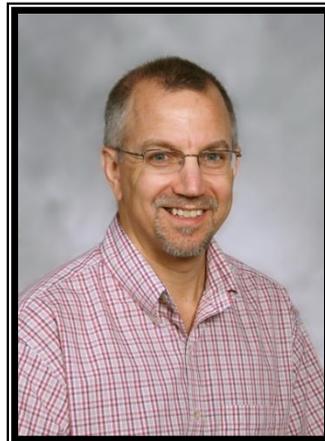


# Fungus Among Us:

The appearance of fungi in  
histologic sections

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# Fungus in tissue sections – basics

Fungus can appear in different forms (e.g. yeast versus mycelial) in tissue compared to natural environment and laboratory cultures. The host response varies depending on the organism and immunologic integrity of the host.

Useful stains in tissue sections include:

Gomori methanamine silver (GMS) – stains yeast and hyphae walls black

Periodic acid Schiff (PAS) – stains yeast and hyphae walls magenta. May stain internal structure also.

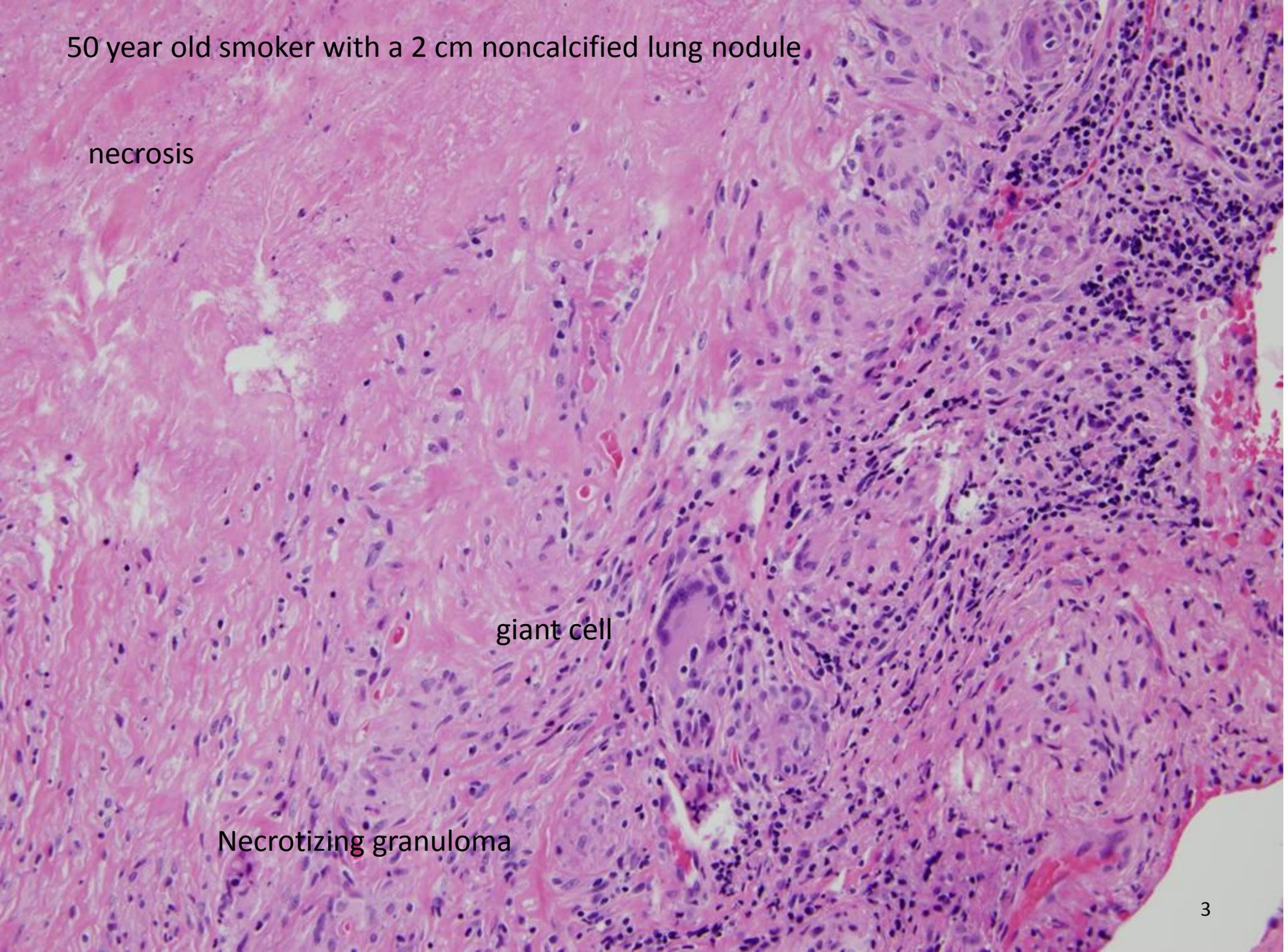
Mucicarmine – stains mucinous capsules of yeast red.

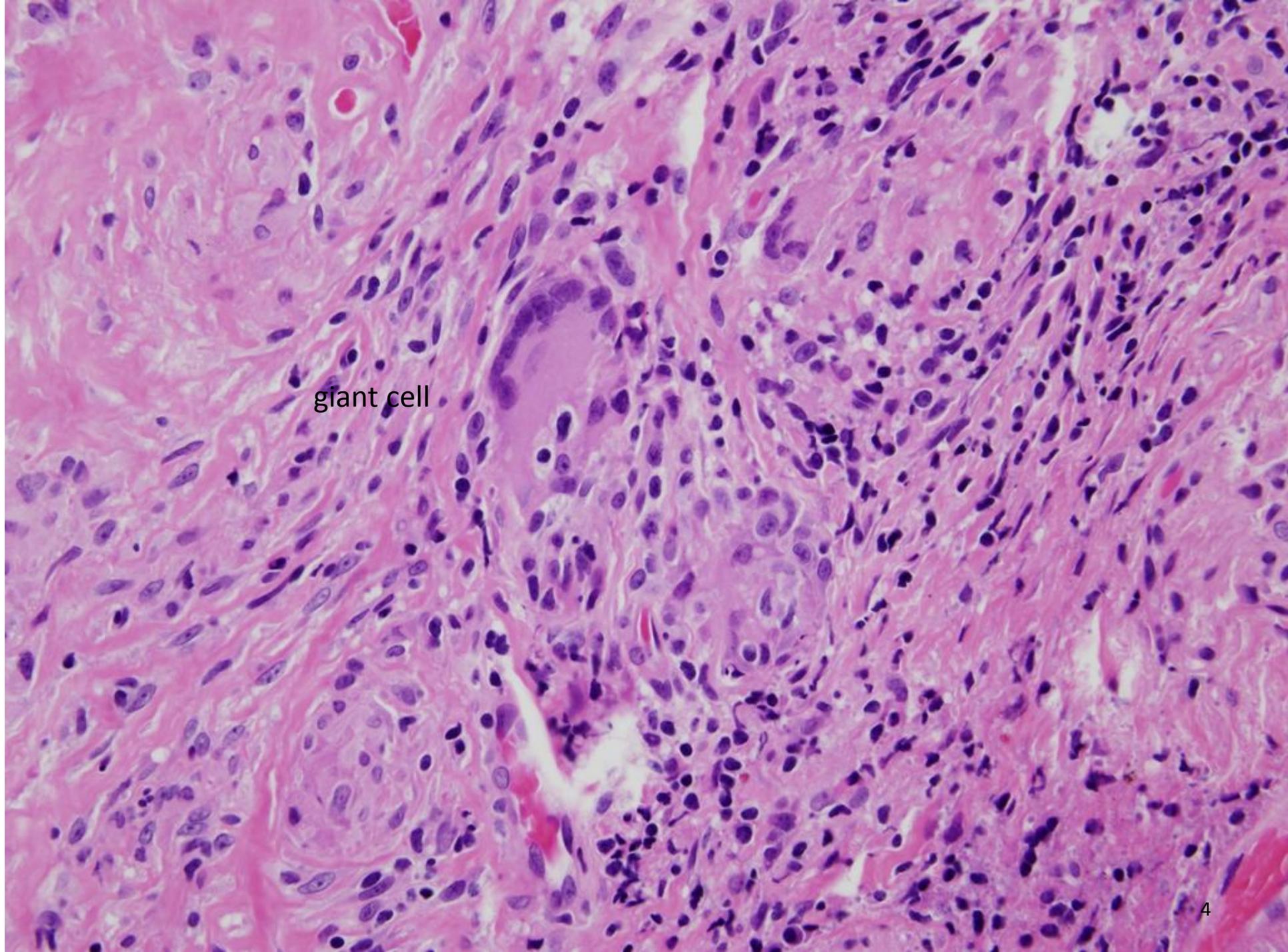
50 year old smoker with a 2 cm noncalcified lung nodule

necrosis

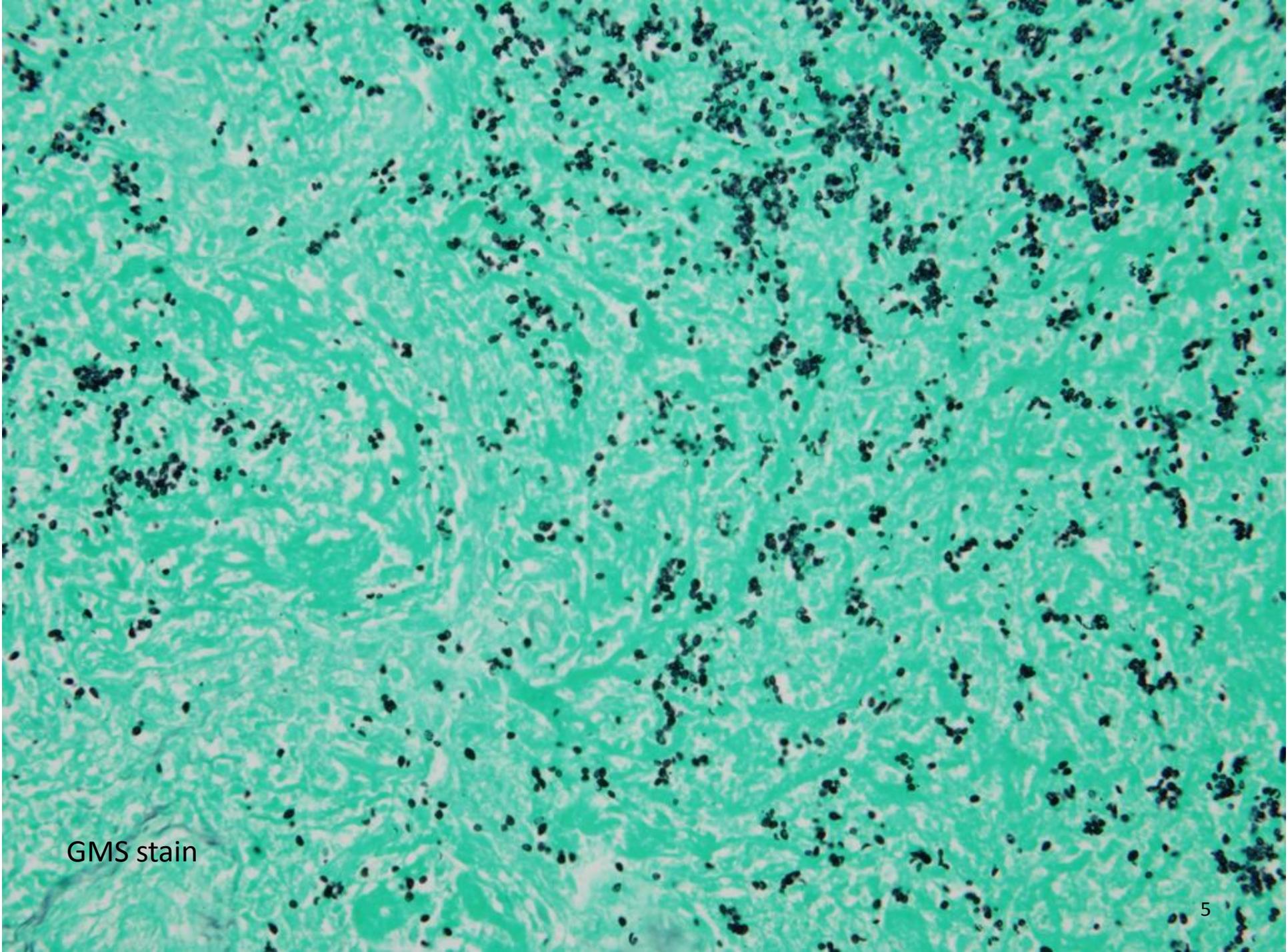
giant cell

Necrotizing granuloma





giant cell



GMS stain

Numerous yeast approx. 2-4 microns

# Histoplasmosis

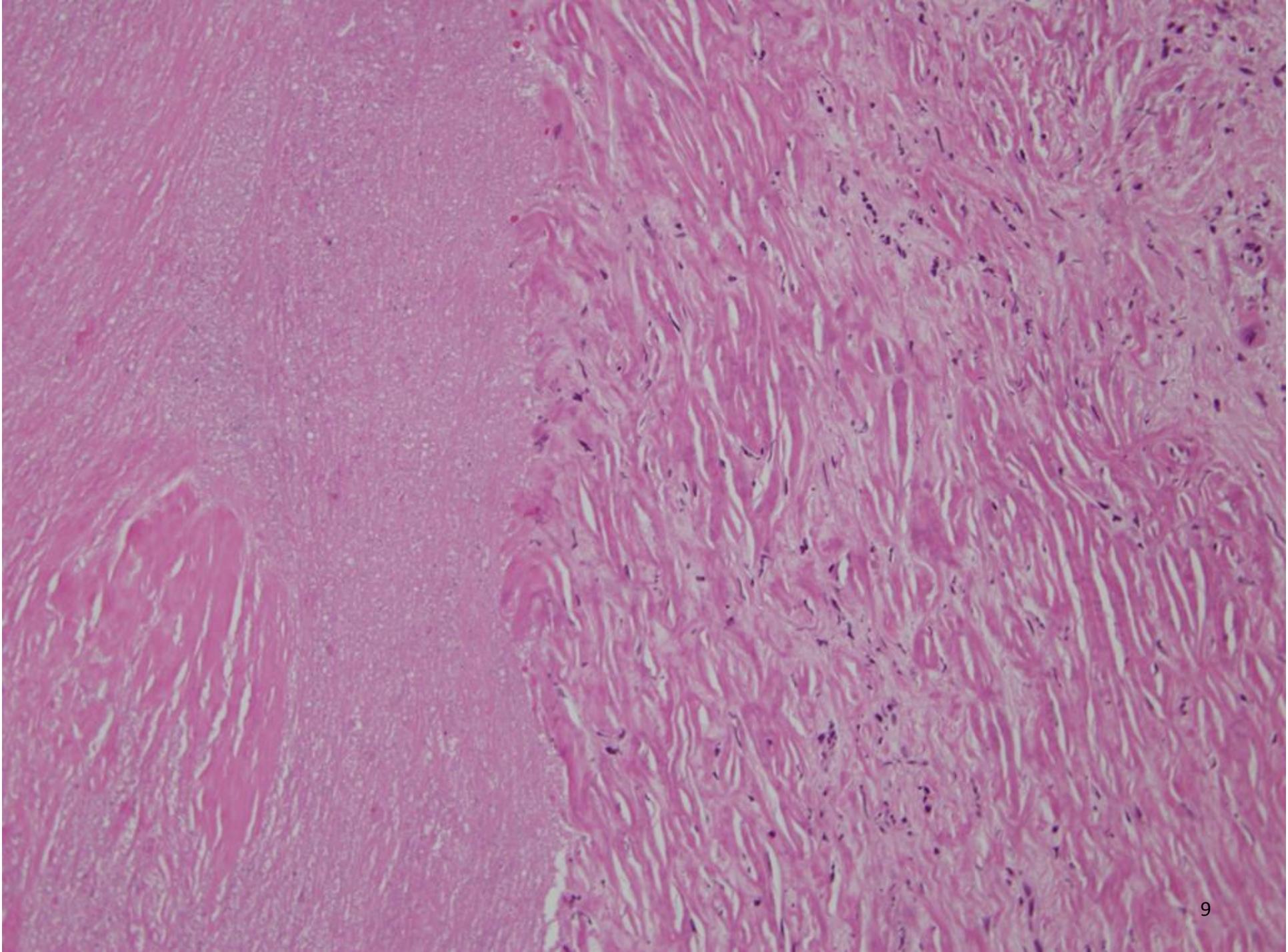
Histoplasmosis is caused by the dimorphic fungus *Histoplasma capsulatum*. It has a natural habitat in soil where it exists in the mycelial form. It is endemic in the south central U.S. especially the Mississippi and Ohio River valley. The disease is caused by inhaling infected dust, commonly from bird droppings, e.g. disturbed chicken coops, bird roosts (pigeons and starlings), caves (bats), attics, etc. In infected individuals the fungus converts from the mycelial to the yeast form. In the lungs the yeast are ingested by alveolar macrophages and may be transported to the regional lymph nodes. The clinical forms of infection include: 1) benign self-limited (most common), 2) acute respiratory illness, 3) chronic with asymptomatic lung nodules, often calcified, 4) progressive nodules with cavitation, 5) progressive disseminated (usually immunocompromised), and 6) mediastinal with compression of structures (airway, blood vessels, etc) by lymph nodes with granulomas or fibrosis (sclerosing mediastinitis).

In tissue the yeast are spherical to oval, 2-4 microns in diameter and have single buds and are often found in the centers of necrotizing granulomas or in histiocytic cells. The yeast may be similar in appearance to *Pneumocystis jiroveci* but have a different type of host response which helps differentiate the two infections.

