17 Histoplasma capsulatum

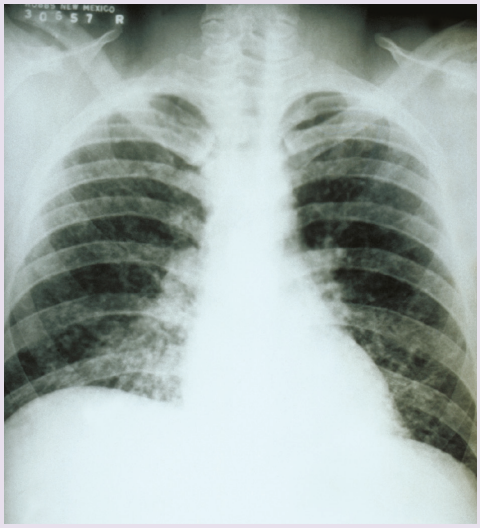

Figure 17.1 Chest radiograph of the patient infected with *H. capsulatum*, revealing bilateral nodular infiltrates. *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #3954. Additional photographic credit is given to M Renz who took the photo in 1963.*

A 60-year-old resident of Louisville (Ohio) had suffered from **rheumatoid arthritis** for 9 years and was currently being treated with 10 mg of methotrexate weekly and 8 mg of methylprednisolone daily followed by monthly injections of 3 mg kg^{-1} infliximab **monoclonal antibody**. Ten weeks after the start of infliximab, he felt severely ill and was hospitalized with the symptoms of **dyspnea** and cough, quickly followed by respiratory failure, requiring mechanical ventilation. A chest radiograph revealed bilateral nodular infiltrates (Figure 17.1). **Bronchoalveolar lavage** fluid contained yeast forms resembling *Histoplasma capsulatum*. Laboratory tests showed normal blood cell counts, but positive *Histoplasma* urine antigen (10.3 U, normal levels <1 U). The findings were confirmed by yeast cell culture and complement fixation titers 1:2048 to the mycelial M antigen and 1:256 to the yeast Y antigen (normal levels < 1:8). The diagnosis of histoplasmosis was further confirmed by immunodiffusion and the patient was given antifungal drugs amphotericin B lipid complex 5 mg kg^{-1} per day for 11 days, followed by itraconazole 200 mg per day for 2 months. Therapy resulted in improvement of the respiratory function, although the patient required ventilation support throughout the treatment.

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

CAUSATIVE AGENT

Histoplasma capsulatum is a dimorphic fungus which causes a systemic endemic **mycosis** called histoplasmosis (sometimes called Darling's disease by the name of a pathologist, Samuel Darling who discovered the disease in 1905). The genus *Histoplasma* belongs to *Ajellomycetaceae* family (order *Onygenales*) and contains one species, *Histoplasma capsulatum*. There are three varieties: *H. capsulatum* var. *capsulatum*, which causes the common histoplasmosis, *H. capsulatum* var. *duboisii*, a cause of African histoplasmosis (histoplasmosis *duboisii*), and *H. capsulatum* var. *farciminosum*, which causes **lymphangitis** in horses.

H. capsulatum is a thermally dimorphic ascomycete, which means that it can survive at two different temperatures. At ambient temperatures below 30°C *H. capsulatum* remains in a saprophytic mycelial mold form, but at mammalian body temperature (37°C) it grows as a parasitic yeast. The two varieties of *H. capsulatum* (*capsulatum* and *duboisii*) that infect humans are similar in **saprophytic** mold form but differ in their parasitic tissue morphology (see below).

The saprophytic mycelial growth of *H. capsulatum* requires an acidic damp soil environment with high organic content. This is provided by bird droppings, particularly those of chickens and starlings, or excrement of bats. The fungus has been found in poultry house litter, caves, areas harboring bats, and in bird roosts. Birds cannot be infected by *Histoplasma* due to the high body temperature of 40°C but can carry *H. capsulatum* on their feathers. In contrast, bats can become infected, and they transmit the fungus through droppings. Contaminated soil is the common natural habitat for *Histoplasma* and it remains potentially infectious for years.

At 25°C on Sabouraud dextrose agar (SDA), or brain heart infusion agar (BHIA) supplemented with 5–10% sheep blood, *H. capsulatum* grows slowly into granular suede-like to cottony colonies, initially white and then brown with a pale yellow-brown or yellow-orange reverse (**Figure 17.2**). The colonies can also be glabrous or verrucose, sometimes with a red pigmented strain. *H. capsulatum* produces hyphae-like short, hyaline, undifferentiated conidiophores, which arise at right angles to the parent hyphae. Macroconidia appear as large (8–14 µm in diameter), thick-walled, round, unicellular, hyaline, and tuberculate with finger-like projections on the surface (**Figure 17.3A**). Microconidia (microaleurioconidia) are small (2–4 µm in diameter), unicellular, hyaline, round with a smooth or rough wall, and borne on short branches or directly on the sides of the hyphae.

However, at 37°C the fungal morphology of *H. capsulatum* is totally different: numerous small round to oval, budding yeast-like cells, 2–4 µm in size can be observed under the microscope (**Figure 17.3B**). Colonies are creamy, smooth, moist, white, and yeast-like (**Figure 17.4**). This change in morphology under temperature-controlled regulation is used as a diagnostic test for *Histoplasma* (see Section 4).

H. capsulatum var. *duboisii* usually grows as a large yeast (7–15µm in diameter) within the cytoplasm of **histiocytes** and multinucleate giant cells, but sometimes can appear as small yeast cells similar to those of var. *capsulatum*. The yeasts may form rudimentary pseudohyphae consisting of four or five cells and aggregates, which can be observed within giant cells and extracellularly following **necrosis** of the host tissue.

As many other fungi, *H. capsulatum* have chitin as the deepest layer of the cell wall and a substantial amount of β-(1,3)-glucan above that layer.

