Case # 1

This case of pulmonary Aspergillosis is another classical opportunistic disease. The case questions and discussion points cover the major points to make.

- MLL
- Chemotherapy
- Profound neutropenia
- Broad spectrum antibiotic therapy

Differentials:
Fungal etiology. High on the list (probably # 1) is yeasts; *Candida sp*

Next on list Aspergillus Sp.

Bacteria

Viral (CMV)

Pneumocystis ??

Broad-spectrum antibiotic Rx - fever persists; lower probability of bacteria.

Amphotericin B Rx -> fever persists ?; lowers probability of many yeasts.

Best guess Aspergillus.

Laboratory Diagnosis:

Opportunistic infection/disease with opportunistic organism requires tissue, which was provided in this case. Bronchoscopy with biopsy is the best/fastest and gives definitive diagnosis.

Procedures to be performed on bronchial washings or brushings:

- KOH/Calcoflor white direct microscopic examination (will have been shown in lecture),
- Cytology stains,
- Culture for fungi,
- Gram stain,
- Bacterial culture,
- Fluorescent Acid-fast stain,
- Mycobacterial culture, if indicated.
Procedures to be performed on lung biopsies:

- KOH/Calcofluor White stain on impression smears,
- Culture for fungi,
- Histopathological stains: H & E and Methenamine Silver,
- Bacterial culture.

NOTE: Emphasize to students that tissue is required for **definitive** diagnosis of opportunistic fungal diseases.

1.0 **Aspergillus species.**

This is a very large group of fungi of several hundred species. Three species cause most disease in the United States: 1. Aspergillus fumigatus, 2. A. flavus and 3. A. niger.

These are molds. They produce septate hyphae of variable size (4-10 μ in diameter). Fruiting bodies called “heads” are produced on the septate hyphae. Large numbers of spores, produced on heads may become airborne and be inhaled.

1.1 **Epidemiology.** With rare exception, Aspergilli only cause disease in compromised patients. Clinical forms of aspergillosis usually occur as: Rhinocerebral, fungus ball, bronchopulmonary plugging and invasive.

1.2 **Common Pathogenic Factors.** Many of the Aspergilli produce toxins, e.g., Aflotoxin, that may play a role in their virulence. Aspergilli also produce various enzymes, e.g., proteases that are virulence factors.

1.3 **Laboratory Diagnosis:**

- As in this case, direct microscopic examination of clinical specimen taken from the diseases site is the fastest method for a presumptive diagnosis. KOH/Calcofluor White stained wet preparation of the specimen will reveal white to green colored septate hyphae. NOTE: Be sure the students understand that seeing hyphae septate hyphae with “acute angle” branching DOES NOT identify Aspergillus sp. Many other fungi including Penicillium sp. Fusarium sp. and Pseudallescheria boydii have these characteristics.
- Culture bronchial washings and tissue for fungi (Students will have seen the Aspergilli in lecture and should know how they are differentiated for other molds).
- Fungal serology. In the absence of direct microscopic evidence of fungal disease, serological tests for antibodies to H. capsulatum, B. dermatitidis, C. immitis and Aspergillus species may be helpful.

NOTE: 1. Antibodies to Aspergillus may or may not have been present in this case.

2. A commercial test, Galactomannan Assay, has been developed and approved for detecting the galactomannans of Aspergillus species in serum. There are conflicting data about the sensitivity and specificity of this test. The efficacy of this test to detect early aspergillosis will require more clinical correlation studies.

- Histopathology. Observation of septate hyphae in tissue defines a case of opportunistic mycosis. A culture is required to identify the organism and finalize the diagnosis.

NOTE: Reemphasize to students that the Methenamine silver stain should be employed to detect fungi in tissue.
• Speciation. Determining the species of Aspergillus is based on morphology and arrangement of components of the sexual and asexual reproductive structures; heads spores, etc. Time will not allow discussion of this material in the small group.

• Blood Culture. Blood for culture is usually collected from compromised patients with fever. Most yeasts grow very well in currently used blood culture systems. Molds usually are not isolated from blood with these systems.

