIV.

The pulmonary or mild to moderate disease treatment is fluconazole for 6–12 months. Lung transplant recipients should receive treatment even if it is only colonization because of the high probability of reactivation. Liposomal amphotericin B plus flucytosine is the first line of treatment for CNS, disseminated, or moderate to severe infection. In some countries, flucytosine is unavailable, so the treatment of amphotericin B plus fluconazole is used with higher mycologic failures [43]. Longer courses of induction therapy may be given to patients with clinical deterioration, persistence of symptoms, or lack of culture clearance. Fluconazole is used for consolidation and maintenance treatment for up to 12 months, but it can be continued depending on the level of persistence of immunosuppression (**Table A1**). Two cases were recently published where the AMBITION regimen was used in SOT patients (single high-dose liposomal amphotericin B (10 mg/kg), 2 weeks of flucytosine (100 mg/kg/day), and fluconazole (1200 mg/day)) and successfully treated [44].

The indications and antifungal susceptibility have been standardized, but the relationship between the minimum inhibitory concentration and the clinical outcomes has not been established. Antifungal susceptibilities are not routinely done, and they are indicated in treatment failure or relapsed infection, patients with previous use of azoles or other potentially susceptible antifungals, and in *C. gattii* genotypes that are related to higher fluconazole MIC, such as VGII [12, 45].

Calcineurin inhibitors must not be suspended. Suspending these drugs is associated with higher mortality and a higher risk of immune reconstitution (IRIS). Azoles should be monitored for interactions, as these antifungals raise levels of tacrolimus and sirolimus. Other immunosuppressants should be lowered carefully to prevent IRIS or graft rejection. Up to 50% of patients with cryptococcal meningitis have

elevated intracranial pressure (ICP). Therapeutic lumbar punctures (LP) have been related to survival protection despite elevated ICP [45, 46]. If the CSF pressure is  $\geq 25$  cm H<sub>2</sub>O or the patient presents with symptoms of high ICP like headache, nausea, vomiting, vision loss, behavior changes, and seizures, LP should be done as frequently as needed (from every 24 hours to every week), taking up to 20–30 ml to achieve an ICP  $< 20$  cm H<sub>2</sub>O and if the pressure is too high at least 50% of the ICP. The clearance of the fungi, documented with an LP after 2 weeks of induction treatment, has been related to a good prognosis in PLHIV. The persistence or failure to lower the ICP has been associated with high morbidity and mortality. If, after daily LP, the ICP continues to be  $\geq 25$  cm H<sub>2</sub>O, temporary ventriculoperitoneal drainage should be considered, and the decision to leave a permanent shunt should be guided by the fact that the ICP persists high despite adequate antifungal treatment and failure of conservatory measures [47].

The use of steroids in PLHIV has been proven not to be beneficial, but there is no precise data in SOT patients except when IRIS is present. IRIS can mimic the worsening of the cryptococcal infection. SNC IRIS has less inflammation in CSF, fewer proteins ( $< 25$  white cells/mcl and  $< 50$  mg/dL), and negative cultures. T-cell-lymphocyte-depleting therapies have been related to a higher risk of IRIS in SOT patients. The incidence of IRIS is 12% in SOT patients [48]. Prednisone of 0.5–1 mg/kg may be considered in severe or worsening clinical scenarios. Antifungal prophylaxis is not recommended in SOT patients [11].

A cohort in China found a 39.6% heteroresistance to fluconazole in cryptococcal meningitis, showing the importance of studying other pharmacologic options [33]. New antifungal treatments are being developed. Rezafungin has limited activity against *Cryptococcus*. Manogepix (the active form of fosmanogepix) has activity against *Cryptococcus*; it has a synergistic effect when combined with fluconazole. Opelconazole is another antifungal formulated for inhalation and is a potential alternative for cryptococcal pneumonia. The oral lipid nanocrystal amphotericin B is a good alternative for cryptococcal meningitis, but it has not been studied in the non-HIV population [49–53].

## 7. Prognosis

In cryptococcosis, serum CrAg is now a well-accepted diagnostic biomarker for detecting or confirming cryptococcal infection; like in HIV patients with cryptococcus infection, high titers herald a poor prognosis [10]. Regarding cryptococcus meningitis in non-HIV patients, over the years there have been described significant prognostic factors, including low CSF glucose, high CSF lactate, high CSF cryptococcal antigen titer ( $> \text{or} = 1:1024$ ), initial level of consciousness, the presence of seizure, hydrocephalus, and central nervous system vasculitis [54, 55]. Non-HIV immunosuppressed hosts have been noted to have a poorer prognosis. In recent years, literature has been published about the prognosis in non-HIV patients with cryptococcus infection.

In a retrospective study that included 302 patients diagnosed with cryptococcosis between 1996 and 2010, mortality was highest in the NHNT group (27%) vs. SOT (14%) and HIV (20%). On univariate analyses, prognostic factors positively associated with 90-day mortality included cancer ( $P = 0.018$ ), fever ( $P = 0.031$ ), altered mental status ( $P = 0.001$ ), positive blood cultures ( $P < 0.001$ ), and high ( $\geq 1:512$ )

serum CrAg ( $P = 0.021$ ). In multivariable logistic regression analyses, cryptococemia [odds ratio (OR) 5.09, 95% confidence interval (CI) 2.54–10.22;  $P < 0.001$ ], baseline opening pressure of 25 cm H<sub>2</sub>O (OR 2.93, 95% CI 1.25–6.88;  $P = 0.013$ ), and altered mental status (OR 1.96, 95% CI 0.98–3.91;  $P = 0.057$ ) were associated with increased odds of mortality [20].

Another retrospective study included 28 confirmed cryptococcal infections between 2005 and 2017. There were nine cryptococcal-attributable deaths. The case fatality rate was highest among patients with underlying hematologic malignancies (63%), followed by SOT (50%) and HIV patients (20%) [56]. A 12-year review at a tertiary care center (University of Kentucky HealthCare system) included 114 patients with cryptococcosis; 23 (20.2%) had HIV infection, 11 (9.6%) had transplants (nine patients had SOT and two had stem cell transplants), and 80 (70.2%) were NHNT patients. Overall, mortality was 28.1% (32/114), and relapse occurred in 10.5% (12/114) of patients. The mortality trend was higher (OR = 2.346,  $p = 0.287$ ) in the transplant group (45.5%, 5/11) than in the HIV (26.1%, 6/23) and NHNT groups (26.3%, 21/80). Chronic liver disease had a large and statistically significant association with mortality on multivariate analysis (OR = 3.583,  $p = 0.013$ ), which was more pronounced than the HIV or transplant groups [57].

## 8. Conclusions

The incidence of *Cryptococcus* infections in non-HIV immunocompromised patients continues to rise around the world. The information in the literature about the clinical course, management, diagnosis, and treatment of these patients continues to be limited and sometimes uncertain. The antifungal treatment studied in the context of people living with HIV has been used in other immunocompromised patients with not-so-favorable outcomes and highly reported infection recurrence. Future investigations focused on this heterogeneous group of patients are needed to better understand cryptococcosis in different hosts, their treatment, and to improve outcomes.

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## Conflict of interest

All authors declare that there are no conflicts of interest.

45 **A. Appendix**

| Clinical Presentation                                                      | Phase         | Therapy                                                                                                                                                                                                                                                                           | Duration                                                            | Renal impairment                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|----------------------------------------------------------------------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Meningitis or disseminated disease or moderate to severe non-CNS infection | Induction     | <ul style="list-style-type: none"><li>• Liposomal AmB (3–4 mg/kg/day IV) or AmB lipid complex (5 mg/kg/day IV) + Flucytosine (100 mg/kg/day in four divided doses)</li><li>• In the absence of Flucytosine: lipid formulation of AmB + Fluconazole 800–1200 mg IV daily</li></ul> | 2 weeks (SOT)<br>4–6 weeks (other immunosuppressed patients)        | 1. Amphotericin has no renal adjustment<br>2. Flucytosine: CrCl > 50 ml/min (100–150 mg/kg/day) CrCl 26–50 ml/min, (75 mg/kg/day) CrCl 13–25 ml/min, (37 mg/kg/day CrCl < 13 ml/min, avoid flucytosine.<br>3. Fluconazol: CrCl ≤ 50 ml/min: 200–400 mg/day (or 50% of the dose) HD: 50–200 mg/day on non-HD days or 100–400 mg after HD. CRRT: 2x dose suggested<br>4. Voriconazole and Posaconazole have no renal adjustment, avoid IV if ≤ 50 ml/min due to cycloextrin |
|                                                                            | Consolidation | <ul style="list-style-type: none"><li>• Fluconazole PO 400–800 mg daily</li></ul> Relapses: <ul style="list-style-type: none"><li>• Fluconazole 800–1200 mg PO daily</li><li>• Voriconazole 200–400 mg PO daily</li><li>• Posaconazole 200 mg PO QID</li></ul>                    | 8 weeks<br>10–12 weeks                                              |                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Mild to moderate non-CNS disease                                           | Maintenance   | Fluconazole PO 200–400 mg daily                                                                                                                                                                                                                                                   | 6–12 months                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|                                                                            |               | Fluconazole PO 400 mg daily                                                                                                                                                                                                                                                       | 6–12 months                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Pretransplant asymptomatic pulmonary nodules                               |               | Fluconazole PO 400 mg daily                                                                                                                                                                                                                                                       | At least 2 weeks before transplant and 6–12 months after transplant |                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

*\*Higher doses of fluconazole may be considered in high fungal burden or in refractory disease. PO: oral, IV: intravenous. CNS: central nervous system, CrCl: creatinine clearance, HD: hemodialysis, CRRT: continuous renal replacement therapy.*  
*\*\*Flucytosine dose monitoring in the first 72 hours: peak concentration < 100 mg/L; trough concentration 20–40 mg/L.*  
*\*\*\*Ref. [5].*

**Table A1.**  
*Antifungal treatment for cryptococcal infection in the immunocompromised population.*

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# Fungal Infections in Critical Illness

Richa Chauhan

## Abstract

Fungal infections, particularly invasive candidiasis (IC) and invasive aspergillosis (IA), are increasingly prevalent in intensive care unit (ICU) patients due to broad-spectrum antibiotic use, immunosuppression, and invasive procedures. Early diagnosis remains challenging, necessitating a structured approach incorporating clinical, radiological, and mycological criteria. IC, affecting 1.56 million individuals annually with 63.6% mortality, requires echinocandins as first-line therapy, with fluconazole as an option for stable patients. Lipid-based amphotericin B is reserved for drug intolerance or resistance. Management includes blood cultures, susceptibility testing, and ophthalmologic exams, with therapy continuing at least 2 weeks post-clearance. IA is best treated with voriconazole, while lipid-based amphotericin B and posaconazole are alternatives. Combination antifungal therapy is considered in select cases but requires careful monitoring. Mucormycosis surged during the COVID-19 second wave, especially in India, with a subsequent rise in dengue-associated cases, possibly due to antibody-dependent enhancement. *Rhizopus oryzae*, the primary pathogen, causes rapid tissue necrosis *via* vascular invasion and thrombosis. Key risk factors include uncontrolled diabetes, corticosteroids, and immunosuppression. Diagnosis relies on histopathology and imaging, while treatment involves amphotericin B, surgical debridement, and risk factor management. Other critical fungal infections include Pneumocystis pneumonia (PCP), affecting 505,000 people annually with a 42.4% mortality rate, primarily in human immunodeficiency virus (HIV)/AIDS and immunosuppressed patients. Trimethoprim-sulfamethoxazole remains the treatment and prophylaxis of choice. Cryptococcosis, caused by *Cryptococcus neoformans* and *C. gattii*, leads to 194,000 cases annually with a 75.8% fatality rate, particularly among HIV/AIDS and transplant patients. With rising fungal infection rates and high mortality, early risk assessment, targeted antifungal therapy, and improved diagnostics are crucial to reducing ICU mortality.

**Keywords:** invasive fungal infections, intensive care units, Candidemia, candidiasis, invasive, invasive pulmonary aspergillosis, Echinocandins, Caspofungin, azoles, fluconazole, Voriconazole, amphotericin B, galactomannan, Mannans, Procalcitonin, Mucormycoses, COVID-19, pneumocystis infections, Cryptococcosis

## 1. Introduction

Fungal infections, particularly invasive candidiasis (IC) and invasive aspergillosis (IA), have emerged as significant causes of morbidity and mortality in critically ill patients admitted to the intensive care unit (ICU). Updated estimates indicate an annual incidence of 6.5 million invasive fungal infections, resulting in 3.8 million deaths. Of

these, approximately 2.5 million (68%; range 35–90) were directly attributed to fungal infections [1]. The increasing incidence of these infections is largely attributed to the widespread use of broad-spectrum antibiotics, immunosuppressive therapies, and invasive medical procedures, such as central venous catheters, mechanical ventilation, and parenteral nutrition. Delayed diagnosis remains a major challenge due to the nonspecific clinical presentation and limitations of current diagnostic tools, often leading to delayed initiation of appropriate antifungal therapy and poor patient outcomes.

Through a series of clinical cases and a comprehensive review of current literature, we explore the epidemiology, clinical manifestations, diagnostic challenges, and therapeutic outcomes associated with invasive fungal infection, namely invasive candidiasis, aspergillosis, and mucormycosis in critically ill patients. By highlighting key risk factors and patterns observed in real-world scenarios, this chapter underscores the importance of early recognition and timely, tailored antifungal therapy to mitigate morbidity and mortality.

## **2. Rising incidence and challenges of invasive fungal infections in ICUs**

Invasive fungal infections (IFIs) are defined as systemic infections resulting from the establishment of yeasts or molds in deep-seated tissues [2]. Once considered uncommon in intensive care units (ICUs), they have seen a sharp rise in their incidence in recent years due to widespread broad-spectrum antibiotic use and improved understanding of fungal diseases. Today, these infections pose a significant challenge for ICUs worldwide [3].

Fungal infections are predominantly caused by *Candida* and *Aspergillus* species, with *Cryptococcus neoformans* and Zygomycetes also being significant contributors. These infections are associated with substantial morbidity and mortality. Notably, the occurrence of candidemia and invasive candidiasis is significantly higher, approximately 10 to 15 times, compared to invasive aspergillosis [4, 5].

### **2.1 Invasive candidiasis in ICUs**

#### *2.1.1 Burden of invasive candidiasis*

Invasive *Candida* infections, including candidemia and deep-seated candidiasis, pose a significant challenge in critically ill patients. Globally, an estimated 1,565,000 people develop *Candida* bloodstream infections or invasive candidiasis each year, resulting in approximately 995,000 deaths, with a mortality rate of 63.6% [1]. The high fatality rate is attributed to delayed diagnosis, antifungal resistance, and underlying comorbidities, particularly in immunocompromised and ICU patients.

*Case study:* Candidemia in a patient with chronic lung disease with prolonged hospitalization.

This 64-year-old critically ill male, on invasive mechanical ventilation for acute exacerbation of chronic obstructive airway disease (COAD) and pneumonia due to pan-drug-resistant *Acinetobacter*, experienced progressive deterioration, developing leukopenia, septic shock requiring inotropic support, and acute renal failure, necessitating renal replacement therapy (RRT). His history of prolonged polymyxin therapy and chronic corticosteroid use increased his susceptibility to opportunistic infections. Despite pre-emptive antifungal therapy with caspofungin, he subsequently developed candidemia.

A chest X-ray revealed a large cavitory lesion in the right lower lobe, but further imaging could not be performed due to his hemodynamically unstable condition (**Figures 1 and 2**).

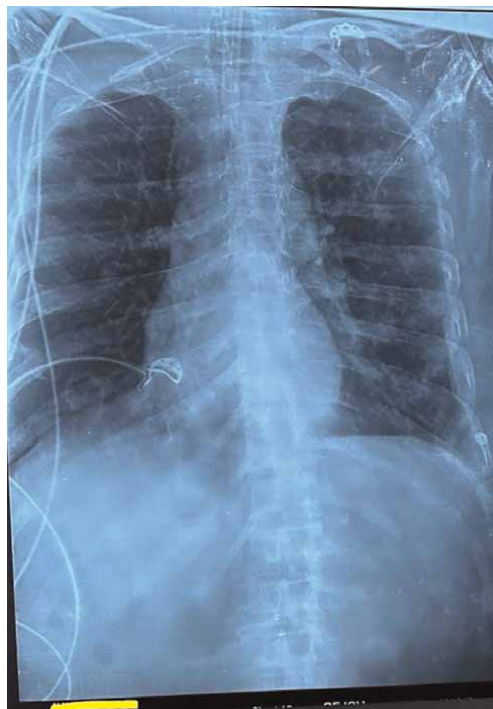


**Figure 1.**  
*Chest X-ray with anteroposterior view showing a large cavitory lesion in the right lower lobe in a COAD patient with candidemia.*



**Figure 2.**  
*Consecutive chest x-ray in 3-day duration showing a rapidly progressive cavitory lesion in the right lower lobe in a COAD patient with candidemia.*

While *Candida* was historically considered a commensal organism, its emergence as a major pathogen in immunocompromised and critically ill patients has led to higher morbidity and mortality rates. It is identified as the third most common cause of bloodstream infections (BSI) in ICU patients and the fourth most common in hospitalized patients [5]. This has an influence on guiding the empirical antibiotic therapy administered. It ranks as the second most lethal organism, following *Pseudomonas aeruginosa*, due to its high mortality rates, as high as 47%, often due to delayed diagnosis and treatment [6].

*Candida* species account for 8% of nosocomial bloodstream infections (BSIs), which account for 10% of all hospital-acquired infections [7]. Candidemia remains a significant clinical challenge due to its high mortality rate of 40.4% [8]. Candidemia is especially common in surgical critically ill patients. According to the Sepsis Occurrence in Acutely Ill Patients (SOAP) study, fungal infections were identified in 17% of sepsis cases among patients admitted to ICUs across Europe [9].

*Candida* causes a wide spectrum of superficial and deep diseases. Superficial infections are frequently community-acquired. Whereas deep-seated and systemic *Candida* infections are usually nosocomial in origin. Invasive candidiasis is the most common fungal disease among ICU patients. It occurs when *Candida* species, which are frequent colonizers of cutaneous and mucosal surfaces, gain access to deeper, normally sterile sites. Invasive candidiasis comprises candidemia and deep-seated tissue candidiasis.

## 2.2 Candidemia

The presence of *Candida* species in at least one blood culture is considered unequivocal evidence of bloodstream infection. These patients are more easily diagnosed because blood cultures directly confirm the infection.

## 2.3 Deep-seated candidiasis

This condition can develop through:

- *Hematogenous dissemination* – *Candida* spreads via the bloodstream to secondary sites, such as the liver, spleen, kidneys, or eyes. In the ICU, intra-abdominal candidiasis constitutes the majority of cases with deep-seated candidiasis.
- *Direct inoculation* – Surgical or invasive procedures introduce *Candida* into normally sterile body sites, leading to localized infections like peritonitis or osteomyelitis.

*Candida albicans* is the most common human pathogen causing fungal infection. But a shift toward *non-albicans Candida* spp. has been observed [6, 10].

*Candida glabrata* has come into the spotlight due to the increasing incidence worldwide and decreased susceptibility to antifungals [11]. *Candida parapsilosis* is the third most common cause of candidemia, and it most commonly causes candidemia in neonatal intensive care units.

The other commonly encountered species in ICU are *Candida tropicalis*, *Candida krusei*. *C. tropicalis* is known for its greater virulence compared to other *Candida* species due to its ability to form biofilms and produce hydrolytic enzymes, contributing to tissue invasion and antifungal resistance. *Candida tropicalis* exhibits the highest

species-specific mortality at 63.6%. Both species are frequently recovered from patients with hematological malignancies, especially those with prolonged neutropenia. ICU admission is a major risk factor associated with candidemia mortality [12].

Non-albicans *Candida* species show higher resistance rates to antifungals adding to their growing clinical significance [10, 13].

## 2.4 Clinical features

The key risk factors for invasive candidiasis are as listed in **Table 1**. Invasive candidiasis has been associated with severe sepsis, septic shock, and multiorgan failure and is clinically indistinguishable from bacterial infection. Thereby, delaying diagnosis and treatment. Despite advancements in antifungal therapy, candidemia remains a serious concern with an estimated mortality rate of 40%, underscoring the ongoing diagnostic challenges. Timely source control and appropriate antifungal management are essential for improving clinical outcomes (**Table 1**) [11].

In recent years, community invasive candidiasis has risen in association with an increase in at-home healthcare [16].

| Risk factor category               | Specific factors                                                                                                                                                                                                                                                                                                                                                                                          |
|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Surgical history                   | Recent surgery within the past 3 months (especially gastrointestinal surgery)                                                                                                                                                                                                                                                                                                                             |
| Invasive devices                   | Endotracheal tubes, central venous catheters (CVC), implantable drug delivery systems (IDDS), urinary catheters                                                                                                                                                                                                                                                                                           |
| Antibiotic exposure                | Antibiotic therapy for more than 5 days within the past month                                                                                                                                                                                                                                                                                                                                             |
| Immunosuppression                  | <ul style="list-style-type: none"><li>• Corticosteroid treatment (prednisolone <math>\geq 0.5</math> mg/kg/day for <math>&gt;1</math> month)</li><li>• HIV infection</li><li>• Cancer chemotherapy (past 3 months)</li><li>• Organ transplant with immunosuppressive therapy</li><li>• Bone marrow or hematopoietic stem cell transplant</li><li>• Use of immunosuppressants or anti-TNF agents</li></ul> |
| Renal impairment                   | Renal failure requiring hemodialysis                                                                                                                                                                                                                                                                                                                                                                      |
| Metabolic disorders                | Type 1 diabetes mellitus                                                                                                                                                                                                                                                                                                                                                                                  |
| Hematologic conditions             | Neutropenia (absolute neutrophil count $\leq 500$ cells/mm <sup>3</sup> )                                                                                                                                                                                                                                                                                                                                 |
| Malignancies                       | Presence of solid tumors or hematologic malignancies                                                                                                                                                                                                                                                                                                                                                      |
| Substance use                      | Active intravenous drug use                                                                                                                                                                                                                                                                                                                                                                               |
| Colonization history               | Previous <i>Candida</i> colonization                                                                                                                                                                                                                                                                                                                                                                      |
| Medication use                     | Gastric acid suppression therapy                                                                                                                                                                                                                                                                                                                                                                          |
| Nutritional factors                | Total parenteral nutrition                                                                                                                                                                                                                                                                                                                                                                                |
| Hospitalization Factors            | Prolonged hospital or ICU stay                                                                                                                                                                                                                                                                                                                                                                            |
| Severity of Illness                | APACHE score $> 20$                                                                                                                                                                                                                                                                                                                                                                                       |
| <i>Candida</i> colonization degree | Assessed using the colonization index                                                                                                                                                                                                                                                                                                                                                                     |

**Table 1.**  
*Risk factors for invasive candidiasis [14, 15].*

*Candida score* - A clinical scoring system that assists clinicians in differentiating between *Candida* species colonization and proven candidal infection, supporting early preemptive antifungal treatment in nonneutropenic critically ill patients [17, 18].

Definitions have been developed according to two levels of probability of invasive fungal disease (IFD) following the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) approach- “proven” and “probable”(Table 2) [19, 20].

| Type of IFD  | Criteria                                                                                                                                                                                                                                                                                                   |
|--------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Proven IFD   | Requires detection through blood culture or confirmation via histological examination or culture of a specimen obtained from a normally sterile site (e.g., pleural fluid, pericardial fluid, and peritoneal fluid). Applies to all individuals, regardless of immune status.                              |
| Probable IFD | Requires three essential criteria:<br>1. Identified host factor indicating patient risk.<br>2. Clinical manifestations consistent with the disease.<br>3. Supporting mycological evidence, including culture, microscopy, antigen detection, or molecular techniques like polymerase chain reaction (PCR). |

**Table 2.**  
*Type of IFDs.*

*Colonization* is defined as the presence of *Candida* species in samples obtained from nonsterile sites, namely the oropharynx, stomach, urine, or tracheal aspirates.

$$\text{Candida Colonisation Index (CI)} = \frac{\text{Total number of distinct body sites tested}}{\text{Number of sites colonized with identical Candida strains}} \quad (1)$$

Assessment with the *Candida score* (CS) should be performed to predict proven candidal infection [17].

#### 2.4.1 Simplified *Candida score*

See **Table 3.**

| Factor                                         | Score (0 = Absent, 1 = Present) |
|------------------------------------------------|---------------------------------|
| Total Parenteral Nutrition (TPN)               | 0 or 1                          |
| Surgery                                        | 0 or 1                          |
| Multifocal <i>Candida</i> species colonization | 0 or 1                          |
| Severe sepsis                                  | 0 or 2                          |
| Total possible score                           | 0 to 5                          |

**Table 3.**  
*Components of the simplified Candida score used to predict proven candidal infection in ICU patients.*

#### 2.4.2 Interpretation

- Cutoff Score:  $\geq 2.5$
- Sensitivity: 81%

- Specificity: 74%
- Patients scoring  $\geq 2.5$  may benefit from early antifungal therapy while reducing the risk of resistance from unnecessary treatment [21].

An alternative scoring system for predicting IFDs (**Table 4**) (by Liao et al. [7]).

| Risk factor                                 | Points  |
|---------------------------------------------|---------|
| Gastrointestinal surgery                    | 5       |
| Diabetes mellitus                           | 5       |
| Hematological malignancies                  | 4       |
| Broad-spectrum antibiotics ( $\geq 4$ days) | 4       |
| Presence of a central venous catheter       | 3       |
| Total Parenteral Nutrition (TPN)            | 3       |
| Mechanical ventilation ( $\geq 2$ days)     | 2       |
| Total possible score                        | 0 to 26 |

**Table 4.**  
*Scoring system by Liao et al. for predicting IFDs.*

2.4.3 Risk categories

See **Table 5**.

| Risk level        | Score range |
|-------------------|-------------|
| Low risk          | $\leq 8$    |
| Intermediate risk | 9–13        |
| High risk         | $\geq 14$   |

**Table 5.**  
*Risk categories on the basis of score obtained from the scoring system by Liao et al.*

2.5 Laboratory diagnosis

Aiming at early and accurate diagnosis of invasive candidiasis, since delay in initiating adequate antifungal therapy is associated with increased mortality [22, 23].

2.5.1 Blood cultures remain the standard method for diagnosing candidemia, but their diagnostic performance has significant limitations

1. Sensitivity - of up to 70%, meaning up to 30% of candidemia cases may be missed.
2. Prolonged Incubation Time- delayed diagnosis increases the risk of mortality.
3. Limited Use in Deep-Seated Candidiasis- In cases of hepatosplenic candidiasis, endocarditis, or peritonitis, *Candida* is often confined to tissues and may not be detected in blood cultures (**Table 6**) [24, 25].

| Parameter                                | Details                                                             |
|------------------------------------------|---------------------------------------------------------------------|
| Number of blood culture pairs            | 2 pairs (10 mL each) for aerobic and anaerobic cultures             |
| Timing of blood culture collection       | Before initiation of antifungal therapy                             |
| Additional blood culture recommendations | Up to 4 blood culture pairs in 24 hours to increase yield above 90% |
| Association with IV catheters            | 40–50% of candidemia episodes are linked to intravenous catheters   |
| Blood culture collection sites           | Central venous line and peripheral site in suspected candidemia     |

**Table 6.**  
*Blood culture guidelines for suspected candidemia.*

### 2.5.2 Serological tests for invasive fungal infections

A dilated fundoscopic examination is important in all candidemia patients (**Table 7**) [28, 29].

*Candida parapsilosis* has been associated to candidemia in neonates and young adults (**Table 8**). exogenously origin and contaminates medical devices, causing central venous catheter-associated candidemias.

*Candida glabrata*, *Candida tropicalis*, and *Candida krusei* are isolated in blood cultures from older patients (>65 years) with important risk factors, such as major abdominal surgery, solid tumors and hematologic malignancies, transplants, and/or prolonged treatment with corticosteroids.

| Test                                      | Description                                                                                                                                | Sensitivity | Specificity   |
|-------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-------------|---------------|
| Galactomannan and 1,3-β-D-Glucan (BDG)    | Fungal cell wall components used as serological markers. BDG ≥ 60 pg./mL distinguishes IFIs from non-IFI cases but lacks sensitivity [26]. | Low [27]    | Varies        |
| Real-time polymerase chain reaction (PCR) | Useful for diagnosing deep-seated candidiasis, especially with negative blood cultures [28].                                               | ~60%        | Not specified |
| Procalcitonin (PCT)                       | PCT < 2 ng/mL suggests candidemia over bacteremia [29, 30].                                                                                | 92%         | 93%           |

**Table 7.**  
*Serological tests for invasive fungal infections.*

| Factor                            | Description                                                               |
|-----------------------------------|---------------------------------------------------------------------------|
| Epidemiology of fungal infections | Prevalence and distribution of fungal pathogens in ICU settings.          |
| Resistance patterns               | Emerging antifungal resistance among ICU pathogens.                       |
| Pharmacokinetics                  | Absorption, distribution, metabolism, and excretion of antifungal agents. |
| Drug interactions                 | Potential interactions with other medications used in ICU patients.       |
| Safety profile                    | Adverse effects and tolerability of antifungal drugs.                     |
| Spectrum of activity              | Efficacy against different fungal species.                                |
| Pharmacy acquisition cost         | Financial considerations for antifungal therapy selection.                |

**Table 8.**  
*Factors influencing antifungal therapy selection [31].*

2.6 Prevention and treatment

The saying “prevention is better than cure” is particularly relevant in this context (Table 5). Candidemia-associated mortality is more effectively prevented than treated, even with advancements in therapy. Implementing rigorous programs to ensure strict adherence to hand hygiene guidelines is crucial in reducing the risk of infection [18].

