Pneumocystis jirovecii Pneumonia Versus Histoplasmosis

53

James A. Mays, Joshua A. Lieberman, and Haodong Xu

Case Presentation

A 57-year-old man with a past medical history of bilateral lung transplant presented with increasing shortness of breath to his outpatient pulmonologist. Chest computed tomography (CT) showed diffuse patchy infiltrates, most prominent in the para-hilar region, with associated lymphadenopathy (Fig. 53.1). The patient underwent bronchoscopy and transbronchial biopsy, which showed granulomatous inflammation with few associated organisms, thought to be consistent with *P. jirovecii*. Prolonged treatment for pneumocystis pneumonia was not effective, and the patient eventually underwent wedge biopsy, which showed necrotizing granulomas and small, narrow-based budding yeasts, morphologically most consistent with *H. capsulatum* (Fig. 53.2). Fungal cultures obtained from fresh tissue confirmed the diagnosis.

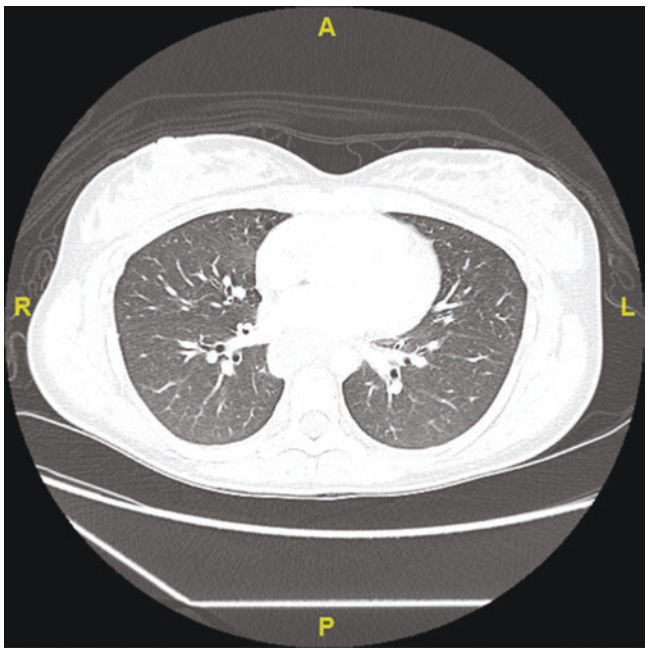

Fig. 53.1 Chest CT showing diffuse patchy infiltrates, most prominent in the para-hilar region, with associated lymphadenopathy

J. A. Mays
Department of Laboratory Medicine and Pathology, University of Washington Medical Center, Seattle, WA, USA

Department of Pathology, Massachusetts General Hospital, Boston, MA, USA
e-mail: jamays@mgh.harvard.edu

J. A. Lieberman · H. Xu (✉)
Department of Laboratory Medicine and Pathology, University of Washington Medical Center, Seattle, WA, USA
e-mail: joshuaal@uw.edu; xu8@uw.edu