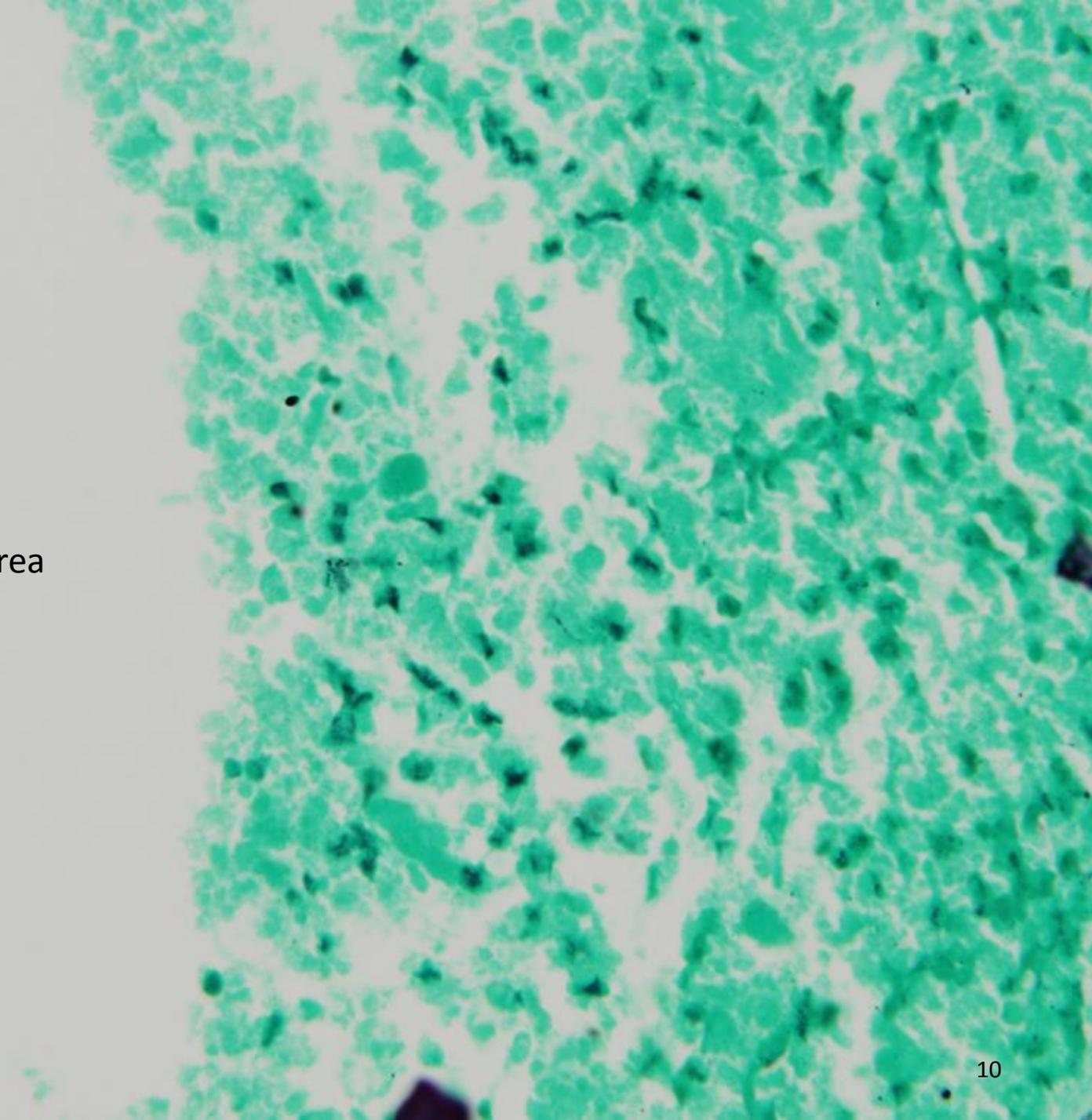
Sclerosing mediastinitis – 30 year old man with enlarging mediastinal mass adherent to aorta and superior vena cava and a 1 cm lung nodule

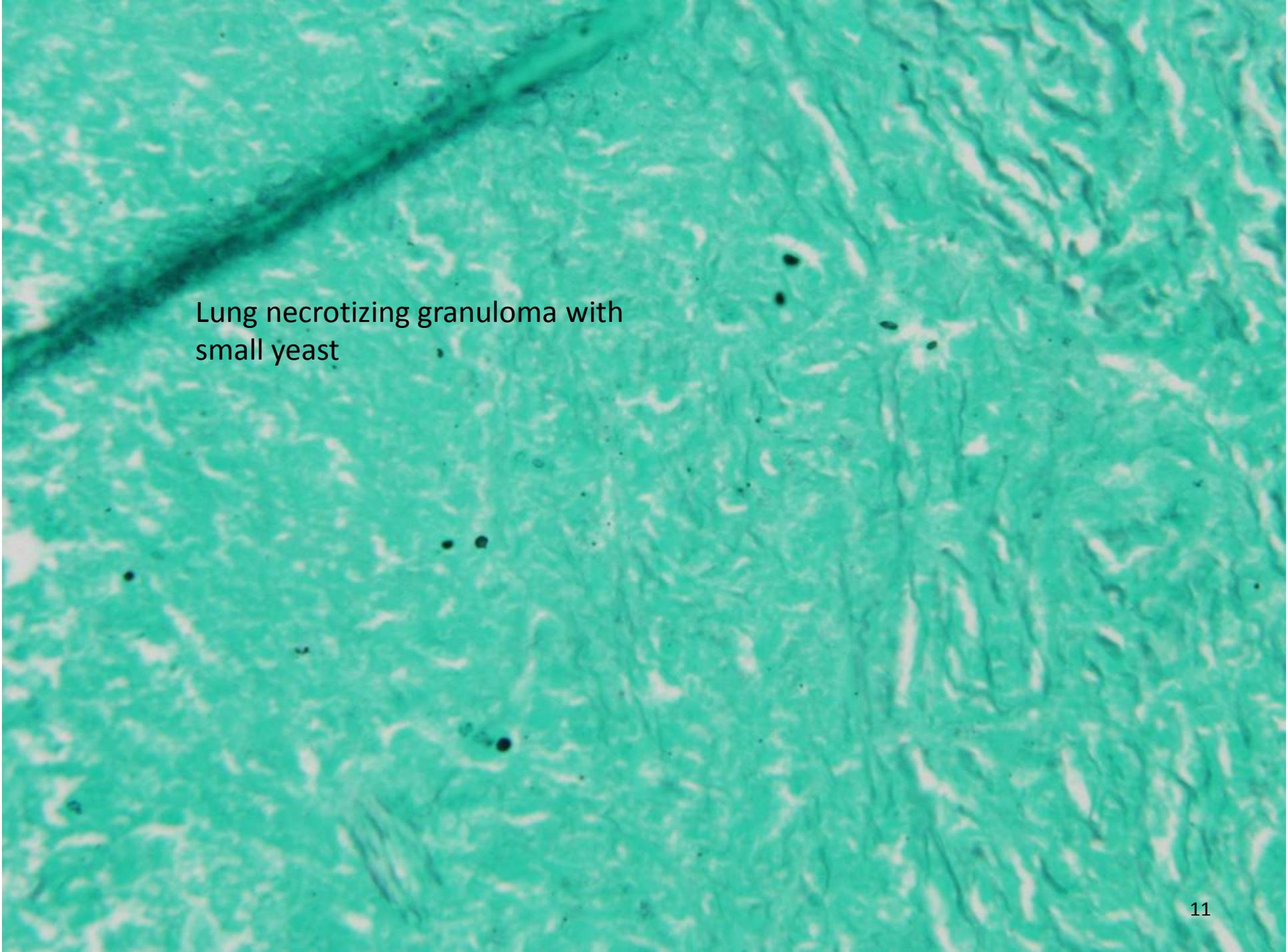
Necrosis

Sclerosis (fibrosis)



GMS stain- small  
yeast in necrotic area



A microscopic image of lung tissue stained with hematoxylin and eosin (H&E). The image shows a large, irregular area of necrosis (dead tissue) in the upper left quadrant. Within and around this necrotic area, there is a granuloma, which is a collection of inflammatory cells. Several small, dark, round yeast cells are visible, some with characteristic thick, double-layered walls. The surrounding lung parenchyma shows some architectural distortion and inflammatory infiltrate.

Lung necrotizing granuloma with  
small yeast

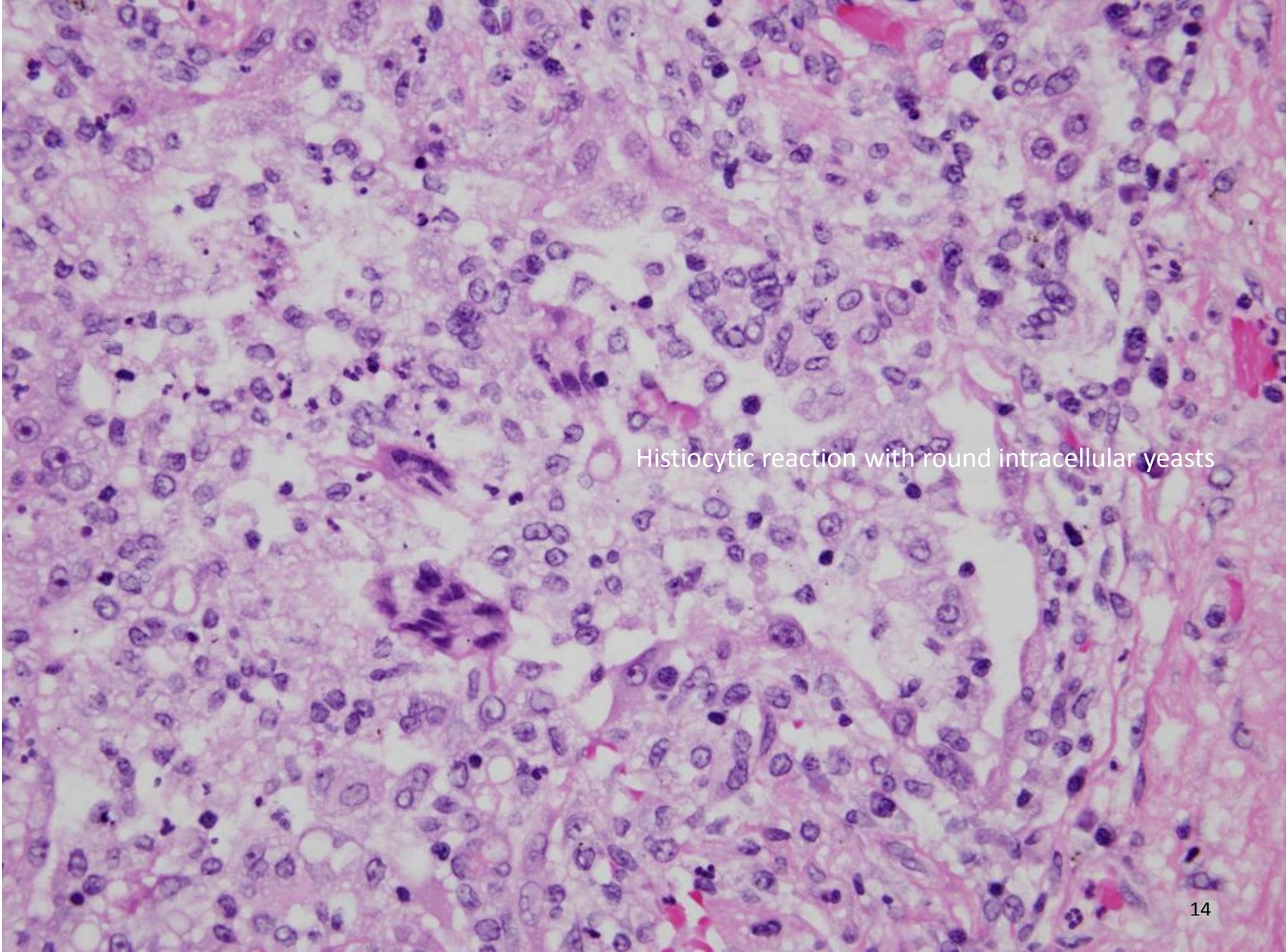
Sclerosing mediastinitis is an excessive fibrotic reaction occurring in the mediastinum. The etiology is unknown but in most cases it is believed to be an exaggerated immune reaction leading to fibrosis (scarring). The triggering mechanism in some cases is felt to be histoplasmosis. The infection involves lymph nodes in the chest and the host response starts with a granulomatous reaction which is followed by fibrosis which not only involves the lymph nodes but also the soft tissues in the mediastinum. Compression of mediastinal structures including airways, major blood vessels, and esophagus may occur. Occasionally necrotizing granulomas are also found in biopsies but only scar tissue may be seen. The fibrosis tends to have a roopy hyalinized appearance. Yeast may be absent or rare. Histoplasma related granulomas may be found in the lung.

Sclerosing mediastinitis appears to be related to other disorders with excessive fibrosis including hyalinizing granuloma of the lung, idiopathic retroperitoneal fibrosis, and Riedel's thyroiditis. There may be a genetic predisposition to an altered immune response leading to excessive fibrosis. Patients with fibrosis in more than one site have been described.

## 30 year old man with new onset fever

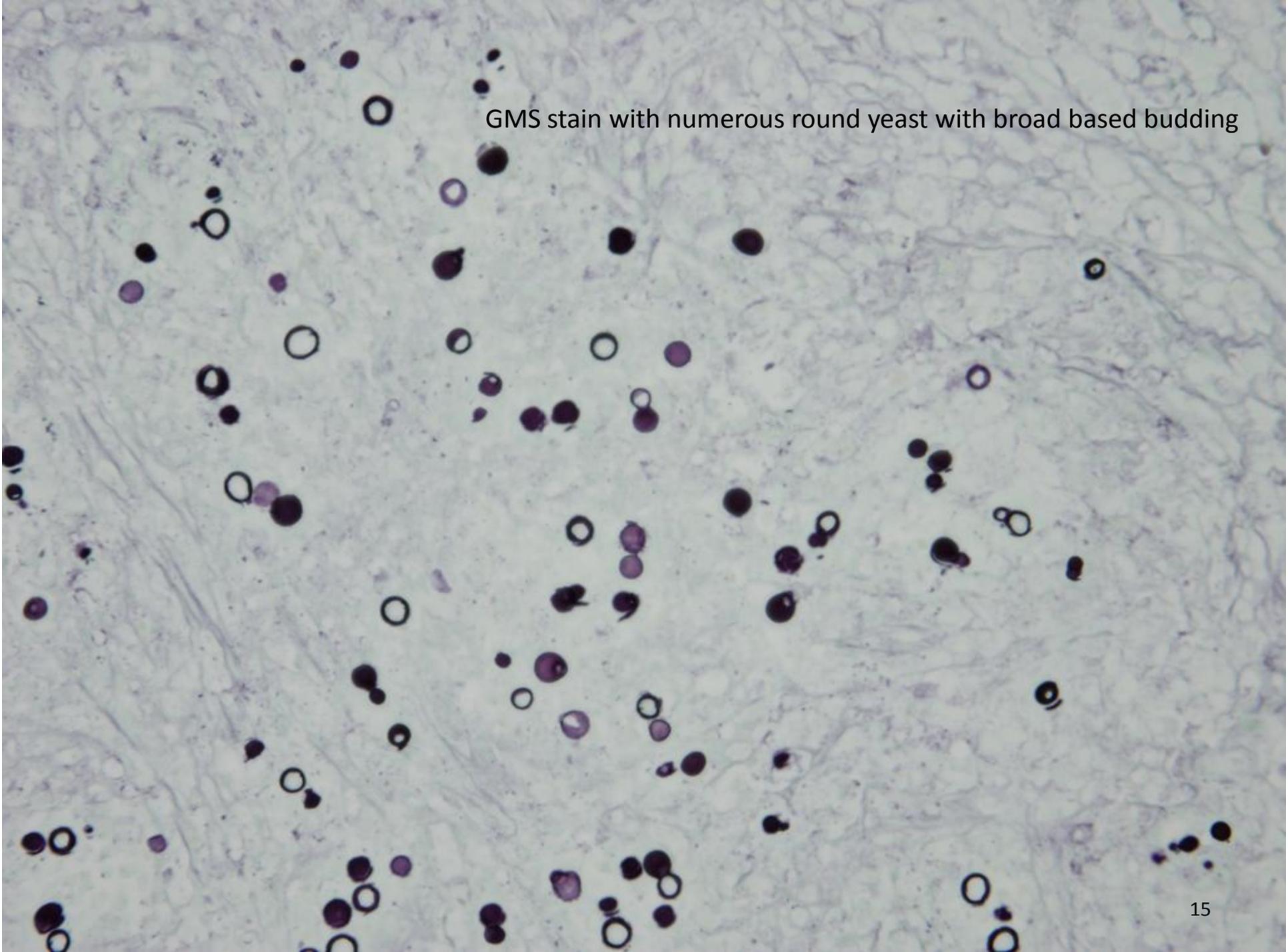
30 year old previously healthy man presented with recent illness including fever and weight loss. Enlarged supraclavicular lymph nodes were present. Lab results included severe anemia and lymphopenia. Chest x-ray showed numerous lung nodules and enlarged lymph nodes in the chest. Tests for HIV were positive.

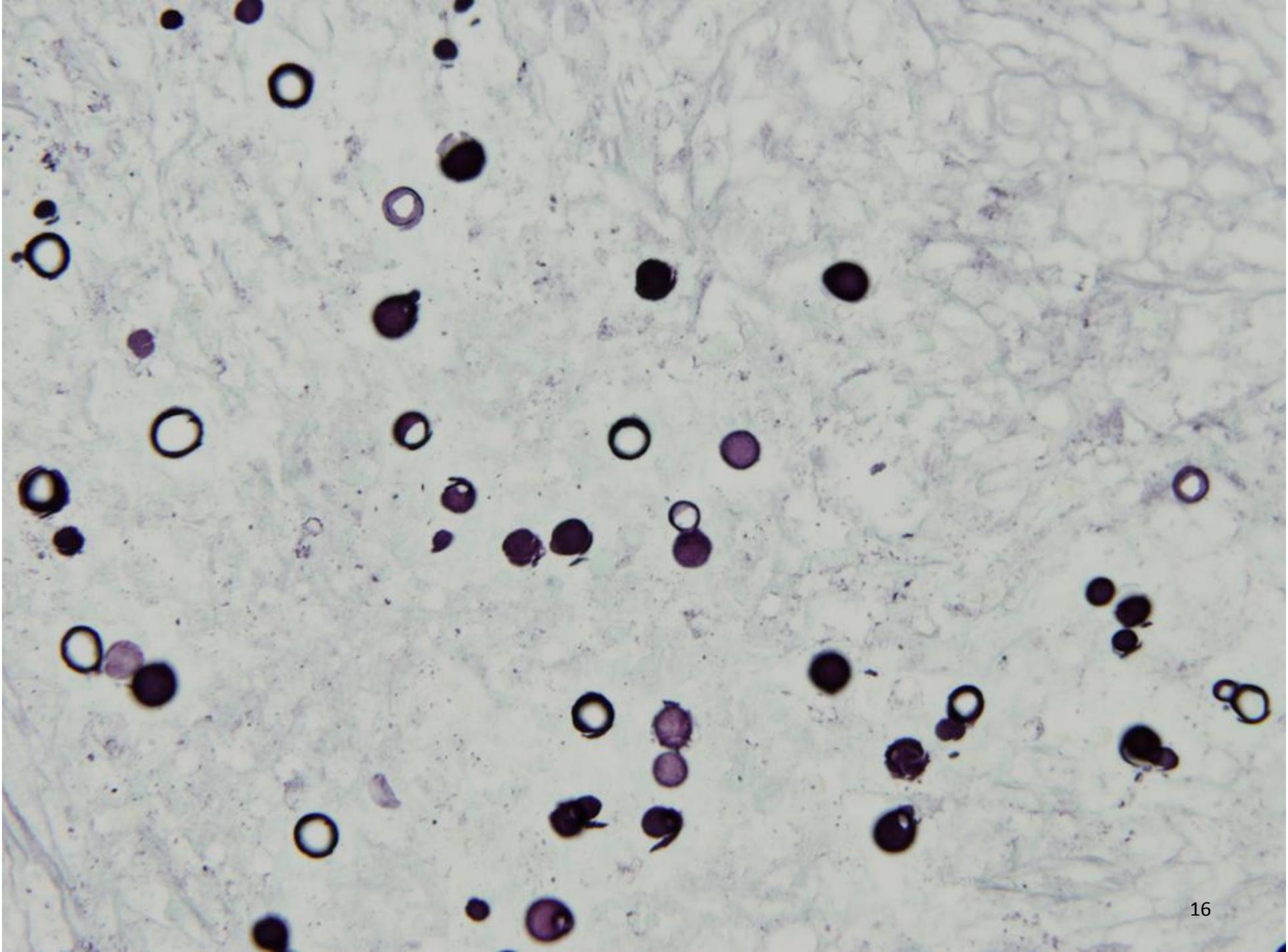
A biopsy of a supraclavicular lymph node was performed.