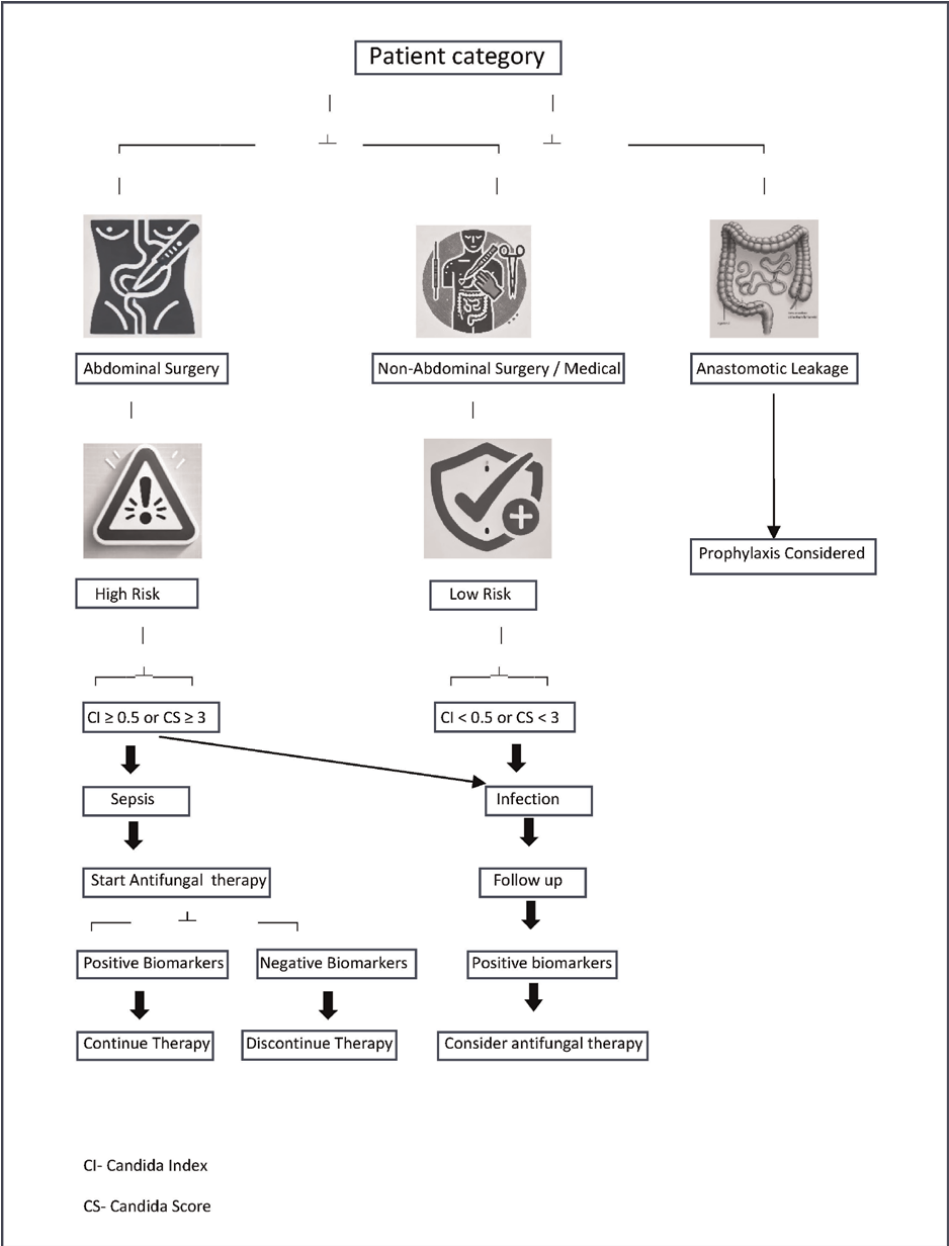


Figure 3.  
An algorithm for systemic antifungal therapy in ICU patients [20].

Ensuring adherence to aseptic precautions during the placement and management of central venous catheters, along with strict control of antimicrobial use, is crucial for preventing nosocomial candidemia [21].

It is crucial to recognize that critical illness induces pathophysiological changes that can significantly alter drug concentrations, leading to differences from those observed in non-critically ill patients. Standard dosing in such cases may result in suboptimal drug levels—either too low, risking treatment failure, or too high, increasing the risk of toxicity. However, antifungal agents are generally less affected by these pharmacokinetic changes due to their lipophilic properties, hepatic metabolism, and high protein binding.

Antifungal therapy approach in ICU patients is based on clinical stratification, *Candida* scores, and *Candida* biomarkers (**Figure 3**).

The 2016 revised guidelines by the Infectious Diseases Society of America (IDSA) for managing candidiasis are summarized below [32]. These recommendations evaluate both the quality of evidence (very low, low, moderate, or high) and the strength of the recommendation (weak or strong).

### 2.6.1 Recommendations for the treatment of candidemia in nonneutropenic patients

See **Table 9**.

Recommendations for central venous catheter (CVC) removal in nonneutropenic patients with candidemia (**Table 10**).

| Recommendation                                  | Details                                                                                                                                                                                                                                                                                                                       | Strength and quality of evidence                 |
|-------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| Initial therapy                                 | <i>Echinocandin</i> <ul style="list-style-type: none"> <li>Caspofungin: 70 mg LD, then 50 mg daily</li> <li>Micafungin: 100 mg daily</li> <li>Anidulafungin: 200 mg LD, then 100 mg daily</li> </ul>                                                                                                                          | Strong recommendation; high-quality evidence     |
| Alternative initial therapy                     | <i>Fluconazole</i> (IV or oral) <ul style="list-style-type: none"> <li>800 mg (12 mg/kg) LD</li> <li>400 mg (6 mg/kg) daily</li> </ul> <i>For non-critically ill patients unlikely to have fluconazole-resistant Candida</i>                                                                                                  | Strong recommendation; high-quality evidence     |
| Susceptibility testing                          | <ul style="list-style-type: none"> <li>Azole susceptibility testing for all significant <i>Candida</i> isolates</li> <li>Echinocandin susceptibility testing for: <ul style="list-style-type: none"> <li>Prior echinocandin treatment</li> <li><i>C. glabrata</i> or <i>C. parapsilosis</i> infections</li> </ul> </li> </ul> | Strong recommendation; low-quality evidence      |
| Transition to fluconazole                       | Recommended within 5–7 days for stable patients with: <ul style="list-style-type: none"> <li><i>Candida</i> isolates susceptible to fluconazole (e.g., <i>C. albicans</i>)</li> <li>Negative repeat blood cultures</li> </ul>                                                                                                 | Strong recommendation; moderate-quality evidence |
| Alternative therapy (if resistance/intolerance) | <i>Lipid-formulation Amphotericin B (AmB)</i> <ul style="list-style-type: none"> <li>3–5 mg/kg daily</li> </ul>                                                                                                                                                                                                               | Strong recommendation; high-quality evidence     |

| Recommendation                     | Details                                                                                                                                                         | Strength and quality of evidence                 |
|------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| Transition from AmB to fluconazole | Recommended after 5–7 days if: <ul style="list-style-type: none"><li>• Candida isolates susceptible to fluconazole</li><li>• Negative repeat cultures</li></ul> | Strong recommendation; high-quality evidence     |
| Ophthalmologic examination         | All nonneutropenic patients should undergo a dilated ophthalmologic exam within the first week of diagnosis                                                     | Strong recommendation; low-quality evidence      |
| Follow-up blood cultures           | Obtain daily or every other day to confirm clearance of candidemia                                                                                              | Strong recommendation; low-quality evidence      |
| Duration of therapy                | Two weeks after: <ul style="list-style-type: none"><li>• Documented clearance of Candida from bloodstream</li><li>• Resolution of candidemia symptoms</li></ul> | Strong recommendation; moderate-quality evidence |

**Table 9.**  
*Recommendations for the treatment of candidemia in nonneutropenic patients.*

| Recommendation | Details                                                                                                                                                              | Strength of recommendation | Quality of evidence |
|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|---------------------|
| CVC removal    | Remove CVC as early as possible if it is suspected to be the source and can be safely removed. The decision should be individualized based on the patient condition. | Strong                     | Moderate            |

**Table 10.**  
*Recommendations for central venous catheter (CVC) removal in nonneutropenic patients with candidemia.*

2.6.2 Recommendations for the treatment of candidemia in neutropenic patients

See **Table 11.**

| Recommendation              | Details                                                                                                                                                                                                | Strength and quality of evidence                 |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| Initial therapy             | <i>Echinocandin</i> <ul style="list-style-type: none"><li>• Caspofungin: 70 mg LD, then 50 mg daily</li><li>• Micafungin: 100 mg daily</li><li>• Anidulafungin: 200 mg LD, then 100 mg daily</li></ul> | Strong recommendation; moderate-quality evidence |
| Alternative therapy         | <i>Lipid-formulation Amphotericin B (AmB)</i> <ul style="list-style-type: none"><li>• 3–5 mg/kg daily</li></ul> Less preferred due to toxicity risk                                                    | Strong recommendation; moderate-quality evidence |
| Step-down therapy           | <i>Fluconazole 400 mg (6 mg/kg) daily</i><br>For stable patients with persistent neutropenia, susceptible isolates, and documented bloodstream clearance                                               | Weak recommendation; low-quality evidence        |
| <i>C. krusei</i> infections | <i>Echinocandin, lipid-formulation AmB, or voriconazole</i>                                                                                                                                            | Strong recommendation; low-quality evidence      |

| Recommendation             | Details                                                                                                                                                                                      | Strength and quality of evidence            |
|----------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Duration of therapy        | Minimum 2 weeks after: <ul style="list-style-type: none"> <li>Documented clearance of Candida from bloodstream</li> <li>Resolution of neutropenia and candidemia-related symptoms</li> </ul> | Strong recommendation; low-quality evidence |
| Ophthalmologic examination | Perform dilated funduscopy examination within 1 week after neutropenia recovery, as ocular signs are often minimal during neutropenia                                                        | Strong recommendation; low-quality evidence |
| Catheter removal           | In neutropenic patients, non-CVC sources (e.g., GI tract) are predominant. CVC removal should be considered on a case-by-case basis                                                          | Strong recommendation; low-quality evidence |

**Table 11.**

*Recommendations for the treatment of candidemia in neutropenic patients.*

### 2.6.3 The role of empiric treatment for suspected invasive candidiasis in nonneutropenic patients in the intensive care unit?

See **Table 12.**

| Recommendation                                                 | Details                                                                                                                                                                                                                      | Strength of recommendation | Quality of evidence |
|----------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|---------------------|
| 1. Consider empiric antifungal therapy                         | For critically ill patients with risk factors for invasive candidiasis and no other identifiable cause of fever. Guided by risk assessment, surrogate markers, and culture results. Initiate promptly in septic shock cases. | Strong                     | Moderate            |
| 2. Preferred empiric therapy (ICU, nonneutropenic patients)    | Echinocandin therapy: caspofungin (70 mg loading dose, then 50 mg daily), micafungin (100 mg daily), anidulafungin (200 mg loading dose, then 100 mg daily).                                                                 | Strong                     | Moderate            |
| 3. Alternative therapy (if no azole resistance/azole exposure) | Fluconazole: 800 mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily.                                                                                                                                                    | Strong                     | Moderate            |
| 4. Lipid-formulation AmB (alternative option)                  | 3–5 mg/kg daily if other antifungal agents are intolerable.                                                                                                                                                                  | Strong                     | Low                 |
| 5. Duration of therapy                                         | Two weeks for suspected invasive candidiasis in improving patients (similar to documented candidemia).                                                                                                                       | Weak                       | Low                 |
| 6. Discontinuation of antifungal therapy                       | If no response after 4–5 days and no evidence of invasive candidiasis or a negative high-negative-predictive-value diagnostic assay.                                                                                         | Strong                     | Low                 |

**Table 12.**

*Recommendations for empiric treatment for suspected invasive candidiasis for nonneutropenic patients in ICU.*

## 2.6.4 Prophylaxis for preventing invasive candidiasis in ICU

See **Table 13**.

| Recommendation                        | Details                                                                                                                                   | Strength of recommendation | Quality of evidence |
|---------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|---------------------|
| 1. Fluconazole for high-risk patients | 800 mg (12 mg/kg) loading dose, then 400 mg (6 mg/kg) daily. Recommended in ICUs where invasive candidiasis incidence >5%.                | Weak                       | Moderate            |
| 2. Alternative: Echinocandin therapy  | Caspofungin (70 mg loading dose, then 50 mg daily), Anidulafungin (200 mg loading dose, then 100 mg daily), or micafungin (100 mg daily). | Weak                       | Low                 |

**Table 13.**  
*Recommendations for prophylaxis for preventing invasive candidiasis in ICU.*

## 2.6.5 Treatment for intra-abdominal candidiasis

See **Table 14**.

| Recommendation                            | Details                                                                                                                                                                       | Strength of recommendation | Quality of evidence |
|-------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|---------------------|
| 1. Consider empiric antifungal therapy    | For patients with clinical signs of intra-abdominal infection and significant risk factors (e.g., recent abdominal surgery, anastomotic leaks, and necrotizing pancreatitis). | Strong                     | Moderate            |
| 2. Ensure source control                  | Appropriate drainage and/or debridement is essential.                                                                                                                         | Strong                     | Moderate            |
| 3. Follow antifungal selection guidelines | Similar to treatment for candidemia or empiric therapy in ICU patients (refer to Sections I & IV).                                                                            | Strong                     | Moderate            |
| 4. Duration of therapy                    | Based on the effectiveness of source control and patient's clinical response.                                                                                                 | Strong                     | Low                 |

**Table 14.**  
*Recommendations for treatment of intraabdominal candidiasis.*

## 2.6.6 Candida isolation from respiratory tract: Need for antifungal therapy?

See **Table 15**.

| Recommendation                           | Details                                                                             | Strength of recommendation | Quality of evidence |
|------------------------------------------|-------------------------------------------------------------------------------------|----------------------------|---------------------|
| Candida growth in respiratory secretions | Usually indicates colonization and does not typically require antifungal treatment. | Strong                     | Moderate            |

**Table 15.**  
*Recommendations for antifungal therapy for Candida isolated from respiratory tract.*

2.6.7 Treatment of urinary tract infections due to *Candida* speciesSee **Table 16**.

| Recommendation                      | Details                                                                                                                                    | Strength of recommendation | Quality of evidence |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|---------------------|
| Elimination of predisposing factors | Remove indwelling bladder catheters whenever possible.                                                                                     | Strong                     | Low                 |
| Antifungal treatment                | Not recommended unless the patient is at high risk for dissemination.                                                                      | Strong                     | Low                 |
| High-risk patients                  | Includes neutropenic patients, very low-birth-weight infants (<1500 g), and those undergoing urologic procedures.                          | Strong                     | Low                 |
| Neutropenic patients                | Should be treated as per candidemia guidelines (see Section III).                                                                          | Strong                     | Low                 |
| Urologic procedures                 | Treat with oral fluconazole (400 mg or 6 mg/kg daily) or amphotericin B deoxycholate (0.3–0.6 mg/kg daily) before and after the procedure. | Strong                     | Low                 |

**Table 16.**Recommendations for treatment of urinary tract infection caused by *Candida* species.2.6.8 Treatment of symptomatic *Candida* cystitisSee **Table 17**.

*C. albicans* was highly susceptible to all antifungals, particularly Voriconazole and Amphotericin B, with 100% susceptibility. In contrast, NAC species exhibited lower susceptibility rates across all antifungals and significant resistance to fluconazole, flucytosine, and caspofungin (**Table 17**) [33].

| Recommendation                              | Details                                                                                                                        | Strength of recommendation | Quality of evidence |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|----------------------------|---------------------|
| Fluconazole-susceptible <i>Candida</i> spp. | Oral fluconazole 200 mg (3 mg/kg) daily for 2 weeks.                                                                           | Strong                     | Moderate            |
| Fluconazole-resistant <i>C. glabrata</i>    | AmB deoxycholate 0.3–0.6 mg/kg daily for 1–7 days<br>OR oral flucytosine 25 mg/kg four times daily for 7–10 days.              | Strong                     | Low                 |
| <i>C. krusei</i>                            | AmB deoxycholate 0.3–0.6 mg/kg daily for 1–7 days.                                                                             | Strong                     | Low                 |
| Catheter removal                            | If feasible, removal of an indwelling bladder catheter is strongly recommended.                                                | Strong                     | Low                 |
| AmB bladder irrigation                      | AmB deoxycholate 50 mg/L in sterile water daily for 5 days may be beneficial for fluconazole-resistant <i>Candida</i> species. | Weak                       | Low                 |

**Table 17.**Recommendations for the treatment of symptomatic *Candida* cystitis.

## 2.7 Invasive aspergillosis

### 2.7.1 Disease burden

Annually, over 2,113,000 people develop invasive aspergillosis in the context of chronic obstructive pulmonary disease, intensive care, lung cancer, or hematologic malignancy, with a crude annual mortality of 1,801,000 (85.2%) [1]. This staggering mortality rate highlights the urgent need for early diagnosis and effective antifungal therapies in ICU settings.

*Case Study:* Invasive pulmonary aspergillosis in a patient with chronic lung disease.

This 55-year-old male with COAD due to industrial exposure presented with acute exacerbation, black expectoration, and type 2 respiratory failure, requiring invasive mechanical ventilation. CT thorax revealed fibrobronchiectatic and fibrocavitary changes, suggesting chronic lung damage. The chest x-ray revealed multiple bilateral opacities, with a cavitary appearance (Figure 4). Tracheal fungal culture grew *Aspergillus flavus*, but galactomannan and candidamannan were negative, making invasive aspergillosis difficult to confirm. He was empirically started on voriconazole (200 mg BD), yet his clinical response remained poor. Simultaneously, he grew MDR and PDR *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, complicating treatment. Despite antifungal and antibiotic therapy, his condition deteriorated over 18 days, highlighting the diagnostic challenges of fungal infections in chronic lung disease and the difficulty in differentiating colonization from invasive disease, ultimately leading to his demise.

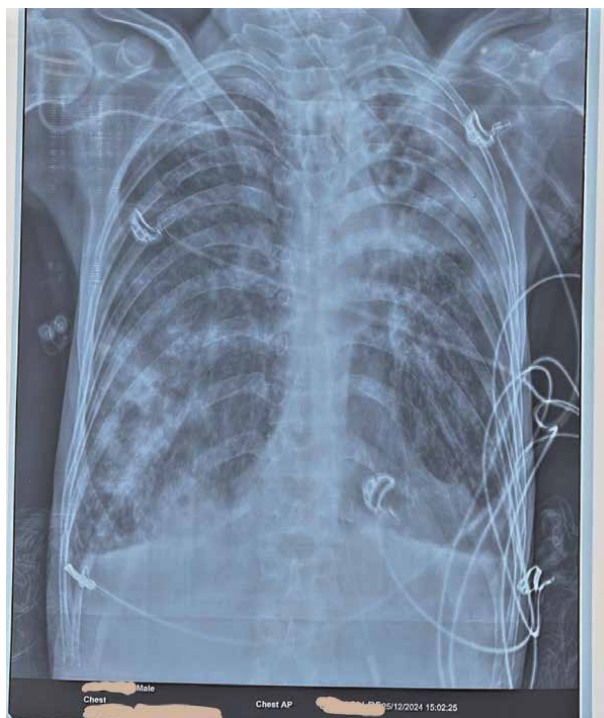


**Figure 4.**  
*Antero-posterior chest X-ray in invasive pulmonary aspergillosis: Multiple bilateral opacities, with a cavitary appearance in the emphysematous lungs of COAD.*

Invasive aspergillosis (IA) is a severe IFD among critically ill patients in the ICU, primarily manifesting as invasive pulmonary aspergillosis (IPA). Although its incidence is much lower than IC, IA is increasingly being reported [34]. Its development is closely linked to a range of established and emerging risk factors that compromise pulmonary defenses (see **Table 18**). Diagnosis remains challenging due to nonspecific signs and symptoms and low clinical suspicion. The mortality rate for IA in the ICU is extremely high, reaching 97% in patients with proven IA (**Table 18**) [35].

Clinical suspicion should arise when there is a combination of persistent or rapidly progressing infiltrative abnormalities on thoracic imaging and/or a persistent pulmonary infection unresponsive to broad-spectrum antibiotics, along with one or more of the aforementioned predisposing conditions (**Table 19**).

Therefore, careful interpretation of results is essential, and clinical correlation remains crucial in guiding treatment decisions (**Tables 20–22**).

Clinical algorithm for diagnosing Invasive Pulmonary Aspergillosis (IPA) in critically ill patients (**Table 23**) [36].

| Risk level        | Risk factors                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| High risk         | <ul style="list-style-type: none"><li>• Neutropenia (<math>&lt;500/\text{mm}^3</math>)</li><li>• Hematological malignancy</li><li>• Allogeneic HSCT</li></ul>                                                                                                                                                                                                                                                                                                |
| Intermediate risk | <ul style="list-style-type: none"><li>• Prolonged corticosteroid treatment before ICU admission</li><li>• Chronic obstructive pulmonary disease (COPD)</li><li>• Liver cirrhosis</li><li>• Solid organ cancer</li><li>• HIV infection</li><li>• Lung transplantation</li><li>• Systemic immunosuppressive therapy</li></ul>                                                                                                                                  |
| Low risk          | <ul style="list-style-type: none"><li>• Severe burns</li><li>• Solid organ transplant</li><li>• Steroid treatment <math>&gt;7</math> days</li><li>• Prolonged ICU stay (<math>&gt;21</math> days)</li><li>• Malnutrition</li><li>• Post-cardiac surgery</li><li>• Near-drowning</li><li>• HIV infection</li><li>• Chronic renal failure with renal replacement therapy</li><li>• Liver cirrhosis/acute hepatic failure</li><li>• Diabetes mellitus</li></ul> |

**Table 18.**  
*Risk factors for invasive pulmonary aspergillosis (IPA) in ICU patients.*

| Diagnostic method | Details                                                                                                                                                                                                                                                                                                                                             |
|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Thoracic imaging  | <ul style="list-style-type: none"><li>• Rapidly growing nodules and/or cavitations.</li><li>• Halo sign: Pulmonary mass with surrounding ground-glass opacification.</li><li>• Air crescent sign: Crescentic radiolucency around a consolidated nodule.</li><li>• Less reliable in ICU patients due to atelectasis and pleural effusions.</li></ul> |

| Diagnostic method                              | Details                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Microbiological examination                    | <ul style="list-style-type: none"><li>• Direct microscopy and culture of sputum or bronchoalveolar lavage fluid (BALF).</li><li>• Differentiates septate molds (<i>Aspergillus</i>) from non-septate molds (<i>Mucorales</i>).</li></ul>                                                                                                                                                                                                                                                                                |
| Bronchoscopy and BALF analysis                 | <ul style="list-style-type: none"><li>• Ulcerative lesions and/or pseudomembranes suggest <i>Aspergillus</i>-related tracheobronchitis.</li></ul>                                                                                                                                                                                                                                                                                                                                                                       |
| Serological tests (galactomannan antigen - GM) | <ul style="list-style-type: none"><li>• GM is a major <i>Aspergillus</i> cell wall component released during fungal growth.</li><li>• Higher sensitivity in BALF compared to serum.</li><li>• False positives can occur due to:<ul style="list-style-type: none"><li>• Gastrointestinal translocation from contaminated food/drink.</li><li>• Antibiotic interference (e.g., piperacillin–tazobactam and amoxicillin–clavulanic acid).</li></ul></li><li>• Reduced sensitivity with itraconazole prophylaxis.</li></ul> |
| PCR for <i>Aspergillus</i> DNA                 | <ul style="list-style-type: none"><li>• Used in serum and BALF for diagnosing IPA.</li><li>• Factors affecting accuracy:<ul style="list-style-type: none"><li>• Antifungal treatment.</li><li>• Respiratory tract colonization.</li><li>• PCR inhibition by ICU anticoagulants.</li></ul></li></ul>                                                                                                                                                                                                                     |
| Fungal culture                                 | <ul style="list-style-type: none"><li>• Low sensitivity</li><li>• Species identification</li><li>• Antifungal susceptibility testing (important due to antifungal resistance).</li></ul>                                                                                                                                                                                                                                                                                                                                |

**Table 19.**  
*Diagnostic evaluation of Invasive pulmonary aspergillosis (IPA).*

|                                                       |                                                                                                                                                                                                                                                   |
|-------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Clinical significance of <i>Aspergillus</i> isolation | <ul style="list-style-type: none"><li>• Active disease</li><li>• True colonization (more common in immunocompetent individuals)</li><li>• Predictive marker for future invasive disease (more concerning in immunocompromised patients)</li></ul> |
|-------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Table 20.**  
*Clinical significance of *Aspergillus* isolation.*

|                     |                                                                                                                                                                                                                                                 |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Diagnostic approach | <ul style="list-style-type: none"><li>• Sequentially positive GM tests in serum or BALF + positive <i>Aspergillus</i> PCR in a high-risk patient = strong suspicion of IPA.</li><li>• Antifungal therapy initiation may be warranted.</li></ul> |
|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

**Table 21.**  
*Diagnostic approach to Invasive Pulmonary Aspergillosis (IPA).*

| Category                     | Criteria                                                                                                                                                                                                                                                                               |
|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Proven IA                    | Requires conclusive histopathological evidence of fungal elements causing tissue damage, along with at least one of the following:                                                                                                                                                     |
| 1. Histopathology/microscopy | Hyphae consistent with <i>Aspergillus</i> spp. observed in a specimen obtained via needle aspiration or biopsy, with tissue damage. Confirmation via culture or PCR is required.                                                                                                       |
| 2. Sterile site culture      | Isolation of <i>Aspergillus</i> spp. from a normally sterile site obtained via a sterile procedure, accompanied by clinical signs of infection or radiological findings consistent with invasive aspergillosis, strongly suggests an active fungal infection rather than colonization. |

| Category                                                          | Criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Probable IA                                                       | Requires mycological evidence + clinical/radiological criteria + host factor criteria.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| 1. Mycological evidence (at least one of the following)           | <p><i>Aspergillus</i> spp. detection in a lower respiratory tract specimen via cytology, microscopy, or culture.</p> <p>Galactomannan antigen index: <math>\geq 0.5</math> in plasma/serum OR <math>\geq 0.8</math> in BALF.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| 2. Clinical/radiological criteria (at least one of the following) | <p>Well-defined, dense pulmonary lesions, with or without a surrounding halo sign, indicating possible angioinvasion.</p> <p>Air crescent sign, typically associated with tissue necrosis and cavitory changes.</p> <p>Presence of cavities.</p> <p>Segmental, lobar, or wedge-shaped consolidation, suggesting vascular compromise and infarction.</p> <p>Tracheobronchial abnormalities observed on bronchoscopy such as ulceration, pseudomembranes, nodules, plaques, or eschar formation).</p>                                                                                                                                                                                                           |
| 3. Host factors (at least one of the following)                   | <p>Glucocorticoid therapy (<math>\geq 20</math> mg/day prednisone equivalent).</p> <p>Neutropenia (absolute neutrophil count <math>\leq 500</math> cells/mm<sup>3</sup>).</p> <p>Chronic respiratory conditions (e.g., COPD and bronchiectasis).</p> <p>Decompensated cirrhosis.</p> <p>Recent (past 90 days) immunosuppressant therapy (e.g., calcineurin inhibitors, mTOR inhibitors, TNF blockers, alemtuzumab, ibrutinib, and nucleoside analogues).</p> <p>Hematological malignancies or hematopoietic stem cell transplantation (HSCT).</p> <p>Solid organ transplantation (SOT).</p> <p>Human immunodeficiency virus (HIV) infection.</p> <p>Severe viral pneumonia (e.g., influenza or COVID-19).</p> |

**Table 22.**  
Defining proven and probable invasive aspergillosis in the ICU [36].

| Step                   | Criteria                                                   | Details                                                                                                                                                                                                                                                                                             |
|------------------------|------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Entry criterion     | <i>Aspergillus</i> -positive culture                       | Detection of <i>Aspergillus</i> in a lower respiratory tract (LRT) sample.                                                                                                                                                                                                                          |
| 2. Diagnostic criteria | Relevant clinical Signs & Symptoms (at least one required) | <p>Fever - Refractory to at least 3 days of appropriate antibiotic therapy.</p> <p>Recurrent fever -Occurs after at least 48 hours of defervescence while still on antibiotics, with no other apparent cause.</p> <p>Pleuritic chest pain</p> <p>Pleuritic rub</p> <p>Dyspnea</p> <p>Hemoptysis</p> |

| Step                                                              | Criteria                  | Details                                                                                                                                                         |
|-------------------------------------------------------------------|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                   |                           | Worsening respiratory insufficiency -<br>Despite appropriate antibiotic therapy and ventilatory support.                                                        |
| 3. Imaging findings                                               | Abnormal imaging          | Detected via portable chest X-ray or CT scan of the lungs.                                                                                                      |
| 4. Additional diagnostic criteria (at least one of the following) | (4a) Host risk factors    | Neutropenia (absolute neutrophil count <500/mm <sup>3</sup> ) at or before ICU admission.                                                                       |
|                                                                   |                           | Underlying hematological or oncological malignancy treated with cytotoxic agents.                                                                               |
|                                                                   |                           | Glucocorticoid therapy (prednisone equivalent >20 mg/day).                                                                                                      |
|                                                                   |                           | Congenital or acquired immunodeficiency.                                                                                                                        |
| OR                                                                | (4b) Mycological evidence | Semiquantitative <i>Aspergillus</i> -positive BAL fluid culture (+ or ++) without bacterial growth.<br><br>Positive cytological smear showing branching hyphae. |

**Table 23.**  
*Clinical algorithm for diagnosing Invasive Pulmonary Aspergillosis (IPA) in critically ill patients.*

2.7.2 Classification based on criteria fulfillment

See **Table 24**.

| Diagnosis                       | Criteria met                                                         |
|---------------------------------|----------------------------------------------------------------------|
| Probable IPA                    | If Criteria 1 + 2 + 3 + (4a or 4b) are met (refer <b>Table 23</b> ). |
| <i>Aspergillus</i> colonization | If at least one of the required criteria is not met.                 |

**Table 24.**  
*Classification based on criteria fulfillment.*

2.7.3 Antifungal therapy for invasive aspergillosis

IPA requires prompt initiation of antifungal therapy, with voriconazole being the first-line agent, alongside management of underlying immunosuppression (see **Table 25**).

| Antifungal agent                           | Role in therapy                      | Advantages                                                                                          | Limitations                                                                          |
|--------------------------------------------|--------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Amphotericin B deoxycholate                | Historically, the standard treatment | Broad-spectrum antifungal activity                                                                  | Significant nephrotoxicity limits its use.                                           |
| Lipid-based formulations of amphotericin B | Developed to reduce renal toxicity   | Improved safety profile                                                                             | Still associated with some nephrotoxicity and infusion-related reactions.            |
| Voriconazole                               | Preferred first-line therapy         | Improves response rates and survival compared to Amphotericin B; fewer drug-related adverse effects | Requires therapeutic drug monitoring (TDM) to optimize dosing and minimize toxicity. |

| Antifungal agent | Role in therapy                                   | Advantages                         | Limitations                                                                                |
|------------------|---------------------------------------------------|------------------------------------|--------------------------------------------------------------------------------------------|
| Posaconazole     | Alternative for refractory or intolerant patients | Broad-spectrum antifungal coverage | Oral administration limits use in critically ill patients due to impaired drug absorption. |

**Table 25.**  
*Antifungal therapy for invasive aspergillosis (IA).*

*2.7.4 Considerations for therapy selection*

- *Disease severity:* More severe cases may require IV formulations for reliable drug delivery.
- *Renal function:* Voriconazole and posaconazole are preferred in patients with renal impairment due to amphotericin B's nephrotoxicity.
- *Drug absorption capacity:* Oral antifungals (e.g., posaconazole) may have reduced efficacy in critically ill patients with impaired gastrointestinal function.