ENTRY INTO THE BODY

Conidia and mycelial fragments of *H. capsulatum* from contaminated disrupted soil can be air-borne, are found in aerosols, and can be inhaled. Once inhaled, they settle

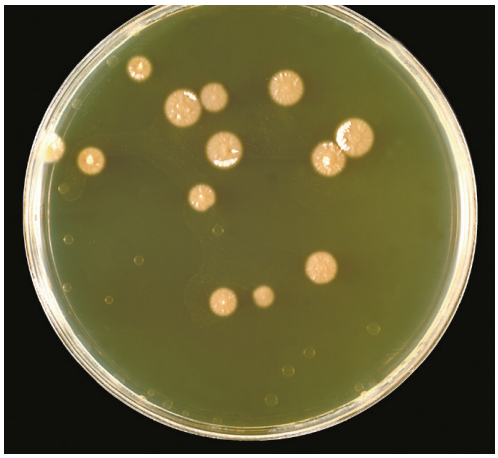

Figure 17.2 *Histoplasma capsulatum* colonies growing at 25°C. *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #3191. Additional photographic credit is given to Dr William Kaplan who took the photo in 1969.*

in bronchioles and alveolar spaces where they encounter phagocytic macrophages. Binding of the microconidia is thought to be through the complement receptor 3 (CR3), pattern recognition receptor (PRR) Dectin-1, which binds to β-(1,3)-glucan, and integrins **CD11/CD18**. **Opsonization** via antibodies and complement is therefore important for uptake of the fungus. Conversion from the mycelial to the pathogenic yeast phase is critical for infectivity of *H. capsulatum* and occurs intracellularly inside the macrophages. The stimuli that drive the conversion are not completely understood, although the temperature change appears to be a major factor. Upon conversion, the intracellular budding yeasts enlarge and reach approximately 3 µm in diameter.

It must be stated that cultures of *H. capsulatum* represent a severe biohazard to laboratory staff and must be handled with extreme caution and under appropriate conditions in a safety cabinet.

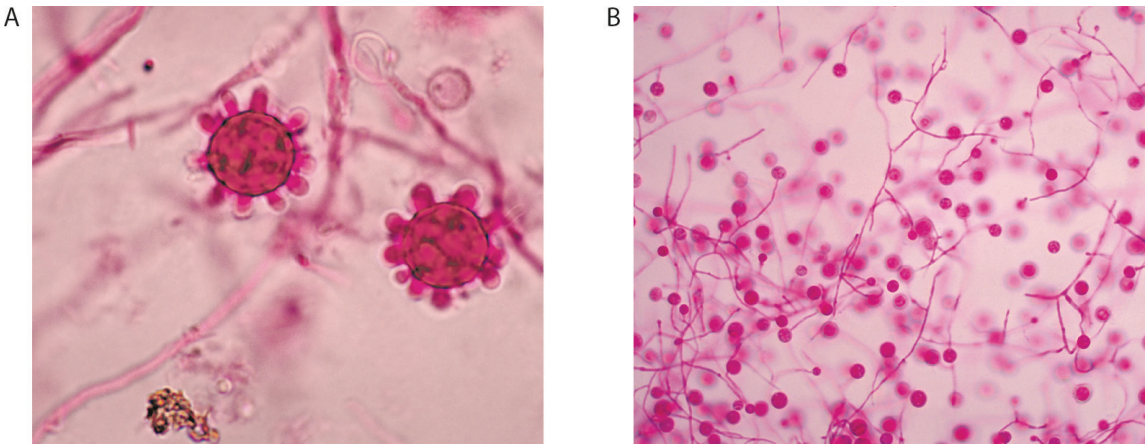

Figure 17.3 Two forms of *Histoplasma capsulatum* demonstrating features of a thermal dimorph. In nature at about 25°C, it grows as a mycelial filamentous form with macroconidia and smaller microconidia (A). At body temperature of 37°C it grows as a yeast (B). *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library (A) #4023 and (B) #4022. Additional photographic credit is given to Dr Libero Ajello who took the photo in 1968.*

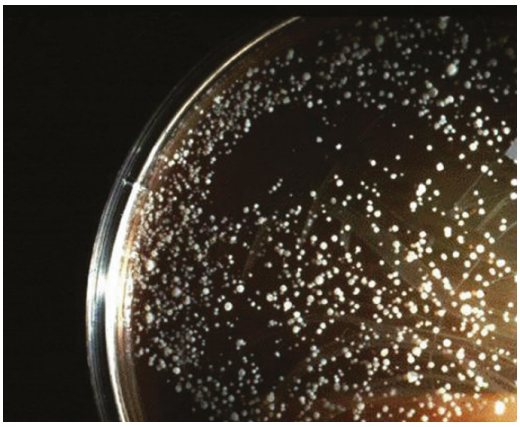

Figure 17.4 Culture of *H. capsulatum* on BHIA with 10% sheep blood, incubated at 37°C. *Courtesy of Professor Michael R McGinnis of DoctorFungus.org. www.doctorfungus.org.*

SPREAD WITHIN THE BODY

Following the initial infection, *H. capsulatum* var, *capsulatum* may spread – carried via pulmonary macrophages in draining lymphatics and the bloodstream – to many organs that contain mononuclear phagocytes. These include the liver and spleen and regional lymph nodes.

Infection with *H. capsulatum* var. *duboisii* rarely involves the lungs but mostly causes cutaneous histoplasmosis. It can also infect the liver, lymphatic system, and subcutaneous and bony tissues. The infection presents as nodular and ulcerative cutaneous and osteolytic bone lesions, disseminated or localized.

PERSON-TO-PERSON SPREAD

This organism is generally not spread from person to person.

EPIDEMIOLOGY

H. capsulatum is found in temperate climates, predominantly in river valleys between latitudes 45° north and 30° south in North and Central America. It is **endemic** to the Ohio, Missouri, and Mississippi River valleys in the US (**Figure 17.5**). In these regions of the US, approximately 90% of residents have been exposed to the fungus, and quite likely on a continuous basis. Between 1938 and 2013, over 100 outbreaks with 3000 cases were reported in 26 states and in Puerto Rico. *H. capsulatum* has five to seven chromosomes, and this lays the foundation for eight clades of *H. capsulatum*, which are distributed in different parts of the world. There are two North American clades, two South American clades, one Australian, one Indonesian, one African, and one Eurasian clade. The genetic differences often define the symptoms. For example, unlike South American clades, those in North America do not cause primary skin disease. The African clade includes all of the *H. capsulatum* var. *duboisii*. It is endemic in Central and West Africa and in the island of Madagascar.

Approximately 250 000 individuals are infected annually with *H. capsulatum* in the US and 500 000 worldwide. Of these, 50 000–200 000 develop symptoms of histoplasmosis and 1500–4000 require hospitalization. Infection is more prevalent in immunocompromised individuals (see Section 2) and therefore *Histoplasma* is regarded as an opportunistic pathogen.

