OPPORTUNISTIC MYCOSES

Opportunistic Mycoses

Infections due to fungi of low virulence in patients who are immunologically compromised
PATHOGENIC FUNGI

• NORMAL HOST
  - Systemic pathogens - 25 species
  - Cutaneous pathogens - 33 species
  - Subcutaneous pathogens - 10 species

• IMMUNOCOMPROMISED HOST
  Opportunistic fungi - 300 species

Opportunistic Fungi

1. Saprophytic - from the environment
2. Endogenous – a commensal organism

Include many species from:

A (Aspergillus)
  To
Z (Zygomycetes)
Opportunistic Mycoses

Opportunistic mycoses are fungal infections that do not normally cause disease in healthy people, but do cause disease in people with weakened immune defenses (immunocompromised people). Weakened immune function may occur due to inherited immunodeficiency diseases, drugs that suppress the immune system (cancer chemotherapy, corticosteroids, drugs to prevent organ transplant rejection), radiation therapy, infections (e.g., HIV), cancer, diabetes, advanced age and malnutrition.

The most common infections are:
- Aspergillosis
- Candidiasis
- Cryptococcosis
- Pneumocystosis (*Pneumocystis carinii*)
- Zygomycosis (*MUCORMYCOSIS*)

Most Serious Opportunistic Infections

- *Candida species*
- *Aspergillus species*
- *Mucor species*
Cryptococcus neoformans

- **Encounter:** Organism is ubiquitous and infections occur worldwide. *C. neoformans* recovered in large amounts in pigeon poop. Does not cause disease in birds.
  - Primary site of human infection is the lungs

- **Spread:** Cryptococcal meningitis is most common disseminated manifestation. Can spread to skin, bone and prostate.

Clinical features of *C. neoformans*

- A mild self-limiting pulmonary infection is believed to be the most common form of cryptococcosis.
- In symptomatic pulmonary infection there are no clear diagnostic features.
- The meningeal form of cryptococcosis can occur in apparently healthy individuals, but occurs most frequently in immunocompromised persons (Patients with AIDS and cryptococcosis generally develop a chronic meningeal form).
- Chronic meningitis or meningo-encephalitis develops insidiously with headaches and low-grade fever, followed by changes in mental state, visual disturbances and eventually coma.
- Although predominantly a disease of the central nervous system, lesions of the skin, bones and other deep sites may also occur; in its disseminated form, the disease may resemble tuberculosis.
Laboratory diagnosis

- *Cryptococcus* is readily demonstrated in CSF or other material by direct microscopy, culture or serological tests for capsular antigen.
- The yeast load is generally higher in patients with AIDS.
- The yeast cells of *Cryptococcus* are round, 4–10 μm in diameter and surrounded by a mucopolysaccharide capsule. The width of the capsule varies and is greatest in vivo and on rich media in vitro.
- In unstained wet preparations of CSF mixed with a drop of Indian ink or nigrosine, the capsule can be seen as a clear halo around the yeast cells.

- Sputum, pus or brain tissue should be examined after digestion in potassium hydroxide and here the capsulate yeasts are often delineated by the cellular debris.
- The yeast is easily cultured from CSF although large volumes or multiple samples may be required in some cases; in patients with AIDS it is also useful to culture blood.
- For examination of tissue sections it is best to use a specific fungal stain such as periodic acid–Schiff and mucicarmine stain the capsular material, enabling the organisms to be differentiated from *H. capsulatum* and *Blastomyces dermatitidis*.
- On Sabouraud agar (without cycloheximide) cultured at 25–30°C and 37°C, colonies normally appear within 2–3 days, but cultures should not be discarded for 3 weeks.
• In culture, *Cryptococcus* appears as creamy white to yellow–brown colonies, which are mucoid in strains with well developed capsules and dry in strains that lack prominent capsules.

• Preliminary identification depends on demonstration of the capsule but this may be absent or difficult to see.

• *Cryptococcus* can be identified with commercial kits or distinguished from other yeasts by its lack of fermentative ability.