Combination antifungal therapy may be considered in specific clinical scenarios, such as refractory infections or breakthrough invasive fungal diseases (IFDs). The combined use of antifungals can result in either synergistic or antagonistic interactions, depending on how one drug influences the target of the other (Table 26).

| Clinical context                                             | First-line therapy                                                                                                         | Alternative therapeutic options                                                                                                   |
|--------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Primary management of invasive pulmonary Aspergillosis (IPA) | Voriconazole: 6 mg/kg intravenously every 12 hours on Day 1, followed by 4 mg/kg intravenously every 12 hours <sup>a</sup> | Liposomal Amphotericin B: 3–5 mg/kg/day intravenously.                                                                            |
|                                                              | or                                                                                                                         | or                                                                                                                                |
|                                                              | <sup>b</sup> Voriconazole 400 mg orally every 12 hours on Day 1, subsequently reduced to 200 mg orally every 12 hours.     | Amphotericin B Deoxycholate: 1 mg/kg/day intravenously                                                                            |
|                                                              |                                                                                                                            | or<br>Caspofungin: 70 mg intravenously as a loading dose on Day 1, followed by a maintenance dose of 50 mg intravenously per day. |

<sup>a</sup>In patients with moderate to severe hepatic failure, dose reduction is recommended to 35 mg/day i.v.  
<sup>b</sup>Oral administration is recommended only in patients with intact intestinal absorption.

**Table 26.**  
*Management of invasive pulmonary aspergillosis in non-neutropenic critically ill patients [37].*

**3. Mucormycosis**

Though the third most common fungal disease, it is a rare and so-called “ultraorphan fungal infection.” The second wave of the COVID-19 pandemic saw an unprecedented increase and surge in superinfections with mucormycosis, especially in developing tropical countries like India [38, 39]. Furthermore, an unexpected increase

was noted in mucormycosis cases among dengue patients as the second wave of COVID-19 receded. This may be explained by the potential interaction between the dengue virus and SARS-CoV-2 *via* antibody-dependent enhancement [40], resulting in a more severe disease and subsequent immunosuppression.

*Case study:* An 18-year-old male presented with left cheek and eye swelling, nasal bleeding, and a history of fever, vomiting, diarrhea, and seizure amid the waning second wave of COVID-19. He tested positive for dengue (NS1) and COVID-19 (RT-PCR), with normal CD3+, CD4+, and CD8+ counts, ruling out overt immunosuppression. However, he developed invasive fungal sinusitis with orbital extension, which was confirmed on imaging, and a KOH mount showed aseptate and septate hyphae, raising suspicion for mucormycosis.

He received 10 cycles of Amphotericin B and underwent subtotal maxillectomy with left orbital decompression. Histopathology confirmed mucormycosis, highlighting the emerging risk of invasive fungal infections in patients with concurrent Dengue and COVID-19, even without diabetes or known immunosuppression (**Figure 5**).

- *Causative agent:* Mold from class *Zygomycetes*, most commonly *Rhizopus oryzae*.
- *Modes of entry:* Respiratory tract (most common). Direct inoculation through abraded skin (primary cutaneous mucormycosis).
- *Clinical features:* Rapid onset with tissue necrosis (due to fungal invasion of blood vessels, leading to thrombosis), resulting in the characteristic “Black Eschar.”

Aggressive progression with high fatality unless treated immediately.

*Association with COVID-19:* Higher incidence (9.6%) in ICU-admitted SARS-CoV-2 patients, increasing risk of fatality due to:

1. *Steroid and immunosuppressive therapy* → Immune dysregulation.
2. *Cytokine storm and IL-6 elevation* → Increased free iron → Endothelial damage → Fungal invasion.
3. *ICU admission and mechanical ventilation* → Higher risk of cross-contamination.
4. *Pancreatic beta-cell dysfunction* → Hyperglycemia and diabetic ketoacidosis.



**Figure 5.**  
*Rhinoorbital mucormycosis in a young male with dengue and COVID-19 infection.*

### 3.1 The major risk factors for mucormycosis

See **Table 27**.

| Risk factor                                    | Associated type of mucormycosis     |
|------------------------------------------------|-------------------------------------|
| Uncontrolled diabetes mellitus                 | Rhino-orbital-cerebral mucormycosis |
| Metabolic acidosis (e.g., DKA)                 | Rhino-orbital-cerebral mucormycosis |
| Corticosteroid therapy (e.g., post-transplant) | Pulmonary/disseminated mucormycosis |
| Hematological malignancies                     | Pulmonary/disseminated mucormycosis |
| Deferoxamine therapy (in dialysis patients)    | Pulmonary mucormycosis              |
| Trauma and burns                               | Cutaneous mucormycosis              |

**Table 27.**

*The major risk factors for mucormycosis and their associated types.*

### 3.2 Diagnostic approach for mucormycosis

See **Table 28**.

| Diagnostic method       | Findings and considerations                                                                                                                                         | Utility                                         |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| Histopathology          | Wide, ribbon-like aseptate hyphal elements branching at right angles (45°–90°), often surrounded by necrotic debris. Stains: H&E, GMS, PAS confirm fungal invasion. | Gold standard for diagnosis.                    |
| Tissue biopsy           | Demonstrates vascular invasion, tissue necrosis, and fungal elements.                                                                                               | Essential for definitive diagnosis.             |
| Blood cultures          | Rarely positive for <i>Mucorales</i> species.                                                                                                                       | Limited diagnostic value.                       |
| Immunochemical tests    | 1,3-β-glucan & Galactomannan are NOT useful for mucormycosis.                                                                                                       | Not recommended for diagnosis.                  |
| PCR (molecular testing) | Can detect DNA of certain <i>Mucorales</i> species, but is not routinely used in clinical practice.                                                                 | Emerging diagnostic tool, not widely available. |
| Imaging (CT/ MRI)       | Sinus CT: Mucoperiosteal thickening, air-fluid levels, bony erosion in rhinocerebral mucormycosis. MRI: orbital, brain involvement.                                 | Supports diagnosis but not definitive.          |
| Empiric therapy         | If orbital mucormycosis is suspected, start amphotericin B immediately while confirming diagnosis.                                                                  | Early treatment improves survival.              |

*H&E, hematoxylin–eosin; GMS, Grocott-methanamine-silver; PAS, periodic acid-Schiff.*

**Table 28.**

*Diagnostic approach for mucormycosis.*

### 3.3 Treatment approach for mucormycosis

See **Table 29**.

| Treatment component              | Recommendations and considerations                                                                                                                                  | Importance                  |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| Early empiric therapy            | If orbital mucormycosis is suspected, start Amphotericin B immediately.                                                                                             | Critical for survival       |
| Reversal of predisposing factors | Control diabetes, reduce immunosuppressants, and manage neutropenia if possible.                                                                                    | Reduces disease progression |
| Surgical debridement             | Aggressive removal of necrotic tissue is essential to prevent fungal spread.                                                                                        | Key to successful treatment |
| First-line antifungal therapy    | Amphotericin B (AmB)<br>Deoxycholate formulation: 1.0–1.5 mg/kg/day (high nephrotoxicity)<br>Lipid formulations (L-AmB): 5 mg/kg/day (preferred, safer for kidneys) | Standard of care            |
| Alternative or adjunct therapy   | Posaconazole, Ravuconazole: Investigational triazoles, active against <i>Rhizopus</i> spp.                                                                          | For refractory cases        |
| Hyperbaric oxygen therapy        | It may improve neutrophil function and enhance antifungal effects.                                                                                                  | Adjunctive therapy          |

**Table 29.**  
*Treatment approach for mucormycosis.*

## 4. Other major fungal infections

### 4.1 *Pneumocystis carinii*

Though traditionally considered a protozoan, has been demonstrated to share similarities with fungi. It is an important cause of pneumonia in HIV/AIDS and other immunosuppressed patients, affecting 505,000 people annually, with a 42.4% mortality rate [1].

Prophylaxis for *P. carinii* pneumonia – with Trimethoprim-sulfamethoxazole most convincingly decreases morbidity and mortality in patients with cancer and HIV.

#### 4.1.1 Laboratory diagnosis of *Pneumocystis carinii* pneumonia

Ref. [40, 41] (see **Table 30**).

| Method                                                   | Description                                                                                                                   |
|----------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| Microscopic staining                                     | Detection of <i>P. carinii</i> cysts or trophozoites in tissue or body fluids using colorimetric or immunofluorescent stains. |
| Bronchoalveolar lavage (BAL) fluid analysis              | Examination of BAL fluid for <i>P. carinii</i> using stains or immunofluorescence. High sensitivity.                          |
| Monoclonal antibody-based immunofluorescence             | Detection of <i>P. carinii</i> in respiratory specimens using monoclonal antibodies.                                          |
| Polymerase chain reaction (PCR) on BAL or induced sputum | Molecular detection using gene targets like mitochondrial rRNA or major surface glycoproteins.                                |
| PCR on oral washes or gargles                            | Detection of <i>P. carinii</i> DNA from oral fluid samples.                                                                   |

| Method           | Description                                                                                                                                               |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| Quantitative PCR | PCR-based method that quantifies <i>P. carinii</i> DNA to differentiate colonization from disease. May help distinguish true infection from colonization. |

**Table 30.**  
*Laboratory diagnosis of Pneumocystis carinii pneumonia (PCP) [40, 41].*

4.1.2 Drug regimens for treatment of *P. carinii pneumonia*

See **Table 31.**

| Agent                             | Dose                                                  | Interval            | Route         |
|-----------------------------------|-------------------------------------------------------|---------------------|---------------|
| Trimethoprim/<br>sulfamethoxazole | Trimethoprim: 5 mg/kg   sulfamethoxazole:<br>25 mg/kg | Every 6–<br>8 hours | IV or<br>Oral |
| Pentamidine                       | 4 mg/kg                                               | Every<br>24 hours   | IV            |

**Table 31.**  
*Drug regimens for treatment of *P. carinii pneumonia*.*

4.2 Cryptococcal disease

It affects 194,000 individuals per year, with a 75.8% fatality rate [1].

4.2.1 Etiology and pathogenesis of *Cryptococcosis*

Ref. [42] (see **Table 32**).

| Aspect                    | Details                                                                                                                           |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Causative agents          | <i>Cryptococcus neoformans</i> and <i>Cryptococcus gattii</i> .                                                                   |
| Source of<br>infection    | Inhalation of fungal spores from soil contaminated with pigeon excreta.                                                           |
| Risk factors              | Immunocompromised states: prolonged steroid use, immunosuppressive therapy (e.g., post-organ transplant), advanced HIV infection. |
| Disease<br>manifestations | Meningitis and pneumonia are the most common presentations.                                                                       |
| Pathogenesis              | Reactivation of latent infection in immunocompromised individuals.                                                                |

**Table 32.**  
*Etiology and pathogenesis of *Cryptococcosis* [42].*

4.2.2 Diagnostic methods for *Cryptococcus infection*

See **Table 33.**

| Method            | Key points                                                                                                 |
|-------------------|------------------------------------------------------------------------------------------------------------|
| Culture           | Growth on Sabouraud's agar, urease production, capsule on India ink stain.                                 |
| Histopathology    | Encapsulated yeasts on Alcian blue, mucicarmine, PAS, GMS stains.                                          |
| Cutaneous types   | Granulomatous – few organisms, scant necrosis. Gelatinous – numerous budding yeasts, minimal inflammation. |
| Antigen detection | Cryptococcal capsular antigen in CSF/serum.                                                                |

**Table 33.**  
*Diagnostic methods for Cryptococcus infections.*

#### 4.2.3 Treatment of Cryptococcal Meningitis, the most common clinical manifestation of *Cryptococcus infection*

See **Table 34.**

| Patient group     | Induction therapy                                        | Consolidation therapy                 | Maintenance therapy    |
|-------------------|----------------------------------------------------------|---------------------------------------|------------------------|
| Non-AIDS patients | Amphotericin B 0.3 mg/kg/day + Flucytosine 150 mg/kg/day | —                                     | —                      |
| AIDS patients     | Amphotericin B 0.7 mg/kg/day for 2 weeks                 | Fluconazole 400 mg/day for 8–10 weeks | Fluconazole 200 mg/day |

**Table 34.**  
*Treatment of Cryptococcal Meningitis.*

## 5. Conclusion

Given the increasing burden of invasive fungal diseases (IFDs) and their high morbidity and mortality, a multidisciplinary approach involving timely risk assessment, early initiation of appropriate antifungal therapy, and close patient monitoring is crucial to improving outcomes in ICU settings. Enhanced awareness and the development of better diagnostic and therapeutic strategies are essential to combat the growing threat of fungal infections in critically ill patients.

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# Sporotrichosis: Quality of YouTube Contents on a Fungal Neglected Tropical Disease

*Ivaan Pitua, Raafidha Raizudheen, Amelia Margaret Namiro, Lorraine Apili and Felix Bongomin*

## Abstract

Sporotrichosis is a chronic fungal infection caused by *Sporothrix* species, commonly associated with plant handling and zoonotic transmission *via* infected animals, particularly cats. With YouTube's growing role as an educational resource, concerns arise regarding the reliability of medical information presented on the platform, particularly for neglected conditions like sporotrichosis. We assessed the quality and reliability of YouTube videos on sporotrichosis evaluated by four independent reviewers using the Global Quality Scale (GQS) and a modified DISCERN tool. The Video Power Index (VPI) was calculated to measure video popularity, and statistical analyses were conducted to explore correlations between video metrics and quality scores. The analysis included 39 videos, with a median GQS of 4.0, indicating high quality, while the median mDISCERN score was 2.83, suggesting moderate reliability. Approximately 37.5% of videos were of low to medium quality, while 60.0% were of high quality; healthcare professionals uploaded 70.0% of the sample videos. There was no significant correlation between video popularity (VPI) and quality scores (GQS or mDISCERN), indicating that popular videos may not provide the most reliable information. While many YouTube videos on sporotrichosis are of high quality, there is a concerning disconnect between video popularity and reliability, highlighting the need for viewers to critically evaluate health information on this platform and for content creators to prioritize accuracy alongside engagement. Sixty percent of YouTube videos on sporotrichosis are of high quality. Seventy percent of high-quality videos were uploaded by healthcare professionals. No correlation was found between video popularity and quality scores. Popular sporotrichosis videos may not be the most reliable.

**Keywords:** sporotrichosis, neglected fungal disease, youtube, quality, reliability

## 1. Introduction

Sporotrichosis is a chronic fungal infection caused by the dimorphic fungus *Sporothrix schenckii* and its related species. Often referred to as “rose gardener’s disease,” it is commonly associated with handling plants and organic material, which serve as primary sources of infection [1, 2]. Sporotrichosis is traditionally associated with the

traumatic implantation of *Sporothrix* species from environmental sources such as soil or vegetation. However, in recent years, its zoonotic transmission, particularly through domestic cats, has emerged as a significant public health concern. In South America, zoonotic outbreaks caused by *Sporothrix brasiliensis* have resulted in severe and widespread human infections linked to direct contact with infected felines. It is thus important to recognize sporotrichosis not only as an environmental disease but also as a zoonotic threat, with implications for both human and veterinary health education [1, 3–6]. The disease primarily affects the skin, subcutaneous tissues, and lymphatic system; however, in severe cases, it can disseminate to other parts of the body, including bones, joints, and the central nervous system [6, 7]. Although sporotrichosis has a global distribution, it is particularly prevalent in tropical and subtropical regions where environmental conditions favor the growth and transmission of *Sporothrix* species [8, 9].

The expansive range of topics and widespread accessibility of YouTube make it a valuable resource for patients seeking information about specific ailments and medical conditions, including sporotrichosis. However, the accuracy and reliability of the information presented in these videos can be a significant concern. Unlike peer-reviewed scientific literature, YouTube videos are not subjected to rigorous editorial processes, and the information presented may be influenced by the creator's expertise, biases, or commercial interests [10, 11]. This variability raises concerns about the potential for misinformation and its impact on public health.

Assessing the quality and reliability of YouTube content is crucial, particularly for medical conditions like Sporotrichosis, where timely and accurate information is vital for disease management and prevention. Our study aims to address this need by analyzing the popularity, quality, and reliability of YouTube videos related to Sporotrichosis. Through this analysis, the study seeks to provide insights into the current state of online health information on the disease and to identify areas for improvement in the dissemination of accurate and reliable content on digital platforms such as YouTube.

## 2. Materials and methods

### 2.1 Search strategy

We retrieved YouTube videos using Sporotrichosis as a search term on August 20, 2024, using a YouTube Data API and an R script, which obtained 200 video URLs. The search results were automatically ranked by YouTube's default "relevance" setting and also included data on view count, like count, comment count, upload date, and video duration. **Figure 1** shows the screening criteria.

### 2.2 Video assessment

All information was saved in a spreadsheet, and four independent reviewers Ivaan Pitua (IP), Raafidha Raizudheen (RR), Amelia Margaret Namiro (AMN), and Lorraine Apili (LA) independently assessed the videos' quality of information and reliability (Appendix A). Reliability was assessed using a 5-point modified DISCERN tool, adapted by Uzun [12]. The quality of the videos was evaluated using the 5-point Global Quality Scale (GQS), developed by Bernard et al. [13] to assess the quality of information presented on websites. Scores of 1–2 points were considered low quality, 3 points indicated moderate quality, and 4–5 points were considered high quality. Disagreements among raters were noted and resolved by an independent opinion

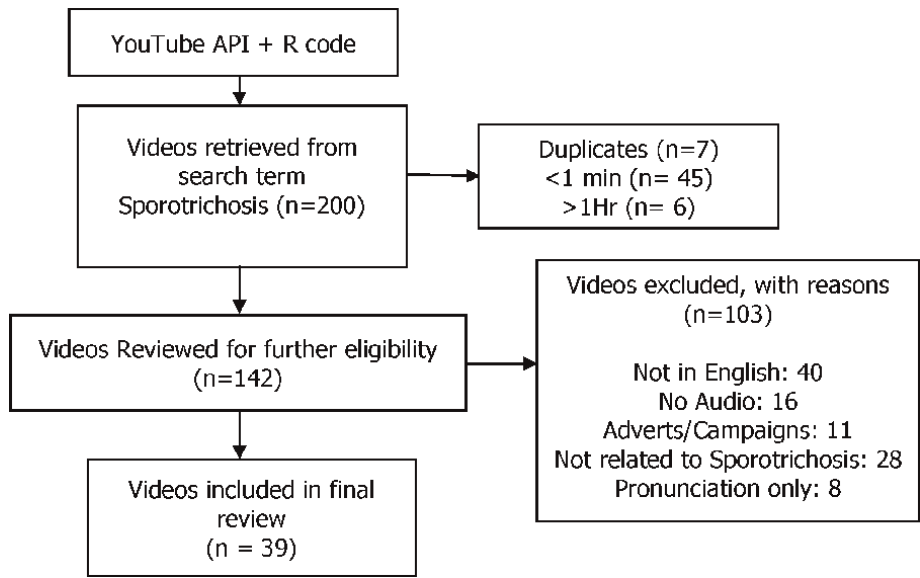


**Figure 1.**  
*Data collection and screening.*

from Felix Bongomin (FB). The Video Power Index (VPI) was calculated for each video using the formula:  $\text{like ratio} \times \text{view ratio} \div 100$ . It has been utilized in prior studies to measure video popularity based on views and likes.

### 3. Statistical analysis

Normal distribution was tested using the Shapiro-Wilk test, which indicated there was no normal distribution, as all  $p < 0.05$ . Variables were summarized as sums, medians, and interquartile ranges. The inter-rater agreement among the four reviewers for the GQS and mDISCERN scores was assessed using the Interclass Correlation Coefficient (ICC). ICC values  $<0.5$  were categorized as indicating poor reliability; values between 0.5 and 0.75 as moderate reliability; values between 0.75 and 0.9 as good reliability; and values  $>0.90$  as excellent reliability. Spearman's rho was also used to evaluate the association between video scores and video metrics. The Mann-Whitney U test was conducted to compare continuous variables between the "Low to Medium Quality" and "High Quality" groups. Statistical significance was set at  $P \leq 0.05$ . All statistical analyses were conducted using R version 4.3.2 [14].

### 4. Results

**Figure 1** shows the data screening of selected videos.

#### 4.1 Descriptive statistics

We included 39 videos in the analysis. The median number of views was 1709.50 (IQR = 6256.75). The median number of likes was 29.50 (IQR = 81.5), while the median number of comments was 3.50 (IQR = 6.00). The median number of days

since upload was 1055 (IQR = 1798.25). The median duration of the videos was 7 minutes (IQR = 9.5). The median GQS was 4.0 (IQR = 1.0), and the median mDISCERN score was 2.825 (IQR = 0.5). The VPI had a median value of 1.70 (IQR = 5.02).

About 37.5% (n = 15) of the videos were low to medium quality, while 60.0% (n = 24) were high quality. 70.0% (n = 28) of the videos were uploaded by health professionals, 10.0% (n = 4) by media and organizations, and 17.5% (n = 7) by others. In 70.0% (n = 28) of the videos, treatment options were mentioned. The discussion of treatment options across the videos shows that Itraconazole was mentioned in 24 videos, Potassium iodide in 17 videos, Amphotericin B in 14 videos, and both Terbinafine and surgery were discussed in four videos each.

**4.2 Correlations between video matrices and VPI, GQS, and mDISCERN**

The ICC of GQS and mDISCERN among the four raters was 0.821 (p < 0.001, 95% CI 0.707–0.897) and 0.786 (p < 0.001, 95% CI 0.650–0.878), respectively. There was a strong positive correlation between VPI and the number of views (r = 0.877, p < 0.001), the number of likes (r = 0.923, p < 0.001), and the number of comments (r = 0.808, p < 0.001). Additionally, the duration in minutes is positively correlated with both GQS (r = 0.522, p = 0.001) and mDISCERN (r = 0.548, p < 0.001) (**Table 1**).

**4.3 Correlations between VPI, GQS, and mDISCERN**

The correlations among the variables indicate that the GQS and mDISCERN scores have a strong positive correlation (r = 0.799, p < 0.001). However, the VPI shows no significant correlation with either GQS (r = 0.095, p = 0.566) or mDISCERN (r = 0.082, p = 0.620).

**4.4 Association between video quality and continuous variables**

There was a significant association between video quality and the GQS (p < 0.001), mDISCERN score (p < 0.001), and video duration (p = 0.001) (**Table 2**).

| Video matrices      |   | Video power Index | Global quality Score | mDISCERN |
|---------------------|---|-------------------|----------------------|----------|
| Views               | r | 0.877             | 0.138                | 0.095    |
|                     | p | <0.001            | 0.401                | 0.566    |
| Likes               | r | 0.923             | 0.063                | 0.051    |
|                     | p | 0.001             | 0.704                | 0.757    |
| Comments            | r | 0.808             | 0.046                | 0.061    |
|                     | p | <0.001            | 0.785                | 0.716    |
| Days since upload   | r | 0.097             | 0.070                | 0.044    |
|                     | p | 0.559             | 0.671                | 0.792    |
| Duration in minutes | r | −0.049            | 0.522                | 0.548    |
|                     | p | 0.768             | <0.001               | <0.001   |

**Table 1.**  
*Correlations between video matrices and popularity, quality and reliability tools used.*

| Video quality, median (IQR) |                              |                     |         |
|-----------------------------|------------------------------|---------------------|---------|
| Variables                   | Low to medium quality N = 15 | High quality N = 24 | p-value |
| Global Quality Score        | 3.00 (.00)                   | 4.00 (.00)          | < 0.001 |
| mDISCERN                    | 2.45 (0.5)                   | 3.00 (0.45)         | < 0.001 |
| Video Power Index           | 2.42 (4.17)                  | 1.14 (4.99)         | 0.919   |
| Views                       | 2029.50 (4910.00)            | 1419.00 (6708.25)   | 0.665   |
| Likes                       | 32.00 (60.75)                | 19.00 (120.00)      | 0.896   |
| Comments                    | 4.00 (5.75)                  | 3.50 (10.00)        | 0.843   |
| Duration of video (mins)    | 3.00 (4.75)                  | 10.00 (8.75)        | < 0.001 |
| Days since upload           | 778.00 (2847.00)             | 1119.00 (1752.75)   | 0.603   |

**Table 2.**  
*Variables associated with video quality.*

## 5. Discussion

This study aims to evaluate the quality and reliability of YouTube videos related to sporotrichosis, a subcutaneous fungal neglected tropical disease of global public health significance [15]. YouTube is one of the most visited websites worldwide and has become a popular platform for individuals seeking health-related information [16]. The accessibility of YouTube allows users to easily find videos on a wide range of medical topics [17], including rare conditions such as sporotrichosis. However, the open nature of YouTube, where anyone can upload content, leads to a mix of reliable information and potential misinformation. This lack of content regulation raises concerns, as YouTube does not require health-related videos to undergo expert review [11]. Consequently, the quality and accuracy of information can vary widely, which is particularly problematic given that about 85 million internet users take online health advice without assessing the quality of the content found on the internet [18]. The consequences of relying on such unvetted information can be severe, especially in cases where treatment decisions are influenced by misleading or incorrect content.

Our findings indicate that the majority of videos on sporotrichosis were not only of high quality, with a median GQS of 4.0, but also less reliable, with a modified DISCERN score of 2.825, suggesting that there is a considerable amount of quality information available on YouTube regarding sporotrichosis, though most of it is most likely less reliable. Despite these encouraging results, the study also revealed that video popularity, as measured by the VPI, was strongly correlated with the number of views, likes, and comments but not with GQS or mDISCERN scores. Popular videos are thus not necessarily those with the most accurate or reliable content. In fact, the pursuit of virality can lead to content that prioritizes engagement over accuracy. This disconnection between popularity and quality has been documented in other studies, where videos with sensationalist, compelling headings or oversimplified content attract more viewers than those that are more thorough or scientifically accurate [19]. The implication is that viewers may be misled by the popularity of a video, assuming it to be more trustworthy or informative than it actually is. There were no significant differences in GQS, mDISCERN, and video duration between high-quality and lower-quality videos. Specifically, higher-quality videos tended to be longer, with a median duration of 10 minutes compared to 3 minutes for lower-quality videos, suggesting that more detailed and comprehensive videos—

which naturally take longer to produce and watch—are more likely to provide accurate and reliable information, a pattern consistent with the idea that quality health information requires time to present and explain thoroughly. However, the challenge is that longer videos may not attract as many viewers, especially in an era where attention spans are often limited [20, 21]. Of late, shorter videos on YouTube, popularly known as “Shorts,” have garnered more engagement than regular videos [22], though the amount of content released in Shorts is quite limited and may not sufficiently convey all health-related information as a regular full-length video would, creating a tension between the need for thoroughness and the demand for brevity, which content creators must navigate carefully. The implications of our findings are significant for both content creators and viewers. For content creators, there is a clear need to balance accuracy with engagement to ensure that reliable information reaches a broad audience. For viewers, the study emphasizes the importance of critically evaluating the source and content of the information they consume, especially when it concerns health-related topics.

This study had some limitations. While GQS and mDISCERN are validated tools for assessing video quality, they are subjective measures and may not capture all aspects of what constitutes high-quality content. The study did not account for other factors that may influence video quality and reliability, such as visual and audio production quality or the qualifications of the presenter. Another key limitation of this study is the inability to conduct a regional analysis due to the absence of geolocation data. As a result, potential variations in public perceptions or discourse across different geographic areas could not be explored, which limits the generalizability of the findings to specific regional contexts. Future research may address these limitations by incorporating additional metrics and considering other factors that contribute to content quality.

## **6. Conclusions**

There is a disconnection between video popularity and reliability despite the relatively high quality of videos on sporotrichosis. Viewers have to critically evaluate health information on YouTube, and content creators need to prioritize accuracy alongside engagement.

### **Funding**

None.

### **Conflict of interest**

None declared.

### **Data availability**

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data on screening is available in Appendix A.