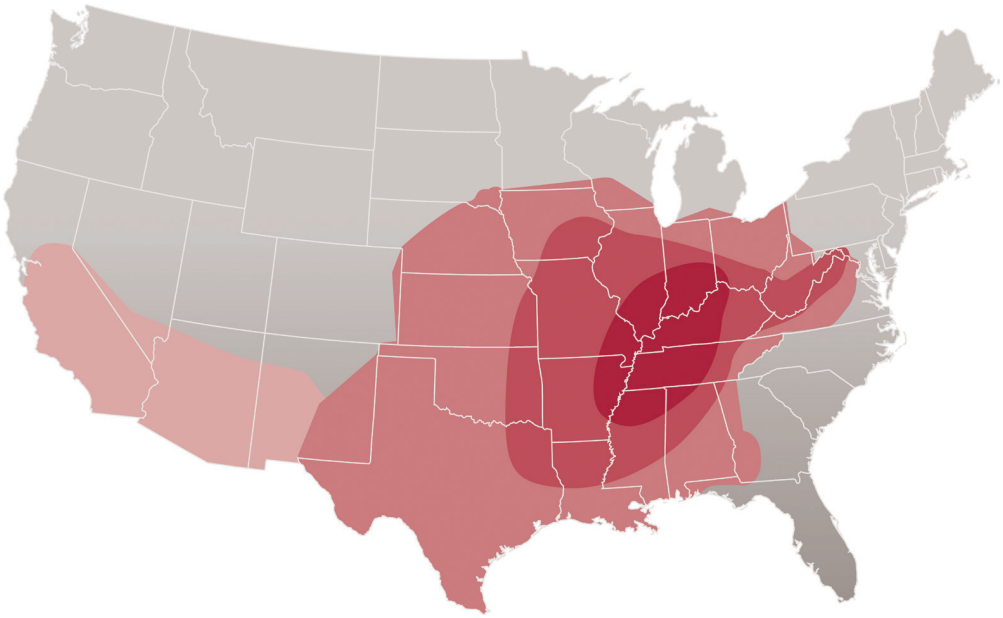

Figure 17.5 Distribution of histoplasmosis in the US, with dark red areas indicating the highest incidence of the disease. *Courtesy of Professor Michael R McGinnis of DoctorFungus.org. www.doctorfungus.org.*

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

INNATE IMMUNITY

As well as being the site of the infection, phagocytes, especially macrophages, are the main cells responsible for removing the fungus (**Figure 17.6**). After ingestion, *H. capsulatum* yeasts reside in phagocytic **phagosomes** and replicate there approximately every 15–18 hours. One mechanism used by the fungus to reduce intracellular killing is by inhibiting phagolysosomal fusion and thus preventing exposure to lysosomal hydrolytic enzymes. The fungus also increases the phagosomal pH to 6.5, which potentially enhances the availability of iron it needs for growth. Decrease in free iron via constitutive and inducible iron and zinc sequestration represents an important host antimicrobial defense. Since β-(1,3)-glucan elicits the strongest immune response, in *H. capsulatum* it is often masked by α-(1,3)-glucan as a part of the pathogen's strategy to avoid immune response.

Human macrophages respond to ingestion of *H. capsulatum* by a vigorous oxidative burst mediated by NADPH oxidase, and the release of nitric oxide (NO) and other nitrogen intermediates, yet the fungus is still able to survive this response and even replicate in the presence of **reactive oxygen species** (**ROS**). It eventually lyses the alveolar macrophage and the released yeasts are ingested by other resident macrophages and polymorphonuclear neutrophils (PMNs) newly recruited to the site of infection. In immunocompromised individuals, the infection/lytic cycle may be repeated several times.

Interaction of Dectin-1 with CR3 triggers the cytokine response to *H. capsulatum* yeasts via the activation of Syk tyrosine kinase. Intracellular growth of the fungus is inhibited by **interleukin** (**IL**)-3, granulocyte-macrophage colony stimulating factor (**GM-CSF**), and macrophage-CSF (M-CSF). GM-CSF limits intracellular *H. capsulatum* growth by producing zinc-sequestering metallothioneines.

PMNs from immunocompetent hosts can inhibit the growth of *H. capsulatum* through release of **defensins**. With the development of immunity mediated by neutrophils and macrophages, yeast growth ceases within 1–2 weeks after exposure.

ANTIBODY-MEDIATED IMMUNITY

Humoral immunity has little or no role in host defense against the fungus. Although exposure to *H. capsulatum* does induce an antibody response, and the **IgG** fraction of the sera from infected individuals contains complement-fixing and precipitating antibodies, these paradoxically are associated with progressive disease. Passive transfer of immune serum has not mediated protection from the fungus and B-lymphocyte-deficient mice were not susceptible to infection. No correlation was found between the titers of antibodies specific to *H. capsulatum* and immunity to the infection.

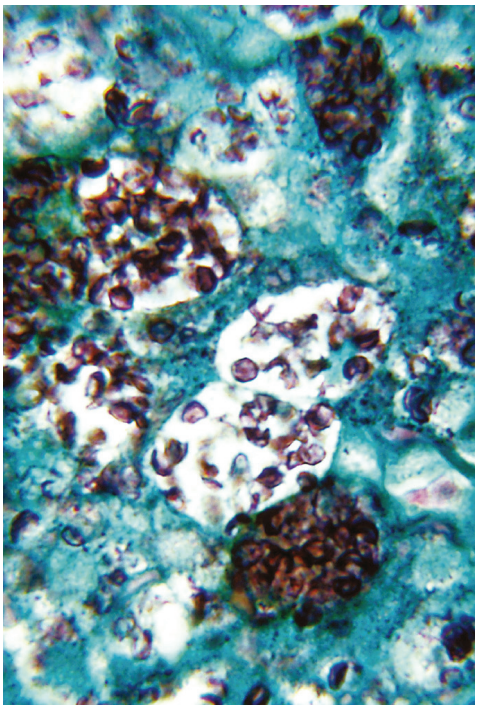

Figure 17.6 Two macrophages with engulfed small yeast-like fungus cells of *Histoplasma capsulatum* var. *capsulatum* (hematoxylin & eosin stain). *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #4223. Additional photographic credit is given to Dr Libero Ajello who took the photo in 1972.*

CELL-MEDIATED IMMUNITY

Of all the human mycoses, histoplasmosis illustrates best the importance of the T-mediated immune system in limiting the extent of *H. capsulatum* infection. Susceptibility to dissemination of *H. capsulatum* is associated with T-cellular immunodeficiency. In immunocompetent individuals, delayed-type hypersensitivity to histoplasma is observed 3–6 weeks after exposure to the fungus and, in up to 85–90% of cases, a positive response to a skin antigen test for *Histoplasma* can be detected (see Section 4).

It is thought that *H. capsulatum*, like other foreign organisms, is taken up by immature **dendritic cells** and processed with antigens then presented to T cells.