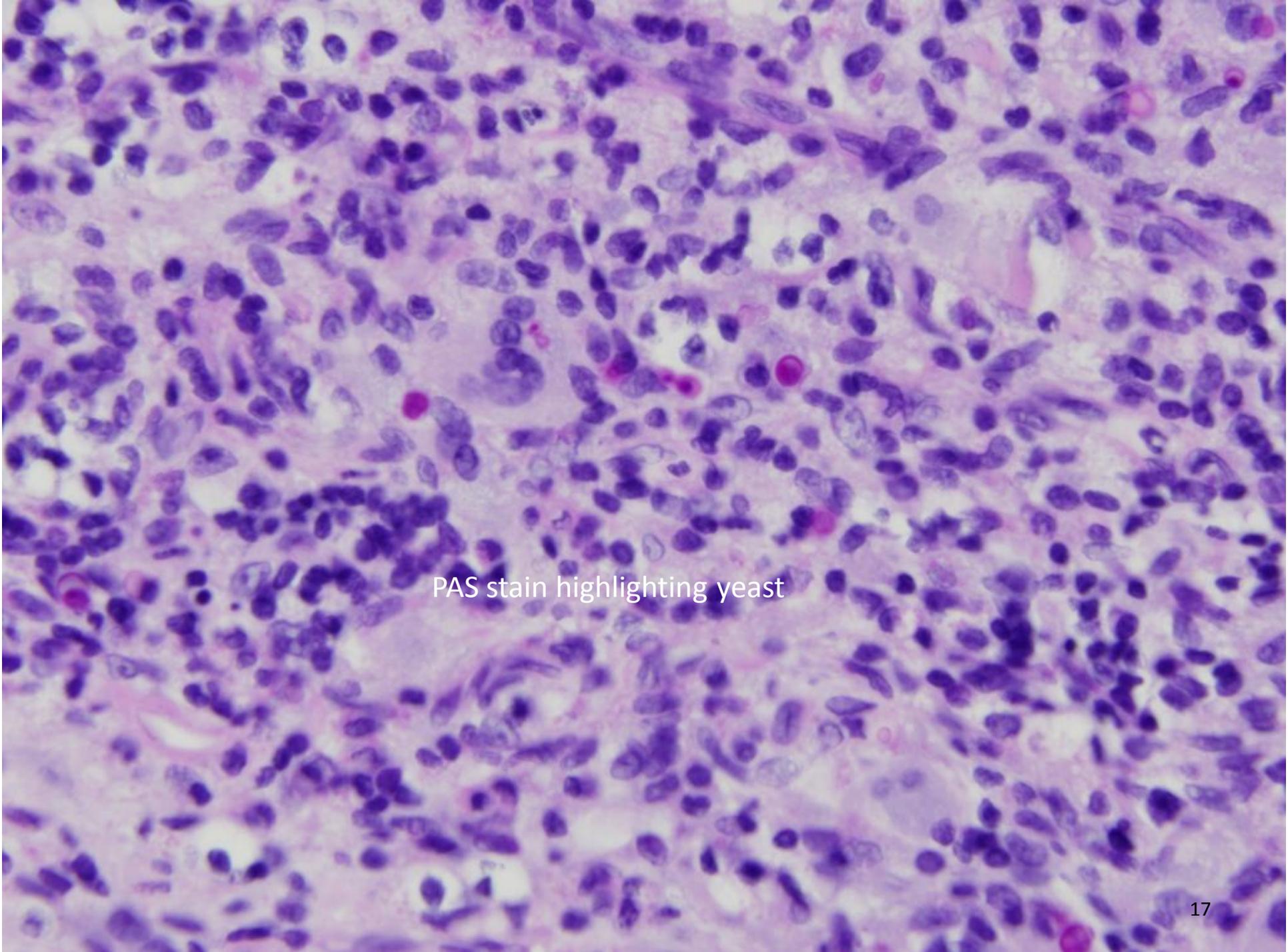


Histiocytic reaction with round intracellular yeasts

GMS stain with numerous round yeast with broad based budding







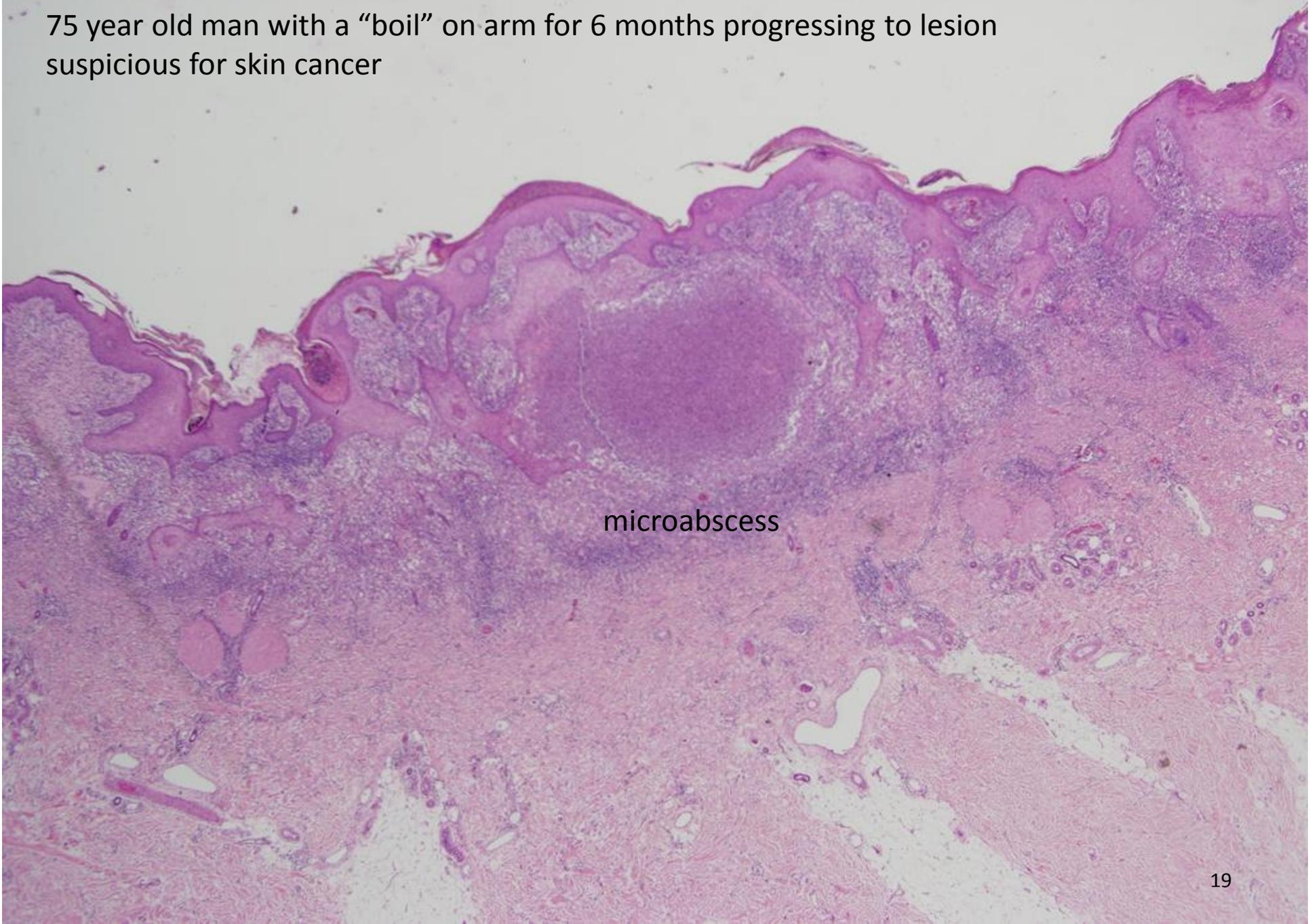
PAS stain highlighting yeast

# Blastomycosis

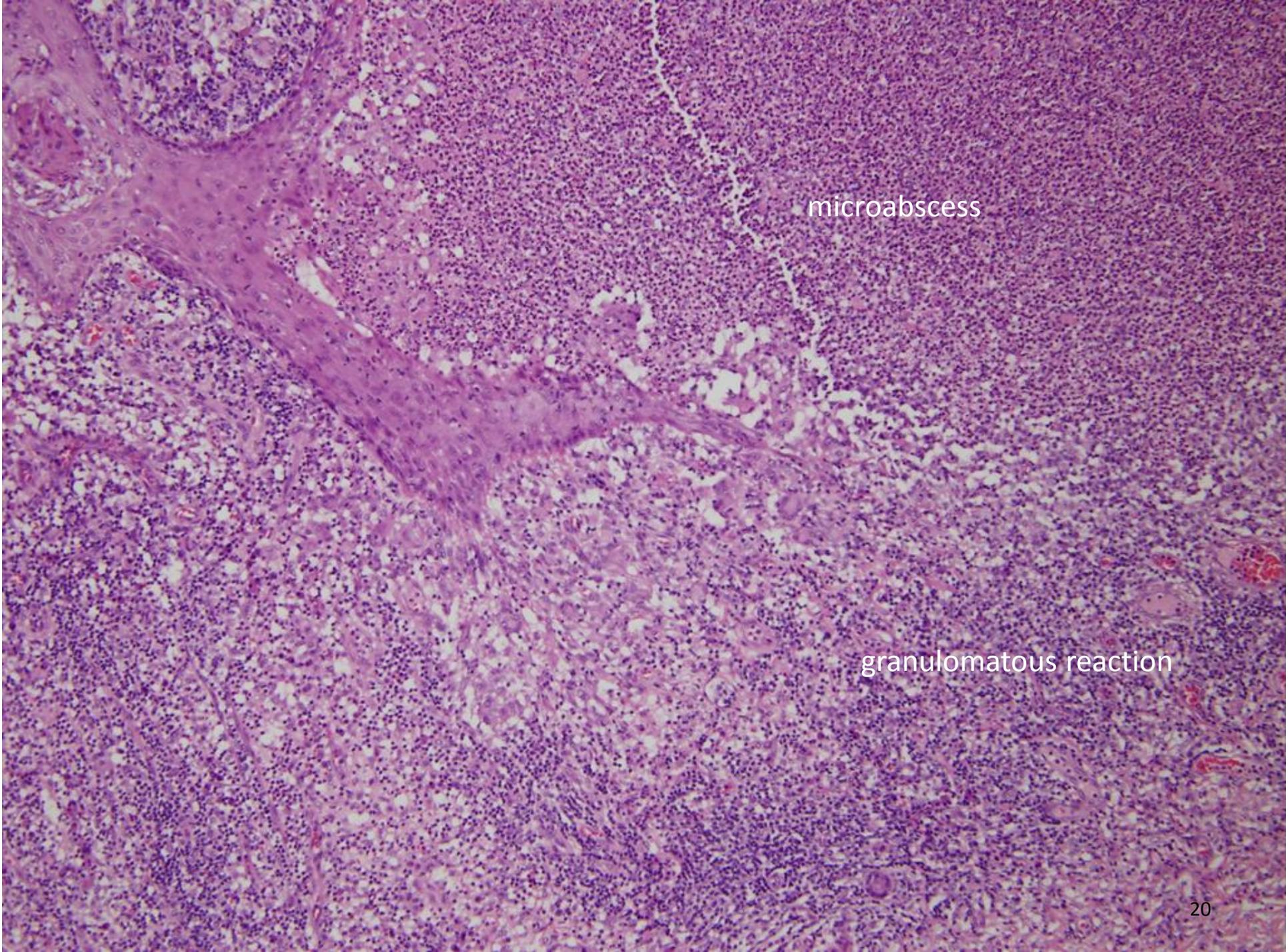
Blastomycosis is an infection caused by inhalation of the dimorphic fungus *Blastomyces dermatitidis*, which is soil organism found primarily in the U.S. in the Mississippi, Missouri and Ohio River valleys, around the Great Lakes and in the southeast. It is also found in parts of Canada. Acute infection resembles bacterial pneumonia. Chronic infection is more common and patients may have productive cough, low grade fever, hemoptysis (coughing up blood), weight loss and chest pain. Extrapulmonary infection can occur to a variety of sites, especially skin and bone. Though uncommon in immunocompromised patients, if it occurs it tends to be more aggressive, especially in HIV patients.

The histologic reaction tends to be suppurative with abscess formation and if progressive, granulomatous inflammation develops. The yeast are spherical to oval measuring 8-15 microns in diameter, have thick refractile walls and have a single broad based bud. Microforms (2-4 microns) have been described. Finding the larger characteristic yeast is helpful.

75 year old man with a “boil” on arm for 6 months progressing to lesion suspicious for skin cancer