### **Appendix**

See **Table A1**.

| Video codes | Views  | Likes | Dislikes | Comments | LikesRatio | ViewsRatio | VPI     | Year | daysAfterUpload | Duration | GQS_R | GQS_A | GQS_P | GQS_L | GQS |
|-------------|--------|-------|----------|----------|------------|------------|---------|------|-----------------|----------|-------|-------|-------|-------|-----|
| 1           | 6836   | 48    | 0        | 7        | 100.000    | 1.508      | 1.508   | 2012 | 4533            | 13       | 4     | 3     | 3     | 4     | 4   |
| 2           | 3275   | 56    | 0        | 5        | 100.000    | 4.338      | 4.338   | 2022 | 755             | 51       | 5     | 4     | 4     | 3     | 4   |
| 3           | 61     | 0     | 0        | 0        |            | 0.051      | 0.00ac0 | 2020 | 1207            | 3        | 2     | 2     | 2     | 2     | 2   |
| 4           | 40     | 4     | 0        | 2        | 100.000    | 0.132      | 0.132   | 2023 | 303             | 1        | 3     | 2     | 2     | 3     | 3   |
| 5           | 235    | 17    | 0        | 0        | 100.000    | 3.052      | 3.052   | 2024 | 77              | 1        | 2     | 3     | 2     | 3     | 3   |
| 6           | 782    | 33    | 0        | 3        | 100.000    | 1.280      | 1.280   | 2021 | 611             | 2        | 3     | 3     | 3     | 3     | 3   |
| 7           | 4912   | 244   | 4        | 12       | 98.387     | 8.850      | 8.708   | 2023 | 555             | 3        | 3     | 3     | 3     | 3     | 3   |
| 8           | 2160   | 28    | 0        | 3        | 100.000    | 3.064      | 3.064   | 2021 | 705             | 6        | 3     | 3     | 4     | 3     | 3   |
| 9           | 5698   | 59    | 4        | 6        | 93.651     | 1.341      | 1.256   | 2012 | 4249            | 3        | 4     | 3     | 3     | 3     | 3   |
| 10          | 10,321 | 52    | 0        | 5        | 100.000    | 9.619      | 9.619   | 2020 | 1073            | 2        | 3     | 3     | 4     | 3     | 3   |
| 11          | 105    | 3     | 0        | 0        | 100.000    | 0.410      | 0.410   | 2023 | 256             | 2        | 4     | 3     | 4     | 3     | 4   |
| 12          | 7410   | 33    | 0        | 11       | 100.000    | 1.778      | 1.778   | 2013 | 4167            | 3        | 4     | 3     | 4     | 3     | 4   |
| 13          | 568    | 4     | 0        | 0        | 100.000    | 0.178      | 0.178   | 2015 | 3186            | 2        | 3     | 4     | 4     | 4     | 4   |
| 14          | 1513   | 7     | 0        | 0        | 100.000    | 0.393      | 0.393   | 2014 | 3852            | 14       | 4     | 4     | 4     | 3     | 4   |
| 15          | 754    | 8     | 0        | 2        | 100.000    | 0.287      | 0.287   | 2016 | 2624            | 10       | 4     | 4     | 4     | 4     | 4   |
| 16          | 4601   | 212   | 0        | 14       | 100.000    | 34.856     | 34.856  | 2024 | 132             | 8        | 4     | 4     | 4     | 4     | 4   |
| 17          | 1325   | 18    | 0        | 2        | 100.000    | 0.780      | 0.780   | 2018 | 1699            | 15       | 4     | 4     | 5     | 4     | 4   |
| 18          | 24,517 | 558   | 13       | 26       | 97.723     | 23.416     | 22.883  | 2020 | 1047            | 10       | 5     | 4     | 5     | 4     | 5   |
| 19          | 528    | 23    | 0        | 3        | 100.000    | 0.126      | 0.126   | 2013 | 4189            | 15       | 3     | 2     | 3     | 3     | 3   |
| 20          | 38     | 0     | 0        | 0        |            | 0.062      | 0.000   | 2021 | 610             | 7        | 4     | 2     | 3     | 3     | 3   |
| 21          | 410    | 6     | 0        | 0        | 100.000    | 0.452      | 0.452   | 2021 | 907             | 10       | 5     | 3     | 4     | 3     | 4   |
| 22          | 32     | 0     | 0        | 0        |            | 0.372      | 0.000   | 2024 | 86              | 6        | 4     | 4     | 4     | 4     | 4   |
| 23          | 578    | 20    | 0        | 2        | 100.000    | 2.524      | 2.524   | 2023 | 229             | 9        | 5     | 4     | 4     | 3     | 4   |
| 24          | 1899   | 31    | 2        | 2        | 93.939     | 1.731      | 1.626   | 2020 | 1097            | 6        | 4     | 3     | 3     | 3     | 3   |
| 25          | 555    | 9     | 0        | 4        | 100.000    | 0.758      | 0.758   | 2021 | 732             | 36       | 4     | 3     | 4     | 3     | 4   |
| 26          | 23,788 | 338   | 15       | 7        | 95.751     | 22.378     | 21.427  | 2020 | 1063            | 20       | 4     | 3     | 4     | 3     | 4   |

| Video codes | Views        | Likes  | Dislikes | Comments | LikesRatio | ViewsRatio         | VPI                | Year               | daysAfterUpload    | Duration   | GQS_R      | GQS_A      | GQS_P      | GQS_L          | GQS            |
|-------------|--------------|--------|----------|----------|------------|--------------------|--------------------|--------------------|--------------------|------------|------------|------------|------------|----------------|----------------|
| 27          | 35           | 0      | 0        | 0        |            | 0.135              | 0.000              | 2023               | 259                | 10         | 5          | 3          | 4          | 4              | 4              |
| 28          | 6752         | 86     | 2        | 27       | 97.727     | 3.231              | 3.157              | 2017               | 2090               | 11         | 4          | 4          | 4          | 4              | 4              |
| 29          | 17           | 0      | 0        | 0        |            | 0.047              | 0.000              | 2023               | 362                | 1          | 2          | 2          | 3          | 3              | 3              |
| 30          | 25,568       | 157    | 4        | 9        | 97.516     | 5.717              | 5.575              | 2012               | 4472               | 4          | 3          | 4          | 3          | 3              | 3              |
| 31          | 7536         | 142    | 3        | 7        | 97.931     | 9.625              | 9.425              | 2022               | 783                | 5          | 4          | 4          | 4          | 4              | 4              |
| 32          | 55           | 0      | 0        | 0        |            | 0.173              | 0.000              | 2023               | 318                | 16         | 5          | 4          | 4          | 4              | 4              |
| 33          | 3455         | 92     | 0        | 8        | 100.000    | 4.986              | 4.986              | 2022               | 693                | 1          | 3          | 3          | 2          | 2              | 3              |
| 34          | 1520         | 23     | 0        | 8        | 100.000    | 1.786              | 1.786              | 2021               | 851                | 11         | 4          | 3          | 3          | 3              | 3              |
| 35          | 13,358       | 219    | 10       |          | 95.633     | 4.737              | 4.530              | 2016               | 2820               | 5          | 3          | 3          | 4          | 4              | 4              |
| 36          | 174          | 9      | 0        | 0        | 100.000    | 0.251              | 0.251              | 2021               | 692                | 16         | 4          | 3          | 5          | 3              | 4              |
| 37          | 570          | 12     | 0        | 3        | 100.000    | 0.412              | 0.412              | 2019               | 1383               | 6          | 4          | 4          | 4          | 4              | 4              |
| 38          | 8076         | 153    | 9        | 14       | 94.444     | 6.081              | 5.743              | 2019               | 1328               | 6          | 4          | 4          | 5          | 3              | 4              |
| 39          | 267          | 8      | 0        | 0        | 100.000    | 0.227              | 0.227              | 2020               | 1175               | 10         | 4          | 4          | 5          | 4              | 4              |
| Video codes | GQS_Category | AIMS_R | AIMS_A   | AIMS_P   | AIMS_L     | RELIABLE_SOURCES_R | RELIABLE_SOURCES_A | RELIABLE_SOURCES_P | RELIABLE_SOURCES_L | BALANCED_R | BALANCED_A | BALANCED_P | BALANCED_L | PT_REFERENCE_R | PT_REFERENCE_A |
| 1           | 2            | 4      | 3        | 3        | 4          | 4                  | 4                  | 4                  | 4                  | 4          | 3          | 3          | 2          | 2              | 1              |
| 2           | 2            | 5      | 4        | 5        | 3          | 4                  | 4                  | 4                  | 4                  | 4          | 4          | 5          | 2          | 2              | 1              |
| 3           | 1            | 2      | 1        | 2        | 3          | 2                  | 3                  | 2                  | 3                  | 2          | 2          | 2          | 4          | 1              | 1              |
| 4           | 1            | 2      | 1        | 2        | 3          | 3                  | 3                  | 3                  | 3                  | 3          | 2          | 2          | 2          | 1              | 1              |
| 5           | 1            | 3      | 1        | 2        | 3          | 4                  | 2                  | 2                  | 4                  | 3          | 3          | 1          | 3          | 3              | 1              |
| 6           | 1            | 4      | 2        | 2        | 1          | 3                  | 3                  | 3                  | 4                  | 3          | 3          | 2          | 2          | 1              | 1              |
| 7           | 1            | 3      | 3        | 2        | 3          | 4                  | 3                  | 4                  | 4                  | 3          | 2          | 2          | 3          | 3              | 1              |
| 8           | 1            | 3      | 3        | 4        | 4          | 4                  | 4                  | 4                  | 4                  | 3          | 4          | 4          | 3          | 2              | 1              |
| 9           | 1            | 4      | 2        | 2        | 2          | 4                  | 3                  | 4                  | 4                  | 3          | 3          | 3          | 3          | 2              | 1              |
| 10          | 1            | 3      | 2        | 4        | 3          | 3                  | 2                  | 4                  | 3                  | 3          | 2          | 4          | 3          | 1              | 1              |
| 11          | 2            | 4      | 3        | 4        | 3          | 4                  | 4                  | 4                  | 4                  | 3          | 3          | 4          | 2          | 3              | 1              |

| Video<br>codes | GQS_<br>Category | AIMS<br>_R | AIMS<br>_A | AIMS<br>_P | AIMS<br>_L | RELIABLE<br>SOURCES_R | RELIABLE<br>SOURCES_A | RELIABLE<br>SOURCES_P | RELIABLE<br>SOURCES_L | BALANCED<br>_R | BALANCED<br>_A | BALANCED<br>_P | BALANCED<br>_L | PT_<br>REFERENCE_R | PT_<br>REFERENCE_A |
|----------------|------------------|------------|------------|------------|------------|-----------------------|-----------------------|-----------------------|-----------------------|----------------|----------------|----------------|----------------|--------------------|--------------------|
| 12             | 2                | 4          | 3          | 4          | 2          | 4                     | 3                     | 4                     | 4                     | 4              | 3              | 4              | 2              | 4                  | 1                  |
| 13             | 2                | 4          | 4          | 4          | 4          | 3                     | 3                     | 4                     | 4                     | 3              | 3              | 3              | 3              | 2                  | 1                  |
| 14             | 2                | 4          | 3          | 4          | 4          | 4                     | 3                     | 3                     | 4                     | 4              | 3              | 3              | 3              | 2                  | 1                  |
| 15             | 2                | 3          | 3          | 4          | 4          | 3                     | 4                     | 4                     | 4                     | 3              | 3              | 4              | 4              | 2                  | 1                  |
| 16             | 2                | 4          | 4          | 4          | 4          | 4                     | 4                     | 4                     | 4                     | 4              | 3              | 4              | 4              | 3                  | 5                  |
| 17             | 2                | 5          | 4          | 5          | 4          | 4                     | 5                     | 4                     | 4                     | 4              | 4              | 5              | 4              | 5                  | 1                  |
| 18             | 2                | 5          | 4          | 4          | 4          | 5                     | 5                     | 5                     | 4                     | 5              | 4              | 4              | 4              | 4                  | 1                  |
| 19             | 1                | 3          | 2          | 3          | 3          | 3                     | 2                     | 3                     | 4                     | 3              | 3              | 4              | 3              | 4                  | 1                  |
| 20             | 1                | 4          | 2          | 4          | 2          | 4                     | 2                     | 3                     | 4                     | 3              | 3              | 3              | 3              | 1                  | 2                  |
| 21             | 2                | 5          | 4          | 4          | 4          | 5                     | 3                     | 4                     | 4                     | 4              | 3              | 3              | 3              | 5                  | 1                  |
| 22             | 2                | 4          | 4          | 4          | 4          | 5                     | 4                     | 4                     | 4                     | 3              | 3              | 2              | 3              | 1                  | 1                  |
| 23             | 2                | 5          | 4          | 4          | 4          | 4                     | 4                     | 4                     | 4                     | 4              | 4              | 4              | 2              | 3                  | 1                  |
| 24             | 1                | 4          | 4          | 3          | 4          | 4                     | 3                     | 3                     | 4                     | 4              | 3              | 3              | 3              | 2                  | 1                  |
| 25             | 2                | 4          | 3          | 4          | 3          | 4                     | 3                     | 4                     | 3                     | 3              | 3              | 3              | 2              | 2                  | 1                  |
| 26             | 2                | 4          | 2          | 3          | 3          | 4                     | 3                     | 3                     | 4                     | 4              | 3              | 3              | 3              | 2                  | 1                  |
| 27             | 2                | 5          | 3          | 4          | 4          | 5                     | 3                     | 4                     | 4                     | 4              | 3              | 5              | 3              | 5                  | 1                  |
| 28             | 2                | 4          | 4          | 4          | 4          | 4                     | 5                     | 4                     | 4                     | 4              | 4              | 3              | 4              | 3                  | 1                  |
| 29             | 1                | 3          | 1          | 3          | 2          | 4                     | 2                     | 4                     | 3                     | 3              | 3              | 2              | 2              | 2                  | 2                  |
| 30             | 1                | 3          | 3          | 4          | 3          | 3                     | 3                     | 3                     | 3                     | 4              | 3              | 3              | 4              | 1                  | 1                  |
| 31             | 2                | 4          | 4          | 4          | 4          | 4                     | 3                     | 4                     | 4                     | 4              | 3              | 3              | 4              | 3                  | 1                  |
| 32             | 2                | 5          | 1          | 4          | 4          | 5                     | 4                     | 4                     | 4                     | 4              | 4              | 4              | 3              | 5                  | 1                  |
| 33             | 1                | 4          | 4          | 1          | 1          | 4                     | 2                     | 3                     | 3                     | 4              | 2              | 2              | 3              | 4                  | 1                  |
| 34             | 1                | 4          | 2          | 3          | 3          | 4                     | 3                     | 4                     | 4                     | 4              | 2              | 3              | 3              | 2                  | 1                  |
| 35             | 2                | 3          | 3          | 4          | 3          | 4                     | 3                     | 5                     | 4                     | 3              | 3              | 4              | 3              | 1                  | 1                  |
| 36             | 2                | 5          | 2          | 4          | 4          | 5                     | 3                     | 4                     | 4                     | 4              | 3              | 4              | 4              | 4                  | 1                  |
| 37             | 2                | 4          | 1          | 4          | 4          | 4                     | 4                     | 3                     | 4                     | 4              | 4              | 3              | 3              | 3                  | 1                  |
| 38             | 2                | 4          | 3          | 4          | 3          | 4                     | 3                     | 4                     | 4                     | 4              | 3              | 4              | 3              | 2                  | 1                  |
| 39             | 2                | 4          | 4          | 5          | 4          | 4                     | 4                     | 4                     | 4                     | 4              | 4              | 4              | 3              | 4                  | 1                  |

| Video codes | PT_ REFERENCE _P | PT_ REFERENCE _L | UNCERTAINTY _R | UNCERTAINTY _A | UNCERTAINTY _P | UNCERTAINTY _L | mDiscern _R | mDiscern _A | mDiscern _P | mDISCERN _L | mDiscern _5 | Treatment Mentioned |   |
|-------------|------------------|------------------|----------------|----------------|----------------|----------------|-------------|-------------|-------------|-------------|-------------|---------------------|---|
| 1           | 1                | 1                | 3              | 1              | 2              | 1              | 17          | 12          | 13          | 13          | 13.75       | 2.75                | 1 |
| 2           | 4                | 1                | 4              | 1              | 4              | 1              | 19          | 14          | 22          | 11          | 16.5        | 3.3                 | 1 |
| 3           | 1                | 2                | 1              | 1              | 1              | 1              | 8           | 8           | 8           | 13          | 9.25        | 1.85                | 0 |
| 4           | 1                | 1                | 1              | 1              | 2              | 1              | 10          | 8           | 10          | 10          | 9.5         | 1.9                 | 1 |
| 5           | 1                | 1                | 3              | 1              | 1              | 1              | 16          | 8           | 7           | 12          | 10.75       | 2.15                | 0 |
| 6           | 1                | 1                | 2              | 1              | 3              | 1              | 13          | 10          | 11          | 9           | 10.75       | 2.15                | 0 |
| 7           | 2                | 1                | 2              | 1              | 2              | 1              | 15          | 10          | 12          | 12          | 12.25       | 2.45                | 1 |
| 8           | 1                | 1                | 3              | 1              | 3              | 1              | 15          | 13          | 16          | 13          | 14.25       | 2.85                | 1 |
| 9           | 1                | 1                | 3              | 1              | 2              | 1              | 16          | 10          | 12          | 11          | 12.25       | 2.45                | 1 |
| 10          | 1                | 1                | 2              | 1              | 4              | 1              | 12          | 8           | 17          | 11          | 12          | 2.4                 | 1 |
| 11          | 1                | 1                | 3              | 1              | 3              | 1              | 17          | 12          | 16          | 11          | 14          | 2.8                 | 1 |
| 12          | 5                | 1                | 2              | 1              | 4              | 1              | 18          | 11          | 21          | 10          | 15          | 3                   | 1 |
| 13          | 2                | 3                | 3              | 2              | 2              | 1              | 15          | 13          | 15          | 15          | 14.5        | 2.9                 | 1 |
| 14          | 1                | 1                | 3              | 1              | 2              | 1              | 17          | 11          | 13          | 13          | 13.5        | 2.7                 | 1 |
| 15          | 1                | 1                | 3              | 2              | 5              | 1              | 14          | 13          | 18          | 14          | 14.75       | 2.95                | 1 |
| 16          | 3                | 4                | 3              | 2              | 4              | 1              | 18          | 18          | 19          | 17          | 18          | 3.6                 | 1 |
| 17          | 4                | 4                | 4              | 2              | 5              | 1              | 22          | 16          | 23          | 17          | 19.5        | 3.9                 | 1 |
| 18          | 4                | 4                | 4              | 1              | 4              | 1              | 23          | 15          | 21          | 17          | 19          | 3.8                 | 1 |
| 19          | 1                | 1                | 3              | 1              | 5              | 1              | 16          | 9           | 16          | 12          | 13.25       | 2.65                | 1 |
| 20          | 1                | 1                | 4              | 1              | 3              | 1              | 16          | 10          | 14          | 11          | 12.75       | 2.55                | 1 |
| 21          | 1                | 1                | 4              | 1              | 3              | 1              | 23          | 12          | 15          | 13          | 15.75       | 3.15                | 1 |
| 22          | 1                | 1                | 3              | 1              | 3              | 1              | 16          | 13          | 14          | 13          | 14          | 2.8                 | 0 |
| 23          | 1                | 1                | 3              | 1              | 4              | 1              | 19          | 14          | 17          | 12          | 15.5        | 3.1                 | 1 |
| 24          | 1                | 1                | 3              | 1              | 2              | 1              | 17          | 12          | 12          | 13          | 13.5        | 2.7                 | 1 |
| 25          | 1                | 1                | 3              | 1              | 4              | 1              | 16          | 11          | 16          | 10          | 13.25       | 2.65                | 0 |

| Video codes | PT_ REFERENCE _P | PT_ REFERENCE _L | UNCERTAINTY _R | UNCERTAINTY _A | UNCERTAINTY _P | UNCERTAINTY _L | mDiscern _R | mDiscern _A | mDiscern _P | mDISCERN _L | mDiscern _5 | Treatment Mentioned |   |
|-------------|------------------|------------------|----------------|----------------|----------------|----------------|-------------|-------------|-------------|-------------|-------------|---------------------|---|
| 26          | 1                | 1                | 3              | 1              | 2              | 1              | 17          | 10          | 12          | 12          | 12.75       | 2.55                | 0 |
| 27          | 5                | 2                | 4              | 1              | 5              | 1              | 23          | 11          | 23          | 14          | 17.75       | 3.55                | 1 |
| 28          | 1                | 1                | 3              | 3              | 3              | 1              | 18          | 17          | 15          | 14          | 16          | 3.2                 | 0 |
| 29          | 3                | 1                | 3              | 1              | 2              | 2              | 15          | 9           | 14          | 10          | 12          | 2.4                 | 0 |
| 30          | 1                | 2                | 3              | 1              | 4              | 1              | 14          | 11          | 15          | 13          | 13.25       | 2.65                | 0 |
| 31          | 2                | 1                | 3              | 1              | 3              | 1              | 18          | 12          | 16          | 14          | 15          | 3                   | 1 |
| 32          | 2                | 2                | 5              | 2              | 5              | 1              | 24          | 12          | 19          | 14          | 17.25       | 3.45                | 1 |
| 33          | 1                | 1                | 3              | 1              | 1              | 1              | 19          | 10          | 8           | 9           | 11.5        | 2.3                 | 0 |
| 34          | 2                | 1                | 3              | 1              | 4              | 1              | 17          | 9           | 16          | 12          | 13.5        | 2.7                 | 1 |
| 35          | 1                | 4                | 2              | 1              | 4              | 1              | 13          | 11          | 18          | 15          | 14.25       | 2.85                | 1 |
| 36          | 1                | 1                | 2              | 1              | 3              | 1              | 20          | 10          | 16          | 14          | 15          | 3                   | 1 |
| 37          | 1                | 3                | 3              | 1              | 3              | 1              | 18          | 11          | 14          | 15          | 14.5        | 2.9                 | 1 |
| 38          | 3                | 1                | 3              | 1              | 4              | 1              | 17          | 11          | 19          | 12          | 14.75       | 2.95                | 1 |
| 39          | 4                | 4                | 4              | 1              | 5              | 1              | 20          | 14          | 22          | 16          | 18          | 3.6                 | 0 |

**Table A1.**  
*Data extraction form summarizing the assesment of included YouTube videos on Sporotrichosis.*

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# Light-Based Therapies for Treatment of Onychomycosis

*Clara Gómez and Enrique Alberdi*

## Abstract

Onychomycosis is a chronic fungal infection of the nail, and its treatment is considered a challenge. The use of systemic antifungals is frequently associated with multiple adverse effects; moreover, topical antifungals are rarely effective when used as a monotherapy. Non-pharmacological alternative treatment modalities are needed. Progress in this direction includes experimentation with light-based therapies: administration of laser radiation through the use of long-pulsed and Q-switched Nd:YAG, CO<sub>2</sub>, infradiode, fractional ablative lasers, and other light sources for photodynamic therapy or intense pulsed light (IPL). These therapies exert their effect through various mechanisms: selective photothermolysis, a non-selective thermal effect that decomposes the infected tissue and has a sterilizing effect, or the induction of photochemical reactions, damaging fungal structures. Well-designed clinical trials are still needed to assess efficacy and establish the best treatment guidelines. It is expected that technological innovation will enable the design of more advanced light devices, enhancing their efficacy and achieving a positive societal impact.

**Keywords:** onychomycosis, laser radiation, Nd:YAG laser, CO<sub>2</sub> laser, diode laser, LED, fractional ablative laser, light, photodynamic therapy, IPL

## 1. Introduction

Onychomycosis (fungal nail infection), and particularly distal and lateral sub-ungual onychomycosis (DLSO), is the most prevalent nail infection worldwide, making up about 90% of toenail infections globally [1]. This condition leads to nail discoloration, detachment from the nail bed (onycholysis), fragility, thickening, and accumulation of scales beneath the nail [1]. While dermatophytes are the primary cause of onychomycosis, non-dermatophyte molds and yeasts are also causative organisms, being dermatophytes responsible for over 60–70% of the cases. Among the different species of dermatophytes, *Trichophyton rubrum* is recognized as the most common pathogen [2]. *Scopulariopsis brevicaulis*, *Acremonium spp.*, *Aspergillus spp.*, *Fusarium spp.*, and *Neoscytalidium spp.* are non-dermatophyte molds also responsible for some cases of the disease [2]. *Candida albicans* accounts for approximately 70% of yeast-related onychomycosis, although fungal nail infections caused by yeasts are rare [2]. Onychomycosis is acquired through direct contact of the nail with these species, as the nail lacks effective cell-mediated immunity [3]. Fungal invasion of the nail

is aided by enzymes produced by fungi, which have proteolytic, keratinolytic, and lipolytic activities that promote the breakdown of keratin in the nail plate [3].

This disorder can negatively impact the quality of life of those affected. Onychomycosis is more likely to occur in toenails than fingernails, with the infection typically developing on the big toe first. It is rare for more than one nail to be affected without a concomitant infection in the toenail unless the patient has suffered trauma or is immunosuppressed [4]. Although its incidence is increasing across all age groups, it is still considered a disorder that primarily affects the older population [5]. Other factors that increase the risk of onychomycosis include persistent nail trauma, psoriasis, diabetes, poor peripheral circulation, HIV, immunosuppression, and smoking. Plantar tinea pedis and interdigital tinea are also risk factors for nail infections and often occur alongside onychomycosis [6]. The condition can be aggravated by moist environments, occlusive footwear, and genetic factors. These factors can also contribute to the recurrence of onychomycosis after treatment, making it a chronic and difficult-to-treat disease. Other risk factors for recurrence include >50% nail involvement at baseline, nail trauma, the presence of infectious organisms that are more difficult to eradicate (nondermatophytes, mixed infections), previous onychomycosis therapy, short-duration therapies, poor treatment success, and poor adherence to treatment [7].

In addition, the drawbacks of conventional therapeutic agents highlight the difficulty of treating this disease. Topical antifungal agents have a low ability to penetrate the nail and reach the infected nail bed, [8] whereas oral antifungals have the potential to cause systemic adverse effects, drug interactions, and the development of antifungal resistance, leading to patient non-adherence [9]. Since the growth of nails is slow, both oral and topical therapies involve longer durations of treatment, leading to poor patient compliance.

Laboratory confirmation is always necessary before treatment even though the diagnosis can be highly probable based on clinical criteria. Nail dermoscopy (onychoscropy) is a valuable, fast, noninvasive, and highly efficient tool that allows for the differential diagnosis of other non-mycotic nail disorders [10]. The current standards for diagnosing onychomycosis include direct microscopic examination with potassium hydroxide (KOH) preparation, which is best for prompt initial diagnosis; histopathologic analysis of the trimmed affected nail plate with a periodic-acid-Schiff (PAS) stain to identify the presence of fungi; fungal culture of collected subungual debris; or molecular detection by means of polymerase chain reaction (PCR) when the pathogen subtype needs to be specified. The ideal diagnostic test would be highly specific and sensitive, capable of assessing fungal viability and identifying the causative species while being easy to perform with a rapid result and low cost [11]. It is recommended to conduct at least two diagnostic tests to identify the infectious species to provide adequate information for determining the appropriate treatment plan.

Endpoints used in clinical trials to evaluate the action of new drugs/devices or strategies in the treatment of onychomycosis include mycologic cure, clinical cure, and complete cure [12]. Long-term treatment is generally required to successfully cure toenail onychomycosis, which can even extend beyond a year. Even then, achieving a complete cure, defined as both a clinical cure (nail clearing) and a mycological cure (negative microscopy and fungal culture), is often unattainable because of the slow growth rate of nails.

Digital images and direct observation can help the physician assess the severity of the disease or its improvement as treatment is applied. The severity of the disease should also be assessed before deciding on a treatment plan. A reproducible and

objective numerical grading system called the Onychomycosis Severity Index (OSI), has been developed to detail the extent and involvement of distal and lateral subungual onychomycosis (DLSO). Points are given based on the percentage of nail area involved, the proximity of disease to the matrix, the presence or absence of dermatophytoma, and subungual hyperkeratosis >2 mm. The OSI can be used to predict responses to antifungal therapy. When cure rates are evaluated, nails with lower OSI scores ( $\leq 5$ ) generally show better responses to conventional therapies, whereas nails with higher scores (such as 35) are accurately indicated as more challenging cases [13]. Another criterion for expected treatment success is the continued growth of the nail, with a growth rate of more than 0.5 mm per week [14].

Onychomycosis is a difficult-to-treat disorder with a high risk for recurrence that requires new approaches beyond pharmacological therapy. Due to their minimally invasive nature, avoidance of systemic side effects, drug interactions, the development of resistance, and the potential to restore clear nail growth, light-based therapies have become a popular option in the treatment of onychomycosis for both physicians and patients [15]. Light-based therapies are safe and can be considered as the first choice for people who cannot tolerate the invasiveness of the systemic antifungal agents. They may also be part of an adjunctive therapy, alongside topical or systemic antifungals, to presumably increase the likelihood of successful fungal elimination. In addition, for its implementation by the physician, an understanding of the light-nail structure interaction is essential. **Table 1** below displays the light devices that are most frequently used in the treatment of onychomycosis.

The main concepts to understand light and nail interactions are wavelength, energy, power, fluence (energy density), and irradiance (power density). Tissue effects occur only when light is absorbed. Based on the wavelength of light, a particular chromophore (such as porphyrin, xanthomagnin, hemoglobin, melanin, or water) can be targeted and absorb that light. Mainly, the behavior of light in its interaction with tissue (nail) can be classified by its wavelength because molecular absorption depends on this parameter (**Table 1**) [16]. The light emitted by a light source can be measured in terms of both energy and power [17]. The intensity of the light beam on the nail is a function of the area of the nail over which it is spread (i.e., the spot size). The physician can adjust the exposure time, fluence, and irradiance of the light source according to the specific clinical requirements [16].