Specific T cells contribute to protective immunity to *H. capsulatum* mainly via production of **cytokines** that activate phagocytes, with **interferon**-γ (**IFN**-γ) and **tumor necrosis factor**-α (**TNF**-α) being particularly important. It has recently been shown that invariant NKT (iNKT) cells, a relatively rare population of T cells, defined by expression of an invariant TCR α-chain, are also instrumental in the production of IFN-γ in response to *H. capsulatum* and IL-12. Deficiency in IFN-γ and TNF-α causes a decrease in NO production by phagocytic cells, which is essential for controlling *H. capsulatum* infection. IL-12 regulates the induction of IFN-γ, which is a critical **Th-1** cytokine in primary host resistance. During the acute phase of the infection IL-12, TNF-α, and IFN-γ are released and

enhance the influx of the myeloid cells into the lungs followed by T and B cells. GM-CSF is also important for the production of TNF-α, IFN-γ, and NO, and is responsible for down-regulation of **Th-2** cytokines IL-4 and IL-10. High levels of Th-2 cytokines weaken the efficiency of protective immunity by inducing inflammatory responses and chronic infection.

For secondary immune responses, TNF-α is critical in both pulmonary and disseminated infections. Its deficiency leads to the dramatic elevation of IL-4 and IL-10 in the lungs. In most immunocompetent individuals, activation of a protective T-lymphocyte-mediated immunity results in containment of the infection. However, even in normal individuals, but particularly in immunodeficient hosts, the organism may not be completely eradicated and may persist as a latent infection. This can erupt as active disease at a later time when and if the host–pathogen balance is disrupted or host immune responses decline with age or through viral infection.

PATHOGENESIS

Chronic infection will lead to the inflammatory responses that can last over weeks to months and result in the development in the affected organs of calcified fibrinous granulomatous lesions with areas of caseous necrosis. *H. capsulatum* may remain latent in healed **granulomas** and recur resulting in impairment of cell-mediated immunity. The function of the granulomas is to contain fungal growth. In case of perturbed cellular immune response, the patient develops progressive disseminated histoplasmosis. Formation of the granulomas requires the generation of IFN-γ, TNF-α, and IL-17. Since X-linked hyper-immunoglobulin M immunodeficiency is associated with defective IL-17 generation, the majority of these individuals develop disseminated histoplasmosis (see Section 3).

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

In about 80–90% of infected immunocompetent individuals, histoplasmosis is asymptomatic, subclinical (showing self-limiting influenza-like symptoms) or benign. Clinical symptoms of histoplasmosis develop mostly in immunocompromised individuals. In addition, the inoculum size often defines symptomatic disease.

ACUTE PULMONARY HISTOPLASMOSIS

The onset of acute pulmonary histoplasmosis (APH) in symptomatic cases develops 7–21 days after exposure to the fungus. Common symptoms include fever, headache, malaise, **myalgia**, abdominal pain, and chills. Individuals exposed to a large inoculum of fungus may develop severe dyspnea due to diffuse pulmonary involvement. Diffuse or localized **pneumonitis** may be severe enough to require ventilation support (see the case). Weight loss, night sweats, and fatigue may persist for weeks after the acute symptoms resolve. A small number of patients may show rheumatologic manifestations such as **erythema multiforme**, **arthritis**, and **erythema nodosum**, which can be of a diagnostic value. Sometimes pulmonary **auscultation** may detect **rales** or wheezes. Severe **hypoxemia** and associated acute respiratory distress syndrome only develop in patients inhaling a high inoculum. In a small group of patients (about 6%), **pericarditis** may be present. More often, the acute pericarditis is due to the granulomatous inflammatory response mounted in mediastinal lymph nodes adjacent to the pericardium. In very rare cases, it is a direct *H. capsulatum* infection that leads to granulomatous inflammation within the pericardial sac. Approximately 10% of patients have asymptomatic pleural **effusions**. Hepatosplenomegaly is rare.

Lymphadenopathy reflected by enlarged hilar and mediastinal lymph nodes is present in 5–10% of patients. In some cases, it can cause local obstructions such as superior vena cava (SVC) syndrome, which results from the compression of the SVC. Obstruction of venous drainage may lead to cerebral symptoms: headache, visual distortion, **tinnitus**, and altered consciousness. Compression of the pulmonary airway and circulation, or of trachea or bronchi presents as cough, hemoptysis, dyspnea, and/or chest pain. Compression of the esophagus with subsequent **dysphagia** is rare.

Rarely, in endemic regions during the healing from APH, the enlarged mediastinal nodes can cause retraction of the airways, leading to post-obstructive pneumonia and bronchiectasis.

CHRONIC PULMONARY HISTOPLASMOSIS

Chronic pulmonary histoplasmosis (CPH) can present as cavitary or noncavitary disease. Cavities occur approximately in 40% of patients. However, if histoplasmosis is pre-conditioned by tuberculosis, the cavities develop in approximately 90% of cases and may lead to necrosis.

Generally, CPH occurs mostly in patients with underlying pulmonary chronic lung disease such as emphysema and may mimic tuberculosis. Histoplasmosis may actually coexist with tuberculosis, actinomycosis, other mycoses, and **sarcoidosis**. In individuals with underlying pulmonary disease chronic cavitary pulmonary histoplasmosis (CCPH) is the condition most commonly observed, with a frequency of 1 per 100 000 persons per year in endemic areas. CPH does not have as strong association with a chronic obstructive pulmonary disease (COPD) as previously believed, although 20% of patients present with COPD.

The main symptoms include cough, weight loss, fever, and malaise. In case of **cavitations**, additional symptoms of hemoptysis, sputum production, and increasing dyspnea develop. Untreated cases may result in progressive pulmonary fibrosis and subsequently in respiratory and cardiac failure and recurrent infections.

Chronic fungal infection leads to the development of well-organized solitary pulmonary nodules with a circumferential

rim of calcification, which allows their identification on chest X-ray (see Figure 17.1). Fungi within these nodules are usually dead. Older lesions show well-developed granuloma and have a central area of **caseation** resembling tuberculosis. These centers are usually occupied by the fungi.

PROGRESSIVE DISSEMINATED HISTOPLASMOSIS

Progressive disseminated histoplasmosis (PDH) occurs in 1:2000 cases of infected immunocompetent adults and in 4–27% of infected immunosuppressed individuals with impaired cellular immunity, children, or the elderly. All infections with histoplasmosis become disseminated as the macrophages are constantly trafficking between the lungs and other organs. Histoplasmosis becomes progressive when uncontrolled growth of the organism occurs in multiple organ systems. Symptoms vary depending on duration of illness. There is an acute and subacute form of PDH.

The ***acute form*** is associated with fever, worsening cough, weight loss, malaise, and dyspnea. Dissemination sites include the central nervous system (CNS; 5–20% of patients) and hematopoietic systems, liver, spleen, and eye. In the bone marrow **anemia**, **leukopenia**, and **thrombocytopenia** are detected. Adrenal glands undergo enlargement, and **Addison's disease** may develop as well. Genitourinary tract symptoms include **hydronephrosis**, bladder ulcers, penile ulcers, and **prostatitis**. Oral ulcers and small bowel micro and macro ulcers develop in the gastrointestinal (GI) tract. Skin symptoms include **papular** to nodular **rash**, while ocular problems include **uveitis** and choroiditis. Among CNS-related conditions chronic **meningitis** and **cerebritis** should be noted. The acute form, if untreated, results in death within weeks. About 10% of individuals develop fatal hyperacute syndrome.