H. Xu et al. (eds.), *Practical Lung Pathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-031-14402-8_53

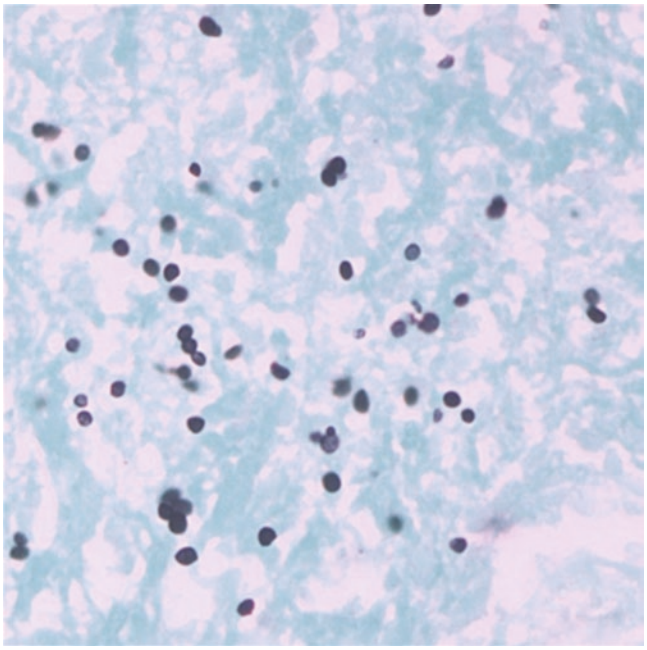

Fig. 53.2 Typical morphology of histoplasmosis. The organisms are small, ovoid yeast forms with narrow-based budding, as contrasted with the folded, umbilicated forms with an argyrophilic dot as seen in *P. jirovecii*

Final Diagnosis: Small Yeasts with Narrow-Based Budding

Comment: These findings are suggestive of *Histoplasma capsulatum* but may be confused with other small yeasts such as *Candida glabrata*, *Pneumocystis jirovecii*, and others. Correlation with microbiology studies is necessary to identify the infectious organism.

Clinical Considerations

Histoplasma capsulatum is an invasive endemic pulmonary fungal pathogen that infects immunocompetent hosts and is seen as narrow-based budding yeast on histologic examination. In North America, *Histoplasma* is most frequently found in the distribution of the Ohio and Mississippi rivers [1], but it is globally distributed and prevalent in sub-Saharan Africa [2, 3] The clinical presentation of histoplasmosis has a wide range of clinical severity: most frequently it is asymptomatic and self-limiting [4] but can produce an acute presentation, a chronic infection akin to mycobacterial disease, or disseminated disease.

P. jirovecii, previously characterized as a protozoan, is now classed as a fungus. It is present worldwide, and although it is overwhelmingly a disease of the immunocompromised, at least one study has found a carrier rate of up to 20% in healthy, immunocompetent adults [5]. *Pneumocystis* pneumonia is one of the most commonly encountered lung infections in people with impaired cell-mediated immunity, such as those with advanced HIV [6], organ transplants, or hematological malignancy. Unlike *Histoplasma* spp. and other fungi, *P. jirovecii* cannot be grown by microbiologic culture [7].

Radiologic Features

Typical radiographic findings in *Pneumocystis* pneumonia are bilateral, diffuse alveolar, and interstitial infiltrates that are typically either lower lobe or hilar predominant but may diffusely involve the lungs. However, multiple atypical radiographic patterns have been reported and include nodular lesions, lobar consolidation, unilateral involvement, cystic spaces, and hilar lymphadenopathy [8]. Up to 20% of patients with AIDS may present with no radiographic abnormalities on chest radiographs, although they will likely have ground-glass opacities on subsequent CT [9]. The key message is that *Pneumocystis* pneumonia is compatible with a wide array of radiographic findings.

In acute histoplasmosis, radiographs most often show patchy consolidation in one or more lobes, frequently with prominent hilar or mediastinal lymph nodes [10]. Severe disease can show diffuse reticulonodular infiltrates, and resolved cases may have calcified nodules. However, a substantial portion of cases may have normal chest radiographs [4, 10]. The variety of radiographic findings reflects the diversity of pathologic manifestations: histoplasmosis can produce an acute presentation, chronic infection akin to mycobacterial disease, disseminated disease, mediastinitis, or a circumscribed nodule of largely resolved infection.