Early studies on the use of lasers (the acronym for Light Amplification by Stimulated Emission of Radiation) for treating onychomycosis began almost 30 years ago with the carbon dioxide laser (CO<sub>2</sub> laser); however, clinical use did not gain popularity until recent years. Additionally, the US Food and Drug Administration (FDA) has currently approved four lasers (long-pulsed Nd:YAG, Q-switched Nd:YAG, diode laser 870/930 nm combination, and 980 nm) for a “temporary increase in clear nail in patients with onychomycosis.” However, lasers have not been established for a cure, and the number of peer-reviewed literature discussing the subject is still too few [18]. Although bibliography evaluating the efficacy of light for onychomycosis are scarce, published studies include the application of a wide variety of therapies and types of devices, such as photodynamic therapy (PDT), intense pulsed light (IPL), CO<sub>2</sub> lasers, fractional ablative lasers, and the Nd:YAG and diode lasers (which have FDA approval). It is important to understand that FDA approval of medical devices used to treat onychomycosis differs from that for medications. While drugs are required to meet the medical endpoints of mycologic and complete cure, devices are required to show an esthetic endpoint of visual improvement [19].

| Source                           | Wavele ngth (nm) | Chromophore       | Mode         | Mechanism                                                                 |
|----------------------------------|------------------|-------------------|--------------|---------------------------------------------------------------------------|
| LED 450                          | 450              | FMN, CU           | cw           | light source for PDT                                                      |
| LED 532                          | 532              | RB                | cw           | light source for PDT                                                      |
| KTP laser                        | 532              | xanthomegnin      | Q-switched   | selective photothermolysis                                                |
| IPL                              | 500–600          | xanthomegnin      | pulsed       | thermal                                                                   |
|                                  | 560–700          | MB                | pulsed       | light source for PDT                                                      |
| LED 635                          | 635              | Protoporphyrin IX | cw           | light source for PDT                                                      |
|                                  |                  | Photofrin         |              |                                                                           |
| Diode laser                      | 670              | MB                | cw           | light source for PDT                                                      |
| Diode laser                      | 870/980          | melanin           | long-pulsed  | thermal disinfection                                                      |
| Nd:YAG laser                     | 1,064            | melanin           | cw           | thermal disinfection                                                      |
|                                  |                  |                   | long-pulsed  | thermal disinfection                                                      |
|                                  |                  |                   | Q-switched   | selective photothermolysis                                                |
| Fractional Er:YAG laser          | 2,940            | water             | pulsed       | nail resurfacing (microablation and disinfection); enhanced drug delivery |
| CO <sub>2</sub> laser            | 10,600           | water             | cw<br>pulsed | thermal resurfacing                                                       |
| Fractional CO <sub>2</sub> laser | 10,600           | water             | pulsed       | nail resurfacing (microablation and disinfection); enhanced drug delivery |

**Table 1.**

*Dermatologic light sources for onychomycosis therapy. Light-emitting diode (LED), flavin mononucleotide (FMN), curcumin (CU), continuous wave (cw), photodynamic therapy (PDT), potassium titanyl phosphate (KTP), rose bengal (RB), intense pulsed light (IPL), methylene blue (MB), neodymium-doped yttrium aluminum garnet (Nd:YAG), erbium-doped yttrium aluminum garnet (Er:YAG), carbon dioxide (CO<sub>2</sub>).*

The different types of light can be able to treat or improve the appearance of onychomycosis through different suggested mechanisms. For example, some exert their effect through a mechanism known as “selective photothermolysis” [20]. The energy is absorbed by a particular fungal chromophore, leaving the surrounding tissue undamaged. This is achieved using light with a wavelength within the absorption spectrum of the target chromophore and with pulse durations shorter than the thermal relaxation time of the target chromophore. This mechanism is, for example, achieved with the Q-switched Nd:YAG laser. Other light devices have a photothermal effect on fungi, meaning that the energy generated when light is absorbed by the fungus is converted to heat, reaching temperatures exceeding 50°C, which can directly lead to thermal killing. Fungal cells have a lower heat capacity than the surrounding human dermal cells, causing them to heat up more rapidly and retain heat better. This property helps inactivate the fungi while safeguarding the dermal cells. The near-infrared diode laser and long-pulsed Nd:YAG laser present this mechanism of action [21]. Alternatively, ablative fractional lasers represent a novel strategy that combines microablation with nail disinfection, promoting its reconstruction and healing. Lastly, in antimicrobial photodynamic therapy, the combination of a photosensitizer, light, and oxygen leads to the generation of reactive oxygen species (ROS), which trigger the death of the fungus responsible for the infection.

Published studies so far show lower cure rates of light-based therapies compared to conventional oral ones, so these treatments are still under development. Protocols (number of sessions, intervals between sessions, and adequate follow-up time) must be established, and the economic cost for the patient and the physician must be estimated as well. The therapeutic landscape is rapidly evolving with new light devices with novel mechanisms of action being investigated. Well-designed clinical trials are necessary to assess efficacy and establish the best treatment guidelines.

Finally, before administering a light-based treatment for onychomycosis, physicians should evaluate the effectiveness, side effects profile, and cost considerations of each option to ensure better patient compliance.

## **2. Carbon dioxide laser (CO<sub>2</sub> laser)**

The carbon dioxide laser (CO<sub>2</sub> laser) was among the first gas laser systems to be developed. This system is the oldest of all laser therapies for onychomycosis. During the 1990s and the early 2000s, the CO<sub>2</sub> laser was the gold standard for skin resurfacing (skin vaporization to stimulate collagen production). This laser system produces a beam of infrared light at 10,600 nm that is selectively absorbed by water in skin cells. Initially, CO<sub>2</sub> lasers operated primarily in continuous-wave mode. With technological advances, the pulsed CO<sub>2</sub> laser was developed, introducing new possibilities in the therapeutic field. Pulsed ablative CO<sub>2</sub> lasers with shorter pulse durations than the thermal relaxation times provided greater precision in tissue vaporization, minimized thermal damage, and facilitated hemostasis [22]. Due to its ablative effects, this laser system was explored as a potential therapy for onychomycosis. CO<sub>2</sub> laser can increase localized temperature, gasify and decompose the infected nail, and have a sterilizing effect. However, the effectiveness of CO<sub>2</sub> devices was limited by their side effect profile. This laser system generates high temperatures and unpredictable thermal side effects, which can be difficult to control, particularly regarding the depth of laser penetration into the nail. This can lead to large wounds, the formation of a brown scab, and an increased risk of bleeding. It is counterproductive to excessively pursue curative outcomes when the treatment itself could cause significant clinical damage to the nail bed and to the plate [23]. Today, the availability of less invasive light treatment options has made the CO<sub>2</sub> laser a less preferred choice for treating onychomycosis.

## **3. Diode lasers and light-emitting diode (LED)**

In addition to the diode laser, a light-emitting diode (LED) also generates light as a result of an electron–hole recombination. Both devices feature a PIN diode at their core, with an active zone, known as the Intrinsic (I) region, sandwiched between the Negative (N) and Positive (P) regions. Light is generated when electrons (negative charge) and electron holes (positive charge, due to the absence of electrons) recombine within the intrinsic region.

In an LED, the intrinsic region is larger than in a diode laser, which causes photon-producing recombinations to occur over a broader area. This promotes better heat dissipation and potentially enhances durability. In LEDs, any photons created escape immediately, emitting “normal” light (incoherent and multidirectional).

For laser light to be generated, population inversion is needed, which can only occur when the density in the upper state is sufficiently high. By making the edges of the intrinsic region smooth and mirror-like, photons are continuously reinjected into the active region. With population inversion, the intrinsic region becomes filled with excited atoms, allowing stimulated emission to occur, which effectively amplifies the reinjected photons. As these photons are repeatedly reinjected and reduplicated, they form a powerful laser beam. One side of the optical cavity (the output) is designed to be partially reflective, allowing the laser to escape. The light emitted here is coherent and unidirectional.

Bornstein et al. observed in an *in vitro* study that near-infrared diode laser reduces mitochondrial membrane potential and increases the production of ROS, which contributes to the photoinactivation of *C. albicans* and *T. rubrum* [24]. There are only a few clinical studies to treat onychomycosis using diode laser systems; however, all have shown positive outcomes. Combined long-pulsed 870/930 nm near-infrared diode laser treatment in patients with mild-to-severe onychomycosis achieved 65% clear nail growth [25]. Long-pulsed 980 nm near-infrared diode laser offers the advantage of contact-free homogeneous heating of the human nail while ensuring adequate temperature rises [26]. The suggested mechanism of action is similar to that of a long-pulsed Nd:YAG laser, where the fungi absorb light energy, leading to their heating and inactivation.

Recent studies show that the application of continuous-wave 405/635 nm dual-band diode laser in patients with mild-to-severe onychomycosis achieved 67% clear nail growth. This laser system acts through a non-thermal mechanism (blue light at 405 nm has antimicrobial effects, while red light at 635 nm promotes wound healing through a photobiomodulation process) [27].

In 1995, LEDs, originally developed for NASA's plant growth experiments in space, were found to have the potential for delivering light deep into tissues of the body. These LEDs were significantly more powerful than the existing ones, with a stable output power, and, most importantly, a quasimonochromatic beam. This made them an excellent new source for phototherapy, offering biologically target-specific wavelengths. LED light sources have been built based on the same effective wavelengths used by diode lasers while benefiting from the inherent advantages of LEDs: they are highly efficient, versatile (able to be mounted in larger planar arrays to treat extensive body areas without hands-on involvement), and then offer a low-cost, less hazardous alternative to diode lasers.

Users of any LED system must select the correct wavelength for the intended target (such as fungal pigments, cytochrome C oxidase for photobiomodulation, or photosensitizers for photodynamic therapy) to ensure optimal absorption. This is crucial because, as the first law of photobiology states, without absorption, no reaction can occur. LED phototherapy at the proper wavelengths is safe, effective, easy to apply, free of pain and side effects, and well tolerated by patients of all ages. As indicated in **Table 1**, in the treatment of onychomycosis, LED lamps are now primarily used to induce the photoactivation of various photosensitizers in photodynamic therapy.

#### 4. Nd:YAG laser

The Nd:YAG laser is a solid-state laser. It is made of a  $Y_3Al_5O_{12}$  (yttrium aluminum garnet) crystal containing 0.1–1% of  $Nd^{3+}$  and emits light at a wavelength of 1.06  $\mu m$  or sometimes at 1.32  $\mu m$ , in the near-infrared region of the spectrum. This laser

system can operate in short-pulse (Q-switched) or long-pulse mode as well as in continuous-wave mode. The Q-switched mode involves a variable attenuator, which stores the beam to maximize energy, delivering a pulse with peak power. This mode can be combined with potassium titanyl phosphate (KTP) crystal to double the laser's frequency, generating laser light at 532 nm (visible green light).

The longer wavelength of the 1064-nm Nd:YAG laser is thought to penetrate tissue more deeply, effectively targeting fungal growth in the nail bed. The cell walls of *Trichophyton* species contain a considerable amount of melanin, which selectively absorbs this wavelength [28]. Furthermore, it is proposed that the 532-nm Nd:YAG laser is effective at targeting xanthomennin (an endogenous fungal pigment), which has an absorption band ranging from 406 to 555 nm [28].

The Q-switched Nd:YAG laser generates high-energy peaks with numerous repetitions, resulting in minimal tissue warming and producing impact energy that mechanically and selectively damages only the fungi. The underlying mechanism, known as selective photothermolysis, depends on fungal chromophore, light characteristics, and pulse duration (which must be shorter than the "thermal relaxation time" of the target chromophore) [29]. Meanwhile, the absorption of light energy from the long-pulsed Nd:YAG laser by the fungus results in a general conversion of the energy into heat. Its microbiocidal effect has been associated with the resulting hyperthermia (as fungi are sensitive to heat above 55°C) and its ability to denature essential cellular proteins and structures [30].

The published results are controversial, with some studies showing positive outcomes, [31, 32] while others report no improvement [33, 34]. It remains uncertain whether the long-pulsed mode is more effective than the Q-switched mode, and no standardized laser protocol has been established yet. A common finding in all the published clinical studies is the high safety profile of this laser system, with no reports of severe or serious side effects. It has been observed, however, that higher energy density and a greater number of sessions tend to result in better clinical and mycological cure rates [35]. With regard to tolerability, patients usually experience mild burning or pain during irradiation sessions, but these sensations do not prevent them from continuing the treatment. Pain during irradiation is commonly reported and is therefore a key limiting factor when increasing laser power.

Valid conclusions have not been reached due to several factors, including the use of different laser protocols (different laser devices with different pulse durations, number of sessions, and intervals between the sessions), short follow-up periods, inadequate mycological diagnosis, recruitment of small and inhomogeneous study populations, and different types of onychomycosis and different causative fungal species. It should be noted that studies without significant results may discourage researchers from submitting their outcomes to prestigious journals. This is unfortunate, as negative results still offer valuable insights that could help redirect research focus toward more promising avenues.

## 5. Ablative fractional laser resurfacing (AFR)

Ablative fractional laser resurfacing (AFR) offers precise and deep tissue ablation with controlled depth of heating and vaporization. AFR was developed to maximize the safety of the ablative procedure while maintaining its effectiveness. Compared to traditional ablative resurfacing, AFR results in faster wound healing, fewer infections, and less

post-operative downtime [36]. In AFR systems, the laser beam is divided into a pattern of microbeams, creating micro-holes due to the ablation and vaporization caused by the microbeams. The depth of the channels through the nail can be controlled by the applied wavelength, fluence, pulse duration, beam profile, and repetition rate.

The mechanism of action of AFR in onychomycosis is unknown. However, some studies suggest that the tissue ablation process induces a direct fungicidal effect. Fungi are highly sensitive to temperatures above 55°C; the photothermal effect of the fractional laser can increase the temperature of the target tissue, directly contributing to the destruction of the fungi in the affected nail. This effect might be further enhanced by alterations in the immune system or modifications in the local microenvironment caused by the micro-holes created through the vaporization of the tissue, ultimately leading to diffuse tissue remodeling and simultaneous destruction of the fungal growth environment [37].

Current AFR systems include erbium-doped yttrium aluminum garnet (Er:YAG) lasers ( $\lambda = 2,940$  nm) and CO<sub>2</sub> lasers ( $\lambda = 10,600$  nm). Both lasers operate in the mid-infrared range, where their wavelengths are absorbed by water (which represents 10–20% of the nail plate's composition), ablating the nail in a fractionated pattern, creating microscopic vertical channels surrounded by a coagulation area [36]. CO<sub>2</sub> lasers penetrate deeper into the tissue due to their longer wavelength, enabling more heat deposition and a greater amount of tissue remodeling. In contrast, Er:YAG lasers emit at a wavelength closer to the water absorption peak ( $\lambda \sim 3,000$  nm), offering a safe profile because most of the energy is absorbed by more superficial layers, resulting in better control over ablation depth and less thermal damage to underlying structures.

The creation of multiple micro-holes increases the contact area between the topical formulations (whose active ingredient can be an antifungal agent or a photosensitizer)



**Figure 1.** Use of a fractional Er:YAG ablative laser to enhance the diffusion and penetration of methylene blue (MB) through the nail, followed by photodynamic treatment. (I) baseline, (II) two sessions, (III) four sessions, and (IV) 36 weeks post-treatment (photographs courtesy of the authors).

and the nail surface, enhancing their diffusion through the nail plate to reach the nail matrix or bed [38]. Consequently, a reasonable number of studies support the benefits generated by AFR in the treatment of onychomycosis, used adjunctively with conventional topical antifungal therapy or photodynamic therapy [39, 40]. **Figure 1** illustrates how an Er:YAG-based AFR system facilitates the diffusion of methylene blue through the nail, enhancing the effectiveness of photodynamic therapy. Additionally, applying a keratolytic agent like urea under occlusion before AFR irradiation increases the ablative and fungicidal effect. Urea, a hygroscopic agent, softens the nail plate and retains water (chromophore for AFR) at deeper levels, promoting ablation and remodeling of the nail or affected areas [41].

It has been demonstrated that combining platelet-rich plasma (PRP) with AFR systems is effective for skin rejuvenation and the treatment of atrophic acne scars. However, further research may be needed to explore its potential for treating onychomycosis. AFR systems enhance the penetration of topically applied PRP, exerting a synergistic effect that promotes faster healing and remodeling of damaged nails, as well as inhibiting microbial colonization.

## 6. Photodynamic therapy (PDT)

With the increasing development of resistance to antimicrobial agents, Photodynamic Therapy (PDT) has regained interest since the 1990s, as it represents a potential alternative for microbial eradication, although the emergence of resistance has not yet been described [42].

PDT is the most researched light-based treatment for onychomycosis, involving the use of a photosensitizing agent (photosensitizer, PS) followed by irradiation with light of a specific wavelength, enabling maximal absorption by the target and leading to the production of reactive oxygen intermediates. PDT relies on three basic elements: 1) the PS; 2) a light source: lamp, laser, or LED, with an appropriate wavelength and power suitable to activate the PS; 3) the presence of intracellular oxygen dissolved in the treated tissue. The success rate of PDT can be affected by the oxygen present in the tissue, the concentration of PS, the incubation time of PS, the time of application of light, and the number of treatment sessions [43].

PDT involves two distinct types of reactions. In the type I reaction, which involves redox (oxidation-reduction) reactions, the excited triplet state of PS interacts with the molecules of the diseased tissue, generating radicals that, in the presence of surrounding oxygen, give rise to ROS, mainly superoxide anion, hydrogen peroxide, and hydroxyl radical. These ROS can oxidize a wide variety of biomolecules. In the type II reaction, the excited triplet state of PS interacts with oxygen, resulting in the formation of singlet oxygen (oxygen in the excited singlet state), a highly reactive molecule that easily damages cells (**Figure 2**) [44]. The ratio of type I and type II processes is influenced by the PS and can happen either separately or together.

ROS produced during the photodynamic process can trigger fungal cell damage or death through three main mechanisms: cell membrane damage, inactivation of essential enzymes and proteins, and/or damage to DNA or RNA. PDT-induced photodamage can lead to significant changes in the morphology and function of microbial structures. Functional damage results from loss of enzymatic activities, protein oxidation, protein-protein cross-link formation, and inhibition of metabolic processes (e.g., DNA synthesis, glucose transport). Direct damage to the cell membrane causes the leakage of cellular contents and disrupts the membrane transport

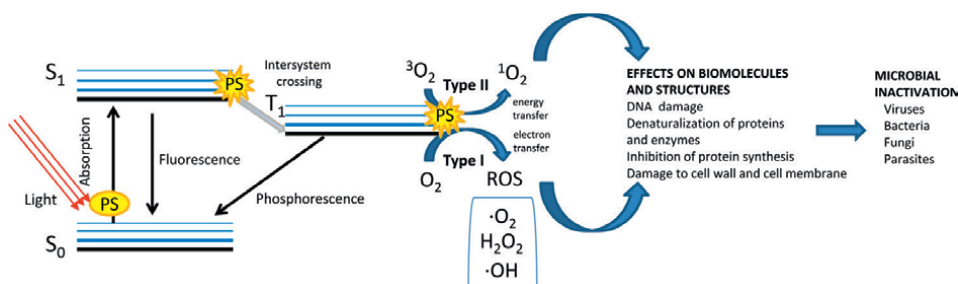


**Figure 2.**  
Mechanism of action of PDT.

system (**Figure 2**) [45]. It is a selective action; the fungus absorbs the PS; then the damage is restricted to its structures without the surrounding healthy part of the nail being affected.

Several PS have been tested for this purpose: porfimer sodium, 5-aminolevulinic acid (ALA), methyl aminolevulinate (MAL), methylene blue (MB), flavin mononucleotide (FMN), rose bengal (RB), and curcumin (CU). The three most tested PS to date for the treatment of onychomycosis are ALA, MAL, and MB. ALA and MAL are precursors of protoporphyrin IX, which is photoactivated by red light in the 630 to 700 nm range. Both of them, applied on the nail in occlusion for 2–5 hours and then exposed to a red light source at 630 nm with a fluence of 37 J/cm<sup>2</sup>, have demonstrated effectiveness in the photodynamic treatment of onychomycosis [46]. MB is another increasingly popular PS. It absorbs light in the 550–700 nm region, with the maximum absorption at 664 nm. MB can induce the formation of type I radicals (ROS) and type II (singlet oxygen), which broadens its uses in PDT, and its widespread availability makes it a commonly used PS [47]. Research conducted with MB uses a concentration of 2% w/w, resulting in a blue discoloration of the nail that can persist for several months following treatment (see **Figure 1**).

For the photoexcitation of porfimer sodium, ALA, MAL, and MB, red light is used, as it is minimally absorbed by endogenous tissue chromophores like hemoglobin and has great penetration in most human tissues. In studies to treat onychomycosis via PDT, the fluence of the red light used typically ranges over the tens of J/cm<sup>2</sup>, values that barely generate thermal effects and that are obtained using continuous low-power lasers, such as diode lasers (635 and 670 nm), and non-coherent light sources like LEDs. FMN and CU can be photoexcited with blue LEDs with a wavelength of around 450 nm [48, 49]. Blue light has less penetration than red light in biological tissue; therefore, for it to be effective, the exposure time is increased compared to the times used with red light. RB absorbs visible light between the wavelengths of 500 nm and 550 nm (encompassing the green light spectrum), so 532 nm solid-state pumped laser or green LED light at 532 ± 10 nm is typically used for RB photoexcitation [50].

For PDT, both coherent and incoherent light sources have been used. Non-laser light sources offer several advantages for topical PDT, including being cost-effective, operator-friendly, low maintenance, and capable of irradiating larger areas. Currently, there is no single light delivery system considered ideal for every indication for topical PDT, and each clinical situation should be assessed individually.

Published research on PDT for onychomycosis demonstrates its effectiveness in eradicating numerous fungal species, including its most common cause, *T. rubrum*.

These studies highlight its safety in terms of the absence of systemic effects and only a low chance of local adverse effects reactions such as temporary pain, burning, and erythema [51].

Studies also suggest that for PDT to be more effective, pretreatment based on the application of 40% urea in occlusion 12 hours/day for 5–7 days before irradiation is advisable. This helps soften the nail and improves the diffusion of the PS, which can somewhat reduce the incubation time of the PS that requires it [52]. AFR with CO<sub>2</sub> and Er:YAG lasers can also be used for this purpose, facilitating the diffusion of PS through the created microchannels [40]. ALA and MAL require incubation times of 3 and 2 hours, respectively, while MB and FMN can be photoactivated immediately after application. For RB and CU, incubation times of 30 min and 1 hour, respectively, have been reported. When ALA, MAL, FMN, and CU are used, the treatment area can be examined with a Wood's light after the designated incubation time to confirm the local accumulation of PS (**Figure 3**).

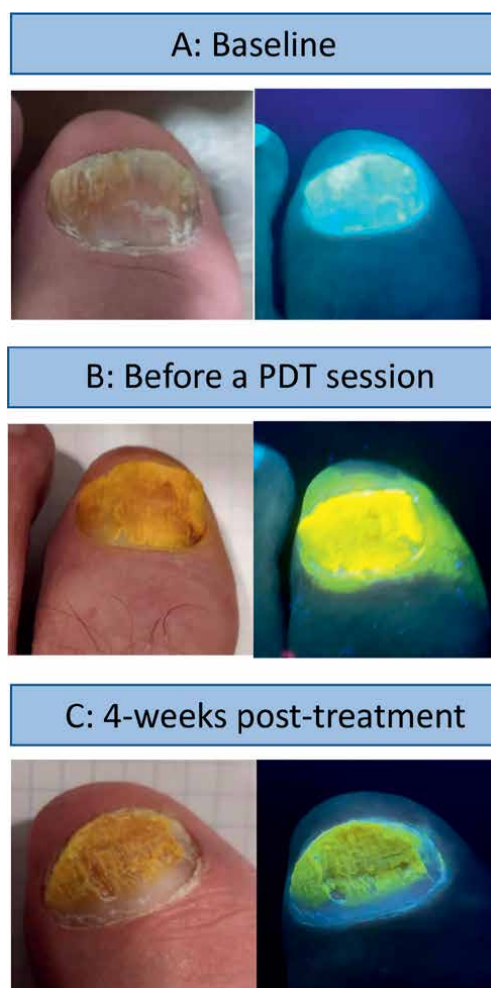


**Figure 3.**  
*Monitoring of nail penetration of FMN incorporated into a semisolid vehicle and applied under occlusion using a Wood's lamp. A: baseline, B: before a PDT session, and C: 4 weeks post-treatment (photographs courtesy of the authors).*

PDT cure rates for mild-moderate DLSO, affecting 20–50% of the entire nail, range from 40–70% [53]. These values are higher than those typically seen with standard topical antifungal agents, which have cure rates of 20–30% [54].

Although onychomycosis caused by non-dermatophyte fungi is not common in the Western world, the therapeutic management of these fungi is considerably more challenging than that of tinea unguium caused by dermatophytes. Some studies have demonstrated the efficacy of PDT in these cases [55].

To date, studies show that PDT is effective for treating mild–moderate DLSO, but they are still limited, making it difficult to draw definitive conclusions. A major disadvantage of treating onychomycosis with PDT is that it is still to be determined which PS is most effective depending on the infectious agent causing it, its optimal concentration, or the best delivery vehicle (liquid/semisolid) to achieve good penetration through the nail. This means that, to date, the only way to obtain good results and avoid recurrences is through the application of multiple PDT sessions, which may interfere with patient compliance.

Many studies using randomized clinical trials are needed to evaluate the efficacy of PDT treatments using a variety of PSs and to gradually delineate a standard protocol for the optimal PDT parameters. This includes determining the appropriate PS for the specific infectious agent (type, concentration, incubation time, administration method), as well as the ideal light source (wavelength, power, irradiance, pulse size, or irradiated area), the number of PDT sessions, and the interval between them, etc. The goal is to achieve the most effective therapeutic outcome based on the severity and type of onychomycosis. Ongoing investigations are focused on addressing these uncertainties.

## **7. Intense pulsed light (IPL)**

This technology operates by emitting a high-intensity, pulsed, non-coherent, polychromatic light through a filter, which can be changed according to the desired target within the skin [56]. IPL devices use a xenon-flash lamp powered by capacitor banks controlled by microprocessors, allowing for adjustments to pulse duration. The wide spectrum of a flash lamp enables energy from multiple wavelengths (400–1200 nm) to be emitted, with pulse durations ranging from 2 to 200 ms. Filters and settings such as fluence, pulse duration, and pulse delay can be adjusted via software to treat different targets, making it a versatile tool [57]. Over the years, technical improvements have made this device safer and easier to use, broadening its clinical applications. Additionally, the large irradiated area per pulse allows for faster treatments. IPL is widely accepted for treating pigmented lesions, among other uses, and can be applied in the treatment of onychomycosis, as wavelengths between 500 and 600 nm can be absorbed by pigments like xanthomennin found in fungi.

IPL has gained popularity as both a cosmetic and medical device for onychomycosis treatment. It is a safe and more affordable option compared to laser devices, both in terms of purchase price and maintenance. IPL and long-pulsed laser radiation have similar mechanisms for onychomycosis treatment, as both are absorbed by specific structures and chromophores, leading to their heating and destruction. While laser radiation is monochromatic and is only absorbed by a specific chromophore, IPL requires the practitioner to select the appropriate filter to ensure the emitted light is absorbed by the target chromophore.

A drawback in treating onychomycosis with IPL is that the procedure can be quite painful, though the discomfort is only felt during the irradiation. A water-based gel is recommended to reduce nail surface damage by decreasing the nail's refraction index of light. This gel also provides a "heat-sink" effect and helps the handpiece glide more easily. The appearance of hematomas has also been reported [58]. Additionally, non-selective thermal damage is a risk, which can be increased when IPL is administered by untrained practitioners. An understanding of the mechanism and design of different IPL devices, along with knowledge of the appropriate parameters and indications, is key to ensuring safe and effective treatment.

There are very few studies on the use of IPL for onychomycosis. One small *in vitro* study using IPL at 420 nm on fungal biofilms developed on nail fragments showed promising results [59]. There is also a clinical study with 20 participants reporting an 80% complete cure rate 12 weeks after treatment, with the remaining 20% showing significant improvement, potentially treatable with adjunctive topical therapy [58]. Finally, IPL has also been used to photoactivate MB in PDT treatment using wavelengths between 560 nm and 700 nm [60].

## 8. Contraindications of light-based therapies

While light-based treatments are generally very safe, there are certain contraindications that must be taken into account: seizure disorders triggered by light, lupus erythematosus, light-sensitive disorder, gold therapy, peripheral neuropathy, and the use of medications that may induce photosensitivity.

## 9. Future directions

There is a clear need for additional research, particularly randomized controlled trials aimed at the eradication and cure of onychomycosis. Light-based therapies, which are noninvasive, are leading the way in addressing this need. Although these therapies are not FDA-approved for a cure, they are already being used in dermatology practices.

Although studies to date show acceptable cure rates only in mild-moderate cases, new devices must be continuously tested as they are introduced or developed for dermatological use, even if used in different contexts. In the field of PDT, new PSs and light sources need to be evaluated. It must be considered that elderly, diabetic, immunocompromised populations cannot tolerate the systemic side effects associated with oral antifungals (the most effective treatment to date). Therefore, light-based therapies should be considered a first-line treatment, not an alternative, for these groups to treat and prevent recurrences. Investing in these therapies will have a positive societal impact.

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### **Conflict of interest**

The authors declare no conflict of interest.

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# Advancing Fungal Diagnostic and Research Stewardship in India: A Prospective Strategic 10-Step Plan for Addressing a National Public Health Challenge

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## Abstract

Invasive fungal infections (IFIs) are silent killers, frequently undiagnosed and untreated. India faces significant challenges with limited diagnostic facilities, scarce data on fungal infection, and rising antifungal resistance. The COVID-associated mucormycosis surge highlighted the urgent need for stronger diagnostic infrastructure for fungal infections. Recognizing this, the Indian Council of Medical Research (ICMR) launched a network of mycology laboratories with advanced diagnostic capabilities across India using a 10-step plan described in this paper. Laboratories were selected using a three-pronged screening approach. Eight Advanced Mycology Diagnostic and Research Centers (AMDRCs) were set up in North, West, Central, South, South-Central, East, and Northeast zones from 2020 to 2022. Each AMDRC received ongoing support in training and quality assurance. The network features a digital data system, fungal repository, and clinical registry to track IFIs in ICU patients. In 2022–2023, the network identified 16.3% of fungal cases, mainly *Candida* spp. (56%,  $n = 5764/10293$ ) and *Aspergillus* spp. (28%,  $n = 2882/10293$ ), with 1.5% ( $n = 227/14861$ ) IFIs and seven rare diseases (Eumycetoma and Chromoblastomycosis) reported in 2023. *Candida auris* showed high resistance to Amphotericin-B (61.2%,  $n = 30/49$ ) and Fluconazole (57.1%,  $n = 28/49$ ). The ICMR-MycoNet has significantly improved access to fungal diagnostics, enabling early detection and management. Comprehensive data on infection trends and resistance patterns provide valuable insights for targeted treatments and outbreak preparedness, strengthening public health efforts.

**Keywords:** laboratory diagnosis, digital database, fungal infections, mycology, diagnostic center

## 1. Introduction

For most of recorded history, fungal infections, unlike other pathogens, were infrequent or had a minimal impact on human health and have emerged more recently as an important public health issue [1]. Human body temperature and a robust immune system, act as barriers against fungal infections. Nonetheless, the rise in global temperatures, coupled with the emergence of immunocompromising conditions, like AIDS, organ transplants, and diabetes, has provided an environment for fungi to adapt and thrive in the human body. Consequently, the increasing incidence of fungal infections and antifungal resistance are becoming a significant concern.

Many fungal diseases go unreported to public health authorities due to the absence of local, national, or regional surveillance systems, which restricts global fungal surveillance efforts by limiting data collection areas and variables [2, 3]. This lack contributes to underdiagnosis and misdiagnosis, exacerbating gaps in understanding their true burden [4]. Enhanced national and regional surveillance systems are crucial for tracking fungal disease distribution and detecting emerging threats early. India, with the largest burden of fungal disease per capita [5, 6], faces the unique challenges of establishing countrywide diagnostic and treatment facilities. In addition to the tropical climate providing an ideal setting for fungal growth, the high burden of immunocompromised patients, insufficiently trained healthcare professionals, and compromised healthcare practices, characterize the challenges outlined above in greater detail. In the era of antimicrobial resistance, the emergence of multidrug-resistant *Candida auris* and azole-resistant *Aspergillus fumigatus* has emerged as a cause of concern [7, 8]. In light of the recent outbreaks of COVID-19-associated *Aspergillosis* and *Mucormycosis*, it is assumed that fungal outbreaks will pose a far greater threat to mankind sooner than anticipated [9]. A lack of advanced mycology laboratories and poor awareness of fungal diseases further exacerbate this problem.