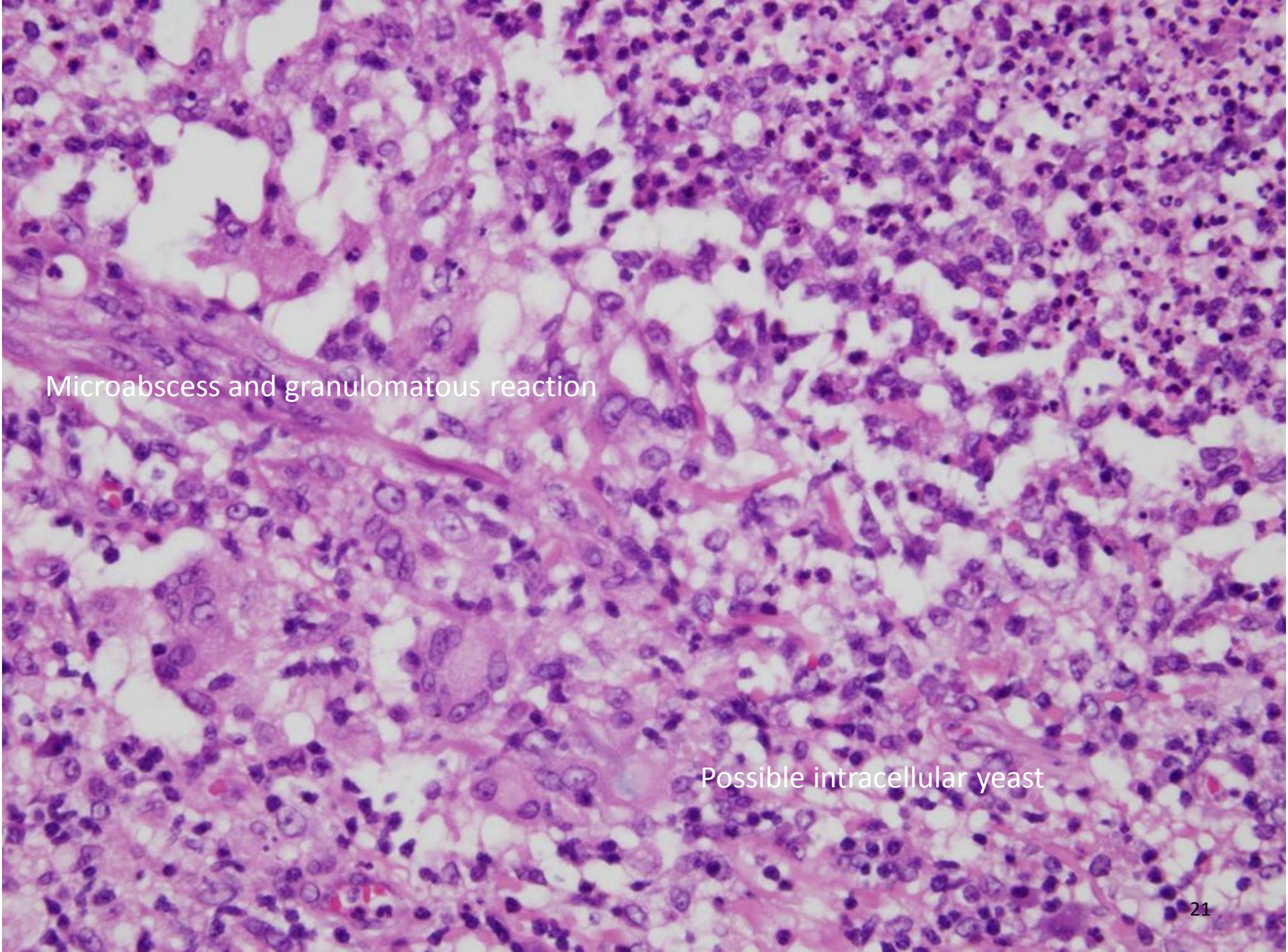


microabscess



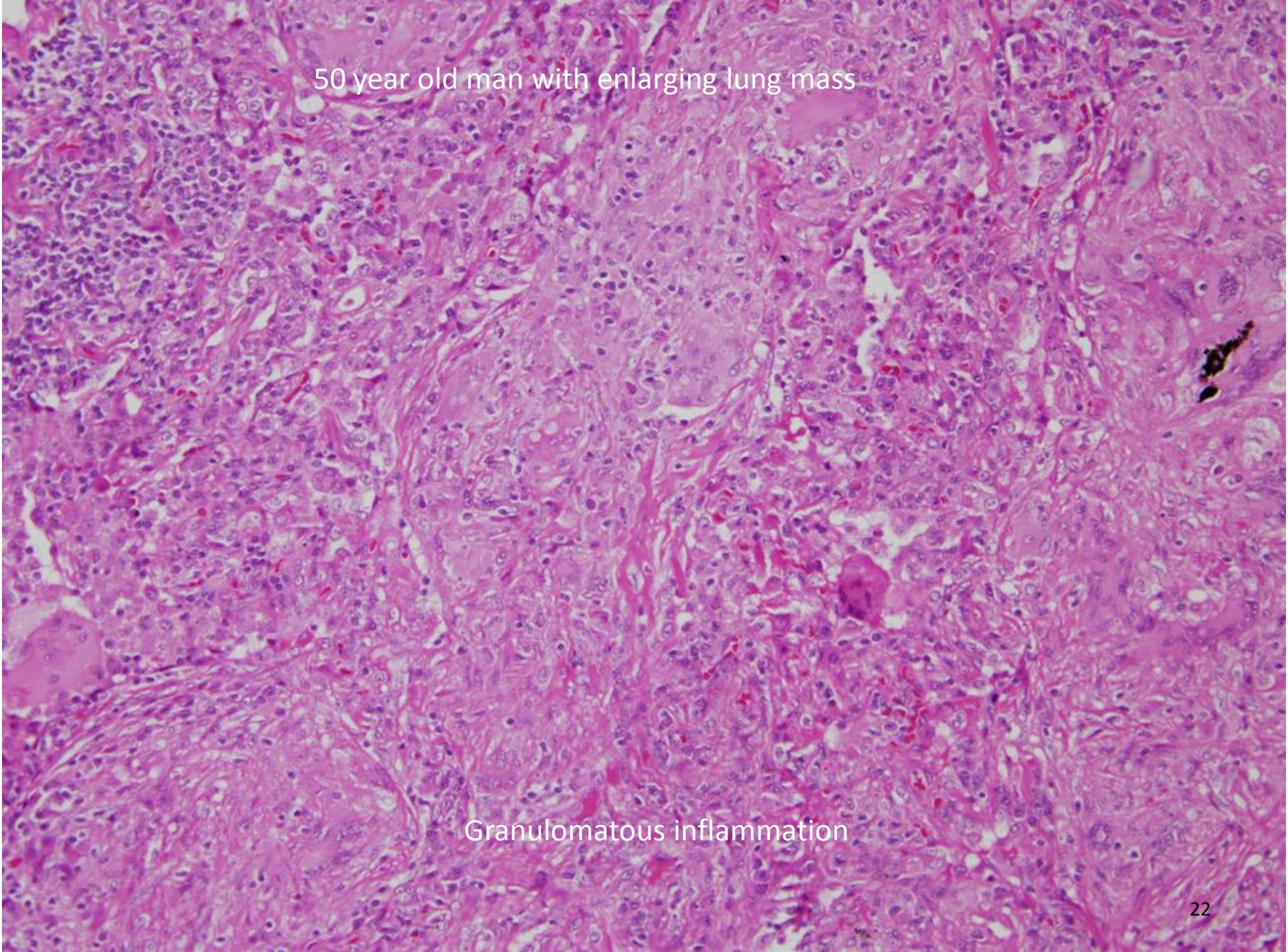
microabscess

granulomatous reaction



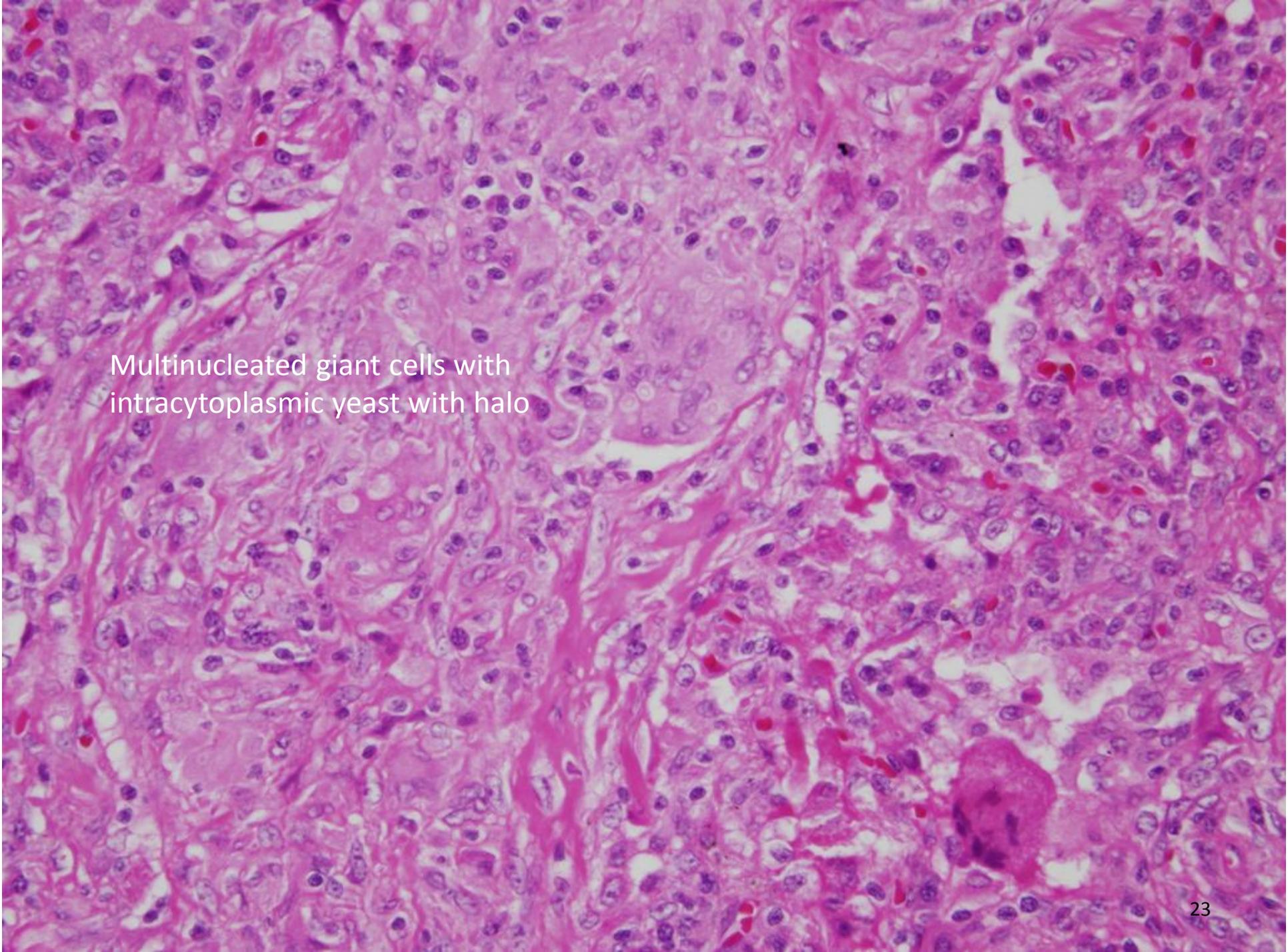
Microabscess and granulomatous reaction

Possible intracellular yeast

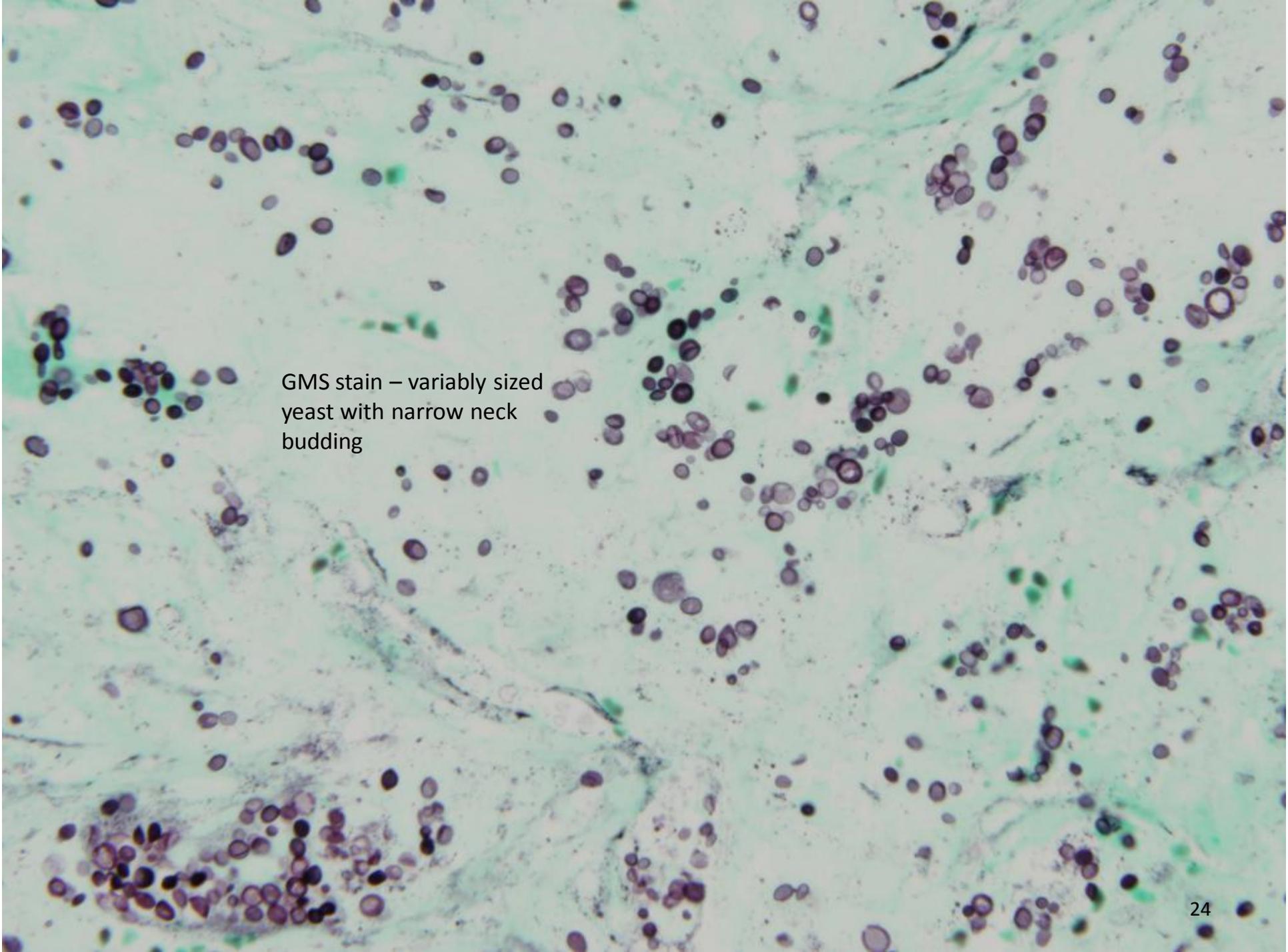
A high-magnification histological micrograph of lung tissue stained with hematoxylin and eosin (H&E). The image displays a dense infiltrate of inflammatory cells, characteristic of granulomatous inflammation. The infiltrate consists of numerous mononuclear cells, including lymphocytes and macrophages, arranged in a somewhat organized pattern. There are also some multinucleated giant cells visible, which are typical of granulomatous reactions. The surrounding lung parenchyma shows some architectural distortion and increased cellularity. The overall appearance is consistent with a chronic inflammatory process, such as sarcoidosis or a fungal infection.

50 year old man with enlarging lung mass

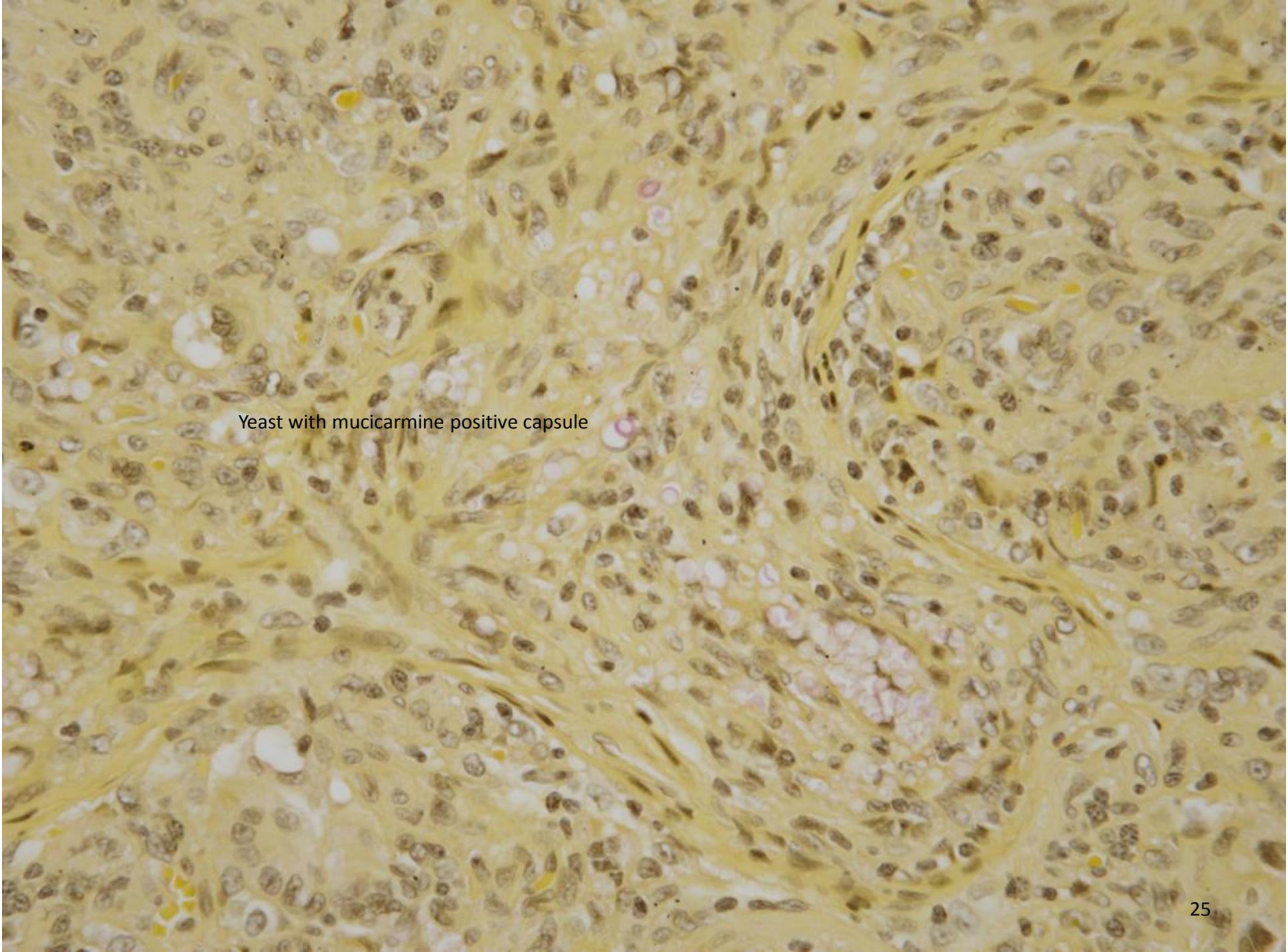
Granulomatous inflammation

A high-magnification histological micrograph of a tissue section stained with hematoxylin and eosin (H&E). The image displays a dense population of cells, including numerous multinucleated giant cells. These giant cells are characterized by multiple nuclei and a foamy or vacuolated cytoplasm. Within the cytoplasm of these cells, there are small, round, clear structures that represent intracytoplasmic yeast organisms, each surrounded by a distinct, thin, eosinophilic (pink) halo. The background consists of a cellular reaction, likely composed of macrophages and other inflammatory cells, with scattered nuclei visible throughout the field.

Multinucleated giant cells with  
intracytoplasmic yeast with halo



GMS stain – variably sized  
yeast with narrow neck  
budding

A high-magnification photomicrograph of a tissue section stained with mucicarmine. The image shows a dense population of yeast cells, many of which are surrounded by a thick, clear, capsule-like structure. These capsules are highlighted in a bright pink color, indicating a positive mucicarmine reaction. The yeast cells themselves are small, round, and have a dark brown or black center. The surrounding tissue is stained a pale yellowish-brown. The overall appearance is that of a highly cellular, possibly inflammatory, response to a fungal infection.

Yeast with mucicarmine positive capsule

# Cryptococcosis

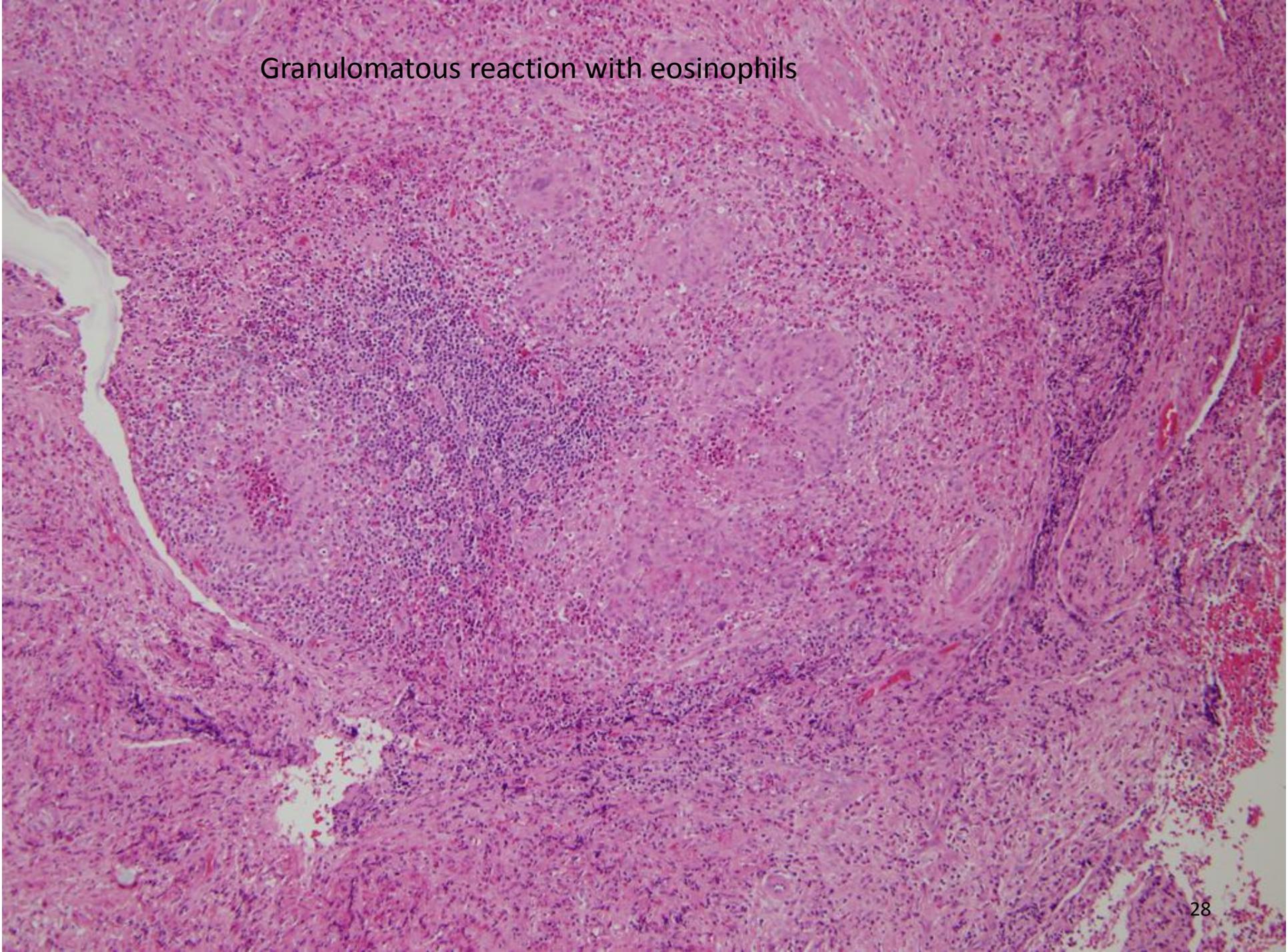
Cryptococcosis is caused by inhalation of the spores of *Cryptococcus neoformans*, a yeast found worldwide in soil, especially soil contaminated with pigeon droppings. There are 2 main clinical forms: pulmonary infection and cerebromeningeal infection due to hematogenous spread from the lung. Immunocompetent individuals with primary lung infections rarely have symptoms. Immunocompromised individuals are at greater risk for both symptomatic lung infections and disseminated disease.

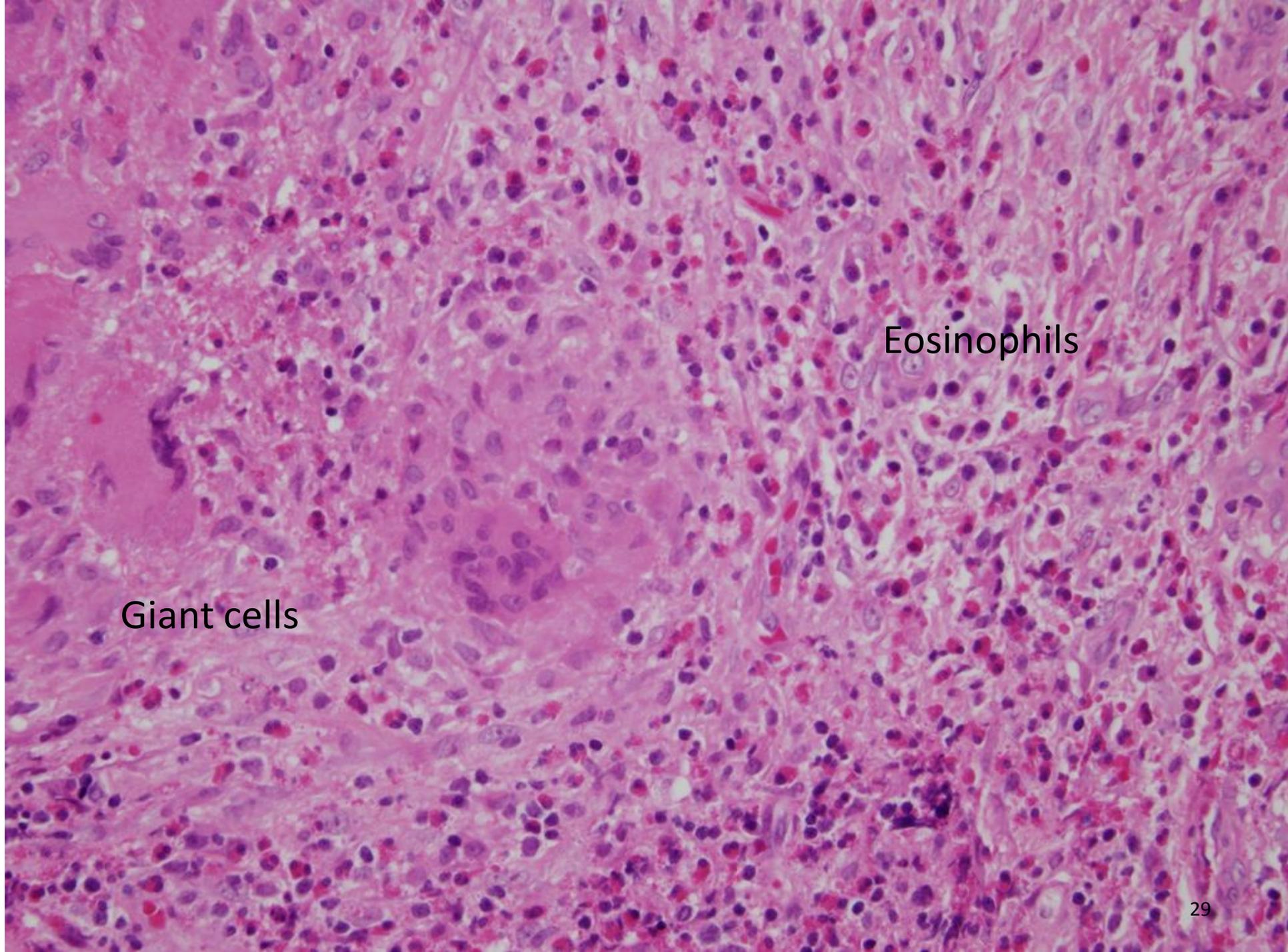
The host response may be granulomatous, similar to histoplasmosis. Immunosuppressed patients may have little host response with extensive proliferation of yeast within tissue. In the lung this may produce a gelatinous appearing pneumonia. The yeast are generally 4-6 microns on average but may range from 2-20 microns. Budding is single and narrow based. The yeast often have a halo due to a thick mucinous capsule which can be highlighted with mucin stains such as mucicarmine. Capsule deficient yeast may be seen in immunocompetent hosts.

# Dead Head West

A 23 year old man returns to Springfield after spending a year following the Grateful Dead tour in the Western U.S. He has fevers, a cough, lung infiltrates and enlarged lymph nodes in the chest. Mediastinoscopy with biopsy of the lymph nodes is performed. Tissue is submitted to Histology and Microbiology.

