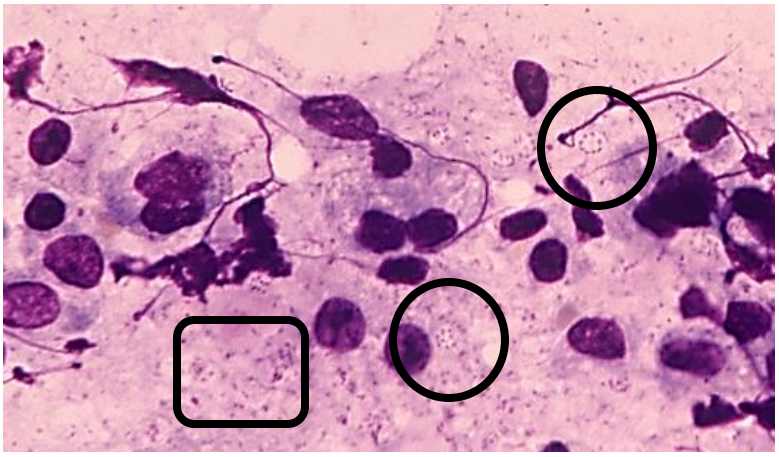
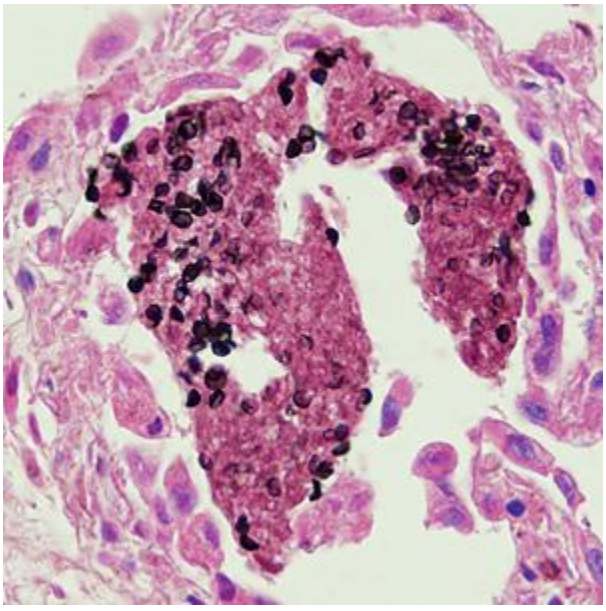
PARASITOLOGY CASE HISTORY 10 (HISTOLOGY)
(Lynne S. Garcia)

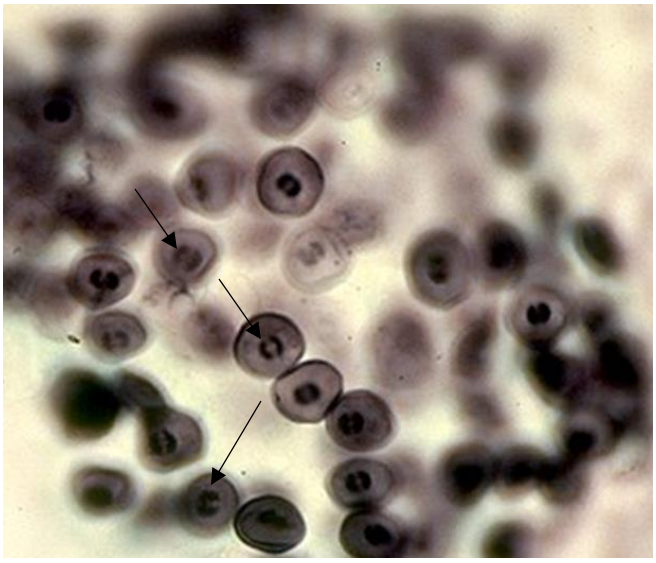
A 46-year-old man with AIDS was admitted to the hospital for complaints of a persisting fever and dry cough. A chest radiograph showed bilateral infiltrate. A sputum specimen was collected and stained with Giemsa, but no parasites were observed and the report was “No Parasites Seen.” Subsequent to this report, a lung biopsy was obtained, sectioned, and stained with both methenamine silver and hematoxylin and eosin (H&E) stains. The images below show what was observed using the oil immersion objective (x100) and/or the high dry objective (x40).