The Indian Council of Medical Research (ICMR), New Delhi, has taken the initiative to establish a network of advanced mycology diagnostic and research centers (AMDRCs), to improve diagnostic capability and laboratory training in fungal disease diagnosis, as well as patient care overall. Data generated *via* the clinical registry through these AMDRCs will also bridge the knowledge gap in understanding and management of fungal diseases and drive further research and policy interventions to strengthen the response to fungal infections in India. Against this background, we present the process of developing the aforementioned network, which is the first dedicated fungal network in the country, and the challenges faced while setting up a national-level network of AMDRCs in India through 10-step plan. Lessons learned through this initial process would help further expand the network in the country. Moreover, this procedural framework can be a valuable tool for other developing nations aiming to establish similar networks to tackle critical public health issues. The aim of the network is to develop fungal mapping of the country along with trends of fungal diseases, emerging fungal infections, and antifungal resistance.

## 2. Methodology

ICMR is an autonomous apex research body under the Ministry of Health and Family Welfare, Government of India, which works on the formulation, coordination, and promotion of biomedical research. ICMR conducts national task force projects, which are large-scale multicenter studies focusing on a public health problem. The ICMR-MycoNet was initiated in March 2020 under a national task force project, aiming at bridging the gap in fungal research, caused by the lack of advanced fungal disease diagnostic facilities across the country.

### 2.1 Ethical considerations

The study was approved by the ethical committee of each participating institute. The Central Ethics Committee on Human Research (ICMR-CECHR) approved the study and the clinical registry (CECHR 009/2022). Memorandums of Agreement (MoA) were signed between the ICMR and all established AMDRCs for shared principles, objectives, and responsibilities.

### 2.2 Setting

ICMR, New Delhi, established a mycology unit in the Division of Development Research, which serves as the nodal coordinating center for planning, funding, and all logistic support for the proposed mycology network (ICMR-MycoNet).

#### 2.2.1 Selection, funding and monitoring of laboratories

The selection of laboratories and development of the network of AMDRCs was achieved through a 10-step plan, as depicted in **Figure 1**. These centers were entrusted to provide diagnostic facilities to their catchment area and serve as the regional reference center for fungal diagnosis and research.

### 2.3 Step 1: Develop a standard protocol, case report form, and guidelines

A standard protocol was developed centrally with defined aims and objectives of the ICMR-MycoNet Taskforce (**Box 1**).



**Figure 1.**  
*10-step plan for establishing a nationwide network of advanced mycology diagnostics and research laboratories in India.*

- Capacity building for complete diagnostic portfolio (microscopic/culture/serology/molecular/therapeutic drug monitoring) for all fungal infections, technical training, and quality assurance to identify cryptic fungal infections and improve overall patient care.
- Generate a centralized digital registry of all invasive fungal infections in ICU patients and study their impact on the patient outcome.
- Collect clinical data on neglected tropical diseases, that is, mycetoma and chromoblastomycosis.
- Regional surveillance of fungal infections for fungal mapping of the country and fungal strain repository.

**Box 1.**

*Objectives of the ICMR-MycoNet taskforce.*

The main aim of our research was to establish a PAN India network of laboratories equipped with advanced fungal diagnostics. This initiative aimed to build capacity for a comprehensive diagnostic portfolio encompassing microscopic, serological, and molecular methods for all fungal infections, alongside providing technical training and ensuring quality assurance to identify cryptic fungal infections, ultimately improving overall patient care.

A secondary objective was to conduct regional surveillance of fungal infections across the country. We sought to generate a centralized digital database of all invasive fungal infections (IFI) in ICU patients and study their impact on patient outcomes. All Intensive care unit or hospitalized patients with proven or probable invasive fungal infections confirmed by culture, histopathology, microscopy, or DNA evidence of any age and gender as per EORTC/MSG (proven cases) or Blot criteria and modified EORTC/MSG (probable cases) [10–12] will be included in the registry. Additionally, all patients with a diagnosis of chromoblastomycosis or mycetoma (eumycetoma) patients will be included. Outpatient department or referral patients, those suspected of invasive fungal infections but without evidence of invasive disease or only colonization, as well as patients with superficial infections, skin-limited infections, and allergic fungal diseases like allergic bronchopulmonary aspergillosis, will not be included in the registry.

The protocol for the registry and the development of the web platform has been published previously [13] by our group including a detailed description of the study design and inclusion criteria for patient selection. All AMDRCs follow the ICMR standard operating procedures for fungal identification and detection of antifungal resistance [14] for uniform reporting and ensuring the quality and comparability of results. A digital Case Report Form (CRF) was also developed for the clinical data collection for the registry, capturing all relevant information related to IFIs including patient demographics, underlying comorbidities, diagnostic methods, antifungal therapies, and clinical outcomes. All documents were discussed, modified, and approved through Technical Review Committee (TRC) meetings.

## **2.4 Step 2: Invite potential investigators and institutes**

To achieve the above objectives, a call for proposals/applications from tertiary medical institutes across the country including different regions for setting up an advanced mycology laboratory was released. All potential centers were listed based on the availability of a dedicated microbiology department, qualified and experienced faculty, and dedicated space for setting up the advanced mycology laboratory, along with, critical care facilities, and a substantial IFI caseload.

### **2.5 Step 3: Design a questionnaire for assessing strengths and areas requiring capacity development in the prospective center**

A questionnaire (**Table A1**) was designed to collect systematic information about the investigator (mycologist) and the institute. A total of 20 questions on (i) willingness to join the ICMR-MycoNet and development of an excellence center, (ii) nodal person's qualification and experience details, (iii) human resources available, (iv) equipment available, (v) diagnostic portfolio, (vi) sample load, (vii) participation in External Quality Assurance (EQA) programs, (viii) networking with other departments/hospitals/state health officials, (ix) number of ICUs, and specialty care departments, and (x) research and publications were included.

### **2.6 Step 4: Questionnaire-based screening for potential investigators and institutes**

A three-pronged approach for was adapted for screening and selection of AMDRCs. The first screening involved a questionnaire-based scoring system to assess all potential institutes in different regions of the country. Scoring was done based on the information provided. Centers with higher scores were shortlisted. Regional preference was also taken into account to cover most major geographical regions of the country.

### **2.7 Step 5: Assessment site visit to evaluate the prospective institute**

For the second screening, a team of experts visited the shortlisted centers to assess the feasibility of establishing AMDRC using a checklist (**Table A2**). The following details were checked: (i) laboratory space and infrastructure, (ii) diagnostic portfolio, (iii) implementation of principles of Good Laboratory Practice (GLP), laboratory manuals, protocol, and guidelines, (iv) laboratory records, equipment, and quality control records, (v) regular and periodic training of staff, (vi) biosafety practices, (vii) interdepartmental engagement and coordination, and (viii) extramural research funding.

### **2.8 Step 6: Review meeting for technical proposals on establishing a reference laboratory**

Thirdly, the shortlisted centers were invited to present their institutional strength and work plans at the TRC meeting with subject experts. The principal investigators (PIs) gave a detailed description of their existing institute infrastructure and facilities, ability to participate in the program, and work plan for developing the mycology excellence center and project execution.

Based on the recommendation of the TRC and approval of DG and ICMR, eight AMDRCs were selected for funding (**Figure 2**). These AMDRCs were funded initially for 3 years and based on satisfactory evaluation and progress, extendable for another 2 years as per ICMR task force policy.

### **2.9 Step 7: Empowering fungal disease diagnostics - Addressing challenges and enhancing capacity**

Due to the nonspecific clinical presentation and radiological findings, IFI are challenging to diagnose [15]. Current diagnostic methods of invasive fungal disease (IFD) have low sensitivity, slow turnaround time, and require invasive procedures

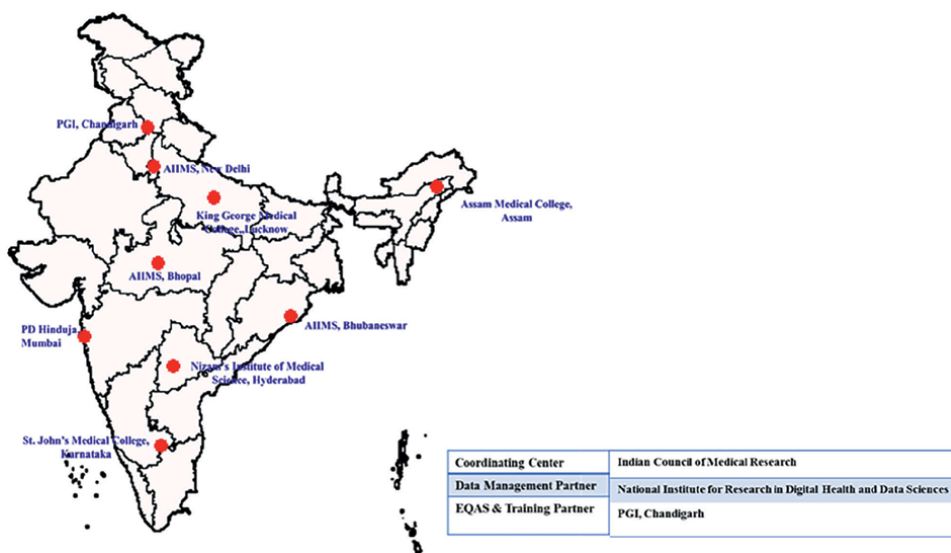


**Figure 2.**  
*Geographical distribution of ICMR advance mycology diagnostics and research laboratories (MycoNet) in India.*

for specimen collection to yield clinically relevant results. The recognition of IFD and its differentiation from other infections with comparable clinical manifestations is also difficult [16, 17]. Microscopic examination, culture, antigen or antibody testing, molecular assays antifungal susceptibility testing (AFST) are methods used in the laboratory to diagnose and manage fungal infections. Through this task force, all AMDRCs have been equipped with laboratory infrastructure for handling infectious fungal pathogens. These labs have been supported with a range of equipment and commercial kits focusing on high-throughput non-culture antigen, antibody and molecular testing, funds for hiring a technician, and operational expenditures.

## 2.10 Step 8: Human resource development through advanced training for the diagnosis of fungal infections and external quality assessment scheme (EQAS)

Trained laboratory staff is the backbone of this program. Post Graduate Institute of Medical Education & Research, Chandigarh (PGIMER), the World Health Organization (WHO) Collaborating Center for Reference and Research on Fungi of Medical Importance, was included in the taskforce as the EQA and training partner. All research and technical staff recruited under AMDRCs are required to take a one-week hands-on training on different laboratory diagnostics. External Quality Assessment Services (EQAS) programs were conducted by PGIMER to periodically assess the quality of performance on the following accounts:

- a. Processing of specimens for the diagnosis of fungal infection, identification of the pathogenic fungi (yeasts or molds) isolated, and interpretation of results.
- b. Serological tests performed for the diagnosis of fungal infections.
- c. Antifungal susceptibility testing of the yeast and molds isolated from the clinical specimen.

Apart from that a team of clinicians was identified and included from different clinical divisions to get the samples from different ICUs.

### **2.11 Step 9: Develop a website and centralized data management and analytics system**

Developing a centralized digital data management portal for swift data visualization and analysis is crucial. The ICMR-MycoNet website was designed with two components: one accessible to the public, and the other serving as a password-protected, web-based tool for data collection and analysis. The ICMR National Institute for Research in Digital Health and Data Science (NIRDHDS) is the center for centralized data management activities and analytics. All AMDRC staff were trained for data collection, recording, and storage using the eCRF platform. Information about all fungal strains stored at the AMDRCs has been cataloged in a digital fungal strain repository. This repository aims to streamline the preservation and distribution of medically significant fungal strains to fellow scientists and researchers across India.

### **2.12 Step-10: Performance monitoring and assessment of the AMDRCs**

Regular performance assessments are essential to uphold laboratory competency. Six months after the initiation of the AMDRC, a team of experts and ICMR officials conducted site monitoring visits to assess the progress in laboratory infrastructure development. Discussions were held with the Institute's Director and state health authority to identify and address ground-level challenges and explore potential solutions.

Performance monitoring indicators are used to assess the impact of the established AMDRCs on public healthcare, focusing on infrastructure development, network and research activities, test portfolio expansion, outbreak investigation, equipment, and human resource training (**Table A3**). The assessment is done in every 6 months. Annual evaluation of the AMDRC by the TRC is done per ICMR policy. PIs present the annual progress report, and continuation for the next year depends upon the successful implementation of the project mandates and satisfactory outcomes.

## **3. Results**

ICMR-MycoNet was initiated in the year 2020, and during the subsequent 3 years, eight mycology centers of excellence were integrated into the network. Initially, a total of 19 institutes were screened from different zones of the country. Ten centers were invited for presentation, and the TRC selected eight centers to develop a mycology network (ICMR-MycoNet). This network was strategically designed to cover diverse zones in the country, and the geographical distribution of the AMDRC (Advanced Mycology Diagnostic and Research Centre) is illustrated in **Figure 2**. All India Institute of Medical Sciences (AIIMS), Bhopal, Madhya Pradesh, covers the central zone; AIIMS Bhubaneswar, Odisha, covers the eastern zone, PD Hinduja Hospital Mumbai, Maharashtra, covers the western zone; AIIMS New Delhi and King George's Medical University (KGMU), Lucknow, Uttar Pradesh covers the northern zone, St. John's Medical College Karnataka covers the southern zone, Nizam's Institute of Medical Sciences (NIMS), Hyderabad, Telangana covers south-central zone, and Assam Medical College, Assam covers the northeastern zone of India.

All the AMDRCs are situated at major hospitals in the country with huge patient loads, and in the first 6 months, infrastructure upgradation was achieved with the development of separate rooms for conventional, serological, and molecular fungal diagnosis with the molecular lab further separated into areas, for master mix preparation, nucleic acid extraction, and DNA template addition, PCR and post PCR processing. Advanced equipment for rapid fungal diagnostics like the fluorescent microscope, Vitek-2 system, Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) Mass spectrometer, biosafety cabinets, kinetic reader, Thermocycler, HPLC system, RT-PCR was installed, and new rapid non-culture based fungal diagnostic tests for detection cryptococcal capsular antigen, Fungal cell wall Galactomannan (GM), 1,3- $\beta$ -d-glucan (BDG), and Histoplasma antigen were added to the diagnostic profiles of all the centers. Antifungal susceptibility testing and therapeutic drug monitoring were also initiated to increase the probability of a successful outcome, minimize drug-related toxicity, and potentially prevent the emergence of antifungal resistance. The impact of the Mycology Network on health infrastructure, research capabilities, and patient outcomes in India is illustrated in **Figure 3**.

To utilize the capacity of the established AMDRCs, ICMR has initiated a comprehensive national-level mycology clinical registry to fill the knowledge gap on the epidemiology of invasive fungal infections, evidence-based decision-making in clinical practice and public health policy, and eventually improve patient management. Through the ICMR-MycoNet Inpatient Clinical Registry, data on clinical features, diagnostics, treatment, and outcomes of ICU patients with Invasive fungal infections (IFIs) will be collected to assess the epidemiology, risk factors, overall trends of fungal diseases, emerging fungal infections, and their clinical pattern. Data will also be collected on chronic subcutaneous infectious, that is mycetoma (eumycetoma) and chromoblastomycosis, which WHO has classified as fungal neglected tropical diseases (NTDs) [17]. Briefly, in 2022–2023, a total of 10,293/63104 (16.31%) positive fungal cases were identified through this network, with *Candida* at 56% (n = 5764/10293)

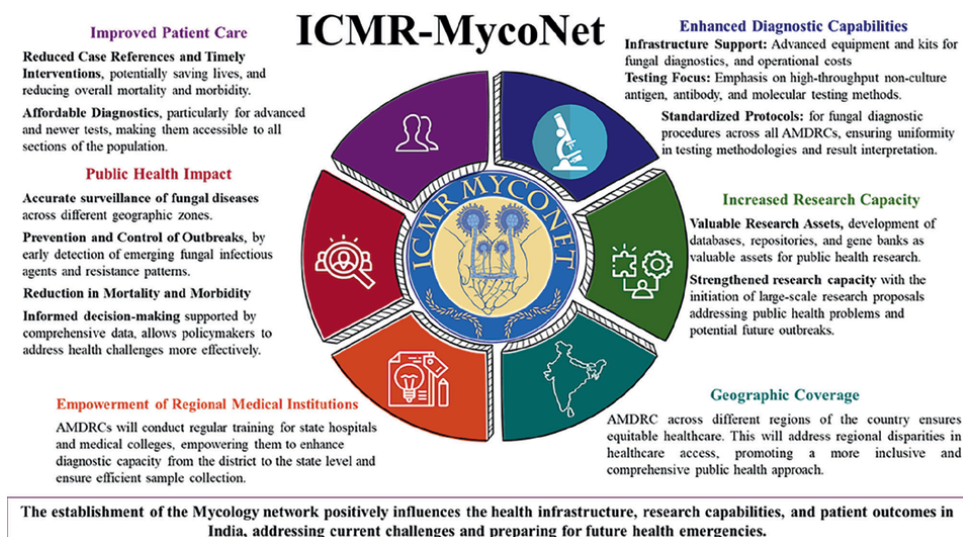


**Figure 3.**  
 The transformative impact of the ICMR-MycoNet in India: Overview of improvements in mycology diagnostics services and patient care services in hospital settings in India since the program's initiation.

and *Aspergillus* at 28% (n = 2882/10293). Since January 2023, 227/14861 (1.52%) IFI cases and seven rare fungal diseases (five chromoblastomycosis and two eumycetoma) were recorded. *C. auris* showed high resistance to Amphotericin-B (61.2%, n = 30/49) and Fluconazole (57.1%, n = 28/49). A total of 5577 fungal strains are deposited in fungal repositories. Six AMDRCs are National Accreditation Board for Testing and Calibration Laboratories (NABL) accredited laboratories [AIIMS Bhubaneswar (MC-6186), PD Hinduja, Mumbai (MC-2513), AIIMS New Delhi (MC-3267), KGMU, Lucknow (MC-2500), St. John's, Bengaluru (MC-2155), and AMC, Assam (MC-2643)] and two are under process for NABL accreditation [AIIMS, Bhopal and NIMS Hyderabad]. An average score of 80/100 was obtained for EQAS activity, and 34 faculties and research scientists/staff trained were at PGI, Chandigarh, in advanced fungal diagnostics.

## 4. Challenges

India's healthcare landscape such as inadequate infrastructure in rural areas, healthcare professionals more inclined toward bacterial infections than fungal infections, and complexities in navigating regulatory requirements for research and diagnostic activities, hindered the establishment of the ICMR-MycoNet. Beyond these challenges, limited funding, a shortage of trained professionals proficient in advanced fungal diagnostics, experienced mycologists, and collaboration with clinicians hampered the implementation and operational effectiveness. In addition, difficulties in the collection of invasive samples such as deep-seated tissues/body fluids, especially from critically ill patients in the ICU, lack of orientation among clinicians regarding early signs and symptoms of fungal infections, often mimics bacterial infections, were the major bottlenecks in data generation.

We faced several challenges in developing this countrywide network. Securing substantial funding for advanced diagnostic equipment like MALDI-TOF, sequencers, and HPLC was hurdle. Identifying institutes with the necessary mycology diagnostic facilities and experienced mycologists was another obstacle. We also encountered difficulties in identifying institutes with ICUs that had a sufficient patient load and in establishing collaborations with clinical divisions and ICUs. Additionally, enrolling eumycetoma and chromoblastomycosis cases from hospitals proved tricky. Lastly, developing a digital data management system and quickly conducting field trials of the CRFs were significant challenges.

## 5. Discussion

### 5.1 Impact of ICMR-MycoNet

As the prevalence of fungal infections varies substantially depending on sociodemographic characteristics, including geography, the proportion of people at risk of infection, and severe disease, the role of dedicated advanced clinical mycology laboratories in different regions of the country has become significant. ICMR has established a first-of-its-kind network of eight advanced mycology diagnostic and research laboratories (MycoNet) covering all geographical zones of India. This task-force program contributes to enhanced diagnostic capabilities supported by a robust infrastructure, featuring advanced equipment, diagnostic kits, and provisions for

operational costs. The AMDRCs have significantly increased the diagnostic capacity for fungal diseases, achieving accuracy levels of up to 95%, focusing on high-throughput non-culture methods such as antigen, antibody, and molecular testing, with standardized protocols ensuring consistency. AMDRCs have accomplished a remarkable 95–100% detection rate for fungal infections, compared to an unidentified isolate rate of approximately 35% before the initiation of this program. Real-time diagnostic reports and reduced diagnostic time allow clinicians to start prompt and accurate therapy, minimizing empiric antifungal treatment and overmedication, leading to a substantial public health gain by preventing untimely deaths. The integration of TDM ensures personalized antifungal management and antifungal stewardship programs and aids clinicians in making informed decisions about antifungal treatment and preventing antifungal resistance.

Furthermore, the empowerment of regional medical institutions is a key outcome, as AMDRCs conduct regular training for state hospitals and medical colleges, empowering them to enhance diagnostic capacity at the district and state levels, and reduce referring patients to higher-tier medical centers. AMDRCs also offer free/reduced-cost diagnostic services, reducing reliance on expensive private laboratories and improving access and affordability of treatment.

The clinical mycology registry established through the network for the data collection on invasive fungal infections and rare fungal diseases plays a pivotal role in assessing the burden of fungal infections. Active surveillance of fungal infections and diseases across different regions enables early detection of emerging agents and resistance patterns. This proactive approach contributes to the prevention and control of outbreaks, ultimately leading to a reduction in mortality and morbidity. Informed decision-making, supported by comprehensive data, allows policymakers to address health challenges effectively.

In the first global effort to systematically prioritize fungal pathogens, considering the lack of research and development (R&D) and perceived public health importance, the WHO released a list of 19 fungal priority pathogens in 2022 [18]. Mycology reference laboratories are pivotal in delivering vital services encompassing the timely detection, accurate identification, characterization, and research of fungal pathogens and actively monitoring emerging fungal pathogens and antifungal resistance. Introduction of newer non-culture-based serological and molecular diagnostic methods in addition to the existing conventional microscopy and culture-based methods, improved facilities for the performance of specialized tests such as antifungal susceptibility testing, TDM, and DNA sequencing have facilitated their early and accurate diagnosis and effective management. These laboratories serve as crucial resources in the field of mycology, offering expertise and specialized facilities for comprehensive analysis and understanding of fungal infections. They can potentially contribute to the development of newer diagnostic tools and the formulation of effective strategies for the management and control of fungal diseases. Apart from that, with improved diagnostic facility of serology, molecular tests along with TDM, AFST, fluorescent microscopy, and sequencing, increased the detection rate and accuracy, reduced turnaround time and drug toxicity and increased efficacy of antifungal drugs.

Despite the need for a reference laboratory network, only a few international mycology reference laboratories have developed around the world, such as (i) Centers for Disease Control and Prevention (CDC) Mycotic Diseases Branch (MDB) Atlanta, Georgia [19], a leading mycology reference laboratory in the United States. It conducts research, surveillance, and outbreak investigations related to fungal infections and also provides diagnostic support, reference materials, and expertise to healthcare

professionals and public health agencies. (ii) Public Health England (PHE) Mycology Reference Laboratory United Kingdom [20] provides training and services for the diagnosis and management of fungal infections and houses the national collection of diverse pathogenic fungi (NCPF), which plays a critical role in diagnostic microbiology by providing reference strains for accurate identification of pathogenic fungi, aiding clinicians in making informed treatment decisions and improving patient outcomes [21].

Additionally, a few fungal registries such as the Fungal Infection Network of Switzerland (FUNGINOS), the European Confederation of Medical Mycology (ECMM) Registry of invasive fungal infections, Transplant Associated Infection Surveillance Network (TRANSNET) on IFI, Mycology Interest Group Registry for invasive fungal infections in Australia and Fungal India Registry (Fung-I-Reg,) a PAN India web-based registry of fungal infections (*Aspergillus*, *Mucor*, and rare fungi). These resources are crucial in tracking and monitoring emerging fungal infections and contribute significantly to understanding, preventing, and managing fungal infections. Their work enhances diagnostic capabilities, supports research efforts, and assists in developing effective antifungal treatments and public health strategies. However, they also have certain limitations in accessibility, geographic representation, variability in quality and standardization, lack of denominator data, uniform CRF, limited diversity of fungal strains, and funding and sustainability for the operation.

Although there are now new diagnostic and treatment choices to address invasive fungal infections, there is an urgent requirement for up-to-date guidelines on utilizing these options in the clinical setting. A few working groups have been established to develop comprehensive recommendations and guidelines on various aspects of mycology to address this need. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID), has developed globally applicable comprehensive guidelines on diagnosing and treating invasive fungal infections. The “One World – One Guideline” initiative, in 2018–2019, has come up with guidelines on Mucorales [22], Rare Molds [23], Rare Yeasts [24], and Endemic Mycoses [25]. The Infectious Diseases Society of America (IDSA) has also issued clinical practice guidelines for managing Cryptococcal disease [26], Aspergillosis [27], and Candidiasis [28]. The guidelines developed by these working groups play a critical role in enhancing the detection, management, and prevention of fungal infections by providing evidence-based recommendations and strategies.

While a network of reference laboratories for better patient care, the development of treatment guidelines and new drugs and diagnostics is the utmost need of a country. An online survey conducted by Asia Fungal Working Group (AFWG) in 2016 in seven countries to assess the competencies of mycology laboratories in Asia covering laboratory structure, equipment, manpower, training, and diagnostic testing availability and identified inadequacies in medical mycology training, advanced diagnostics, limited access to antifungal drugs, and unavailability of local guidelines as targets for improvement [29, 30]. Contrary to the above study, a recent survey in the Eastern and South-Eastern regions showed increasing trends in diagnostic capabilities and facilities for fungal infections [31]. However, the condition of developing countries, that is, in Asia, Latin America, and Africa, depicts a need for constant efforts toward developing mycology laboratories and infrastructure [32–34].

The methodical program management of ICMR-MycoNet ensures that any operational setbacks are promptly addressed, maintaining smooth and sustainable operations. Regular meetings with the PIs, program coordinators, and expert members are conducted to discuss any micro-level issues and provide timely resolution.

To ensure the sustainability and systematic expansion of the reach of the program to more regional locations, including district levels there are plans of integration of this network into the existing laboratory networks under the Department of Health Research, Ministry of Health & Family Welfare, Government of India.

## **5.2 Limitations**

Although this ICMR initiative has substantial public health benefits, it has faced several limitations. One significant constraint is funding, which has resulted in the establishment of the AMDRCs in a staggered, phased manner. Currently, the network covers eight states across seven geographical zones of the country. However, there is significant potential expansion of the network given the vast geographical landscape and climate variations, which impact the epidemiology of fungal infections in India. Additionally, the recruitment and retention of skilled personnel, experienced in fungal diagnostics is challenging. Moreover, the lack of advanced infrastructure in certain regions also hampers the rapid expansion of the network. There is also a need for collaboration with state health authorities to support national programs as well as during any outbreak situation.

## **6. Conclusion**

The establishment of the ICMR Advanced Mycology Diagnostic and Research Network positively influences India's health infrastructure, research capabilities, and patient outcomes, addressing current challenges and preparing for future health emergencies. There is a need for continuous training and education programs for healthcare professionals to ensure they remain updated on the latest diagnostic techniques and treatment protocols; ICMR-MycNet contributes to our understanding of fungal pathogenesis and epidemiology. Furthermore, it can guide resource prioritization and shape national policies to address the lack of awareness among the general public about fungal infections and the importance of early diagnosis and treatment.

## **Acknowledgements**

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## **Authors' contributions**

MD developed and conceptualized the study and contributed to review and revise the manuscript. VA contributed to writing and revising the paper. SS, GS, VH, UP, and KJS critically reviewed and revised the manuscript. TK, IX, JS, RN, PG, and AS reviewed the manuscript. All authors have read and approved the manuscript.

## **Conflict of interest**

The authors declare no conflict of interest.

Data availability

The data sets used or analyzed during the study will be made available from the corresponding author on reasonable request.