Granulomatous reaction with eosinophils



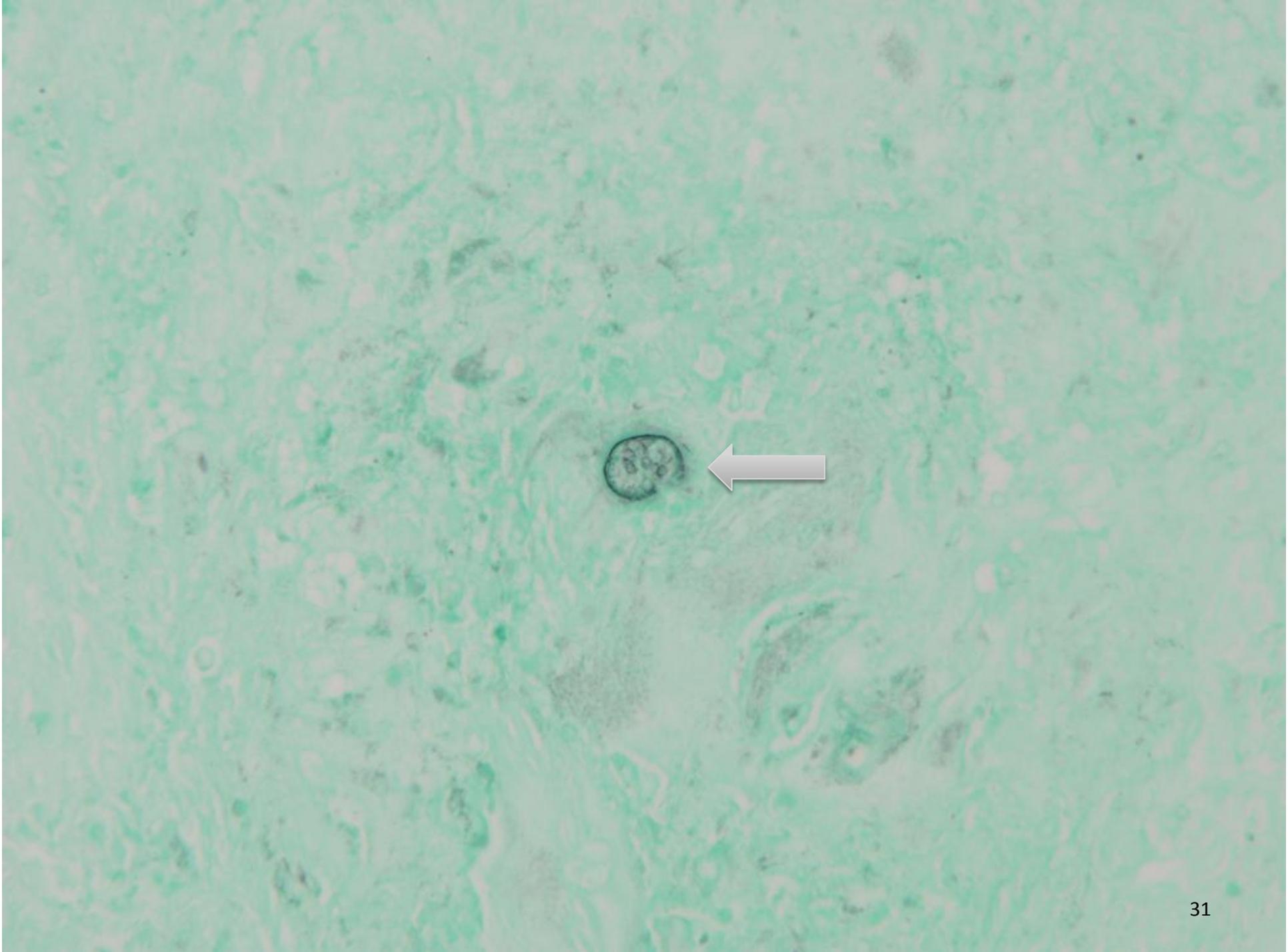


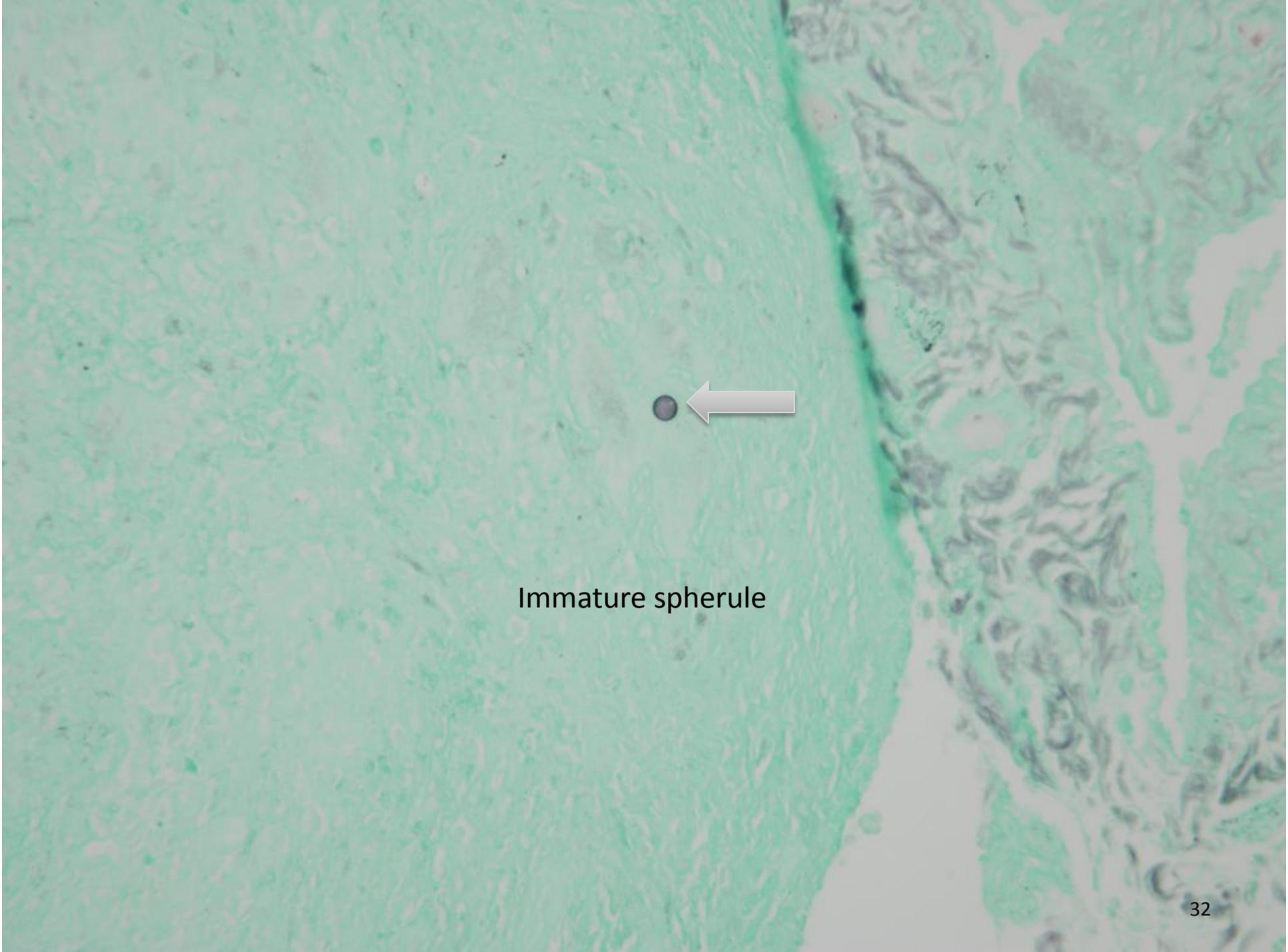
Eosinophils

Giant cells



Spherule with endospores





Immature spherule

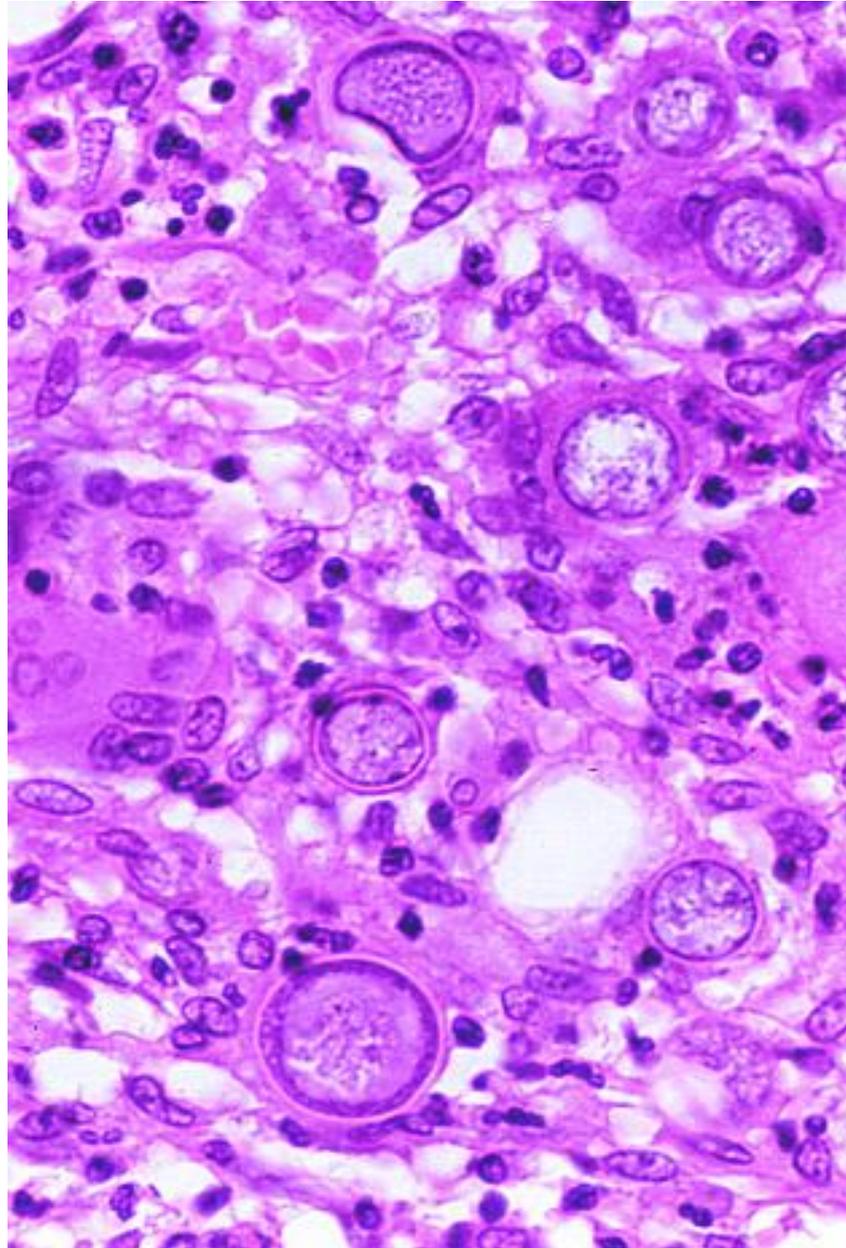
# Diagnosis – Coccidioidomycosis

Coccidioidomycosis is caused by inhalation of fungal spores of *Coccidioides immitis*, a diphasic, multimorphic fungus which is endemic throughout the SW U.S., northern and central Mexico, and regions of Central and South America. It grows optimally in sandy, alkaline soil and the mycelial form is present in desert soil. Wind and rain help disperse the spores. The disease is also known as San Joaquin Valley fever.

Most infections are asymptomatic while others have a mild flu-like illness. Occasional infections persist and progress primarily with pulmonary symptoms and rarely dissemination in the body. In tissues *C. immitis* consists of immature and mature spherules and endospores. The mycelial form may be seen when the organisms are exposed to air such as in cavitary lesions in the lung. The tissue reaction is often suppurative and granulomatous with necrosis. Eosinophils may be prominent in the inflammatory reaction.

Culture grew *C. immitis*. The culture plate was not opened in our laboratory.

Numerous spherules with endospores

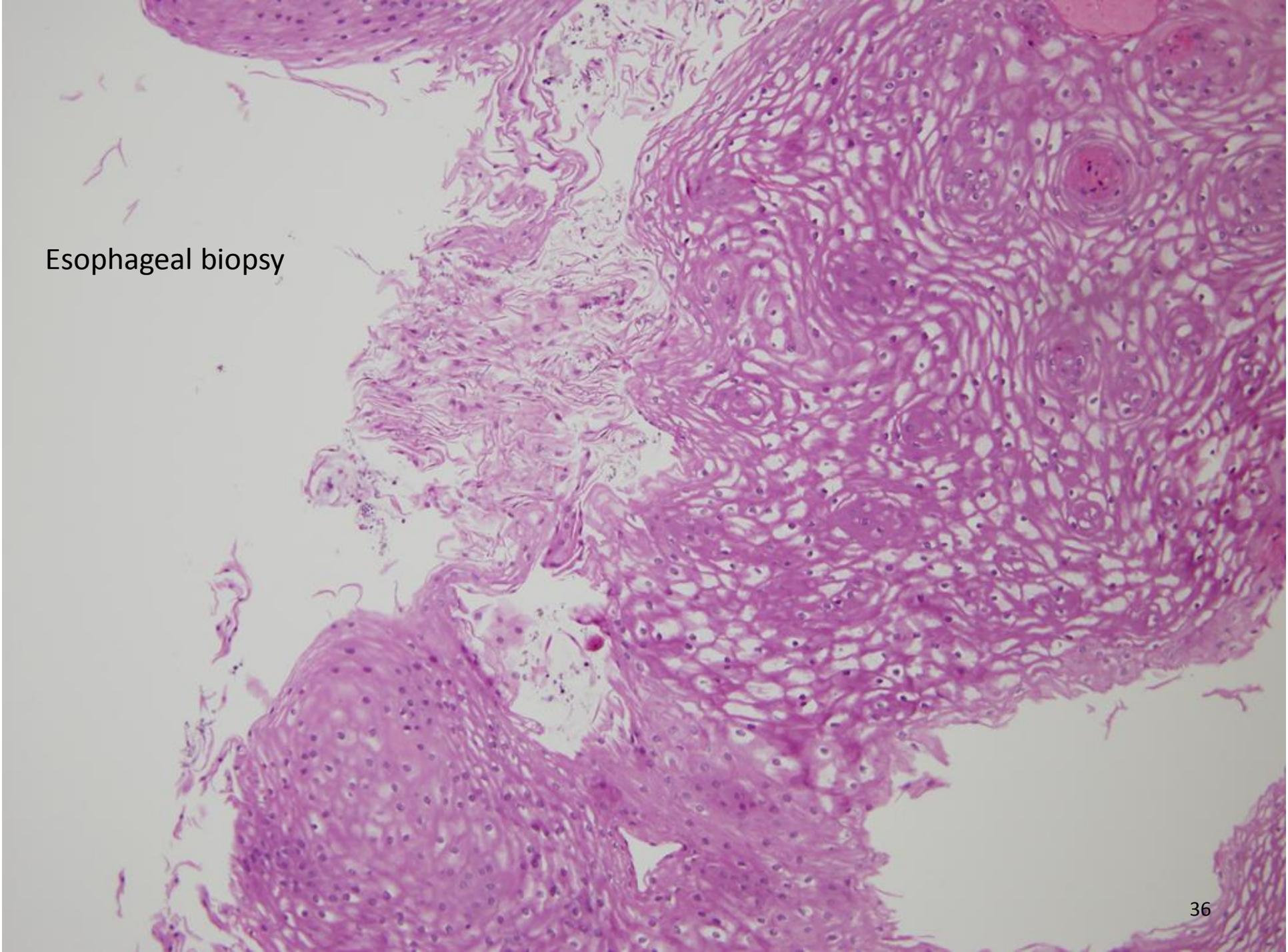


# White patches in the esophagus

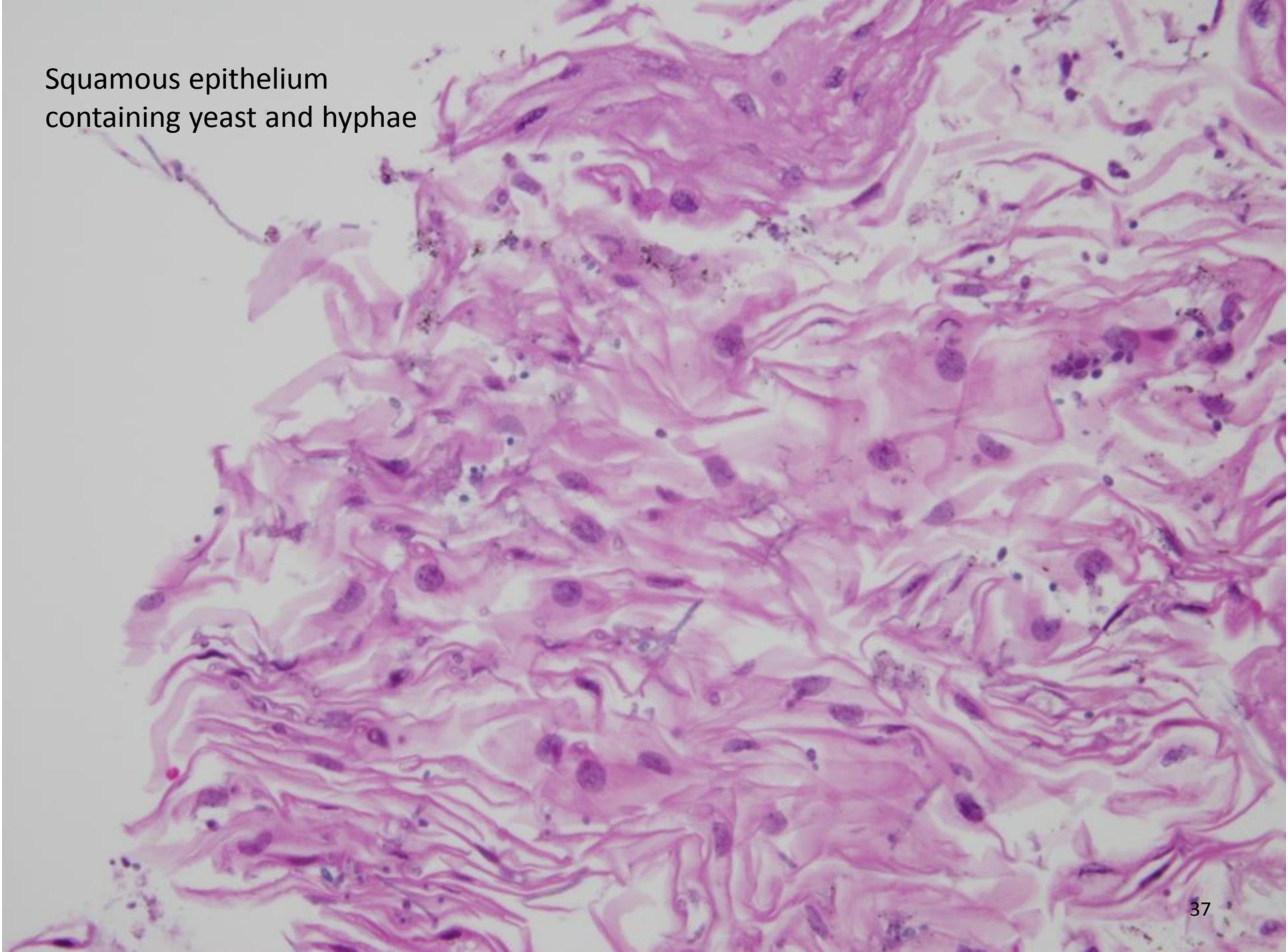
60 year old woman presents with dysphagia (difficulty swallowing) and acid reflux (heartburn). She has a history of chronic obstructive pulmonary disease (COPD), diabetes mellitus, and was recently treated with prednisone and antibiotics due to an exacerbation of her COPD.

At endoscopy, white stuck on patches were seen throughout her esophagus. Biopsies were obtained and sent for histologic examination.