The ***subacute form*** presents with a wide spectrum of symptoms based on dissemination in the affected organs. GI involvement leads to diarrhea and abdominal pain, while cardiac dissemination results in valvular disease, cardiac insufficiency, dyspnea, peripheral edema, angina, and fever. Pericarditis may develop, often with pleural effusions. CNS involvement is reflected by headaches, visual and gait disturbances, confusion, **seizures**, altered consciousness, and neck stiffness or pain. If untreated, the subacute form is fatal within 2–24 months. Approximately 5–10% of patients, treated or not, develop adrenal insufficiency.

In 50–60% of patients with the chronic form of disseminated histoplasmosis constitutional symptoms such as mouth and gum pain are present due to mucosal ulcers. Extensive ulceration including the oropharyngeal area, buccal mucosa, tongue, gingiva, and larynx is characteristic of chronic PDH (Figure 17.7). Rare, isolated lesions can be found in individuals who are immunocompetent.

The patients susceptible to the development of disseminated and potentially fatal histoplasmosis are mostly immunocompromised individuals, particularly HIV-infected patients in the endemic areas (see Section 1), children less than 2 years old, elderly persons, and people exposed to a very large inoculum.

PRESUMED OCCULAR

This syndrome is only found in 1–10% of individuals who live in endemic areas. Ocular histoplasmosis damages the retina of the eyes and leaves scar tissue leading to retinal leakage and a loss of vision similar to macular degeneration. Atrophic scars with lymphocytic cell infiltration (histo spots) may be seen posterior to the equator of the eye. The condition is bilateral in approximately 10% of patients. In the case of macular scarring, retinal hemorrhage, detachment or edema may be present.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

A social and occupational history is of great help for the initial evaluation. This includes travel to or residence in an endemic area, interaction with birds or bats, either recent or past. Any possible immunodeficiency in patients due to a medical condition or drug treatment must be taken into account (see the case).

IMAGING

A chest X-ray may be taken to help in the diagnosis of the kind of histoplasmosis. It usually does not show any irregularities in APH. However, diffuse pulmonary involvement caused by exposure to a large inoculum may present with a reticular nodular or **miliary** pattern. Pleural effusions are found in fewer than 10% of uncomplicated cases. Cavitations are very rare. Histoplasmosis can be detected as lesions and residual nodules, 1–4 cm in diameter.

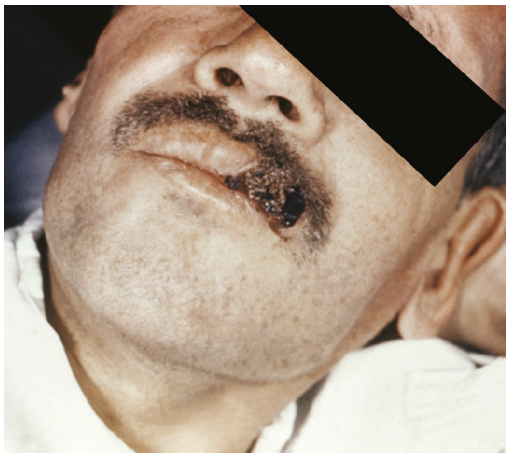

Figure 17.7 Mouth lesions due to *Histoplasma capsulatum* dissemination into oral cavity. *Courtesy of the Centers for Disease Control & Prevention, Atlanta, Georgia. Image is found in the Public Health Image Library #6840. Additional photographic credit is given to Susan Lindsley, VD, who took the photo in 1973.*

In CPH hilar lymphadenopathy is rare. The cavitations are located in the upper lobes of the lungs. Often **emphysematous** changes are present as well as fibrotic scarring, particularly in long-standing cases.

Around 50% of patients with acute PDH have hilar lymphadenopathy with diffuse nodular infiltrates. At the onset of the disease, no chest radiography changes are detected in 33% of patients but, as the disease progresses, pulmonary involvement becomes gradually more apparent. In contrast, chronic PDH does not present with chest radiographic changes.

Head **CT scanning** helps to detect cerebral histoplasmosis before performing a lumbar puncture. Abdominal CT can confirm subacute PDH, which leads to adrenal infection in 80% of patients and presents with bilateral adrenal enlargement on the scan.

RESPIRATORY FUNCTIONAL TESTS

Pulmonary functional tests help characterize the scale of pulmonary involvement. They include evaluation of the restrictive defect, detection of a small airway obstruction, diffusion impairment, and hypoxemia. The functional tests are also used for monitoring the progression of pulmonary disease in patients with CPH.

LABORATORY TESTS

Specimens obtained for analysis include: blood, sputum, bronchial lavage, and cerebrospinal fluid (CSF), by lumbar puncture, if CNS involvement is suspected. Tissue biopsies may be taken of pulmonary lesions and lymph nodes by **bronchoscopy** or thoracoscopy. Biopsies can be also taken from oropharyngeal ulcers.

Serology

Complement-fixing (CF) antibody tests for the presence of M and H precipitin bands with the antigen extract of the Histoplasma mycelial form called histoplasmin. The titers of CF antibodies to yeast and mycelial-phase antigens (Y and M, see the case) are considered positive at dilutions greater than 1:8. A titer of 1:32 or more suggests active histoplasmosis infection. CF antibody to Y antigen is detectable in primary infection, while CF antibody to M antigen appears later. An M band develops with acute infection, persists for months to years and is also present in chronic forms. H band appears after M band and may disappear early. Thus, the presence of both M and H bands suggests active histoplasmosis. Positive results are usually found in 5–15% of cases of acute pulmonary infection 3 weeks after exposure and in 75–95% of cases at 6 weeks. The test usually turns negative in the course of months with resolution of infection. However, in chronic infection, the CF test may remain positive for a long time in 70–90% of patients with CPH or chronic PDH.

Cross-reactivity with *Blastomyces dermatitidis* and *Coccidioides immitis* must be ruled out for differential diagnosis.

Antigen Detection in Urine and Other Fluids

Antigen detection by **ELISA** is useful in individuals who are immunocompromised when antibody production may be impaired. This method is more sensitive for urine samples compared with serum, plasma, CSF, and bronchoalveolar lavage fluid. Detection of the capsular antigen of *H. capsulatum* in urine or serum is a very powerful tool for the diagnosis of the disseminated forms of histoplasmosis. In cases of acute PDH, detection rates are 50% with serum assay and 90% with urine assay. Lower detection rates are observed in APH or CPH.