Appendix

| Questionnaire headings                                         | Information                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                      |
|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| Willingness for Regional Reference Mycology Laboratory Upgrade | Desire for ICMR-supported upgrade: Yes/No<br>Supporting Points (Bullet points (4–5) justifying the upgrade)                                                                                                                                                                                                                                                                                                                                   |                                                                      |
| Information on Nodal Person in Mycology Laboratory             | Name, Designation, Experience                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                      |
|                                                                | List all the publications in Medical Mycology (Number of papers and 10 best manuscripts)<br>Mycology Projects Handled in the Past (Number of Projects, Funding Agencies, Duration, Amount Received)                                                                                                                                                                                                                                           |                                                                      |
| Human Resource Capacity of the Laboratory                      | Faculty Members in Department<br>Full-time faculty dedicated to mycology<br>Part-time faculty for mycology<br>Permanent Technicians in Mycology Laboratory                                                                                                                                                                                                                                                                                    |                                                                      |
| Equipment Availability in the Laboratory                       | BOD Incubators: 1/2/3/more<br>Biosafety Cabinet: Number<br>Spectrophotometer: Number<br>ELISA Reader: Number<br>PCR Machine (Conventional/Real-time): Number<br>Gel Electrophoresis: Yes/No<br>Gel Documentation System: Yes/No<br>Deep Freezer (–80, –20): Number<br>HPLC: Yes/No<br>Vitek 2 system/Other Automated System: Yes/No<br>MALDI-TOF: Yes/No<br>DNA Sequencer: Yes/No<br>Lyophilizer: Yes/No<br>Other Equipment (Specify): Yes/No |                                                                      |
| Availability of critical care units (ICU)                      | Number of ICU and specialty<br>Average patient load in ICU                                                                                                                                                                                                                                                                                                                                                                                    |                                                                      |
| Monthly Sample Distribution                                    | Average Monthly Samples<br>Distribution by Type (Blood, Skin Scrapings, Other Body Fluids, Tissue)                                                                                                                                                                                                                                                                                                                                            |                                                                      |
| Diagnostic Facilities available                                | Yeast Identification: Method<br>Mold Identification: Yes/No<br>Galactomannan Detection: Yes/No<br>Beta D Glucan Detection: Yes/No<br>Cryptococcal Antigen: Yes/No<br>Histopathology: Yes/No<br>Molecular Techniques (PCR/Real-time PCR): Yes/No<br>Other Tests (List): Yes/No                                                                                                                                                                 | Average Sample Load and percentage of positivity (for mycology only) |
| Antifungal Susceptibility Testing                              | Yeast/Mold: Yes/No<br>Tests done with average Monthly samples<br>Data for the previous year                                                                                                                                                                                                                                                                                                                                                   |                                                                      |

| Questionnaire headings                                                                   | Information                                                                                                                                                            |
|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Preservation of Clinical Isolates and Reference Strains                                  | Details of stored isolates                                                                                                                                             |
| Laboratory Accreditation                                                                 | Accrediting Body/Institution and Tests Accredited                                                                                                                      |
| External Quality Assurance Scheme                                                        | Accrediting Body/Institution<br>Tests Accredited<br>Last 2 Years' Scores                                                                                               |
| Reference Service to Other Laboratories/Institutes                                       | Type of diagnostic services offered<br>Average Number of Referral samples tested in a month<br>Distribution by Type (Blood, Skin Scrapings, Other Body Fluids, Tissue) |
| Top five most common diagnoses of fungi identified in the laboratory                     |                                                                                                                                                                        |
| Any rare or significant identification of fungal infections conducted in the laboratory. |                                                                                                                                                                        |

**Table A1.**

*Questionnaire-based screening for potential Investigators and institutes for ICMR-MycoNet.*

| Laboratory component                | Components                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Laboratory space and infrastructure | Total lab space (area)<br>Water supply/ distillation /Milli-Q water<br>Measures taken to combat fungal contamination /Fumigation/sterility testing<br>Equipment maintenance and cleaning<br>Specimens receiving and handling<br>Microscopic and culture preparation<br>Reporting and positive culture handling<br>Antifungal susceptibility testing area<br>Separate serological lab space<br>Molecular laboratory with separate DNA extraction, Master mix preparation and template addition area<br>Electricity back up<br>Maintenance of Refrigerator temperature chart<br>Fire safety management |
| Laboratory investigations           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Microscopy Techniques and Stains    | KOH wet mount<br>KOH with Calcofluor white<br>India ink wet mounts<br>Modified ZN stain<br>Gram stain<br>Grocott-Gomori's methenamine-silver stain<br>Periodic Acid Schiff (PAS)<br>Wright-Giemsa<br>Fluorescent antibody stain (DFA)<br>Lactophenol cotton blue                                                                                                                                                                                                                                                                                                                                     |

| Laboratory component                             | Components                                                                                                                                                                                                                                                                                                                                                                                                    |
|--------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Culture positive isolate identification          | Phenotypic /Chromogenic medium /biochemical/slide culture<br>MALDI-ToF<br>DNA Sequencing                                                                                                                                                                                                                                                                                                                      |
| Antifungal Susceptibility Testing                | Disk diffusion method/ E-test<br>Micro broth dilution<br>Sensititre<br>Vitek-2                                                                                                                                                                                                                                                                                                                                |
| Antigen/Antibody detection test                  | Cryptococcal capsular polysaccharide antigen detection (Latex/LFA)<br>Aspergillus Galactomannan antigen test (ELISA/LFA)<br>1,3-b-D-glucan (G) test<br>Histoplasma GM assay (ELISA/LFA)<br>Candida mannan-anti mannan assay<br>Aspergillus antibody detection (IgG/IgE)<br>Immunodiffusion assay (Inhouse/commercial Antigen)<br>ID Skin test for Aspergillus/Histoplasma etc.<br>Any other serological tests |
| Molecular Diagnosis                              | Conventional PCR<br>Real Time PCR<br>DNA sequencing<br>Typing techniques (RFLP/AFLP/MLST)                                                                                                                                                                                                                                                                                                                     |
| Therapeutic drug monitoring practice             | Bioassay<br>HPLC/UV-DAD<br>LC-MS                                                                                                                                                                                                                                                                                                                                                                              |
| Quality control and records                      | EQAS participation<br>Internal QC (Media/stains/kits/TDM)<br>Procedural control (For ex.- Back up CSF culture on antigen positive)<br>Maintenance of daily lab log notebook/temperature recording<br>Autoclave                                                                                                                                                                                                |
| Biosafety practices                              | Class 2A or higher-class Biosafety cabinets (BSCs)<br>Spill management training<br>Waste management guidelines<br>Good laboratory practices                                                                                                                                                                                                                                                                   |
| Laboratory response capacity and training        | Adequate qualified laboratory personnel<br>Dedicated permanent staff for Mycology laboratory<br>Regular competence evaluation and training<br>Receive referrals for identification or confirmation                                                                                                                                                                                                            |
| Interdepartmental engagement and coordination    | Complete detailed clinical requisition Performa for mycology investigation<br>Clinical correlation of laboratory results and communication to clinicians<br>Frequent lab to clinic interaction and vice-versa                                                                                                                                                                                                 |
| Manpower development and knowledge dissemination | Short/long term training for mycology staff<br>CME/Hands on workshop in the region<br>Use of print/social media for community awareness building and knowledge dissemination<br>MycNet website activity                                                                                                                                                                                                       |
| Research and publication                         | Identify priority research of your area and research gaps<br>Surveillance and outbreak investigation if any<br>Sourcing for research funding<br>Publications                                                                                                                                                                                                                                                  |

**Table A2.**  
*Checklist for site visit assessment to evaluate the prospective institute.*

| <b>State:</b>                                                       |                                                                                                      |                                         |          |          |           |          |           |          |
|---------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|-----------------------------------------|----------|----------|-----------|----------|-----------|----------|
| <b>Centre Name:</b>                                                 |                                                                                                      |                                         |          |          |           |          |           |          |
| <b>Date:</b>                                                        |                                                                                                      |                                         |          |          |           |          |           |          |
| Domain 1: Infrastructure, Networking Research (average of 6 months) |                                                                                                      |                                         |          |          |           |          |           |          |
| S.No.                                                               | Quantitative measures                                                                                | At the beginning of the project (0 day) | 6 months | % change | 12 months | % change | 18 months | % change |
| D.1.(a) Infrastructure development                                  |                                                                                                      |                                         |          |          |           |          |           |          |
| 1                                                                   | Total space allocated to AMDRC                                                                       |                                         |          |          |           |          |           |          |
| 2.                                                                  | Development of separate labs for serology, molecular, culture/AFST/TDM etc.                          |                                         |          |          |           |          |           |          |
| 3                                                                   | Fungal repository (total number of isolates stocked along with methods used)                         |                                         |          |          |           |          |           |          |
| 4                                                                   | Development own mycology website and online data repository                                          |                                         |          |          |           |          |           |          |
| D.1.(b) Network development                                         |                                                                                                      |                                         |          |          |           |          |           |          |
| 5                                                                   | Total number of Referrals/requests from other Institutes (include details)                           |                                         |          |          |           |          |           |          |
| 6                                                                   | Awareness programmes organized-<br>• For physicians<br>• General public                              |                                         |          |          |           |          |           |          |
| 7                                                                   | Number of trainings imparted/ conference/ CME /seminar etc.                                          |                                         |          |          |           |          |           |          |
| D.1.(c) Research activities                                         |                                                                                                      |                                         |          |          |           |          |           |          |
| 8                                                                   | Collaborative projects (total number and institute wise):<br>-Surveillance study<br>-any major study |                                         |          |          |           |          |           |          |
| 9                                                                   | Extramural Projects in Mycology (mention number along with funding and name of funding agency)       |                                         |          |          |           |          |           |          |
| 10                                                                  | Intramural Projects (total number and funds allocated)                                               |                                         |          |          |           |          |           |          |

| <b>State:</b>                                                       |                                                                                                                                                                                                                                 |                                                    |          |           |           |           |           |          |
|---------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|----------|-----------|-----------|-----------|-----------|----------|
| <b>Centre Name:</b>                                                 |                                                                                                                                                                                                                                 |                                                    |          |           |           |           |           |          |
| <b>Date:</b>                                                        |                                                                                                                                                                                                                                 |                                                    |          |           |           |           |           |          |
| Domain 1: Infrastructure, Networking Research (average of 6 months) |                                                                                                                                                                                                                                 |                                                    |          |           |           |           |           |          |
| S.No.                                                               | Quantitative measures                                                                                                                                                                                                           | At the beginning of the project (0 day)            | 6 months | % change  | 12 months | % change  | 18 months | % change |
| 11                                                                  | Number of samples received within your institute over 6 months period<br><ul style="list-style-type: none"><li>• Superficial fungal infections</li><li>• Systemic fungal infections (mention details department wise)</li></ul> |                                                    |          |           |           |           |           |          |
| 12.                                                                 | Publications                                                                                                                                                                                                                    |                                                    |          |           |           |           |           |          |
| D. 1. (d) Quality Indicators                                        |                                                                                                                                                                                                                                 |                                                    |          |           |           |           |           |          |
| 13                                                                  | Turnaround time (in hrs)<br><ul style="list-style-type: none"><li>• KOH</li><li>• CrAg</li><li>• GM</li></ul>                                                                                                                   |                                                    |          |           |           |           |           |          |
| 14                                                                  | Performance in external QC schemes (mention name of scheme along with score)                                                                                                                                                    |                                                    |          |           |           |           |           |          |
| 15                                                                  | Any Additional inputs:                                                                                                                                                                                                          |                                                    |          |           |           |           |           |          |
| Domain 2: Test portfolio and outbreak investigation                 |                                                                                                                                                                                                                                 |                                                    |          |           |           |           |           |          |
| S. No                                                               | Name of Test                                                                                                                                                                                                                    | Availability at the beginning of the project (Y/N) | 6 months | 12 months | 18 months | 24 months | 36 months |          |
| D.2.(a) Test portfolio                                              |                                                                                                                                                                                                                                 |                                                    |          |           |           |           |           |          |
| 1                                                                   | KOH-Calcofluor                                                                                                                                                                                                                  |                                                    |          |           |           |           |           |          |
| 2                                                                   | CrAg testing                                                                                                                                                                                                                    |                                                    |          |           |           |           |           |          |
| 3                                                                   | Automated fungal blood culture                                                                                                                                                                                                  |                                                    |          |           |           |           |           |          |
| 4                                                                   | Vitek 2 ID & AST                                                                                                                                                                                                                |                                                    |          |           |           |           |           |          |
| 5                                                                   | MALDI-TOF based ID                                                                                                                                                                                                              |                                                    |          |           |           |           |           |          |
| 6                                                                   | Galactomannan testing                                                                                                                                                                                                           |                                                    |          |           |           |           |           |          |

| <b>Domain 2: Test portfolio and outbreak investigation</b>    |                                                                                                          |                                                    |          |           |           |           |           |           |
|---------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|----------------------------------------------------|----------|-----------|-----------|-----------|-----------|-----------|
| S. No                                                         | Name of Test                                                                                             | Availability at the beginning of the project (Y/N) | 6 months | 12 months | 18 months | 24 months | 36 months |           |
| 7                                                             | GM LFA/ OLM Asp Ag                                                                                       |                                                    |          |           |           |           |           |           |
| 8                                                             | Aspergillus Ig M/IgG Antibody                                                                            |                                                    |          |           |           |           |           |           |
| 9                                                             | Beta-D-Glucan testing                                                                                    |                                                    |          |           |           |           |           |           |
| 10                                                            | Histoplasma Ag testing                                                                                   |                                                    |          |           |           |           |           |           |
| 11                                                            | AFST by BMD (CLSI)                                                                                       |                                                    |          |           |           |           |           |           |
| 12                                                            | AFST by Sensititre                                                                                       |                                                    |          |           |           |           |           |           |
| 13                                                            | AFST by Disk diffusion (CLSI)                                                                            |                                                    |          |           |           |           |           |           |
| 14                                                            | Therapeutic Drug Monitoring <ul style="list-style-type: none"> <li>• Bioassay</li> <li>• HPLC</li> </ul> |                                                    |          |           |           |           |           |           |
| 15                                                            | Molecular tests                                                                                          |                                                    |          |           |           |           |           |           |
| <b>D.2.(b) Outbreak investigation</b>                         |                                                                                                          |                                                    |          |           |           |           |           |           |
| 16                                                            | Outbreaks investigated, if any (brief description and findings)                                          |                                                    |          |           |           |           |           |           |
| 17                                                            | Rapid situation & Response Assessment (RSRA) report/Critical call alerts (avg. of 6 months)              |                                                    |          |           |           |           |           |           |
| 18                                                            | Any other                                                                                                |                                                    |          |           |           |           |           |           |
| <b>Domain 3: Equipment, human resource/manpower, training</b> |                                                                                                          |                                                    |          |           |           |           |           |           |
| S. No                                                         | Quantitative measures                                                                                    | Beginning of the project                           | 6 months | 12 months | 18 months | 24 months | 30 months | 36 months |
| <b>D.3.(a) Equipments:</b>                                    |                                                                                                          |                                                    |          |           |           |           |           |           |
| 1                                                             |                                                                                                          |                                                    |          |           |           |           |           |           |
| 2                                                             |                                                                                                          |                                                    |          |           |           |           |           |           |
| 3                                                             |                                                                                                          |                                                    |          |           |           |           |           |           |
| 4                                                             |                                                                                                          |                                                    |          |           |           |           |           |           |

| Domain 3: Equipment, human resource/manpower, training |                       |                          |          |           |           |           |           |           |
|--------------------------------------------------------|-----------------------|--------------------------|----------|-----------|-----------|-----------|-----------|-----------|
| S. No                                                  | Quantitative measures | Beginning of the project | 6 months | 12 months | 18 months | 24 months | 30 months | 36 months |
| 5                                                      |                       |                          |          |           |           |           |           |           |
| 6                                                      |                       |                          |          |           |           |           |           |           |
| 7                                                      |                       |                          |          |           |           |           |           |           |
| 8                                                      |                       |                          |          |           |           |           |           |           |
| D.3.(b) Manpower recruitment:                          |                       |                          |          |           |           |           |           |           |
| 1                                                      | Faculties             |                          |          |           |           |           |           |           |
| 2                                                      | Technical staff       |                          |          |           |           |           |           |           |
| 3                                                      | Scientists            |                          |          |           |           |           |           |           |
| 4                                                      | PG students           |                          |          |           |           |           |           |           |
| 5                                                      | PhD students          |                          |          |           |           |           |           |           |
| 6                                                      | IT person             |                          |          |           |           |           |           |           |
| 7                                                      | any other             |                          |          |           |           |           |           |           |
| D.3.(c) Manpower training:                             |                       |                          |          |           |           |           |           |           |
| 1                                                      | Clinicians            |                          |          |           |           |           |           |           |
| 2                                                      | Technical staff       |                          |          |           |           |           |           |           |
| 3                                                      | Scientists            |                          |          |           |           |           |           |           |
| 4                                                      | PG students           |                          |          |           |           |           |           |           |
| 5                                                      | PhD students          |                          |          |           |           |           |           |           |
| 6                                                      | Any other             |                          |          |           |           |           |           |           |

**Table A3.**  
 ICMR-MycoNet performance monitoring indicator tool.

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# Perspective Chapter: Zhuxiang Syndrome – A Novel Mechanism Linking Chronic Fungal Infections to Systemic Inflammatory Diseases in Aging

*Xiujuan Yan, Jian An and Charles Xiaoxiang Zhu*

## Abstract

Aging is a complex biological process characterized by progressive physiological changes that elevate the risk of various diseases. One of the most notable consequences of aging is “inflammaging,” a chronic, low-grade inflammatory state linked to the development of several age-related diseases, including cardiovascular and musculoskeletal disorders, as well as skin conditions. Chronic fungal infections, particularly those caused by *Aspergillus sydowii*, have emerged as significant contributors to exacerbating systemic inflammation in aging populations. This study focuses on a 58-year-old male with a 43-year history of onychomycosis and tinea pedis, who developed recurrent fungal infections leading to systemic inflammation manifesting as arthritis, dermatitis, and cardiovascular abnormalities. Over a 3-year period, we examined the impact of environmental factors, fungal-secreted proteins, and antifungal therapies on his condition. Our findings suggest that *Aspergillus sydowii* can form composite fungal-human cells, termed “zombie cells,” within the foot skin. These cells, which integrate fungal components with anucleated human keratinocytes, are not dormant but exhibit fungal-like characteristics that trigger systemic inflammatory responses. This phenomenon is proposed to underlie the newly identified Zhuxiang syndrome, which highlights a novel mechanism linking chronic fungal infections to systemic inflammatory diseases in the elderly. By shedding light on the role of chronic fungal infections in the pathogenesis of systemic diseases, this study offers insights into the interplay between aging, inflammation, and fungal pathogenesis, while suggesting potential therapeutic avenues for the management of fungal-related inflammatory conditions in older adults.

**Keywords:** inflammaging, *aspergillus sydowii*, Zhuxiang syndrome, chronic fungal infection, systemic inflammation, arthritis, dermatitis, aging, aspergillus skin infection, fungal-secreted proteins, chronic inflammation, environment factors of arthritis, Zhuxiang syndrome

## 1. Introduction

Aging is a complex biological process characterized by progressive physiological changes that increase susceptibility to various diseases [1, 2]. A prominent feature of aging is “inflammaging,” a chronic low-grade inflammatory state marked by elevated levels of pro-inflammatory cytokines and immune markers [3]. This persistent inflammation is implicated in the development and progression of numerous age-related diseases [4], including cardiovascular diseases [5], metabolic disorders [6], musculoskeletal ailments [7], respiratory conditions [8], skin disorders [9], and heightened vulnerability to infections [10].

The human body harbors a vast microbiome, estimated to consist of over 30–50 trillion microbial cells, including bacteria and fungi, comparable to the number of human cells [11]. As individuals age, this balance is often disrupted, leading to an increased incidence of microbial infections [12–14]. Among the elderly, particularly those aged 70 and above, the prevalence of infections such as bloodstream infections and dermatological conditions like onychomycosis and tinea pedis can reach as high as 50% in the United States [15].

A significant gap in knowledge exists regarding the role of chronic fungal infections in exacerbating systemic inflammation and contributing to age-related diseases [16]. Recent studies have highlighted the pathogenic potential of certain fungal species, such as *Aspergillus sydowii*, which can form fungal-human composite cells termed “zombie cells” within the foot skin [17]. These “zombie cells” represent a novel and complex entity where the fungus integrates with anucleated human keratinocytes, effectively reanimating these cells with fungal characteristics. This integration results in cells that are capable of growth and division, leading to the formation of hyperkeratosis-like lesions known as Xiangya lesions [18]. These lesions are not only resistant to conventional antifungal treatments but are also capable of secreting fungal proteins, which may contribute to systemic inflammation.

This study addresses a crucial gap in knowledge by exploring the correlation between environmental factors, recurrent fungal infections, and systemic inflammatory symptoms over a 3-year period in a 58-year-old male with a 43-year history of onychomycosis and tinea pedis. Our observations suggest that persistent onychomycosis and Xiangya lesions may trigger the relapse of active fungal infections, potentially leading to systemic diseases such as chronic arthritis, dermatitis, and cardiovascular abnormalities. By introducing the concept of Zhuxiang syndrome, we aim to describe a potentially under-recognized mechanism that links chronic fungal infections to systemic inflammation and disease progression, particularly in the elderly. This study is important as it sheds light on the complex interplay between chronic fungal infections, inflammation, and the aging process, offering new insights into the pathogenesis of systemic diseases and the development of targeted therapeutic strategies.

## 2. Methods

The patient was a 58-year-old male who experienced flare-ups of arthritis on multiple finger joints and dermatitis after moving to Wuhan. At age 15–22, he had a history of joint stiffness, swelling, and pain in fingers and elbows during winters in Changsha. He also had a history of tinea pedis, onychomycosis, and plantar callus for over 43 years. In December of 1982, he suddenly developed a low fever with itchy and painful skin lesions over his back and limbs after consuming alcohol and experiencing

insomnia. He was diagnosed with erythrodermic psoriasis and treated with topical hydrocortisone ointment and oral antibiotics, which had little effect. However, 2 weeks later, he moved to Beijing, where the ambient temperature was higher and humidity was lower than in Changsha. The lesions disappeared without treatment in 3–4 weeks and did not recur since then. His arthritis symptoms improved after moving to Beijing and Michigan for the last 30 years, but finger stiffness remained. He had a 40-year smoking history but was otherwise healthy. When he moved to Wuhan in February 2015, he provoked an arthritis attack while receiving aggressive treatment for onychomycosis and plantar hyperkeratotic lesions. His arthritis symptoms subsided gradually within a month following the improvement of foot lesions. He had two aunts with psoriasis vitiligo and ankylosing spondylitis after their seventies but no other family history of arthritis.

On foot examination, the patient had onychomycosis of seven toenails ranging from distal and lateral subungual form to total dystrophic form, with two on the left and five on the right foot. He had three plantar callus-like hyperkeratosis lesions on both feet that caused pressing pain, which combined to approximately 30 square centimeters. On hand examination, he had proximal interphalangeal joints fusiform with tenderness on the middle and index fingers. The patient's fingers had stiffness with gaps of 3–5 millimeters between distal and proximal phalanges at maximum flexing, which became worse in the morning, and bumpy nodules on the digital skin.

*Ethical considerations:* Informed consent was obtained from the patient prior to the commencement of any procedures or treatments described in this study. All procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

*Observations of weather and fungal infections relapse:* To investigate the relationship between weather conditions and relapse of fungal infections, historical weather data for Wuhan City from May 1, 2015, to April 30, 2018, was obtained from Weather Underground. Patients with foot lesions were examined every 1–3 days, and photographs were taken. Relapse was defined as the re-emergence of visible skin inflammation (redness, scaling, or vesicles) in previously treated areas or the appearance of new lesions adjacent to treated areas. To determine the occurrence of relapses, skin inflammation was retrospectively assessed based on the photographs. The 7-day period preceding the date of each photograph was considered as the time needed for fungal growth to become visible as skin inflammation. The means of temperature, humidity, barometer, visibility, wind, and days of rain during this 7-day period were compared to the annual weather record. Statistical analysis was performed using SPSS Statistics 17.0 software (SPSS, Chicago, IL). Specifically, we used independent samples t-tests to compare the means of weather variables (temperature, humidity, etc.) during relapse periods with the means of the same variables during non-relapse periods. Pearson correlation coefficients were calculated to assess the relationship between individual weather variables and the frequency of relapse. A p-value of less than 0.05 was considered statistically significant.

*Assessment of finger stiffness with caliper:* Joint stiffness, particularly morning stiffness, is a hallmark symptom reported by arthritis patients and is used clinically as an indicator of inflammatory disease activity. Joint stiffness severity is often subjectively rated by patients on visual analog scales to quantify daily variations and treatment response. However, subjective measures can be prone to variability and lack sensitivity to detect subtle changes. An objective, standardized method to directly measure joint stiffness could provide a more precise, reproducible metric to monitor arthritis progression and treatment efficacy. A method for quantitatively assessing finger joint

stiffness in hand arthritis was developed for this study. This involved using a caliper with 0.02 mm precision to measure the interphalangeal joint stiffness of the second through fifth fingers (F2-F5) of the hands. The external jaw of the caliper was pushed until pain or resistance occurred under pressure, and the distance between the nail and the backside of the proximal phalanx was measured at maximal flexion. The measurements were taken at the same time on different days due to the characteristic fluctuation of hand stiffness, and repeated measurements were avoided to prevent affecting the joints and reducing the stiffness. Finger stiffness is calculated by subtracting finger thickness, which includes proximal and distal phalanx thickness, from the measured reading. This method measures the stiffness of the proximal and distal joints of the fingers, excluding the metacarpophalangeal joint.

To evaluate the reliability of the caliper measurement, an evaluation test was conducted five times a day for 25 days during the summertime when arthritis symptoms were stable. For a single measurement, the intra-class correlation coefficient (ICC) was 0.924, with a 95% confidence interval of 0.890 to 0.953. The average of 25 measurements had higher intra-rater reliability with an ICC of 0.997 and a 95% confidence interval of 0.995–0.998, indicating high reproducibility.

*Fungal-secreted proteins:* Foot *Aspergillus sydowii* infection of this patient was characterized previously [18]. For fungal-secreted proteins preparation and analysis, *Aspsy* primary cultures of skin lesions were subsequently cultured using human cell culture medium (ATG293, Shenzhen Yisheng Branch Co., Ltd., China) and collected by centrifugation and filtered twice through a 0.22  $\mu$ M cut-off syringe filter and The filtered medium was used for subcutaneous injection, and the remaining medium was concentrated 33 times on centrifugal filter and quantified on SDS-PAGE. Thirty protein bands ranging from 5kD to 200kD were detected and quantified with gel image analysis software, resulting in equivalent concentrations of the proteins in the original medium ranging from 0.01 to 1.5  $\mu$ g/mL. The concentrated protein was diluted with phosphate-buffered saline (PBS) to five times the concentration of the original medium and then further sterilized by filtering through a syringe filter with a cut-off size of 0.2  $\mu$ M before being stored in a refrigerator at 4°C.

*Subcutaneous injection of fungal proteins:* In this study, we administered fungal-secreted protein *via* subcutaneous injection to a human subject, following skin allergy testing guidelines [19]. We selected the subcutaneous route to simulate natural skin infections. Two observational trials were conducted over 3 years: the first trial lasted for 193 days and involved daily injections for 46 days, while the second trial lasted 116 days with injections given for 9 days. Finger stiffness was measured using calipers five times daily, at waking, 12 pm, 4 pm, 8 pm, and midnight. Joint pain and tenderness were self-assessed and recorded once daily using a visual analog scale [20]. Clinical laboratory tests, including full blood cell count, liver and kidney function tests, rheumatoid factor, anti-cyclic citrullinated peptide (anti-CCP) and C-reactive protein autoantibodies, and erythrocyte sedimentation rates, were conducted, and X-ray examinations were also performed in outpatient settings. Statistical analysis of the visual analog scale data was performed using repeated measures ANOVA to assess changes in pain and tenderness scores over time in relation to the subcutaneous injections.

*Treatment of fungal infection:* The aim of this study was to effectively control an active fungal infection and observe how the treatment affected arthritic symptoms. Topical antifungal treatments, such as terbinafine and ciclopirox creams, were applied twice daily for 12 weeks. Daily oral terbinafine 125 mg was administered for 4 weeks [21]. Additionally, methotrexate 5 mg/vial was mixed with terbinafine cream to form a 0.2% methotrexate cream for topical use, applied on trimmed lesions four

times a day. Eighteen vials of this mixture were applied to foot lesions over a 4-month period within a year. To isolate lesion cells from the atmosphere, prevent Zombie cell growth, and alleviate topical irritation symptoms, cyanoacrylate glue was also applied to freshly trimmed lesions to form a thin film [22, 23].

*Definition of Xiangya Lesions:* For the purposes of this study, “Xiangya Lesions” refers to the hyperkeratotic plantar lesions observed on the patient’s feet. These lesions are characterized by thickened, hardened skin resembling calluses and were identified by visual examination and palpation. They are described as “plantar callus-like hyperkeratosis lesions” in the patient’s initial presentation.

### 3. Results

#### 3.1 Weather conditions and fungal infection relapses

During a period spanning from May 1, 2015, to April 30, 2018, ten instances of relapse of plantar fungal infection were observed and recorded through photographs. All ten relapses occurred at the hyperkeratosis-like Xiangya lesion, with eight of them happening between October and March, which corresponds with winter and early spring. The remaining two relapses occurred in August and September of 2017, when the subject was exposed to air-conditioning facilities for more than 20 hours per day. During the relapses, the seven-day weather conditions prior to each occurrence were significantly different from the average data over 3 years, showing higher pressure ( $p < 0.0005$ ), lower temperature ( $p < 0.01$ ), rainy days ( $p < 0.01$ ), and higher humidity ( $p < 0.025$ ) (**Table 1**). The relapses occurred predominantly during winter and early spring months, with the highest frequency in November through March. This suggests that colder, humid weather conditions exacerbate fungal infections, supporting the idea that environmental factors play a crucial role in the progression of Zhuxiang syndrome.

#### 3.2 Relationship between fungal infections and arthritis

Some instances of relapsed fungal infections caused topical inflammatory symptoms such as redness, warmth, pain, and swelling, accompanied by 3–5 days of

| Weather conditions <sup>*</sup>    |         | Temperature<br>(°C) | Humidity<br>(%) | Barometer (hPa) | Visibility<br>(km) | Wind<br>speed<br>(km/h) | Rain<br>(days) |
|------------------------------------|---------|---------------------|-----------------|-----------------|--------------------|-------------------------|----------------|
| Seven days<br>prior to<br>relapses | Average | 11.5                | 81.4            | 1023            | 6.1                | 8.5                     | 4              |
|                                    | (Range) | (−1 ~ 19)           | (62 ~ 92)       | (1017 ~ 1030)   | (3 ~ 9)            | (6 ~ 11)                | (2 ~ 5)        |
| 3 year                             | Average | 17.6                | 75.3            | 1015.7          | 7.2                | 8.5                     | 3              |
|                                    | (Range) | (−3 ~ 34)           | (31 ~ 100)      | (997 ~ 1046)    | (1 ~ 25)           | (2 ~ 26)                | (0 ~ 7)        |
| P-value                            |         | <0.01               | <0.025          | <0.0005         | <0.05              | <0.5                    | <0.01          |

<sup>\*</sup>The daily averages of temperature, humidity, barometer, visibility, wind, and rainy days in the 7 days before the photograph date of the relapses were investigated for their associations with the occurrences. The results showed that the weather conditions preceding the relapses were significantly different from the three-year average data. 3 years from May 1, 2015, to April 30, 2018.

**Table 1.**  
*Impact of weather on eight relapses of foot fungal infection.*

fatigue. These symptoms were followed by the onset of arthritis symptoms within a few days, which included stiffness, pain, tenderness, and swelling around the joints of fingers, toes, limbs, and lumbar area (**Figures 1 and 2**), along with a painful bumpy nodules on near the joint of the right fourth finger. Arthritis persisted throughout the course of the fungal infection. Although no anti-arthritis remedy was administered, arthritis symptoms subsided in a week to 2 weeks after plantar inflammation resolved.