Giemsa, 1000x



H&E/methenamine silver, 400x (black cysts)



Methenamine Silver, 1000x

- Based on these images, what is your diagnosis? Why do you think the first image stained with Giemsa stain was interpreted as a negative?

Scroll Down for Answer and Discussion

Answer and Discussion of Histology Quiz #10

This was a case of pneumonia caused by *Pneumocystis jirovecii* (previously classified as *Pneumocystis carinii*). Because this case showed images taken from a lung biopsy stained with a combination of hematoxylin and eosin (H&E) and methenamine silver, only the cyst walls stained black and intracystic bodies are not visible. However, in the top image stained with Giemsa, the actual trophozoite nuclei are visible (see circles and small B/W image); many freed/individual trophozoite nuclei are also visible in the background (see square). Confirmation of these organisms can be difficult without the use of careful microscopic examination and/or the use of methenamine silver stains (stains the cyst walls, not the trophozoite nuclei). Another potential difficulty involves a low fungal burden, thus impeding the microscopic observation

especially in non-HIV patients' cases, which may lead to a false-negative diagnosis. The trophozoite nuclei can also be stained using periodic acid-Schiff stain (PAS) and Papanicolaou stained pulmonary specimens (nuclei are seen as dark dots).

History. *Pneumocystis*, initially considered to be a protozoan, later has been assigned to the kingdom of fungi, due to its high genetic sequence homology with these organisms, as demonstrated by molecular studies. Nevertheless, despite many similarities, *Pneumocystis* is an atypical fungus which differs in several respects from its relatives. One of such distinctive features, among others, is the presence of cholesterol in the *Pneumocystis* cell membrane, instead of ergosterol, which is the target of amphotericin B and the ketoconazoles. Therefore these drugs, commonly used as therapeutics in infections caused by other fungi, are ineffective in treatment of symptoms triggered by *P. jirovecii*.

Further DNA analyses have revealed that *Pneumocystis* species infecting lungs of various mammalian species are quite different and their infection is host specific. After this discovery, *P. jirovecii*, formerly *Pneumocystis* f. sp. *hominis*, has been identified as a separate subspecies characteristic for humans. The name was given in honor of the Czech parasitologist Otto Jirovec, due to his important contribution in describing this organism in humans.

Life Cycle. *Pneumocystis* spp. has a biphasic life cycle with two distinct forms: haploid trophozoites, constituting the proliferative stages, being asexual phase of the lifecycle, and cysts, representing a reproductive stage. Cysts are generated during the sexual phase, as a result of conjugation of trophozoites. Trophic forms predominate in lungs during the infection, while cysts have the major role in *Pneumocystis* propagation.

The transmission of *Pneumocystis* occurs via an airborne route. It has been demonstrated that immunocompetent hosts could serve as a reservoir of the fungus in population through transferring it from one individual to another when they are within sufficiently close distance, without causing symptomatic disease, until the pathogen reaches the immunocompromised host, in which *Pneumocystis* pneumonia (PcP) may develop.