Histology and Cytology

In rare cases, a tissue biopsy may reveal the presence of large yeast cells with a false capsule. Special stains may reveal budding yeast in areas of necrosis from histoplasmosis and calcified lymph nodes. Direct microscopic evidence of histoplasma infection showing characteristic yeast-like cells from any specimen taken from the patient is considered significant diagnostic proof.

Cell Culture

Isolation of *H. capsulatum* in culture remains the gold standard method for diagnosis of histoplasmosis. However, the fungus takes up to 4 weeks to grow *in vitro*. Specimens are inoculated onto SDA or BHIA supplemented with 5–10% sheep blood and observed for morphology of the growing colonies (see Section 1). A positive culture of diagnostic value has to show conversion of the mold form to the yeast phase by growth at 37°C. Sputum culture results are usually positive in 60% of patients with CPH and in approximately 10–15% of patients with APH. Blood cultures are positive for histoplasma in 50–90% of patients with acute PDH. Cultures of CSF are positive in 30–60% of patients with histoplasmal meningitis.

Skin Tests

Positive histoplasmin skin tests were demonstrated by almost 80% of the people living in areas endemic for *H. capsulatum* and therefore their diagnostic value is very low. During the acute pulmonary infection, a skin test may be positive in more than 90% of cases; while during the chronic pulmonary infections, in 70–90% of cases, and during PDH, in 3–55% of cases.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) tests have been developed for the detection of *H. capsulatum* but are not used in clinical practice. The usual target is *H. capsulatum* ribsomal DNA (rDNA), but it is similar to 18-S rDNA of other fungi. Hence, a nested PCR has been developed with multiple primer sets. This includes both *H. capsulatum* rDNA and a 100-kDa-like protein unique to Histoplasma (Hcp100); or N-acetylated α-linked acidic dipeptidase (NAALAD) and the internal

transcribed spacer (ITS) region. This assay can be modified to accommodate fluorescent markers and has the potential to be used in a low budget setting. Fluorescent *in situ* hybridization (FISH) targeting Histoplasma rRNA may be clinically useful.

DIFFERENTIAL DIAGNOSIS

A differential diagnosis for *H. capsulatum* with *Chrysosporium* and *Sepedonium* species that also inhabit soil and plant material should be carried out.

The conidia of *Chrysosporium parvum* morphologically resemble those of *H. capsulatum*. *Chrysosporium* species are also dimorphic, but this is a false dimorphism since they do not convert from mold to yeast at 37°C. Macroconidia of *Sepedonium* species also resemble those of *Histoplasma*.

However, Histoplasma galactomannans (GM) are similar to antigens of other dimorphic fungi such *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Talaromyces marneffei*, and Sporothrix species, and cross-reactivity of these fungi with the antigen test is common. Aspergillus galactomannan testing also cross-reacts with Histoplasma GM.

In the case of *H. capsulatum* var. *duboisii*, differential diagnosis must be made from *B. dermatitidis* since they both occur in Africa.

Importantly, in 2017, a separate genus *Emergomyces*, formerly classified under the genus *Emmonsia* was placed in the *Ajellomycetaceae* family. Emergomyces species is also a dimorphic fungus mostly found among immunocompromised individuals in Asia, Europe, Africa, and North America. The number of emergomycosis cases might be underestimated considering the high incidence of HIV/AIDS. Differential diagnosis remains challenging, with histoplasmosis due to clinical and histopathologic overlap between the two entities. The gold standard for identification would be sequencing the ITS region of ribosomal DNA but this requires substantial resources.

Some patients with acute histoplasmosis may have high serum levels of angiotensin-converting enzyme. This may cause a diagnostic confusion with sarcoidosis, particularly if the patient with histoplasmosis also has hilar **adenopathy**.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

As discussed in Section 3, in immunocompetent individuals infection with *Histoplasma* species in most cases is self-limiting and does not require therapy. Therapeutic intervention is recommended in cases of prolonged infection, systemic infection or if patients are immunocompromised.

MANAGEMENT

APH: Mild symptoms in immunocompetent individuals need monitoring only. In patients with prolonged symptoms or extensive pulmonary involvement, therapy is recommended.

CPH: No treatment is required for asymptomatic immunocompetent individuals without serious underlying disease. Mild interstitial pneumonitis and/or thin-walled (<3 mm) cavities with serial chest radiographs require monitoring for 2–4 months. Medical treatment is recommended if the lesions are persistent or if patients develop cavities with thick walls (>3 mm). Treatment is indicated for all immunocompromised patients.

PDH: All patients with the symptoms of meningitis require medical therapy. In the case of severe CNS infection, intravenous (IV) antifungal therapy should be supplemented with **intrathecal** or intraventricular injections.

Cutaneous and rheumatologic lesions are usually self-limiting and medical intervention is recommended only for prolonged course of the disease episodes or in immunocompromised patients.

Maculopathy in ocular histoplasmosis is treated with steroids.

Surgery may be required in some cases for resection of pulmonary cavitation lesions, repair of infected heart valves, and **aneurysms**.

Antifungal Drugs

Severe cases of acute histoplasmosis, and all cases of symptomatic chronic and disseminated disease, require treatment with antifungal drugs, if the symptoms persist for more than 4 weeks. The recommended regimen is a 3-month course of itraconazole. In patients with CPH, noncavitary disease, a 6-month therapy is recommended instead, whereas for a cavitary disease the treatment might be prolonged for one year. Patients with PDH require induction therapy with amphotericin-B for 2–4 weeks followed by one year of itraconazole. There are some reports of patients being treated with posaconazole instead of both, itraconazole and amphotericin B. Amphotericin B (AmB) is used to treat APH, CPH, all forms of PDH, meningitis, and endovascular histoplasmosis. There is evidence that the liposomal AmB has better outcomes, particularly in patients with AIDS.

In adults, as well as in children, 0.7–1 mg kg^{-1} per day of AmB or up to a total dose of 35 mg kg^{-1} is used IV. A minimum total dose should be no less than 2 g. In the case of underlying severe immunodeficiency (such as AIDS) when a life-long antifungal maintenance therapy is required to treat acute PDH, the induction dose of 0.7–1 mg kg^{-1} per day AmB to a total of 20–25 mg kg^{-1} is used, followed by a maintenance therapy of 50 mg once per week.

Itraconazole is a fungistatic drug that suppresses fungal cell growth. It is administered 200 mg three times daily for 3 days, then 200 mg twice daily in adults and 5–10 mg/kg (maximum 400 mg daily) in children. However, several studies have indicated that fluconazole is characterized by higher response rates and lower relapse rates.