• The latex agglutination test for the detection of cryptococcal polysaccharide antigen in **CSF or blood** is highly sensitive and specific for the diagnosis of cryptococcal meningitis, and gives **better results than microscopy and culture**. In AIDS, the test is positive in well over 90% of infected patients.

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**Cryptococcus neoformans**

• **Diagnosis**: Lumbar puncture and microscopic examination of cerebrospinal fluid is diagnostic. **(India ink or nigrosine staining).**

• Culture of organisms from blood or CSF

• **(LAT)** Cryptococcal antigens in Cerebrospinal fluid (CSF) and serum.

  Microscopic appearance of encapsulated *Cryptococcus* cells in an Indian ink preparation of CSF.

**Treatment**: Amphotericin B & 5FC. Followed by oral fluconazole.
Candidiasis

*C. albicans* is a member of the indigenous microbial flora of humans.

1. Found in the gastrointestinal tract, upper respiratory tract, buccal cavity, and vaginal tract.
2. Growth is normally suppressed by other microorganisms found in these areas.
3. Alterations of gastrointestinal flora by broad spectrum antibiotics or mucosal injury can lead to gastrointestinal tract invasion.
4. Skin and mucus membranes are normally an effective barrier but damage by introduction of catheters or intravascular devices can permit *Candida* to enter the bloodstream.

*In vitro* (25°C): mostly yeast;

*In vivo* (37°C): Yeast, hyphae and pseudohyphae
Microscopical appearance of *Candida albicans* yeast cells and pseudohyphae in a Gram-stained vaginal smear.

**Candidiasis**

Vaginal candidiasis is the most common clinical infection. Local factors such as pH and glucose concentration (under hormonal control) are of prime importance in the occurrence of vaginal candidiasis. In mouth: normal saliva reduces adhesion (lactoferrin is also protective).

**Immune Response**

Hyphae are too big for phagocytosis but are damaged by **polymorphonuclear leukocytes** (PMNs) and by extracellular mechanisms (myeloperoxidase and β-glucuronidase). Resistance to invasive infection by *Candida* is mediated by phagocytes. Patients with defects in phagocytosis function and myeloperoxidase deficiency are at risk for disseminated (even fatal) *Candidiasis*. 
Candidiasis

**Risk factors for candidiasis**
- Post-operative status
- Cytotoxic cancer
- Chemotherapy
- Antibiotic therapy
- Burns
- Drug abuse
- Gastrointestinal damage.

**Laboratory diagnosis of Superficial or cutaneous candidosis**
- Specimens of skin and nail are collected in the same way as for suspected dermatophytosis.
- For infections of the mouth or vagina, scrapings taken with a blunt scalpel or a spatula from areas with white plaques or erythema are better than swabs if the material is to be processed immediately.
- However, swabs are more convenient for transport to the laboratory, and they are better for collecting vaginal discharge. Swabs should first be moistened with sterile water or saline before taking the sample and should be sent to the laboratory in ‘clear’ transport medium.
- In Gram-stained smears of mucous membrane samples the fungus is seen as budding Gram-positive yeast cells; pseudohyphae are usually present except in the case of *C. glabrata*.
- The presence of *Candida* pseudohyphae in clinical material does not confirm infection with the organism, particularly as it may have developed in the period between collection and processing of the sample.
• *Candida* species grow well on Sabouraud medium or on blood agar at 25–37°C; typical yeast colonies appear within 1–2 days.

• *C. albicans* isolates can be identified by the germ tube test: after incubation in serum at 37°C for 1.5–2 h, *C. albicans* produces short hyphae known as germ tubes.

• Other yeasts may be identified with one of the commercial kits, or by fermentation and assimilation tests.

• **Also can use the chromogenic media for identification of most common *Candida* sp.**

• Quantification of growth, especially in the case of vulvovaginal samples, may help the clinician to distinguish between commensal carriage and infection.

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**Laboratory diagnosis of invasive candidosis**

• *Candida* species may be present as commensals in the absence of infection, so that isolation from clinical material, except from sites that are normally sterile, is of little significance.

• In suspected invasive candidosis, samples from as many sources as possible should be examined by **direct microscopy and culture.**

**Direct microscopy**

• Appropriate samples are examined microscopically in potassium hydroxide or after Gram staining.