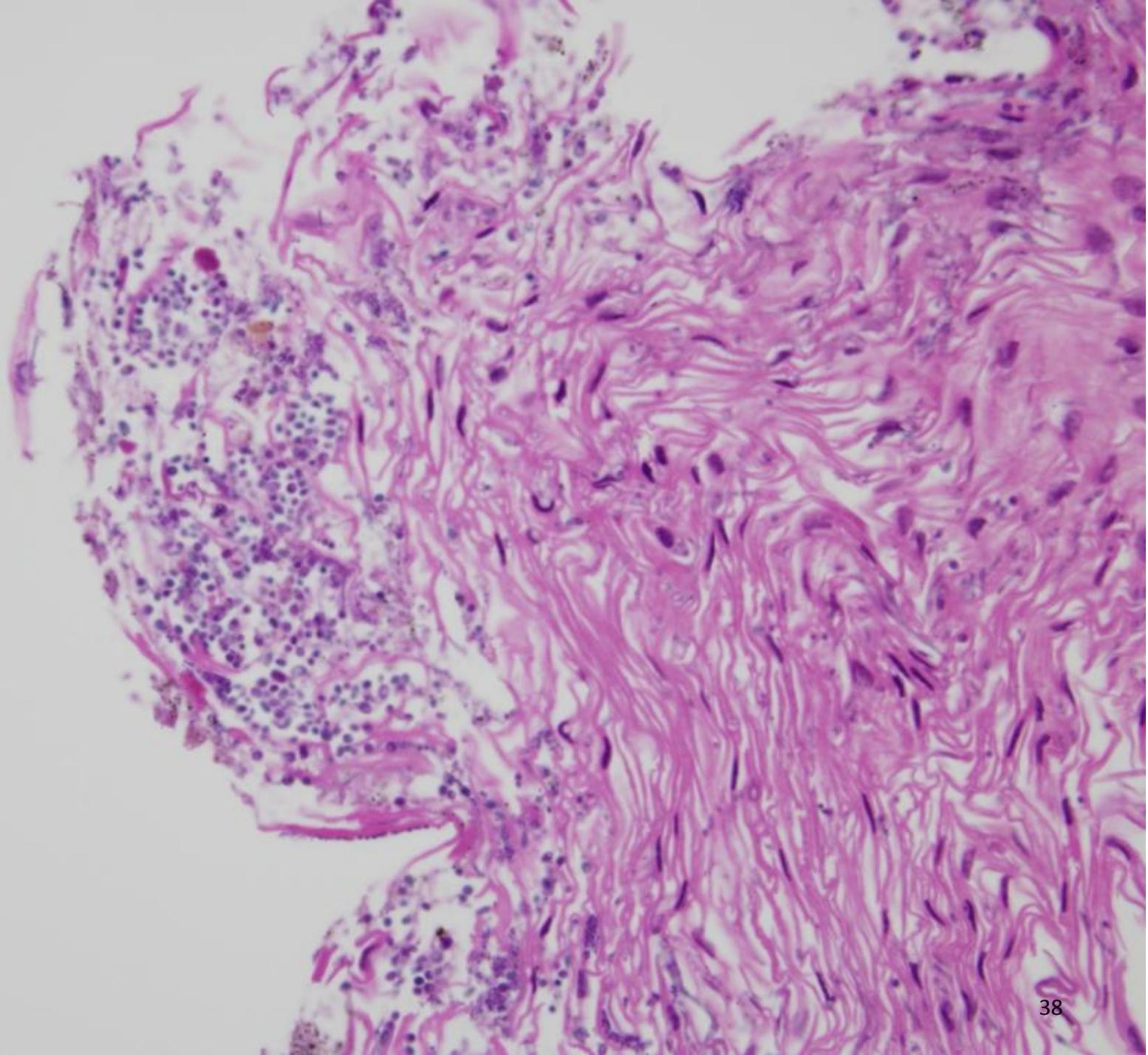
Esophageal biopsy



Squamous epithelium  
containing yeast and hyphae

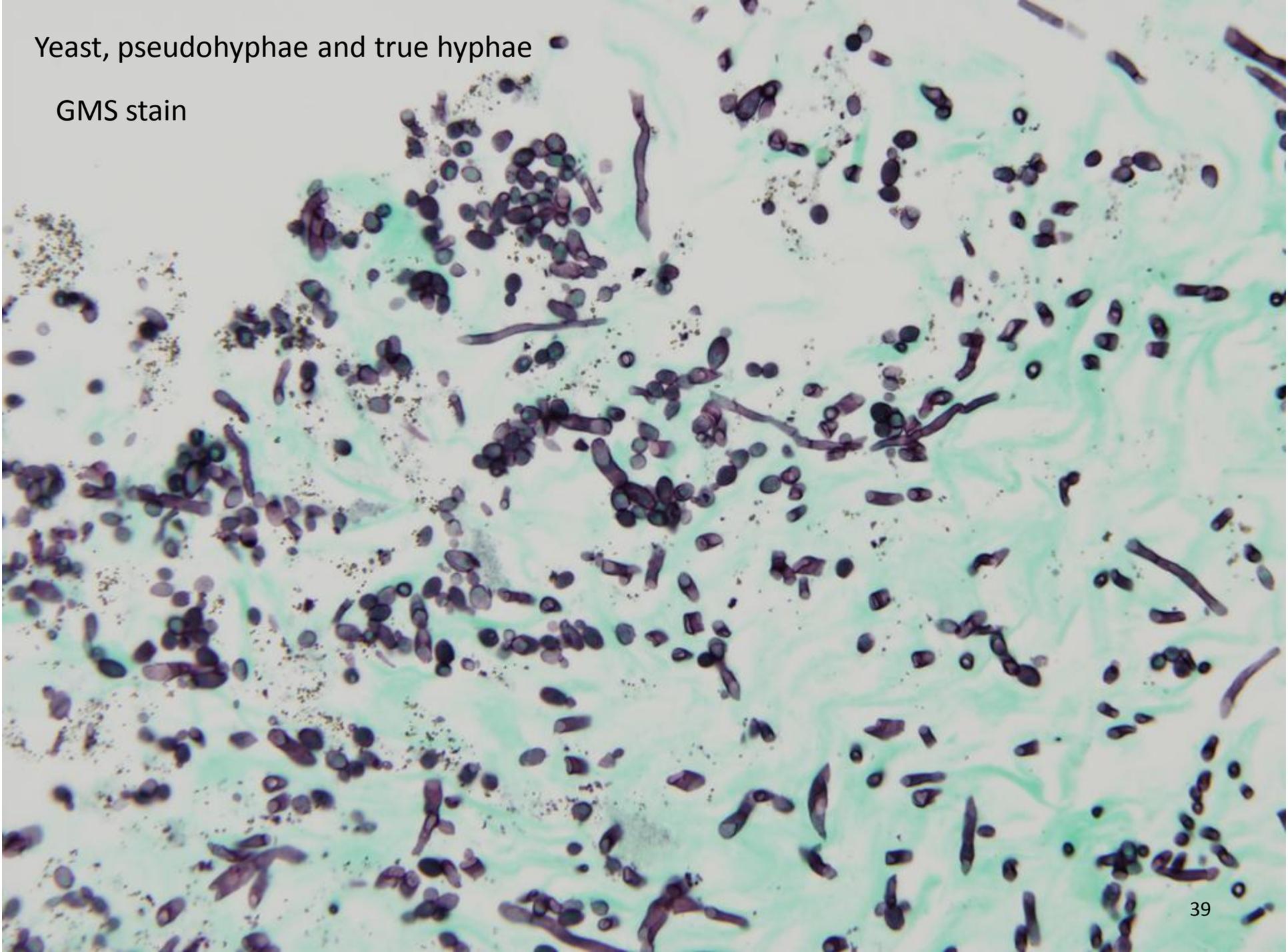


Numerous yeast

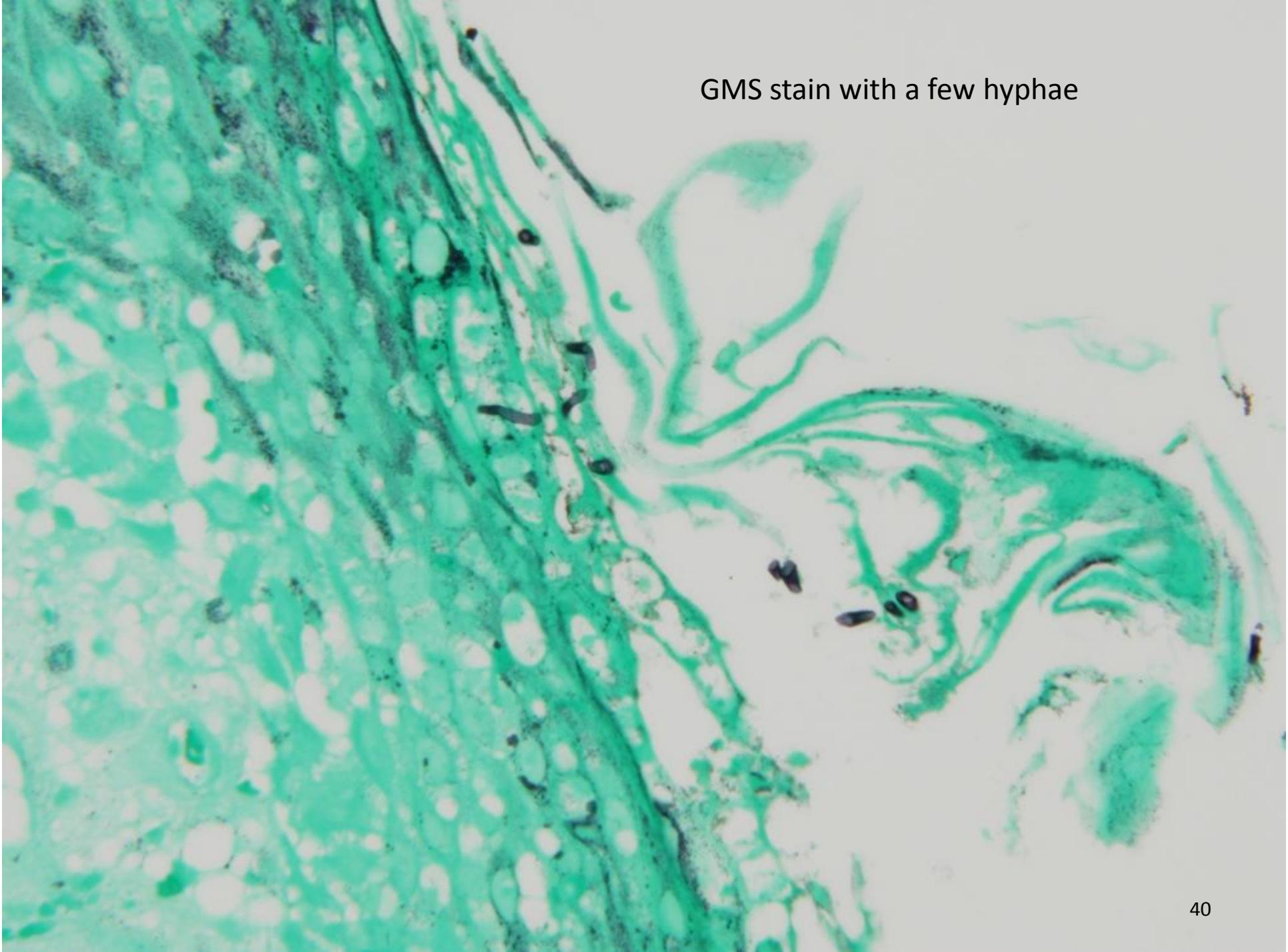


Yeast, pseudohyphae and true hyphae

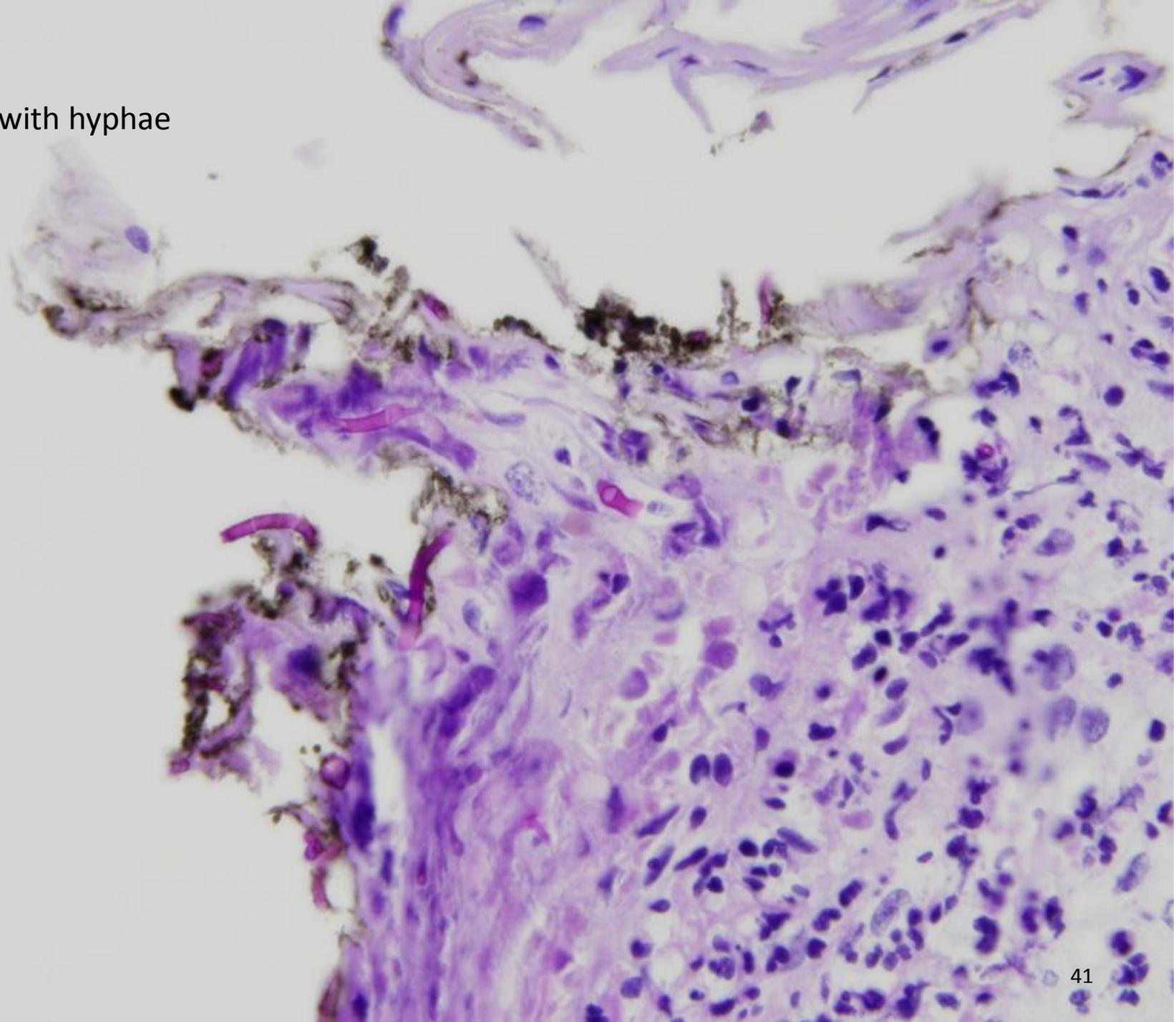
GMS stain



GMS stain with a few hyphae



PAS stain with hyphae



# Candidiasis

Candidiasis is an infection caused by *Candida* sp. There are 81 recognized species of *Candida* but only a small number are pathogenic in humans, the most common being *C. albicans*. *Candida* is a common commensal organism in the respiratory, gastrointestinal and genitourinary tracts. They are not pathogens unless the organisms invade tissue, generally in immunodeficient patients.

Oropharyngeal candidiasis is called thrush and presents with whitish plaques on an inflamed background. This can occur in patients being treated with antibiotics, chemotherapy and radiation therapy. In severely immunocompromised patients *Candida* can invade deeper into tissues and potentially disseminated hematogenously.

*Candida* may be seen in tissue as yeast-like cells, 2-6 microns in diameter, which are accompanied by pseudohyphae and true hyphae. Pseudohyphae line up like sausage links with constrictions instead of true septae.

ally larger and more variable in size with a broader-based bud than *H. capsulatum*. Rarely, *Candida* sp may be mistaken for *H. capsulatum*, but the presence of pseudohyphae aids in the separation. *Penicillium marneffei* can also be mistaken for *H. capsulatum* since they measure 2.5 to 5.0  $\mu\text{m}$  or larger and are often intracellular. However, no budding is seen and the organisms reproduce by schizogony (fission), forming a conspicuous transverse septum. In addition, they have short hyphal forms, and elongated oval and curved sausage-shaped forms up to 20  $\mu\text{m}$  long with rounded ends and one or more septa.

The cytoplasmic inclusions of cytomegalovirus (CMV) could be mistaken for *H. capsulatum*, however, the presence of intranuclear viral inclusions helps make this distinction. *Toxoplasma gondii* could also be mistaken for *H. capsulatum*, however, they are more often seen in pulmonary epithelial cells rather than histiocytes, are easily seen with the H&E stain, and do not usually stain with the GMS stain.

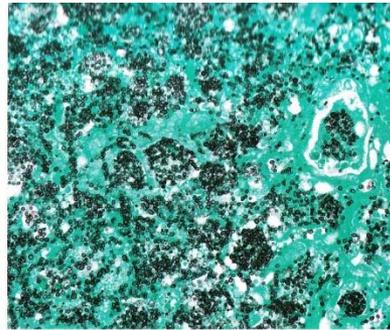


Figure 12-78

*HISTOPLASMA CAPSULATUM*: PROGRESSIVE DISSEMINATED INFECTION IN HIV PATIENTS  
There are sheets of organisms infiltrating the pulmonary interstitium (GMS stain).

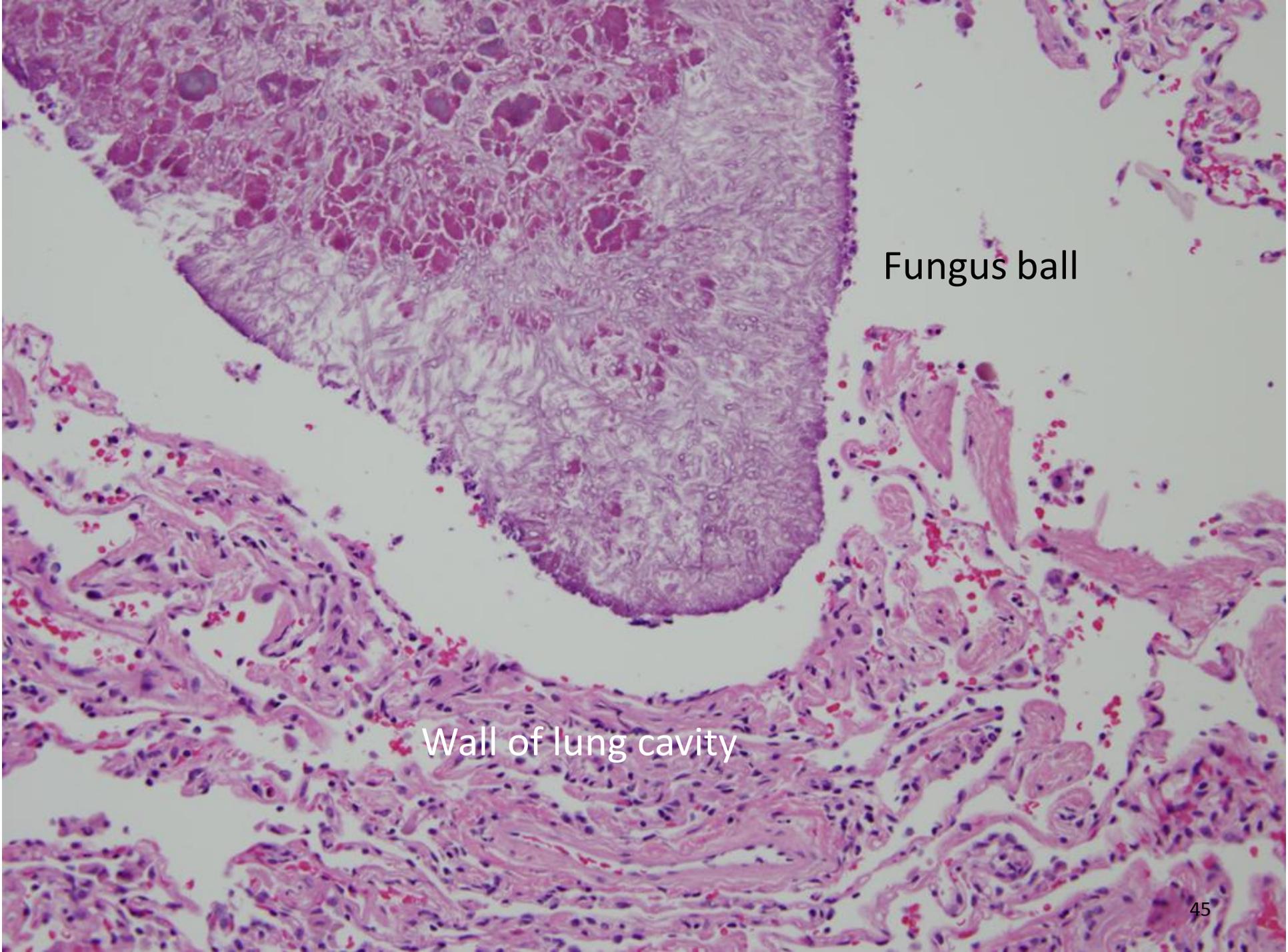
Table 12-7

MORPHOLOGIC CHARACTERISTICS OF YEAST-LIKE FUNGI

	Size	Shape	Budding	Cell Wall	Pseudohyphae/ Hyphae	Nuclei	Mucicarmine Staining
<i>Coccidioides immitis</i>	Spherules, 30-100 $\mu\text{m}$ (rarely up to 200 $\mu\text{m}$ ), endospores 2-5 $\mu\text{m}$	Spherical to oval	Endosporulation	Thin	Rare	Single	Negative
<i>Histoplasma capsulatum</i>	2-4 $\mu\text{m}$	Spherical to oval	Single, narrow based	Thin	Rare	Single	Negative
<i>Cryptococcus neoformans</i>	2-20 $\mu\text{m}$	Spherical to oval	Single, rarely multiple, narrow based	Thin (thick mucinous capsule)	Rare	Single	Positive
<i>Blastomyces dermatitidis</i>	8-15 $\mu\text{m}$	Spherical	Single, broad based	Thick	Rare	Multiple	Negative
<i>Candida</i> sp	2-6 $\mu\text{m}$	Spherical to oval	Single, chains, narrow based	Thin	Pseudohyphae (rare true hyphae)	Single	Negative
<i>Torulopsis galbrata</i>	2-5 $\mu\text{m}$	Spherical or oval	Single, narrow based	Thin	Absent	Single	Negative