Ketoconazole can be used as an alternative to AmB in the treatment of CPH or chronic and subacute progressive disseminated histoplasmosis. However, since these drugs do

not cross the blood–brain barrier, they are not effective in the treatment of fungal meningitis.

Anti-Inflammatory Drugs

Apart from the fungicidal and fungistatic drugs, nonsteroidal anti-inflammatory drugs (NSAIDs) are used with analgesic, anti-inflammatory, and antipyretic action. They are particularly recommended for patients with pericarditis. Among the most used is ibuprofen given orally at 400 mg every 4–6 hours, 600 mg every 6 hours, or 800 mg every 8 hours, with a maximal dose of 3.2 g per day while symptoms persist. The pediatric dose is 20–70 mg kg^{-1} per day with a low induction dose and maximal dose of 2.4 g per day. Usual NSAIDs contraindications apply.

Among other anti-inflammatory drugs, corticosteroids, mostly prednisone, can be used to decrease hypersensitivity to *Histoplasma*. High-dose steroids are used in patients with extensive maculopathy.

Since IFN-γ augments antifungal activity of PMNs and macrophages it is considered for immunotherapy of *H. capsulatum*.

PROGNOSIS

Prognosis for a complete recovery is good for APH. The relapse rate in CPH is 20%, while in treated acute PDH it is 50%. If lifelong antifungal maintenance is administered, the relapse rate drops to 10–20%. The course of chronic PDH can last for years with long asymptomatic periods.

Fatal outcome of acute PDH and subacute PDH is imminent without treatment.

Full recovery from histoplasmal meningitis with therapy is 50%.

PREVENTION

Prevention includes chemical disinfection and respiratory barrier protection in high-risk areas or during high-risk activities. Since the outbreaks of histoplasmosis are associated with disruption of the soil in endemic areas, decontamination of the infected soil with a 3% formalin solution is highly recommended.

Individuals residing in or traveling to endemic areas, particularly those with a history of immunodeficiency (AIDS, lymphoma, immunosuppressive treatment), must be educated/briefed about exposure risks. The elderly and children are also at risk.

In some areas, special precautions are taken by placing warning signs around particularly contaminated soil.

VACCINES

Although it has been shown that there is no immunity to *Histoplasma* by prior infection and no effective vaccine is currently available, recent studies have indicated that vaccination with highly immunogenic recombinant rHSP60 protein or its F3 fragment protects mice from lethal and sublethal histoplasmosis by stimulating **CD4**+ T lymphocytes. Other approaches to development of a vaccine are associated with targeting the histone H2B-like protein on the surface of *H. capsulatum* (see Section 2) and studies based on glucan nanoparticles extracted from *S. cerevisiae*.

SUMMARY

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- The genus *Histoplasma* contains one species, *Histoplasma capsulatum*, with three varieties: *H. capsulatum* var. *capsulatum*, which causes the common histoplasmosis, *H. capsulatum* var. *duboisii*, which causes African histoplasmosis, and *H. capsulatum* var. *farciminosum*, which causes lymphangitis in horses.
- *H. capsulatum* is thermally dimorphic and can survive at two different temperatures. Below 30°C, *H. capsulatum* remains in a saprophytic mycelial mold form, but at an average mammalian body temperature 37°C, it grows as a parasitic yeast.
- For the saprophytic mycelial growth, *H. capsulatum* requires an acidic damp soil with high organic content provided mostly by bird droppings or excrement of bats. Bats can become infected, and they transmit the fungus through droppings.
- In a saprophytic form at 25°C, *H. capsulatum* colonies grow slowly into granular suede-like to cottony colonies brown with a pale yellow-brown or yellow-orange reverse. Macroconidia are large and tuberculate, microconidia are small and round. At 37°C, *H. capsulatum* grows as creamy, smooth, white and yeast-like colonies.
- In the lungs, inhaled mycelial fragments and microconidia of *H. capsulatum* are ingested by resident phagocytes. Conversion from the mycelial to the pathogenic yeast phase occurs inside the macrophages, mostly due to the temperature change.
- Binding of the microconidia is thought to be through the complement receptor 3 (CR3), pattern recognition receptor (PRR) Dectin-1, which binds to β-(1,3)-glucan, and integrins CD11/CD18. The initial pulmonary infection with *H. capsulatum* var. *capsulatum* disseminates to many organs that contain mononuclear phagocytes and produces extrapulmonary manifestations in the liver and spleen, or in regional lymph nodes. Infection with *H. capsulatum* var. *duboisii* mostly involves cutaneous, subcutaneous, liver, lung, lymphatic, and bony tissues. *H. capsulatum* is endemic to the Ohio, Missouri, and Mississippi River valleys in the US. Approximately 250 000 individuals are infected annually with *H. capsulatum* in the US and 500 000 worldwide. Of these, 50 000–200 000 develop symptoms of histoplasmosis and 1500–4000 require hospitalization.

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2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- After ingestion, *H. capsulatum* yeasts reside in phagosomes and replicate approximately every 15–18 hours. The yeasts inhibit phagolysosomal fusion, prevent exposure to the lysosomal hydrolytic enzymes, block accumulation of vacuolar ATPase, and increase phagosomal pH to 6.5.
- In response to ingestion of *H. capsulatum*, macrophages enhance oxidative burst and the release of nitrogen intermediates. The yeasts are ingested by newly recruited PMNs, which release fungistatic defensins.
- T cells contribute to protective immunity via production of cytokines that activate phagocytes, particularly IFN-γ and TNF-α. GM-CSF is important for the production of TNF-α, IFN-γ, and NO, and down-regulation of Th-2 cytokines IL-4 and IL-10, which are involved in pathogenesis.
- In most immunocompetent individuals, protective T-mediated immunity results in containment of the infection. However, even in normal individuals, and particularly in immunodeficient hosts, the organism may persist as a chronic infection and may lead to inflammatory responses and fibrinous granulomatous lesions with necrosis.
- Humoral immunity has little role in host defense against *H. capsulatum*, although exposure to it induces antibody response.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- In about 80–90% of infected immunocompetent individuals, histoplasmosis is asymptomatic and subclinical with self-limiting influenza-like symptoms or benign.
- The symptoms of severe acute pulmonary syndrome are nonspecific: fever, chills, myalgias, cough, and chest pain. The syndrome can be mild (lasting 1–5 days) or severe (lasting 10–21 days).
- In a small group of the infected individuals (5–10%), histoplasmosis presents as chronic progressive lung disease, chronic cutaneous or systemic disease or an acute fatal systemic disease. The patients are mostly immunocompromised, children less than 2 years old, the elderly, and people exposed to a very large inoculum.
- Chronic pulmonary histoplasmosis (CPH) occurs in patients with underlying pulmonary chronic lung disease (emphysema) and may mimic tuberculosis. The symptoms include cough, weight loss, fever, and malaise. In case of cavitations, hemoptysis, sputum production, and increasing dyspnea develop and lead to necrosis.
- The acute form of progressive disseminated histoplasmosis (PDH) is associated with fever, worsening cough, weight loss, malaise, and dyspnea. The symptoms related to the organ of dissemination include: anemia, leukopenia and thrombocytopenia (bone marrow); hydronephrosis, bladder and penile ulcers, prostatitis (genitourinary tract); oral ulcers, small bowel ulcers (gastrointestinal tract); papular to nodular rash (skin); uveitis and choroiditis (eyes); chronic meningitis and cerebritis (CNS). The subacute form of PDH presents with diarrhea and abdominal pain, valvular disease, cardiac insufficiency, dyspnea, peripheral edema, angina, fever, pericarditis, CNS involvement, and adrenal insufficiency. If untreated, the subacute form is fatal within 2–24 months.
- In 50–60% of patients with the chronic form of PDH, painful granulomatous mucosal ulcers appear as nodular ulcerative or vegetative lesions localized on the oral mucosa, tongue, palate, or lips.
- Presumed ocular histoplasmosis syndrome is only found in 1–10% of individuals who live in endemic areas.