• In tissue sections, the fungus is seen best in stained preparations.
Culture

- *Candida* species grow readily in culture at 37°C on common isolation media, such as Sabouraud dextrose agar.
- Blood cultures provide the most reliable evidence of invasive infection,
- Isolation of the yeast from otherwise sterile sites provides reliable evidence for the diagnosis,
- but cultures obtained from urine, faeces and sputum are of less value unless done quantitatively over a period of time.
- Cell counts of the yeast in urine in excess of $10^4$ per mL are usually taken to indicate urinary tract infection, except in those with an indwelling urinary catheter.
- As *Candida* species multiply rapidly in clinical material it is important that specimens are processed as soon as possible after collection.

Treatment and prevention of Superficial or Cutaneous candidosis

- Most superficial infections respond well to topical therapy with an imidazole. In oral candidosis, nystatin, amphotericin B or miconazole may be effective in gel form.
- Most patients with vaginal candidosis can be treated successfully with a single application of a topical imidazole, or with oral fluconazole or itraconazole.
- For nail care and avoidance of prolonged exposure to water by use of protective gloves; patients should dry their hands carefully after washing.
- Regular application of an azole lotion or an azole given orally, sometimes in conjunction with a topical steroid and an antibacterial agent, is the most appropriate therapy but it may take several months to cure the condition; antifungal creams or ointments are less effective.
The treatments of invasive candidosis

• The treatments of choice for most forms of invasive candidosis are:
  • intravenous echinocandin (anidulafungin, caspofungin or micafungin)
  • intravenous amphotericin B (conventional or liposomal)
  • intravenous or oral fluconazole.
  • Amphotericin B can be used in combination with flucytosine,
Aspergillosis

- There are more than **200 species of Aspergillus** but fewer than **20** have been implicated in human disease;
- the most important are *A. fumigatus, A. flavus, A. terreus, A. niger* and *A. nidulans*.
- *Aspergillus* species are ubiquitous in the environment, growing in the soil, on plants, and on decomposing organic matter. These moulds are often found in the outdoor and indoor air, in water, on food items, and in dust.

Aspergillosis

- Genus occurs worldwide and contains hundreds of species.
- These species constitute the most commonly found fungi in any environment.

Major portal of entry is the respiratory tract. Dissemination can occur from the lungs and involve other areas of the lung, brain, skin, bone, GI tract, and kidney.
Aspergillosis

- Aspergillosis is the most common fatal infection seen in patients with chronic granulomatous disease of childhood.
- In immunosuppressed hosts: invasive pulmonary infection, usually with fever, cough, and chest pain.
- In immunocompetent hosts: localized pulmonary infection in persons with underlying lung disease. Also causes allergic disease.

Epidemiology

- Inhalation of *Aspergillus* conidia is the usual mode of infection, infection follows the traumatic implantation of spores as in corneal infection, or inadvertent inoculation as in endocarditis.
- Progressive and disseminated disease can complicate neoplastic diseases, especially acute leukemia, stem cell, children with chronic granulomatous disease bone marrow and solid organ transplant recipients, (not necessarily AIDS).
- In non-immunocompromised persons, these moulds can cause localized infection of the lungs, sinuses and other sites.
- the most host factors important of which is the level of immunosuppression. The mortality rate is high, ranging from 50–100% in almost all groups of immunocompromised patients.
Microscopical appearance of an *Aspergillus fumigatus* conidiophore.

Radiological appearance of an aspergilloma.

Sputum culture *A. Fumigatus* (left) *A. Flatus* (right)

Sputum microscopic analysis

Corticosteroid-induced immunosuppression: PMN recruitment and tissue damage

Sporulation

Inhalation of airborne conidia

Conidial germination in absence of sufficient pulmonary defenses

Neutropenia: excessive hyphal growth and dissemination
Clinical features of Aspergillosis