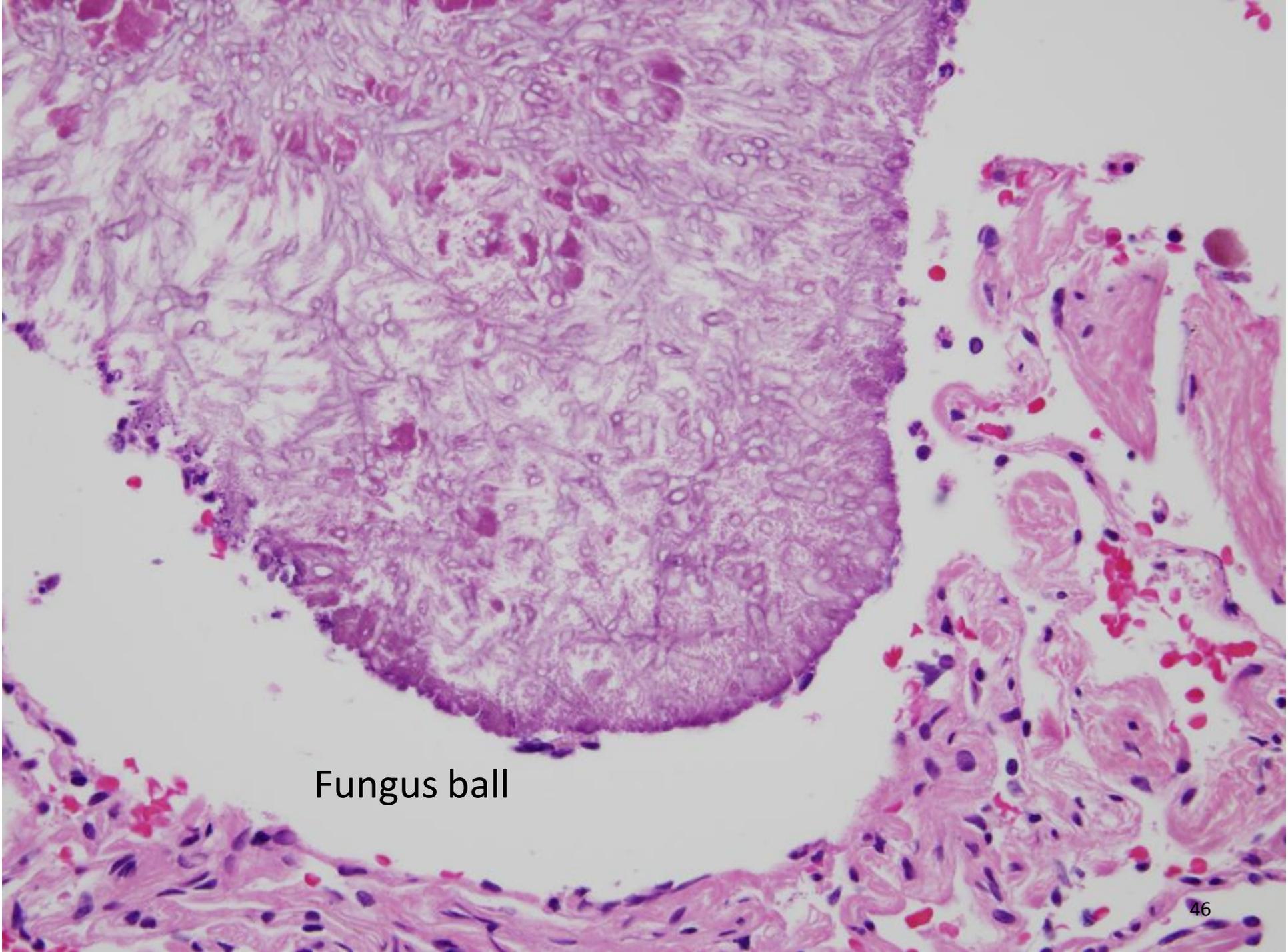
55 year old woman presents with cough and hemoptysis (coughing up blood). Chest x-ray and CT scan reveal a 3 cm cavitory lesion in the right lower lobe.

Fungal organisms were identified on a needle biopsy of the lesion and surgery was performed to resect the involved portion of lung.

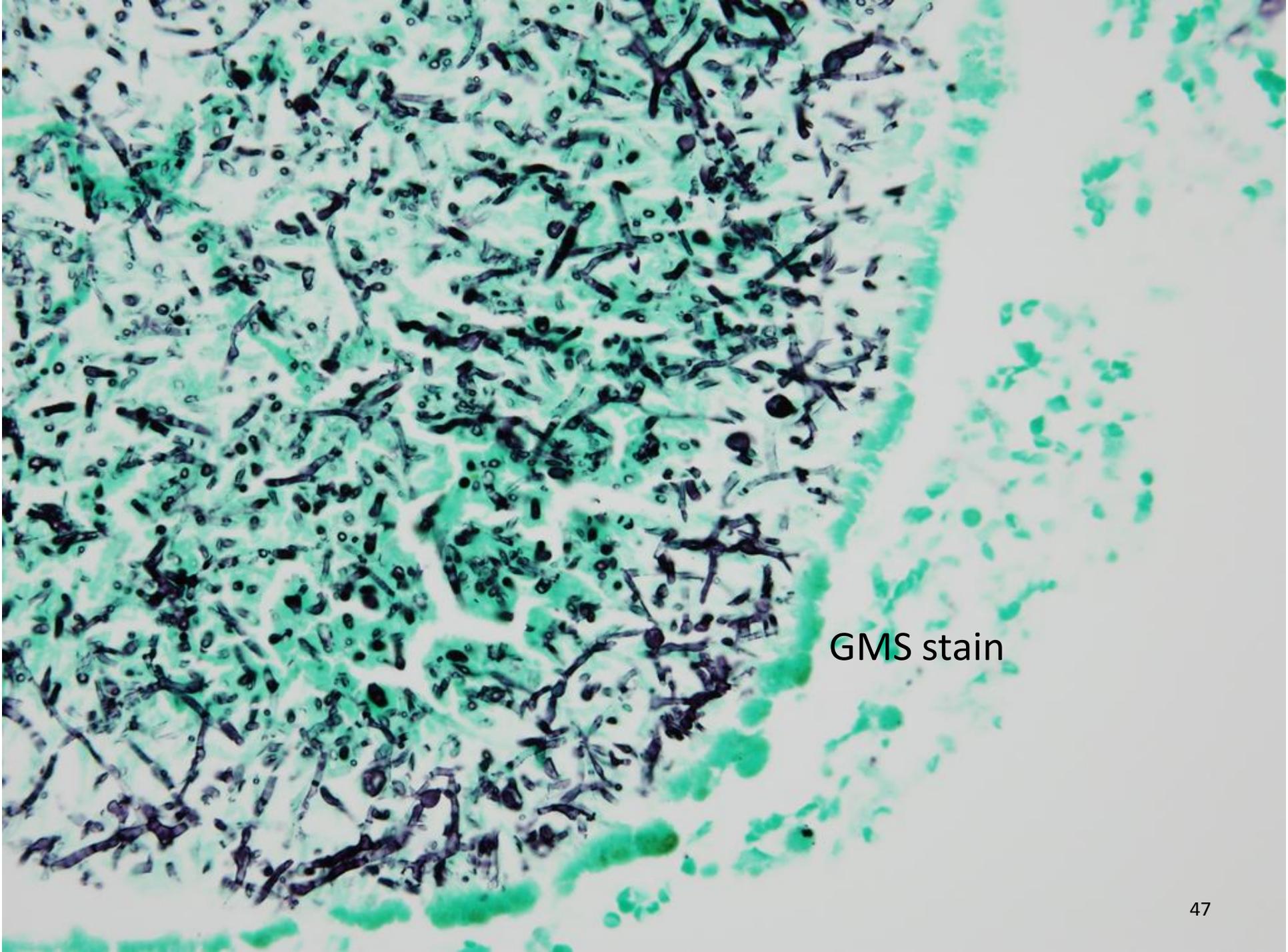


Fungus ball

Wall of lung cavity



Fungus ball



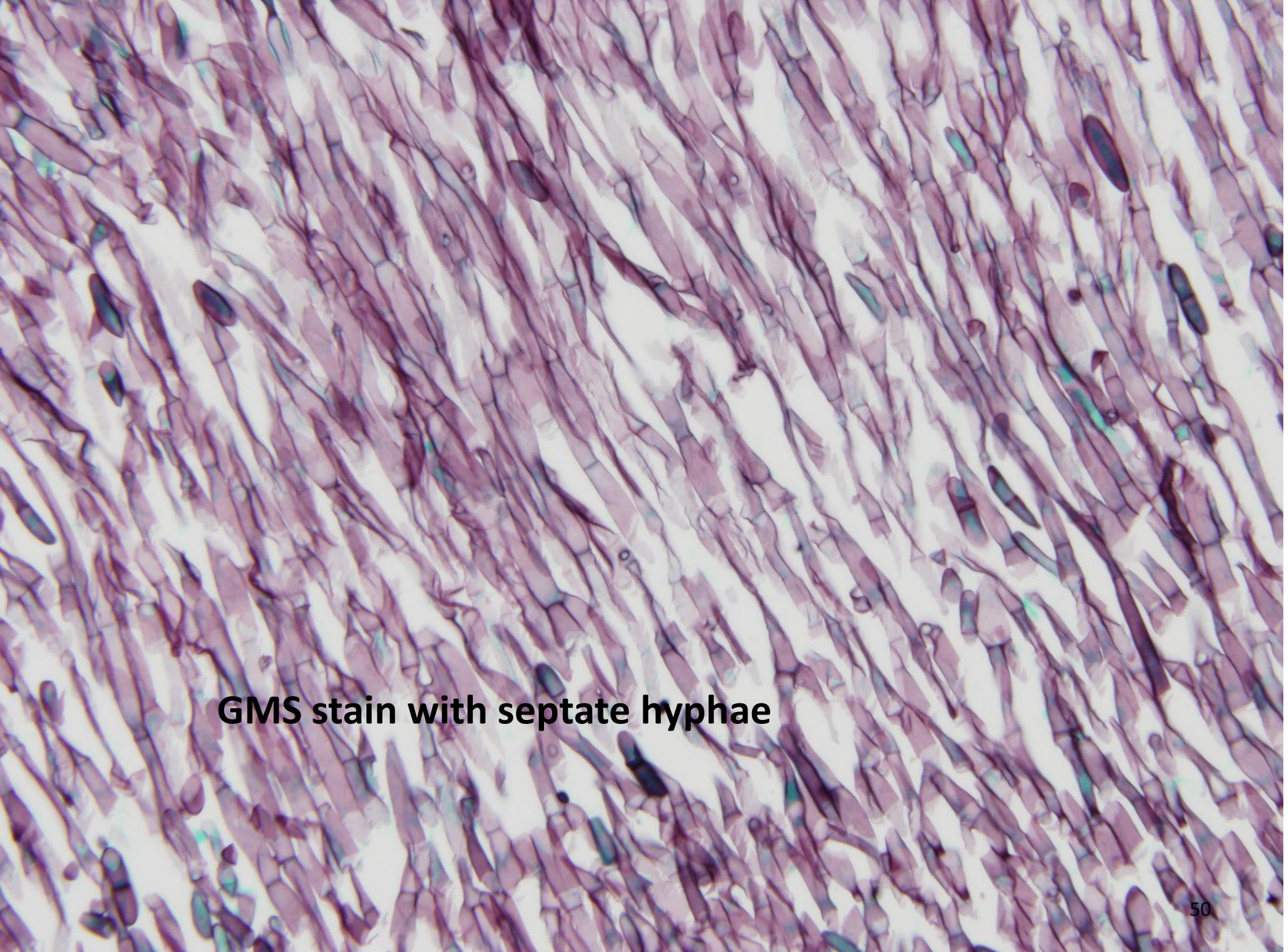
GMS stain

Septate branching hyphae

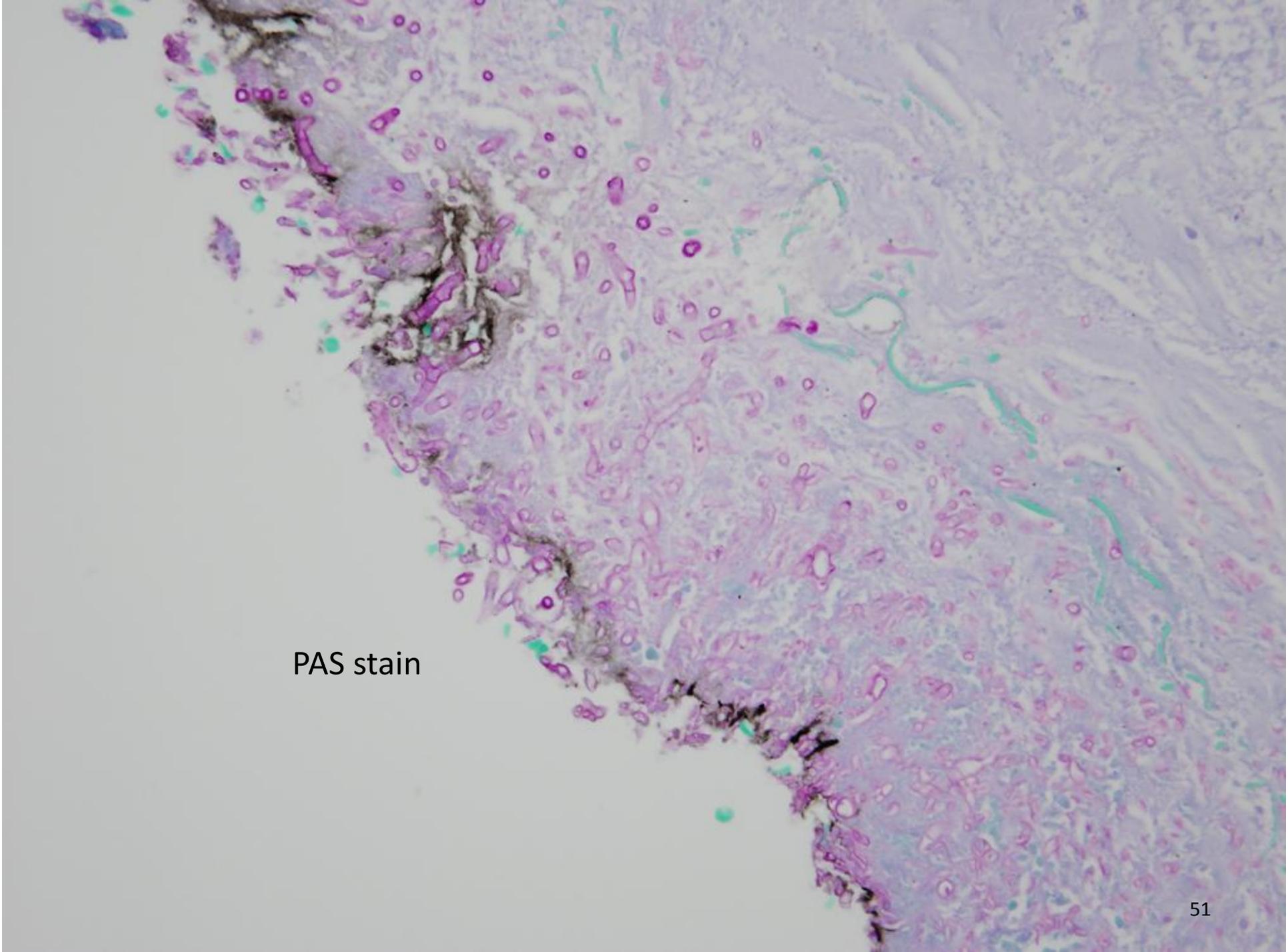




GMS stain with hyphae



**GMS stain with septate hyphae**



PAS stain

# Aspergillosis

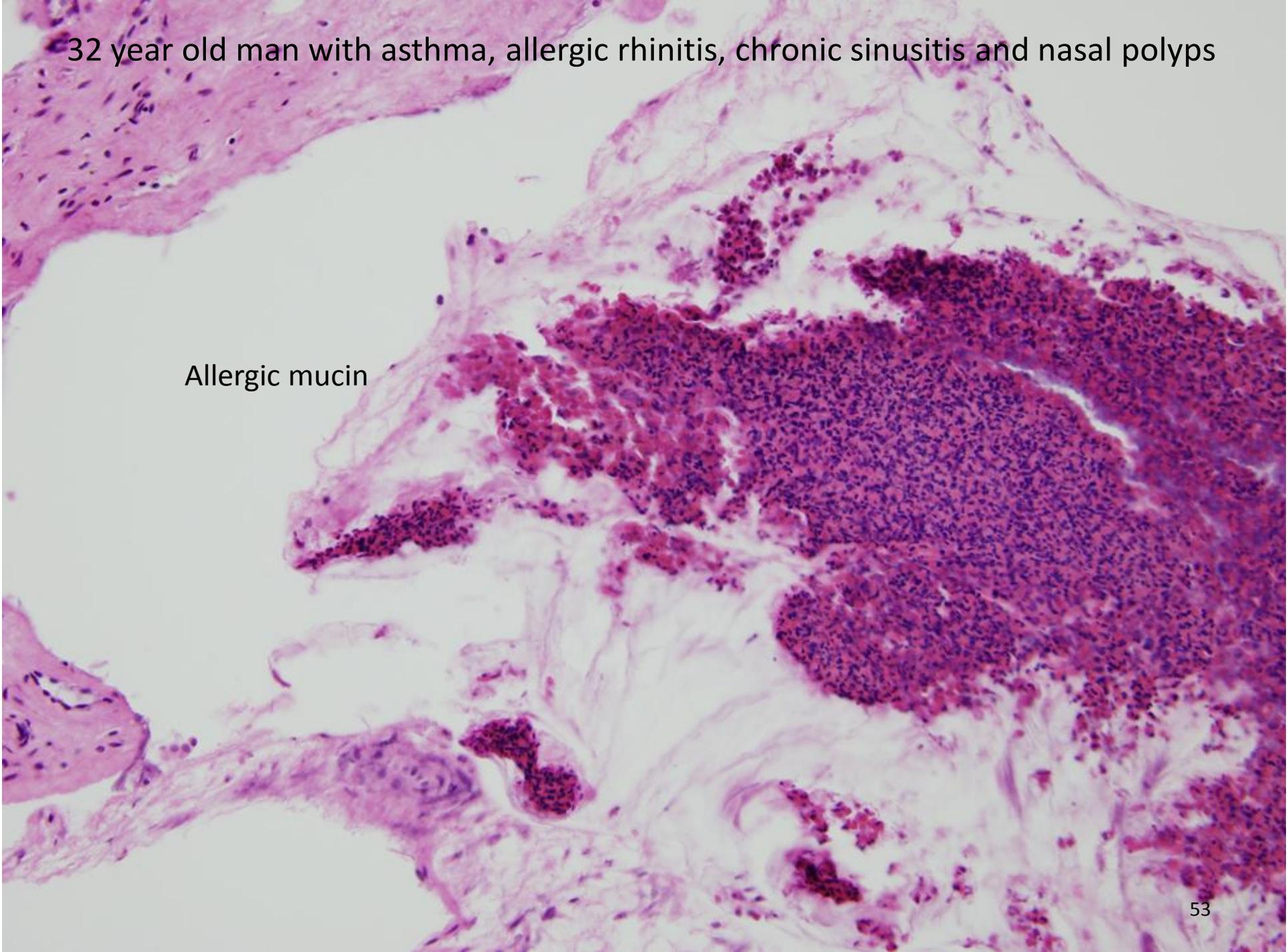
Aspergillosis is caused by infection with *Aspergillus*, especially *A. fumigatus*, *A. flavus*, or *A. niger*. *Aspergillus* is a ubiquitous mold and its spores are inhaled by everyone. Colonization of the airways may occur especially after massive exposure. Pulmonary aspergillosis may take several forms, most commonly colonization of airways or preexisting cavities, including the formation of fungus balls (mycetoma, aspergilloma). Other forms include allergic bronchopulmonary aspergillosis, noninvasive and invasive infections, and involvement of the pleural cavity with empyema.

Fungus balls may occupy in cavities caused by a variety of disease processes including tuberculosis, bronchiectasis (dilated airways), chronic abscess, and malignancy. Allergic bronchopulmonary aspergillosis represents a hypersensitivity reaction and can be seen in asthmatics. Invasive aspergillosis is most common in patients who are immunosuppressed and myelosuppressed. The hyphae can invade blood vessels leading to necrosis due to lack of blood supply and hematogenous dissemination.