Histologic Features

The diagnosis of *Pneumocystis* pneumonia can be accomplished by recognition of characteristic histomorphologic features of the organism and intra-alveolar exudates (Fig 53.3a). Four developmental forms of *P. jirovecii* are described, in keeping with its former protozoan classification: trophozoites, precysts, cysts, and sporozoites (also known as intracystic bodies). For the purpose of pathologic identification, cysts and trophozoites are the most relevant. The cyst is the largest, most easily recognized stage, and is easily demonstrated with GMS stain. The vegetative forms of *Pneumocystis*, trophozoites, are 1–5 μm in diameter and characteristically attach to type I pneumocytes. The cyst is 5–7 μm in diameter and most contain up to eight intracystic bodies (sporozoites), each 1–2 μm in diameter. These sporozoites have a single nucleus. Some cysts will contain internal

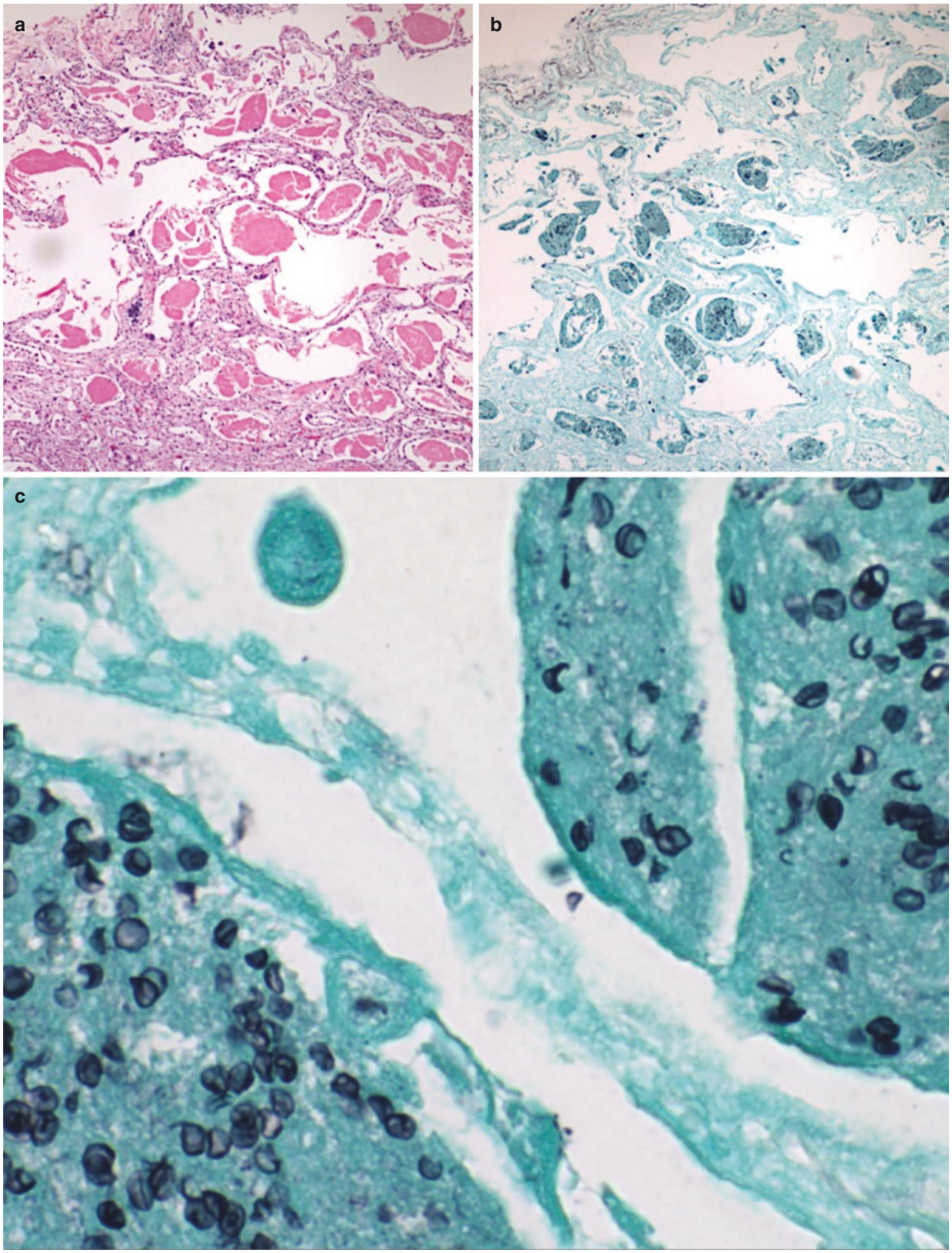

Fig. 53.3 Typical morphology and alveolar filling pattern of *P. jirovecii* pneumonia. Low (100×) magnification sections of a postmortem lung stained with H&E (**a**) and GMS (**b**) demonstrate an alveolar filling pattern with eosinophilic, proteinaceous exudate typical for *P. jirovecii* pneumonia. The opportunistic pathogens are abundant within intra-alveolar exudates and easily seen with a silver stain (**b**, **c**). Note the central umbilication and slightly folded appearance of the organisms at high (400×) magnification (**c**)

comma-shaped structures, either singular or in pairs, that are localized thickenings of the internal layer of the cyst wall. Ruptured cysts that have released their sporozoites will have a characteristic cup or crescent shape when collapsed. Intracystic sporozoites are not visible on histopathologic stains. The cysts are round to oval, focally curved, with crushed "boat" forms present (Fig. 53.3c). These structures are useful in identifying *P. jirovecii* as compared to budding yeast pathogens. When found separately from the cysts, sporozoites and trophozoites are difficult to identify and distinguish from background debris or tissue, but they can have an amoeboid appearance in tissue sections.