Invasive aspergillosis

- This form occurs in severely immunocompromised individuals.
- *A. fumigatus* is the species most frequently involved.
- The most common initial presentation in the neutropenic patient is an unremitting fever (>38°C), without any respiratory tract symptoms, that fails to respond to broad-spectrum antibiotics.
- The lung is the sole site of infection in 70% of patients, but dissemination of infection to other organs often occurs (brain, skin, bone, GI tract, and kidney);
- the central nervous system is involved in 10–20% of cases.
- There is widespread destructive growth of *Aspergillus* species in lung tissue and the fungus invades blood vessels, causing thrombosis and infarction.
- Invasive aspergillosis has a poor prognosis;
Aspergilloma

- In this form of aspergillosis, also referred to as *fungus ball*, the fungus colonizes pre-existing cavities in the lung and forms a **compact ball of mycelium**, eventually surrounded by a dense fibrous wall.
- Aspergillomas are usually solitary.
- Patients are either asymptomatic or have only a moderate cough and sputum production. Occasional haemoptysis may occur, especially when the fungus is actively growing, and haemorrhage following invasion of a blood vessel is one of the fatal complications.
- Surgical resection is most often used to treat this condition.

Sinusitis

- *Aspergillus* species, particularly *A. flavus* and *A. fumigatus*, may colonize and invade the paranasal sinuses; the infection may spread through the bone to the orbit of the eye and brain.
- Acute invasive sinusitis is a rapidly progressive disease, most commonly seen in immunocompromised persons.
Allergic bronchopulmonary aspergillosis

• inhalation of spores can cause allergic symptoms
• Allergy to *Aspergillus* species is usually seen in atopic individuals
• The condition is a form of asthma with pulmonary eosinophilia
• the fungus *grows in the airways to produce mucous plugs of fungal mycelium that may block off segments of lung tissue* and that, when coughed up.

Laboratory diagnosis

*Direct microscopy*

• In potassium hydroxide preparations (preferably with Calcofluor to enhance detection) of sputum the fungus appears as non-pigmented septate hyphae, 3–5 μm in diameter, with characteristic dichotomous branching and an irregular outline;
• In allergic aspergillosis there is usually abundant fungus in the sputum and mycelial plugs may also be present.
• In aspergilloma, fungus may be difficult to find on microscopy.
• In invasive aspergillosis, microscopy is often negative.
• Biopsy may provide a definitive diagnosis,
• In tissue sections *Aspergillus* species are best seen after staining with periodic acid–Schiff or methenamine–silver.
Culture

- *Aspergillus* species grow readily at 25–37°C on Sabouraud agar without cycloheximide; colonies appear after 1–2 days. Isolates can be identified by their colonial appearance and micromorphology.
- As *aspergilli* are among the most common laboratory contaminants,
- quantification of the amount of fungus in sputum helps to confirm the relevance of a positive culture.
- However, all isolates from Immunocompromised patients must be taken seriously and acted upon.
- Large quantities of fungus are usually recovered from the sputum of patients with allergic aspergillosis, but cultures from those with aspergilloma or invasive disease are commonly negative or only a few colonies.
- Blood cultures are negative in invasive disease.

Skin tests

- Skin tests with *A. fumigatus* antigen are useful for the diagnosis of allergic aspergillosis. All patients give an immediate type I reaction and 70% of those with pulmonary eosinophilia also give a delayed type III Arthus reaction.

Serological tests

- Immunodiffusion and ELISA are widely used for the detection of antibodies in the diagnosis of all forms of aspergillosis, particularly aspergilloma and allergic bronchopulmonary aspergillosis.
- Tests for *Aspergillus* antibodies are seldom helpful in the diagnosis of invasive infection in immunocompromised patients.
Treatment

- In invasive aspergillosis, the historical standard of treatment is intravenous amphotericin B.
- Voriconazole proved superior to amphotericin B in a large clinical trial, and is now used to treat many patients with this disease.
- Aspergilloma is treated by surgical excision because antifungal therapy is of little value.
- Allergic forms of aspergillosis are treated with corticosteroids.

Conclusions

- Most fungal infections affect our surface not our contents
- A few dimorphic fungi can cause systemic infections in otherwise healthy people.
  - Endemic areas
  - Contact by inhalation
- Candida species inhabit our guts and usually stay there, but, given the right (wrong) conditions can disseminate to infect almost any organ.
- In immune compromised people, any fungus can be a deadly pathogen