2.0 Zygomycetes

Zygomycosis is a disease caused by one of the Zygomycetes. The most common genera involved are: Rhizopus and Mucor.

The students should be aware of this and know that all information above applies to the Zygomycetes. The Zygomycetes can be differentiated from the Aspergilli by the following:

• The Zygomycetes have large (8-20μ diameter), non-septate hyphae.
• The colonies of Zygomycetes grow more rapidly than those of Aspergilli.
• The morphology of the reproductive structures are different. The medical students will have seen these in lecture and laboratory. The dental students will have seen them in lecture. All these images are available on the IID Blackboard website.
• There is no serology for the Zygomycetes.
This case of blastomycosis should be used as an example of "classical" (my terminology) systemic mycoses, i.e., blastomycosis, coccidioidomycosis and histoplasmosis. The fungi causing these diseases: Blastomyces dermatitidis, Coccidioides immitis, and Histoplasma capsulatum, may cause infection and disease in Immunocompetent people.

The discussion points of this case are relatively good, but if time permits, I suggest reading chapter 48 of Sherris.

The students will have had lectures on the "classical" systemic fungal diseases and have seen the fungi in laboratory. Each student should have made a wet. (KOH) preparation of sputum and observed B. dermatitidis.

Remind the students to review, and remember, the cases of tuberculosis, which are similar to these cases.

1.0 Blastomyces dermatitidis is a dimorphic fungus, which grows as a mold in the environment (or in the laboratory when incubated at 25-30C). This fungus, like H. capsulatum and C. neoformans, has been found in soil, but unlike the others, has been associated with decaying wood and wood products. Unlike H. capsulatum, it can not be consistently isolated from the soil from a point source. Multiple isolations have been made from soil where outbreaks have occurred in Minnesota and Wisconsin.

1.1 Epidemiology. Blastomycosis is endemic in the East-Central and South-Southeastern part of the United States and Southeastern Canada. This is roughly the same endemic area of histoplasmosis (a consideration in making a differential diagnosis). The disease occurs most commonly in adult males. Recent epidemiological studies related to outbreaks of disease indicate that infection occurs equally in male and female. The studies also show that there is asymptomatic infection and mild pulmonary disease.

There is no reliable skin test antigen to use for epidemiological studies.

1.2 COMMON PATHOGENIC FACTORS. Relatively little is known about the pathogenesis of this disease, although considerable research is now being done.

1.3 LABORATORY DIAGNOSIS.

Direct Microscopic Examination. The yeast form of B. dermatitidis can be readily observed in liquid or semi-liquid specimens. The specimen can be examined directly without stain; however, it is much preferable to use the Calcofluor White stain on the wet preparation. This stain stains the fungal cell, which fluoresces white to apple green when appropriate filters are used.

Blastomyces dermatitidis appears as a large, 5-19μ, budding yeast with a wide bud base. This morphology is unique among the dimorphic fungi. Cryptococcus species and other nonmorphogenic yeasts, e.g., Histoplasma capsulatum is a small, 2-4μ yeast, with a narrow bud base; Coccidioides immitis appears as a large, 15-200μ, spherule containing multiple endospores; Sporothrix schenckii is a small, 4-6 X 6-8 μ, oval to elongate (cigar-shaped) yeast; Cryptococcus neoformans is a variable-sized, 4-10μ, yeast with a pinched bud base and surrounded by a mucopolysaccharide capsule; Candida glabrata is a small, 4-6μ, budding yeast that may closely resemble H. capsulatum and all other Canida species appear as pseudohyphae. These fungi have the same morphological characteristics when stained in tissue, with various tissue stains.