### 3.3 Laboratory test results

During the period of arthritis symptoms, blood monocyte counts were elevated at  $0.61\text{--}0.79 \times 10^9/\text{L}$ , returning to  $0.51\text{--}0.57 \times 10^9/\text{L}$  (norm  $0.10\text{--}0.60 \times 10^9/\text{L}$ ) during remission. Serum galactomannan (GM) levels were 0.04 (invasive aspergillosis  $>0.5$ ). Other laboratory tests, including complete blood count, liver and kidney function tests, rheumatoid factor ( $<20.0$  IU/mL, norm  $<20.0$  IU/mL), anti-CCP antibody ( $<0.5$  U/mL, norm  $0\text{--}5$  U/mL), erythrocyte sedimentation rates (2 mm/H, norm  $0\text{--}15$  mm/H), and C-reactive protein ( $0.6\text{--}0.9$  mg/L, norm  $0.1\text{--}3.0$  mg/L) all yielded results within normal ranges during relapses or remissions. X-rays showed asymmetric increased soft tissue thickness on the affected fingers and interphalangeal joints but revealed no bone or cartilage deformities on the joints of the hands and fingers (data not shown).

### 3.4 Fungal-secreted proteins induce arthritis

The amount of fungal-secreted proteins used for subcutaneous injection was equivalent to 1 mL of fungal culture medium (either straight medium or  $200 \mu\text{L}$  of



**Figure 1.**  
*Clinical manifestations of fungal infection, arthritis, and dermatitis.*

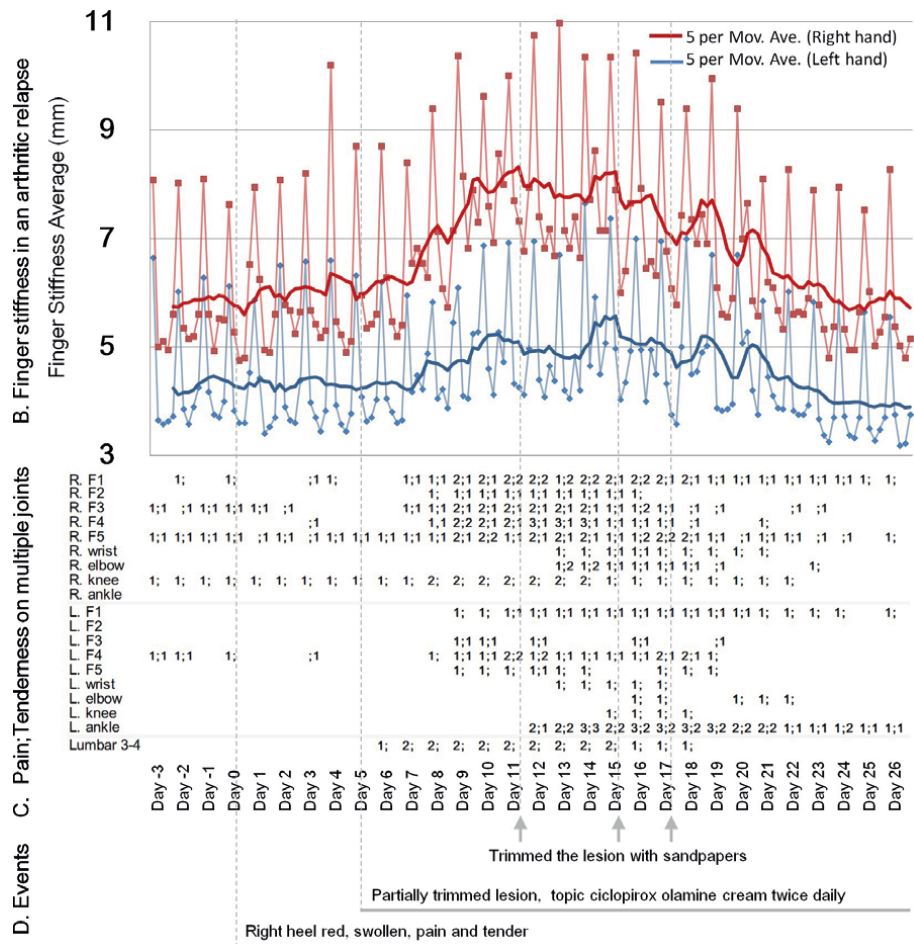


**Figure 2.**  
*Quantitative analysis of arthritis symptoms during a fungal infection flare-up.*

five times concentrated protein solution in PBS), which consisted of approximately 30 secreted protein peptides at concentrations ranging from 0.01 to 1.5  $\mu\text{g/mL}$ . The causative protein(s) appeared rather stable as the protein solution used for injections was stored at 4°C for 14 months and still induced arthritis.

Subcutaneous injections to the front of the thighs caused local skin reactions, including redness, swelling, pain, and tenderness in areas of 4–8 cm in diameter, starting 3–4 hours after injection and lasting 2–4 days (**Figure 1E**). This reaction resembled the appearance of skin reactions to fungal infection or trimming Xiangya lesions. Arthritis symptoms, as measured by finger stiffness, started 2–3 days after the initial injection. Within 7–9 days of ceasing injections, symptoms quickly improved (**Figure 3B** and **C**). Nine consecutive days of once-daily injection induced arthritis symptoms for 15 days (**Figure 3**). Prolonged injection periods extended the duration of arthritis accordingly (**Figure 4**). Elevated blood monocyte counts of  $0.65 \times 10^9/\text{L}$  also occurred. The secreted protein(s) causing arthritis was highly potent, requiring no more than 2  $\mu\text{g/day}$  in each subcutaneous injection—100,000 times less than the 200 mg of anti-TNF $\alpha$  antibody used per injection dose to treat arthritis.

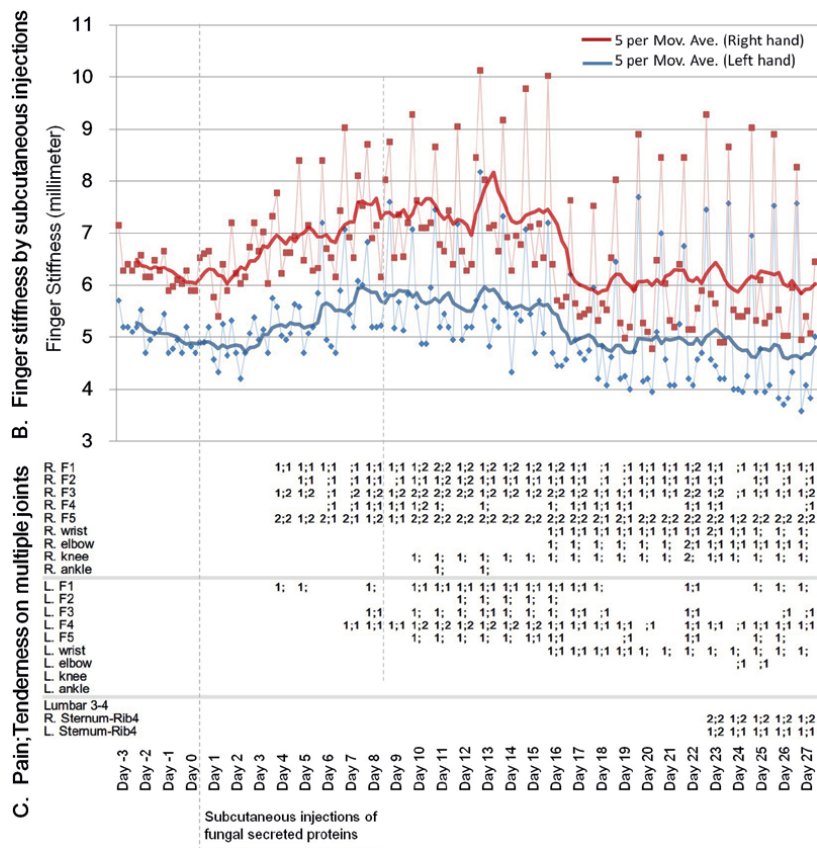


**Figure 3.**  
*Quantitative analysis of arthritis symptoms during subcutaneous injection of fungal-secreted proteins.*

Before self-experimentation with subcutaneous injection of fungal proteins, 100  $\mu$ L of culture medium from each of six fungal strains and fresh medium as control were injected subcutaneously into seven groups of three mice each for 8 consecutive days. Observations continued for 10 days after the injections. No apparent adverse effects or abnormalities of the skin or joints resulted from the injections (data not shown).

### 3.5 Activation of Xiangya lesions exacerbate arthritis

Excessive growth of plantar hyperkeratosis-like lesions (Xiangya lesions) caused walking pain under the lesions, requiring trimming of the lesions every 2 months for pain relief. Repeated trimming/debridement of the lesions led to rapid growth of lesion cells and local stimulation sensations for 2–3 days from fungal proteins, followed by arthritis symptoms within 2 days (**Figure 4**). Twice-daily topical application of methotrexate to the lesion immediately after trimming/debridement provided local symptom relief and prevented worsening of arthritis symptoms (**Figure 4**). Similarly, covering the trimmed lesions with cyanoacrylate glue also relieved local symptoms and lessen arthritis symptoms.

Dermatitis is a skin manifestation associated with arthritis relapses caused by foot fungal infections and activation of Xiangya lesions. It was less noticeable when proteins from fungal cultures were used in subcutaneous injection. Dermatitis appeared

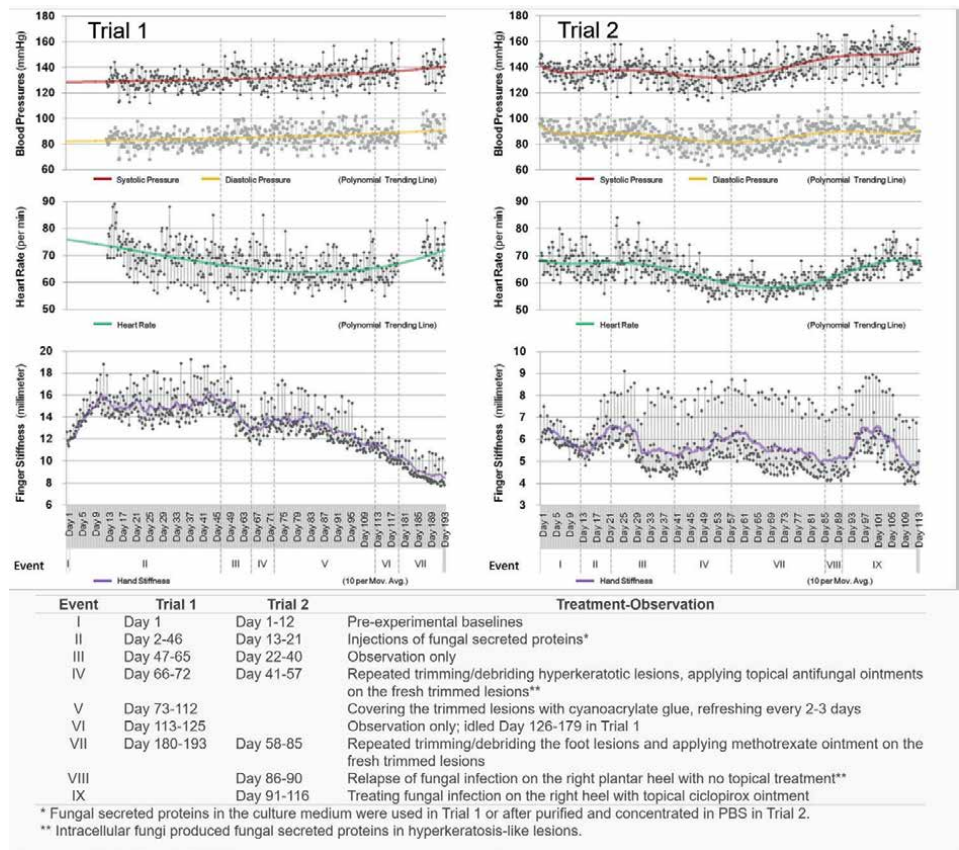


**Figure 4.**  
*Longitudinal analysis of hand stiffness, blood pressure, and heart rate during fungal infection relapses and treatments.*

gradually on various parts of the body following relapses of foot fungal infections and arthritis, lasting longer. It presented as red, itchy, hyperkeratotic, rough, and pigmented skin with bumpy nodules occurring on or near arthritic joints, which were red and painful during active arthritis and painless in remission phase (**Figure 1**). Peeling of the dermatitic skin occurred months after arthritis symptoms subsided. This type of dermatitis is common in the general population and considered a cosmetic problem. The skin returned to normal without any topical treatment, but residual finger stiffness, dermatitis on the back of fingers, finger nodules, and high blood pressure persisted throughout the year.

### 3.6 Cardiovascular abnormalities

Edema, high blood pressure, and bradycardia were associated with skin fungal infections (**Figure 4**). Edema manifested as swollen digital joints, finger stiffness, and pitting edema of the plantar soles, especially in the morning or after walking for 1 hour (**Figure 4**). Its appearance coincided with arthritis but lasted longer, becoming less severe and eventually disappeared in late summer and autumn.

An increase in blood pressure (BP), especially systolic pressure, was associated with foot fungal infections and fungal-secreted proteins in the two trials. The 30-day

average BP (systolic/diastolic pressure) increased from 129/83 mmHg in the early phase of the first trial (N = 150) to 150/89 mmHg at the end of the second trial (N = 150) over 1.5 years (**Figure 4**). The 30-day average BP was 147/83 mmHg over 4 months without antihypertensive medication after the second trial ended. Systolic pressure increased more than diastolic pressure, indicating decreased vascular compliance, possibly due to atherosclerosis or a mechanism similar to that causing nodule formation on the fingers. Further research is needed to determine the cause of hypertension.

The subject's heart rate gradually decreased from 70 to 80 beats per minute to below 60 beats per minute during the two trials (**Figure 4**). Sinus bradycardia was seen on an electrocardiogram. After the first trial, the daily average heart rate slowly returned to 65–75 beats per minute (N = 35 in 7 days) over 4 months. After the second trial, the rate was 65–70 beats per minute (N = 35 in 7 days) over 4 months and 70–82 beats per minute (N = 35 in 7 days) over 8 months with remission of the fungal infection.

### **3.7 Methotrexate treatment and effects**

The topical application of 0.2% Methotrexate ointment twice daily for 7 days on freshly trimmed hyperkeratotic lesions showed slow growth of the lesion, relief of topical irritation, and prevention of arthritis symptoms induced by trimming/debriding the Xiangya lesions (see **Figure 4**). Methotrexate may also reduce arthritis symptoms when applied to lesions of fungal infection. However, methotrexate did not appear to cure Xiangya lesions, reduce lesion size in the long term, or prevent relapses of fungal infection after cessation.

### **3.8 Antifungal treatments and impacts on systemic health**

Various treatments were used to manage chronic foot fungal infections and associated systemic symptoms over a 14-month period. Topical/oral antifungals and topical methotrexate suppressed acute infections (**Figure 2**) but did not preclude recurrence, highlighting pathogen persistence. Frequent retreatment was required, although none of the interventions achieved a cure.

Caliper use and exercise were associated with temporary gains in finger joint function (**Figure 4**), offering symptom relief between acute flare-ups. However, Xiangya lesions, onychomycosis, and *\*Aspergillus\** colonization persisted, and treatment could not clear these completely.

The average 14-day finger stiffness decreased from 13.5 mm to 6.08 mm over a two-year period, indicating a 7.4-millimeter (54.9%) improvement through oral terbinafine (45 days, three times in 2 years), topical antifungals/methotrexate, and regular caliper use/exercise (**Figure 4**). However, the need for frequent retreatment due to infection recurrence indicates ongoing disease activity and impacts. More recently, in the last year, through regular preventive antifungal regimens, such as 1 week per month of itraconazole 100 mg/day, the relapse of fungal infection has not recurred, and residue of arthritis, dermatitis, and hypertension are also gradually returning to normal (data not shown).

### **3.9 Formation of Xiangya lesions and “zombie cells”**

Xiangya lesions, characterized by hyperkeratosis and fungal invasion, were consistently observed in the patient's feet, particularly on the toenails and plantar skin. These lesions were resistant to conventional antifungal treatments and showed

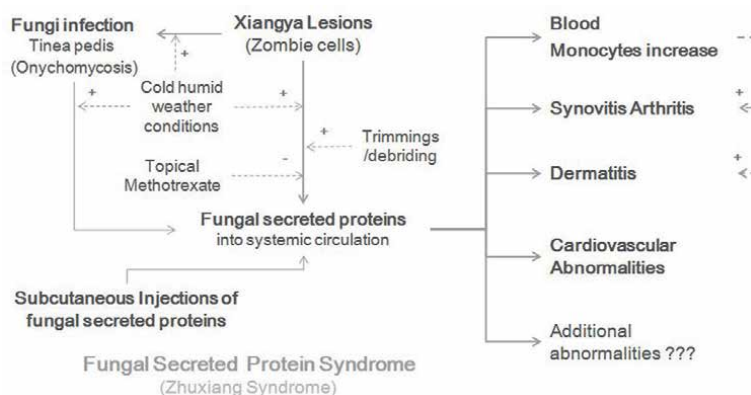


**Figure 5.**  
 Summary of fungal secreted protein-induced chronic systemic abnormalities.

persistence over time. Microscopic analysis revealed the presence of “zombie cells,” where *Aspergillus sydowii* fungal cells integrated with anucleated human keratinocytes, forming composite cells capable of growth and division. These lesions secreted fungal proteins, which were hypothesized to contribute to systemic inflammation and trigger the symptoms of Zhuxiang syndrome (Figure 5).

The severity, chronicity, and systemic influence of this fungal infection remained evident through arthritis, dermatitis, and potential cardiovascular effects observed during active lesions or withdrawal of treatment. While interventions limited acute symptoms, they neither prevented recurrence nor eliminated lesions, onychomycosis, pathogen colonization, or downstream health impacts – which is the primary focus of this longitudinal analysis.

Overall, frequent multidrug therapy, exercise, and monitoring provided limited disease control and temporary functional gains over 14 months but did not cure the infection or prevent recurrent systemic symptoms. Persistent fungal lesions, pathogens, and associated health issues highlight chronic infection mechanisms that continued to drive morbidity in between periods of retreatment and remission – the key objective and outcome demonstrated through this three-year self-study case report of interventions, observations, and impacts.

## 4. Discussion and conclusions

### 4.1 Discussion

#### 4.1.1 Zhuxiang syndrome: A proposed mechanism linking chronic fungal infections to systemic inflammation

This study presents a compelling case for “Zhuxiang syndrome,” a novel concept linking chronic fungal infections, particularly those involving *Aspergillus sydowii*, to systemic inflammatory diseases in aging individuals. The proposed mechanism involves the formation of “zombie cells” – fungal-human composite cells within Xiangya lesions – and the subsequent secretion of fungal proteins. These factors, exacerbated by cold and humid weather, trigger a systemic inflammatory response,

manifesting as arthritis, dermatitis, and cardiovascular abnormalities. Subcutaneous injection of fungal-secreted proteins in our case study induced Zhuxiang syndrome, further supporting a direct link between fungal components and systemic inflammation.

#### *4.1.2 Interpretation in the context of existing literature*

Our findings build upon the established concept of “inflammaging” by highlighting the previously underexplored role of chronic fungal infections. While previous research has focused on bacterial or viral triggers of systemic inflammation, our study identifies fungal-secreted proteins and “zombie cell” formation as potential drivers. This aligns with recent research linking microbial persistence to chronic inflammatory states, extending the literature by offering mechanistic insights into how fungal infections may exacerbate conditions such as arthritis, dermatitis, and cardiovascular abnormalities. The patient’s history of psoriasis and family history of rheumatic diseases also suggests a possible genetic predisposition to inflammatory responses [24], which may be amplified by chronic fungal infections.

#### *4.1.3 Potential mechanisms of fungal-induced systemic inflammation*

We propose that *Aspergillus sydowii* integration with anucleated keratinocytes leads to “zombie cell” formation and the active secretion of fungal proteins. The persistent presence of onychomycosis and Xiangya lesions in this patient provided a continual source of these proteins for antigenic stimulation. This chronic stimulation can lead to sustained inflammatory responses, contributing to systemic inflammation. Elevated monocyte counts during symptomatic phases further support this role. Monocytes are pivotal in the innate immune response, and their persistent elevation suggests ongoing immune activation, a hallmark of chronic inflammatory states.

The temporal association between fungal infection relapses and arthritis flare-ups suggests a possible causal relationship. Fungal-secreted proteins may act as antigens, stimulating immune cells and exhibiting a high affinity for collagen in joints, skin, and blood vessels, leading to inflammation. These proteins may also act as molecular mimics or directly stimulate immune cells, leading to the production of pro-inflammatory cytokines and autoantibodies that target joint tissues. The patient’s improvement following antifungal treatment and the resolution of arthritis symptoms upon cessation of fungal protein injections strengthen the hypothesis that fungal antigens play a pivotal role in driving joint inflammation.

The observed cardiovascular abnormalities, specifically hypertension and sinus bradycardia, suggest a direct role for fungal-secreted proteins rather than these effects being solely secondary to chronic systemic inflammation. The temporal sequence, with bradycardia preceding hypertension, hints at a specific, potentially immune-mediated mechanism. We hypothesize that fungal-secreted proteins may bind to collagen within arterial walls, acting as antigens and triggering an immune response. This pathological change in the arteries, which is frequently observed in primary hypertension, likely reduces vascular compliance, ultimately contributing to elevated blood pressure. Initially, the body may compensate for the reduced compliance by decreasing heart rate, resulting in bradycardia. However, as this compensatory mechanism is overwhelmed, or the blood pressure regulatory loop is pushed beyond its capacity, hypertension develops. The correlation between fungal infection

relapses and increased blood pressure in this patient further reinforces the systemic impact of chronic fungal-induced inflammation and the potential for a direct, immune-mediated vascular pathology.

Chronic inflammation, often termed “inflammaging,” is a significant driver of various age-related diseases, including cardiovascular diseases, neurodegenerative disorders, and metabolic syndromes. Emerging research suggests that persistent microbial dysbiosis, involving not only fungi but also bacteria and other microorganisms, can act as continuous and fluctuating sources of inflammatory stimuli, accelerating the aging process and increasing susceptibility to age-associated pathologies [25]. This case highlights a potential mechanism where chronic colonization by these microbes can contribute to systemic inflammation. Specifically, we hypothesize that microbial-secreted proteins, including fungal and bacterial components, may bind to tissue components such as collagen within various tissues, including arterial walls, acting as antigens and triggering an immune response. This process, analogous to the observed arterial changes in hypertension, could contribute to a generalized state of chronic inflammation and tissue dysfunction associated with aging. The patient’s long-term exposure to chronic inflammation, potentially through this mechanism, likely contributed to the development of hypertension and may predispose him to other age-related conditions, although such manifestations were not explicitly detailed in this report.

The analysis of weather conditions revealed that increased humidity and sea-level pressure were significant predictors of fungal infection relapses. These findings align with the natural requirements for fungal growth and proliferation, which include specific nutritional and environmental conditions such as temperature and humidity [26]. The environmental factors observed in this study likely enhanced fungal growth and persistence, thereby exacerbating the chronicity of infections. Cold and humid conditions created favorable niches for fungal colonization, consistent with the observed seasonal patterns of infection relapse. This underscores the importance of environmental modulation in managing chronic fungal infections and mitigating associated systemic inflammation.

#### *4.1.4 Mechanistic insights: The role of microbial antigens in inflammaging*

The observation that injection of fungal-secreted proteins induces arthritis, along with evidence suggesting a broader role for other microbes, indicates that specific microbial antigens can directly activate immune pathways involved in inflammation. This extends beyond joint inflammation, as the proposed mechanism of microbial protein binding to collagen and other tissue components and triggering inflammation could occur in multiple tissues. Potential mechanisms include:

**Molecular Mimicry:** Microbial antigens, including those from fungi and bacteria, may share structural similarities with human proteins, such as collagen and BiP, leading to autoantibody production and autoimmunity. This could explain why the immune response is not limited to the site of infection but can have systemic effects, as seen in rheumatoid arthritis.

**Direct Activation of Immune Cells:** Microbial proteins, especially those bound to collagen or other tissue components, could directly stimulate monocytes and other immune cells to produce pro-inflammatory cytokines (e.g., TNF- $\alpha$  and IL-6) [27], perpetuating inflammation in various tissues. This chronic activation can contribute to the systemic inflammation seen in inflammaging [28].

**Persistent Antigen Exposure:** Continuous exposure to microbial antigens, particularly those bound to collagen and other tissue components, may prevent the resolution of inflammatory responses, maintaining a state of chronic inflammation [29]. This persistent inflammation, potentially driven by collagen-bound microbial proteins, could be a key factor in the development of age-related diseases.

**Dysregulation of Immune Balance:** Chronic microbial infections can dysregulate immune balance, such as decreasing Treg cells and increasing pro-inflammatory cytokines like IL-23 and IL-17 [30], as observed in fungal infections. This imbalance can contribute to the development of autoimmune diseases and exacerbate inflammaging [2].

## **4.2 Clinical implications**

The recognition of Zhuxiang syndrome as a distinct clinical entity has significant implications for patient care. First, it suggests that conventional antifungal therapies may need to be complemented by targeted anti-inflammatory or immunomodulatory treatments to manage systemic manifestations effectively. Second, screening for chronic fungal infections in elderly patients with unexplained systemic inflammatory symptoms might lead to earlier diagnosis and intervention. This integrated approach could potentially reduce morbidity by preventing the chronic inflammatory cycle triggered by persistent fungal infection. This case underscores the importance of addressing chronic microbial dysbiosis, including fungal and bacterial infections, not only for localized symptom relief but also for mitigating systemic inflammatory consequences, particularly in the context of aging. Clinicians should be aware of the potential for chronic infections and the resulting microbial antigens to contribute to systemic diseases, particularly in patients with a predisposition to autoimmune conditions or age-related pathologies [31, 32]. Comprehensive management strategies that include effective antimicrobial therapies, regular monitoring of inflammatory markers, and interventions to control environmental triggers are essential for reducing the burden of chronic inflammation, preventing the progression of related systemic diseases, and improving lifespan and health span [33].

The introduction of an objective, caliper-based method to quantify joint stiffness represents a significant advancement in the clinical assessment of arthritis. This method provides a precise and reproducible metric, reducing the subjectivity inherent in visual analog scales. Such objective measurements are crucial for accurately monitoring disease progression and the efficacy of therapeutic interventions, particularly in chronic conditions with fluctuating symptomatology.

## **5. Limitations**

This study is based on a single case, which inherently limits the generalizability of our findings. The observational nature of the data and reliance on self-reported symptoms further restrict the scope of our conclusions. Moreover, while the correlation between fungal infections and systemic inflammation is compelling, causal relationships require further validation in larger, controlled studies. The regression model used to identify predictors of fungal infection relapses explained only 73% of the variance, indicating that other unmeasured factors may significantly contribute to infection dynamics. Additionally, the causal relationship between fungal proteins and systemic inflammation requires further elucidation through mechanistic studies.

## 6. Future research directions

To build upon these findings, future research should focus on:

*Expanding cohort studies:* Including larger patient populations to confirm the prevalence and clinical impact of Zhuxiang syndrome.

*Mechanistic studies:* Employing *in vitro* and *in vivo* models to elucidate the molecular pathways by which fungal-secreted proteins trigger systemic inflammation, including the specific molecular pathways by which fungal-secreted proteins interact with collagen and trigger immune responses [34].

*Therapeutic trials:* Evaluating the efficacy of combined antifungal and anti-inflammatory treatments in reducing the systemic manifestations associated with chronic fungal infections, including potential immune-mediated vascular changes [35].

*Biomarker development:* Identifying specific biomarkers that can reliably indicate the formation of “zombie cells” and predict systemic inflammatory responses [36].

*Environmental interventions:* Investigating the impact of environmental modifications on fungal infection relapse rates [37].

*Longitudinal studies:* Examining the long-term impact of chronic fungal infections on aging-related inflammatory markers and disease outcomes.

*Microbiome analysis:* Exploring the specific roles of different microbial species and their interactions with the host immune system.

*Collagen binding studies:* Investigating the role of microbial-secreted proteins binding to collagen and other tissue components in various tissues as a potential mechanism contributing to inflammation and age-related diseases [38].

## 7. Conclusion

This case report highlights the significant role chronic microbial infections, including fungal infections, may play in driving systemic inflammatory responses and contributing to the pathogenesis of aging-related diseases. The interplay between persistent microbial pathogens, environmental factors, and immune activation underscores a novel perspective on chronic inflammation and its systemic manifestations, including potential immune-mediated vascular changes. The observation of bradycardia preceding hypertension suggests a specific mechanism involving microbial antigens binding to arterial collagen. The introduction of an objective measurement method for joint stiffness and the identification of environmental predictors for microbial infection relapses provide valuable tools for clinical assessment and management. However, the findings necessitate further investigation through comprehensive studies to confirm the proposed mechanisms, including the role of microbial antigens in vascular pathology, and evaluate potential therapeutic interventions. Understanding the relationship between chronic infections and systemic inflammation holds promise for developing strategies to mitigate inflammation and its associated morbidities, ultimately enhancing the quality of life in aging populations [39, 40].

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## **Competing interests**

The authors declare no competing interests with any financial or non-financial entities related to the subject matter or materials discussed in this manuscript.

## **Consent to publish**

Written informed consent was obtained from the patient, who is also the researcher and author of this manuscript, for publication of clinical details and/or images. A copy of the consent form is available for review by the Editor of this journal.

## **Ethics statement**

All methods were carried out in accordance with relevant guidelines and regulations. Ethical approval was obtained from the Ethics Committee of Xiangya Hospital,

Central South University (Approval Number: IRB {C} NO. 202501002, file Ethical Approval 20,251,002.pdf). We, the researchers and authors of this manuscript, provided full, informed consent to participate in this self-experimentation research. The research was conducted with due consideration of ethical principles, including the minimization of harm and adherence to established scientific practices.

## Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files and are also available from the corresponding author on reasonable request.

## Abbreviations

|              |                                     |
|--------------|-------------------------------------|
| <i>Aspsy</i> | <i>Aspergillus sydowii</i>          |
| ICC          | intra-class correlation coefficient |
| anti-CCP     | anti-cyclic citrullinated peptides  |
| BP           | blood pressure                      |

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