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- A social and occupational history is important for the initial evaluation, such as travel to or residence in an endemic area, interaction with birds or bats, possible immunodeficiency.
- CPH can be detected through pulmonary auscultation as rales, wheezes with a history of underlying pneumonitis, consolidation, or cavitation.
- In acute progressive disseminated histoplasmosis, the leading symptoms are hepatosplenomegaly and lymphadenopathy. CNS-related symptoms include a mass lesion, encephalopathy, and meningitis. Subacute PDH presents as abdominal mass or intestinal ulcers and lesions. CNS dissemination may lead to mass lesions or meningismus, muscle weakness, ataxia, altered consciousness, or focal deficits. Endocarditis, murmurs, peripheral edema, petechiae indicate cardiac dissemination.
- Extensive ulceration in the oropharyngeal area, buccal mucosa, tongue, gingiva, and larynx is characteristic of chronic PDH.
- In presumed ocular histoplasmosis syndrome, atrophic scars with lymphocytic cell infiltration (histo spots) may be seen posterior to the equator of the eye. The condition is bilateral in approximately 10% of patients.
- Around 50% of patients with acute PDH have hilar lymphadenopathy with diffuse nodular infiltrates. As the disease progresses, pulmonary involvement becomes more apparent on radiography.
- Abdominal CT can confirm subacute PDH in 80% of patients, presenting with bilateral adrenal enlargement on the scan.
- Direct microscopy evidence demonstrating characteristic yeast-like cells from any specimen taken from the patient is considered significant diagnostic proof.
- Using sputum and blood samples, diagnosis is made on the basis of conversion of the mold form to the yeast phase by growth at 37°C. The titer of complement-fixing antibody is considered positive at dilutions greater than 1:8. Positive results are usually found in 5–15% of cases of acute pulmonary infection 3 weeks after exposure and in 75–95% of cases at 6 weeks. In chronic infection, the test may remain positive for a long time in 70–90% of patients.

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- Differential diagnosis needs to be carried out on *H. capsulatum* with *Chrysosporium* and *Sepedonium* species. Since Histoplasma GMs are similar to antigens of other dimorphic fungi such as *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Talaromyces marneffei*, Aspergillus and Sporothrix species, cross-reactivity of these fungi with the antigen test is common.
- In 2017, a separate genus *Emergomyces*, formerly classified under the genus *Emmonsia* was placed in *Ajellomycetaceae* family. Emergomyces species is also a dimorphic fungus mostly found among immunocompromised individuals in Asia, Europe, Africa, and North America. The number of emergomycosis cases might be underestimated considering the high incidence of HIV/AIDS. Differential diagnosis remains challenging.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- In most cases, infection with Histoplasma species of **immunocompetent individuals** is **self-limiting** and does not require therapy, which is recommended in cases of prolonged infection, systemic infection or immunocompromised patients.
- All patients with progressive disseminated histoplasmosis and the symptoms of meningitis require medical therapy.
- Surgical resection of pulmonary cavitary lesions is required when repeated relapses or progressive disease occurs despite repeated intensive medical therapy.
- Severe cases of acute histoplasmosis, and all cases of chronic and disseminated disease, require treatment with antifungal drugs. The recommended regimen is a 3-month course of itraconazole. In patients with CPH, noncavitary disease, a 6-month therapy is recommended instead, whereas for a cavitary disease, the treatment might be prolonged for one year. Patients with PDH, require induction therapy with amphotericin-B for 2–4 weeks followed by one year of itraconazole.
- Amphotericin B (AmB) is used to treat acute pulmonary histoplasmosis, chronic pulmonary histoplasmosis, all forms of progressive disseminated histoplasmosis, meningitis, and endovascular histoplasmosis. In adults, as well as in children, 0.7–1 mg kg^{-1} per day of AmB or up to a total dose of 35 mg kg^{-1} is used IV. A minimum total dose should be no less than 2 g. In the case of underlying severe immunodeficiency (such as AIDS) when a life-long antifungal maintenance therapy is required to treat acute progressive disseminated histoplasmosis, the induction dose of 0.7–1 mg kg^{-1} per day AmB to a total of 20–25 mg kg^{-1} is used, followed by a maintenance therapy of 50 mg once per week.
- Itraconazole is administered 200 mg three times daily for 3 days, then 200 mg twice daily in adults and 5–10 mg/kg (maximum 400 mg daily) in children.
- Ketoconazole can be used as an alternative to AmB in the treatment of chronic pulmonary histoplasmosis or chronic and subacute progressive disseminated histoplasmosis.
- Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen are used with analgesic, anti-inflammatory, and antipyretic action, particularly for patients with pericarditis. Corticosteroids, mostly prednisone, can be used to decrease hypersensitivity to *Histoplasma*. High-dose steroids are used in patients with extensive maculopathy.
- Prognosis for a complete recovery is good for acute pulmonary histoplasmosis. The relapse rate is 20% in chronic pulmonary histoplasmosis and 50% in treated acute progressive disseminated histoplasmosis, which drops to 10–20% in case of a life-long antifungal maintenance.
- Prevention includes chemical disinfection and respiratory barrier protection in high-risk areas or during high-risk activities. Individuals residing in or traveling to endemic areas, particularly those with a history of immunodeficiency, the elderly, and children, must be educated/briefed about exposure risks.

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Students can test their knowledge of this case study by visiting the Instructor and Student Resources: [www.routledge.com/cw/lydyard] where several multiple choice questions can be found.