Grossly, in patients with *P. jirovecii* pneumonia, the lungs are typically heavy with a gray or tan consolidated cut surface. The most typical histologic features are that of mild interstitial mononuclear inflammation, type II pneumocyte hyperplasia, and eosinophilic foamy intra-alveolar exudates that expand the alveolar spaces (Fig. 53.3a). On higher magnification, the exudates can be punctuated by round basophilic dots that correspond to sporozoite and trophozoite nuclei. These help to distinguish the findings from pulmonary edema or alveolar proteinosis. In tissue sections, there is often characteristic retraction of the exudate from the adjacent alveolar wall.

Although the above features are the most common histologic pattern, *P. jirovecii* can provoke essentially any lung injury reaction pattern. In chronic infection, interstitial fibrosis may be seen. In an acute and progressive infection, diffuse alveolar damage, hyaline membranes, and reactive epithelial proliferation can be seen. There is often robust lymphoplasmacytic inflammation and type II pneumocyte hyperplasia that can be misinterpreted as lymphocytic interstitial pneumonia (LIP) or nonspecific interstitial pneumonia (NSIP). In one study, 19% of patients lacked the characteristic alveolar exudates, and many showed atypical features such as interstitial inflammation (63%), fibrosis (50%), numerous alveolar macrophages resembling desquamative interstitial pneumonia (9%), granulomatous inflammation (5%), hyaline membranes (4%), and interstitial pneumonitis (3%) [11]. Due to this multitude of possible histologic patterns, the differential diagnosis of opportunistic lung infections frequently includes both *Pneumocystis* pneumonia and endemic mycoses such as *H. capsulatum*. In such cases, diagnosis may be delayed without definitive histologic or microbiologic evidence of organisms.

Several infectious etiologies could be confused for *P. jirovecii*. These include *Histoplasma capsulatum*, *Candida glabrata*, and *Cryptococcus* spp. Each of these entities has characteristic findings to suggest it. *H. capsulatum* has small, ovoid yeast forms (2–5 μm) that show narrow-based budding, which is absent in *Pneumocystis*. Yeast forms of *H. capsulatum* typically stain poorly in routine preparations but stain well with GMS (Fig. 53.1). In addition, *H. capsulatum* is primarily an intracellular pathogen, whereas *P. jirovecii* is primarily an extracellular organism within alveolar spaces. *Cryptococcus* yeasts (5–15 μm) are larger than either *H. capsulatum* or *P. jirovecii* and typically have a capsule. Pseudohyphae production by a small yeast, like *C. glabrata*, would argue strongly against the above pathogens.