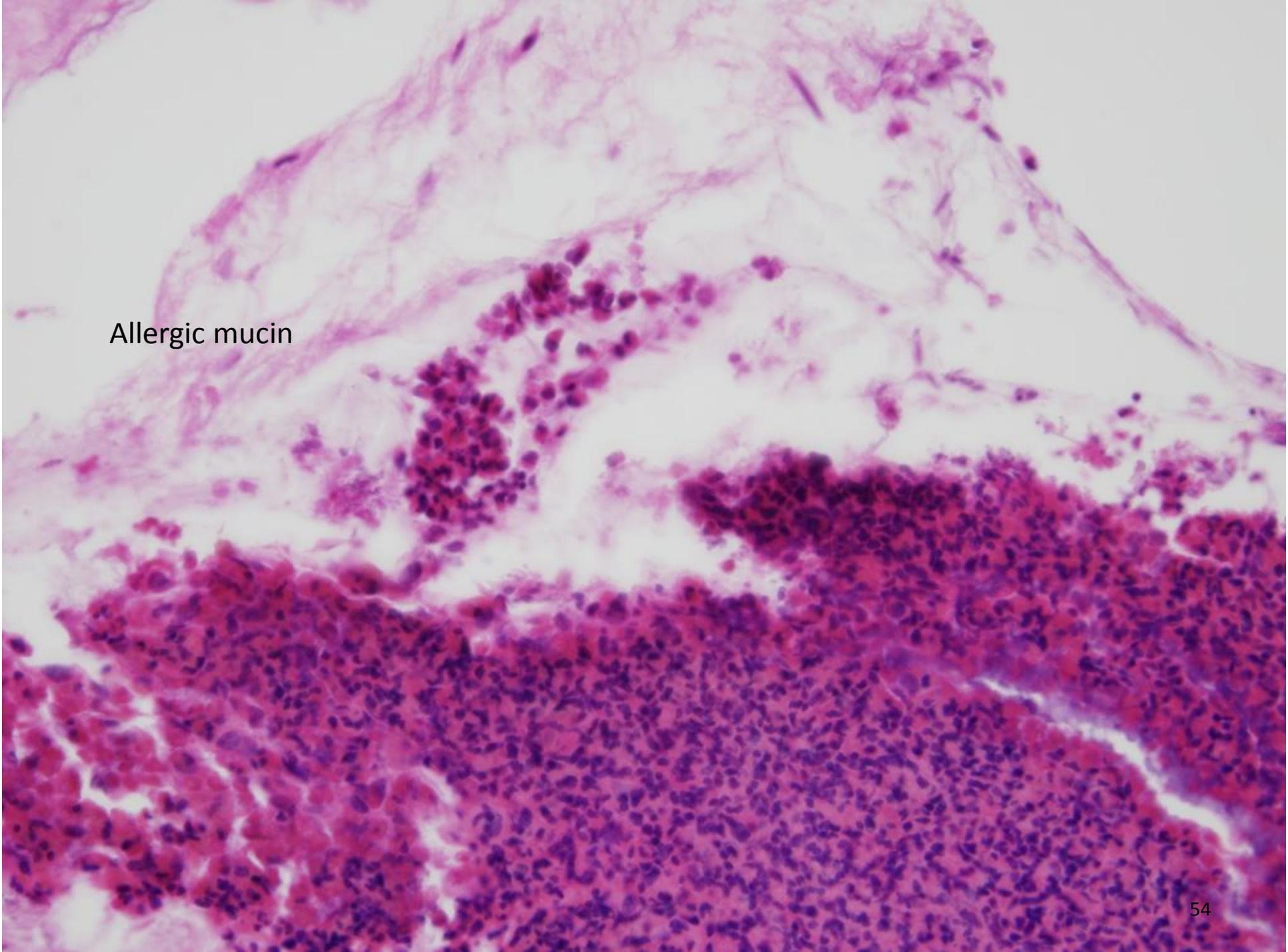
The hyphae of *Aspergillus* are septated and branching and 3-6 microns in width with parallel walls. The branching is usually at acute angles, often 45 degrees. Degenerative changes occur and can mimic mucormycosis. Swollen globular hyphal elements without septae can be seen. Conidial heads (fruiting bodies) can be seen with air exposure.

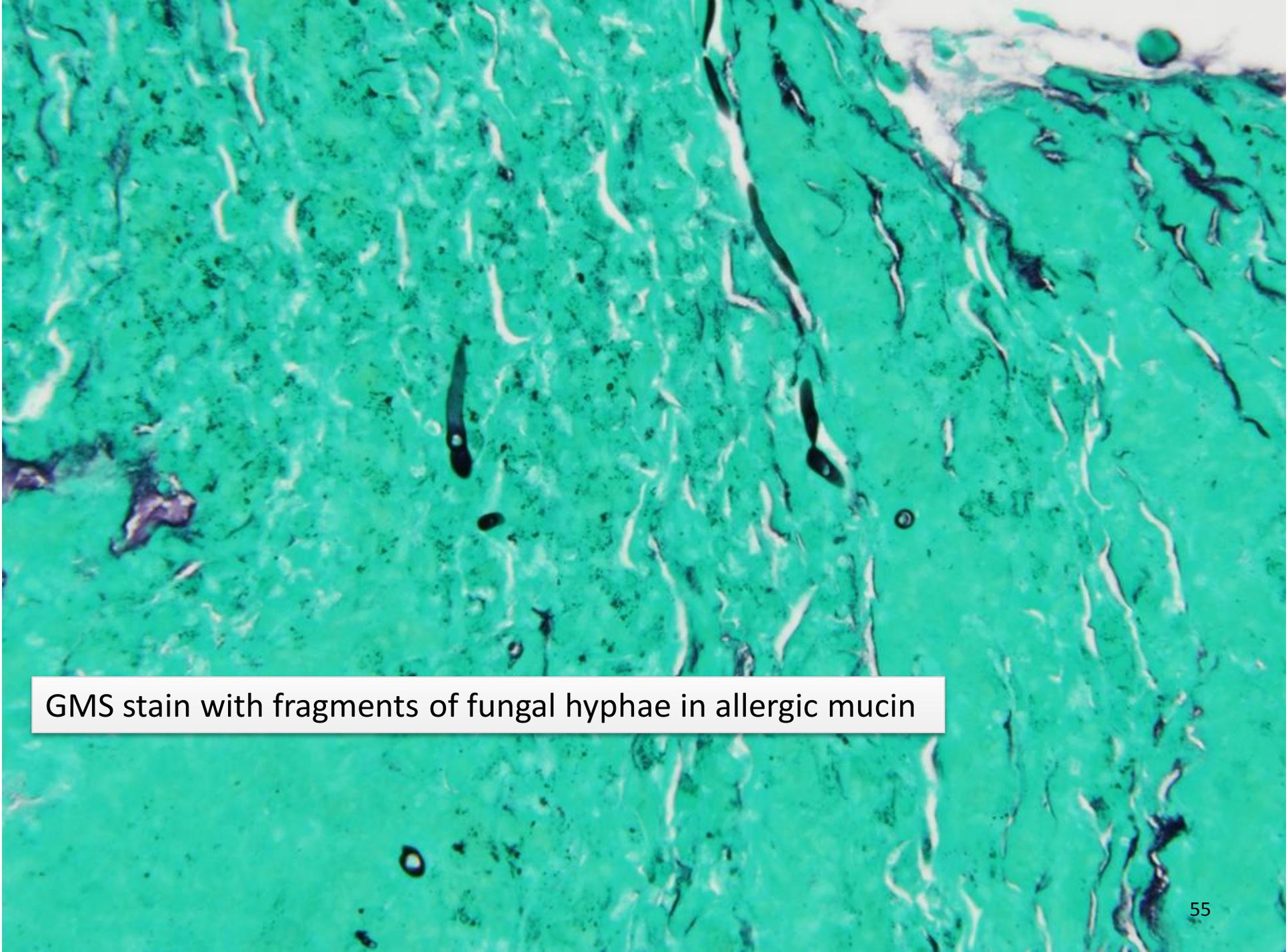
32 year old man with asthma, allergic rhinitis, chronic sinusitis and nasal polyps

Allergic mucin

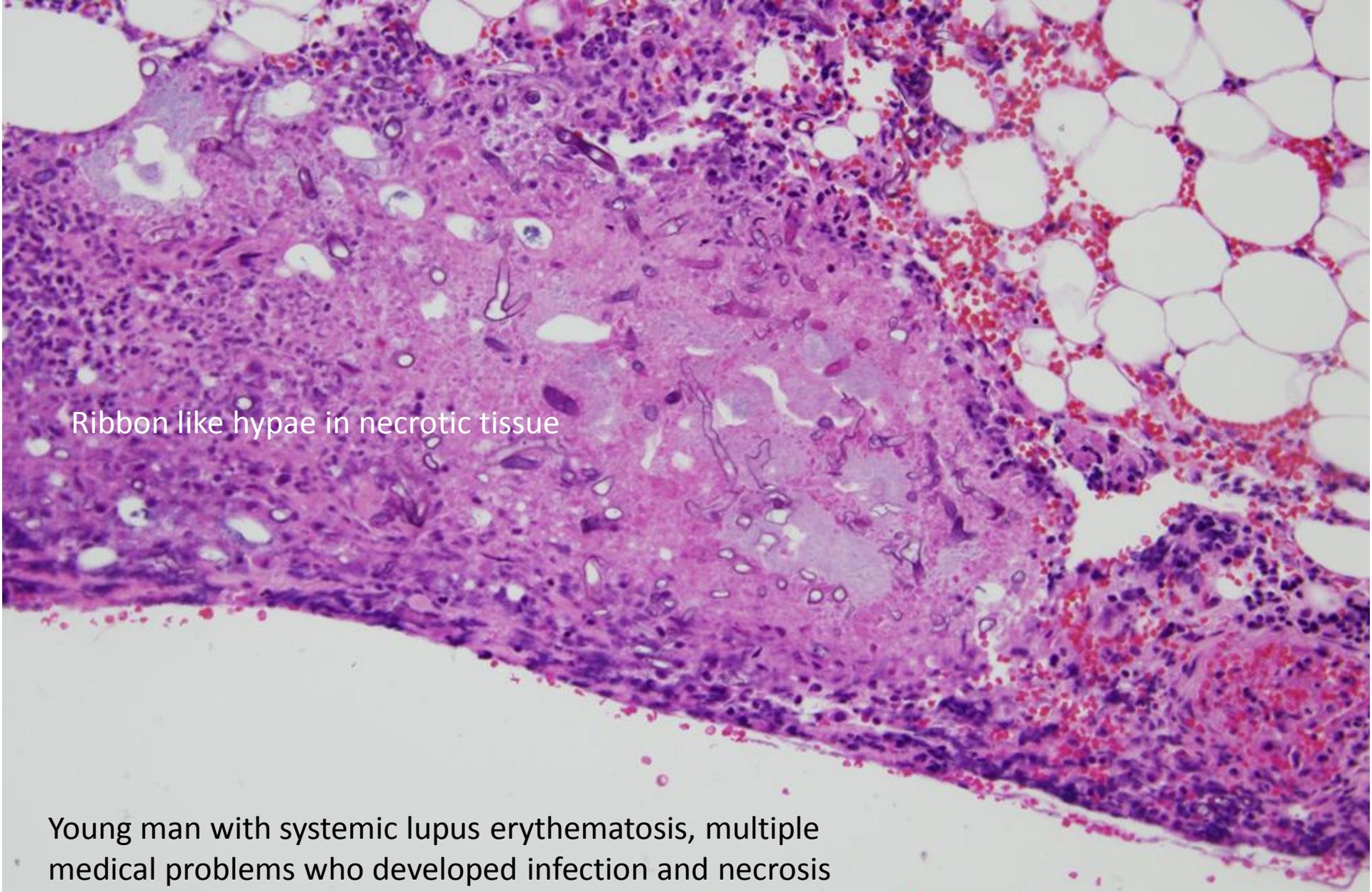


Allergic mucin



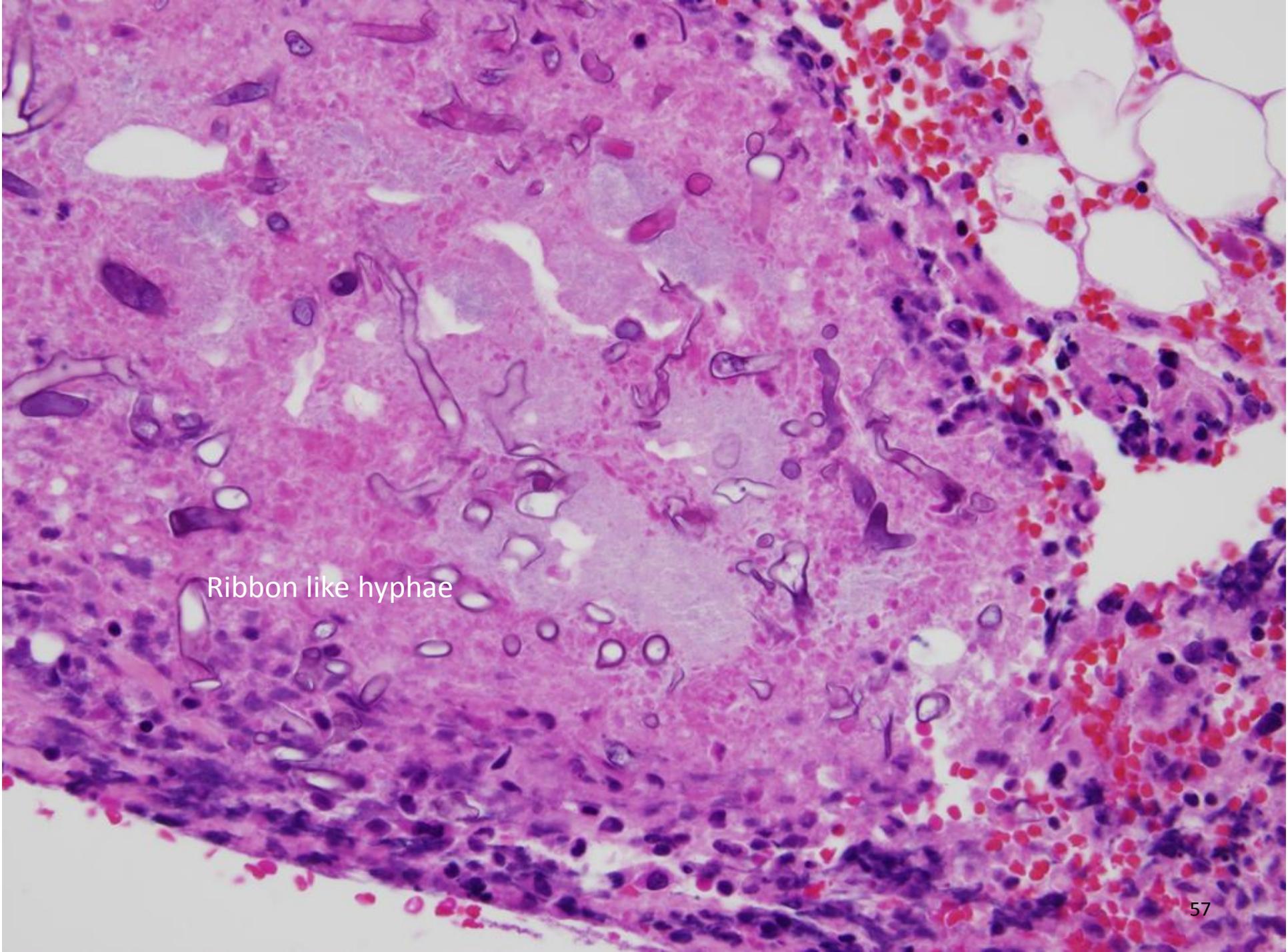


GMS stain with fragments of fungal hyphae in allergic mucin



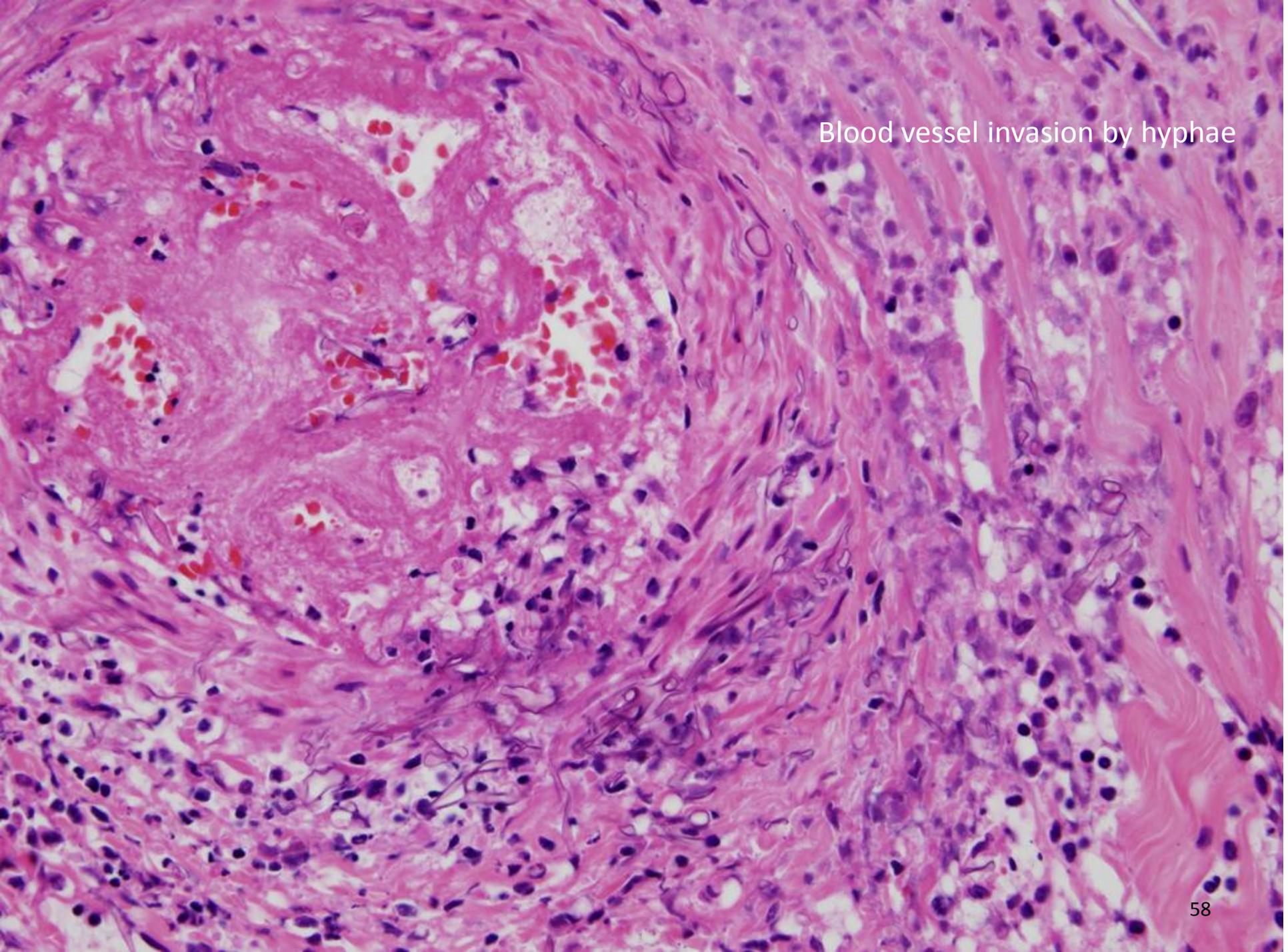
Ribbon like hypae in necrotic tissue

Young man with systemic lupus erythematosus, multiple medical problems who developed infection and necrosis near indwelling vascular access line. Diagnosis – mucormycosis (zygomycosis) due to Rhizopus.



Ribbon like hyphae

Blood vessel invasion by hyphae



# Zygomycosis (Mucorcyosis)

Zygomycosis is an uncommon and often fatal infection caused by a variety of fungi in the order Mucorales and class Zygomycetes. Infection may be caused by inhalation of spores, percutaneous routes, or ingestion. Most patients are immunosuppressed. Infection can occur due to direct inoculation of wounds.

The Zygomycetes have broad hyphae ranging from 5-25 microns. The walls are thin such that the hyphae often appear twisted or folded. Branching is irregular and frequently is at 90 degrees. There is inconspicuous to absent septation. Vascular invasion with resulting tissue necrosis is common. The hyphae do not stain with GMS but are usually easily identified in routine tissue sections.