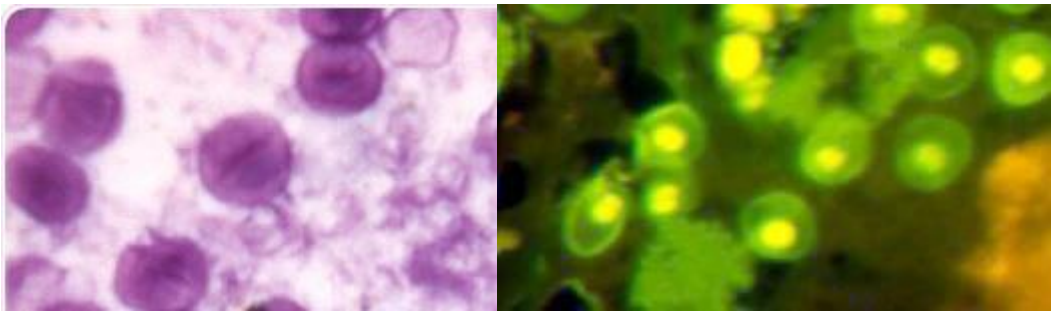
Clinical Disease. *Pneumocystis* pneumonia is an opportunistic disease caused by invasion of unicellular fungus *Pneumocystis jirovecii*. Initially, it was responsible for majority of morbidity and mortality cases among HIV-infected patients, which later have been reduced due to the introduction of anti-

retroviral therapy, as well as anti-*Pneumocystis* prophylaxis among these patients. *Pneumocystis* pneumonia, however, is still a significant cause of mortality among HIV-negative patients being under immunosuppression caused by different factors, such as transplant recipients as well as those undergoing cancer chemotherapy. Rapid onset and fast progression of severe symptoms result in high mortality rate among these patients, who thereby represent the group of highest risk of developing *Pneumocystis* pneumonia. In contrast, fungal invasion in immunocompetent people usually leads to asymptomatic colonization, which frequent incidence among healthy infants has even suggested the possibility of its association with sudden unexpected infant death syndrome.

Asymptomatic carriage of *Pneumocystis* is a phenomenon known as colonization and is important for several reasons. Besides transmission of the pathogen to other people, colonized individuals are also at risk of PcP development in case of decreased immunity. Furthermore, colonization in individuals receiving anti-*Pneumocystis* prophylaxis for a long time may lead to selection of drug-resistant strains. Even minor amounts of pathogen present in lungs may provoke host inflammatory response, which in turn leads to lung damage and plays a role in progression of lung disorders, such as chronic obstructive pulmonary disease or lung cancer.

Diagnosis. Detection methods used in most laboratories involve molecular techniques; however, the standard cutoff value to distinguish colonization from active pneumocystosis has not been established yet. Therefore, when colonized patient experiences pneumonia with a different etiology, positive results of *Pneumocystis* detection may be misleading. Confirmation of the presence of the organism can be obtained from the examination of stained histologic smears.

The cyst forms contain single or paired discrete foci of enhanced staining that measure 1-2 microns in size (often referred to as the parentheses – see arrows in the image above of methenamine silver stain). The morphology of these structures by light microscopy is characteristic, and their recognition is helpful in identifying *P. jirovecii* cysts and in differentiating them from yeast-form fungi and argyrophilic tissue elements in histologic sections and cytology specimens. Toluidine Blue O/sulphation and Calcofluor white can also be used to reveal the cysts (see images below). Also, sulphation of smears before staining with Giemsa allows cysts to be visualized.



Toluidine blue O/sulphation, cysts Calcofluor white, cysts

Prevention. Although PcP prophylaxis for susceptible, immunocompromised patients has become the standard of care, the optimal scheme and duration of therapy have not been defined. In case of organ recipients, the early post-transplant period is the considered highest risk time for infection, albeit there is a growing evidence of late PcP recognition, even above one year after transplantation.

The lack of appropriate routine systems for culturing *P. jirovecii* is an impediment in research enabling expanding our knowledge about this organism, which can be instead bypassed by more specific molecular studies, including research on *Pneumocystis* epidemiology.

References:

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3. **Roux A, Canet E, Valade S, et al.** *Pneumocystis jirovecii* pneumonia in patients with or without AIDS, France. Emerg Infect Dis. 2014;20:1490–1497. doi: 10.3201/eid2009.131668.

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