Special Stains

Special stains for *Pneumocystis* can be grouped into two general categories: those that highlight the cyst wall and its internal structures and those that stain the nuclei of trophozoites and sporozoites. Only cytologic specimens are usable in the case of the latter, and multiple stains, including Giemsa, Wright, and Diff-Quik, can highlight the vegetative state nuclei. More commonly used in clinical practice are stains that highlight the cyst wall. Of these, GMS is the most reliable and sensitive and will highlight the walls of round to oval 5–7 μm cysts and any "crescent" or "boat" forms (Fig. 53.3b) within the exudates. Some cysts have a unique argyrophilic peripheral dot-like structure and are helpful to distinguish cysts from budding yeast forms. This feature is also present as a basophilic dot in cyst forms when seen on hematoxylin and eosin (H&E) preparations. Taken together (Fig. 53.3a), these findings are highly suggestive of *Pneumocystis* pneumonia. In cases where *Cryptococcus* is in the differential diagnosis, a mucicarmine stain is a useful adjunct to visualize capsule; however, capsule-deficient forms exist.

Molecular and Microbiologic Features

While *H. capsulatum* grows reliably in culture, *P. jirovecii* cannot be grown on cell-free media. The inability to culture *P. jirovecii* underlines the importance of clinical suspicion, awareness of key morphologic features, and importance of ancillary detection methods when *P. jirovecii* pneumonia is suspected. Both *H. capsulatum* and *P. jirovecii* can be detected and distinguished from each other by broad-range fungal PCR with sequence-based identification or species-specific PCR. Species-specific assays employ primer sets optimized to bind the target organism's DNA, such as the *Pneumocystis jirovecii* gene *cdc2*, a conserved species-specific cell division cycle2 [12, 13]. Optimized primer binding is thought to have higher analytical sensitivity than broad-range PCR assays. Clinical sensitivity is influenced by pretest probability of infection, tissue volume [14], organism burden, and target gene copy number.

Some microbiology laboratories continue to offer direct fluorescent antibody (DFA) staining of respiratory fluids for *Pneumocystis* detection, although a lack of control material threatens the continued use of these assays (personal communication). The clinical sensitivity of DFA staining is also subject to organism burden and sample volume but performs well in high-prevalence populations, with sensitivity ~55% in HIV-infected patients with suspected PJP [15]. DFA is therefore an excellent assay when PJP is suspected; however, the addition of a species-specific PCR increases sensitivity [12, 13] and should be considered when clinical suspicion is high but DFA is negative. PCR is of particular value when cytopathology or histopathology specimens are the only available diagnostic material.

Key Points for Differentiating *P. jirovecii* and *H. capsulatum*

Both *Pneumocystis jirovecii* and *Histoplasma capsulatum* Cause Pulmonary Infections in Overlapping Patient Populations. What Histopathologic Features Help Distinguish Between *P. jirovecii* and *H. capsulatum*?

Two important differences are very helpful in distinguishing these two infections: tissue distribution and tissue reaction pattern. *P. jirovecii* is predominantly found in the alveolar spaces and is often accompanied by a "frothy" eosinophilic exudate that fills the alveolar space. *H. capsulatum*, on the other hand, germinates from inhaled conidia inside alveolar macrophages and is primarily intracellular with lymphatic dissemination. Eosinophilic exudates in the alveoli are not a typical feature of *H. capsulatum.* Confusion in diagnosis can arise when the typical alveolar exudate of *P. jirovecii* is absent, when atypical tissue reaction patterns—such as granulomatous inflammation or interstitial fibrosis—are present, or when the organisms cannot be well-visualized.

What Morphologic Features Help Distinguish These Two Organisms from Each Other?

These two organisms have similar morphology but can often be distinguished from each other using several morphologic features. First, *P. jirovecii* cysts are slightly larger (5–7 μm) than *H. capsulatum* (2–5 μm) and have a characteristic boat- or cup-like forms and perinuclear dot-like structures indicative of sporozoite release. These morphologic features are important to note since the sizes of the organisms are similar. Second, *P. jirovecii* lack the narrow-based budding typical of *H. capsulatum* dividing yeasts.