Culture. When grown on non-enriched media, Blastomyces dermatitidis appears as white to tan colonies. Visible growth appears usually within 2-3 days; however, speculation may not occur for several more days.
Serology. Serological tests are often helpful to identify a patient with a systemic fungal disease. Antibodies to B. dermatitidis, C. immitis, and H. capsulatum may be detected using the Immunodiffusion (ID), Complement Fixation (CF) and Latex Agglutination tests. The ID test is qualitative and is not highly sensitive, but specific. The CF test is quantitative and is relatively sensitive, but non-specific. There is common cross reaction between H. capsulatum and B. dermatitidis. The Latex Agglutination test is used primarily to detect IgM so is limited to early stage of disease. In general, high antibody titers indicate increasing severity of disease. It should be emphasized that serological tests are not helpful in patients who cannot produce antibodies. An Enzyme Immuno Assay (EIA) test has been developed to detect and quantitate antigen of Histoplasma capsulatum and Blastomyces dermatitidis in urine, serum and other body fluids. These tests are useful in determining if these fungi have disseminated from the lungs. Cross reactivity has limited the use of this test for making definitive diagnoses.
Case # 3

This is a case of disseminated cryptococcosis in an HIV positive patient, who is an IV drug user, is rather straight-forward. The students will have had a lecture on cryptococcosis and will have seen the organism in the laboratory.

The following are some additional points the student should note:

- Skin lesions from *C. neoformans* are relatively rare, but do occur. They may also occur in non-AIDS patients. (Old literature of Cryptococcosis mentions this often.)

- Reemphasize the importance of CSF cell count and chemistry in diagnosing meningitis.

- Reemphasize the fact that pulmonary cryptococcosis may occur in immunocompetent people and is more prevalent than cryptococcal meningitis.

- Emphasize importance of cell-mediated immunity in Cryptococcosis.

1.0 *Cryptococcus* neoformans

*C. neoformans* is a variable-size (4-10 μ), budding yeast, with a narrow (pinched-off) bud base. The cell is usually surrounded by a mucopolysaccharide capsule. The capsule may vary in size from very small to large (sometimes exceeding the diameter of the cell).

1.1 Epidemiology

Cryptococcosis occurs throughout the world. It is acquired by inhaling yeast cells from the environment. The organism grows in soil, particularly soil enriched with bird manure; especially pigeon manure. *C. neoformans* has been isolated from sites all over the world. The disease is primarily found in adults. We have no knowledge of the prevalence of infection, because we have no skin test, or other tool, for epidemiological study.

Cases of Cryptococcosis have increased with the increase in AIDS cases.

1.2 Pathogenic factors

- The capsule of *C. neoformans* inhibits phagocytosis.

- Studies show the yeast cell wall induce delayed hypersensitivity; however, in natural infection this is variable.
Case # 4

This is a clear case of opportunistic fungal disease; an elderly person; major surgery; use of intravenous, broad-spectrum antibiotics; central venous catheter.

A differential diagnosis would include Gram-negative bacteria if broad-spectrum antibiotics had not been given. The most likely source of infection is *Candida albicans*; now the 4-5th cause of nosocomial infections in many hospitals. Other *Candida* species that may have been the etiological agent are *C. parapsilosis* and *C. tropicalis with C lusitaniae, C krusei* and *C guillermondii* being less likely. *Candida* (Torulopsis) glabrata is also a strong candidate. Other yeast and yeast-like organisms, such as *Cryptococcus neoformans, Histoplasma capsulatum and Blastomyces dermatitidis* may cause disease in compromised patients. The following points may be helpful when reviewing the comments about this case.

1.0 *Candida albicans* is a yeast of variable size that generally reproduces by budding when grown in aerobic conditions, especially in liquid medium. This yeast may also produce pseudohyphae when growing in tissue or on culture media. Pseudohyphae are chains of blastoconidia (blastospores) that have not separated from the mother cell.

NOTE: All *Candida* species (except glabrata) may produce pseudohyphae.

*C albicans* produces germ tubes within 2 hours when incubated with serum, at 35-37°C. The student should understand that this is not a foolproof test. When properly performed, the test is 92-95% specific.

NOTE: We now have more rapid tests (30 minutes) that detect enzymes specific to *C albicans*. These tests are more expensive that the germ tube.

*C albicans* is definitively identified by biochemical tests.

There are no reliable serological tests for candidiasis.