What Laboratory Tests Are Available to Confirm the Diagnosis?

Culture is always important in the evaluation of infectious diseases, but only *H. capsulatum* will grow in microbiologic culture. Two other types of assays in the clinical microbiology laboratory are both sensitive and specific: PCR (both organisms) and DFA (*P. jirovecii* only).

References

1. Manos NE, Ferebee SH, Kerschbaum WF. Geographic variation in the prevalence of histoplasmin sensitivity. Dis Chest. 1956;29(6):649–68.
2. Houston S. Tropical respiratory medicine. 3. Histoplasmosis and pulmonary involvement in the tropics. Thorax. 1994;49(6):598–601.
3. Oladele RO, Ayanlowo OO, Richardson MD, Denning DW. Histoplasmosis in Africa: an emerging or a neglected disease? PLoS Negl Trop Dis. 2018;12(1):e0006046.
4. Goodwin RA, Loyd JE, Des Prez RM. Histoplasmosis in normal hosts. Medicine (Baltimore). 1981;60(4):231.
5. Medrano FJ, Montes-Cano M, Conde M, de la Horra C, Respaldiza N, Gasch A, et al. Pneumocystis jirovecii in general population. Emerg Infect Dis. 2005;11(2):245–50.
6. Roux A, Canet E, Valade S, Gangneux-Robert F, Hamane S, Lafabrie A, et al. Pneumocystis jirovecii pneumonia in patients with or without AIDS, France. Emerg Infect Dis. 2014;20(9):1490–7.
7. Liu Y, Fahle GA, Kovacs JA. Inability to culture Pneumocystis jirovecii. MBio. 2018;129(3):e00939–18.
8. Kuhlman JE, Kavuru M, Fishman EK, Siegelman SS. Pneumocystis carinii pneumonia: spectrum of parenchymal CT findings. Radiology. 1990;175(3):711–4.
9. Kennedy CA, Goetz MB. Atypical roentgenographic manifestations of Pneumocystis carinii pneumonia. Arch Intern Med. 1992;152(7):1390–8.
10. Gurney JW, Conces DJ. Pulmonary histoplasmosis. Radiology. 1996;199(2):297–306.
11. Travis WD, Pittaluga S, Lipschik GY, Ognibene FP, Suffredini AF, Masur H, et al. Atypical pathologic manifestations of Pneumocystis carinii pneumonia in the acquired immune deficiency syndrome. Review of 123 lung biopsies from 76 patients with emphasis on cysts, vascular invasion, vasculitis, and granulomas. Am J Surg Pathol. 1990;14(7):615–25.
12. Wilson JW, Limper AH, Grys TE, Karre T, Wengenack NL, Binnicker MJ. Pneumocystis jirovecii testing by real-time polymerase chain reaction and direct examination among immunocompetent and immunosuppressed patient groups and correlation to disease specificity. Diagn Microbiol Infect Dis. 2011;69(2):145–52.
13. Church DL, Ambasta A, Wilmer A, Williscroft H, Ritchie G, Pillai DR, et al. Development and validation of a Pneumocystis jirovecii real-time polymerase chain reaction assay for diagnosis of Pneumocystis pneumonia. Can J Infect Dis Med Microbiol. 2015;26(5):263–7.
14. Gomez CA, Budvytiene I, Zemek AJ, Banaei N. Performance of targeted fungal sequencing for culture-independent diagnosis of invasive fungal disease. Clin Infect Dis. 2017;65(12):2035–41.
15. Choe PG, Kang YM, Kim G, Park WB, Park SW, Kim HB, et al. Diagnostic value of direct fluorescence antibody staining for detecting Pneumocystis jirovecii in expectorated sputum from patients with HIV infection. Med Mycol. 2014;52(3):326–30.