

Immunohistochemical Identification of *Pneumocystis jirovecii* in Liquid-based Cytology of Bronchoalveolar Lavage – Nine Cases Report –

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Pneumocystis pneumonia (PCP) is caused by the yeast-like fungus *Pneumocystis jirovecii*, which is specific to humans. PCP could be a source of opportunistic infection in adults that are immunosuppressed and children with prematurity or malnutrition. The diagnosis should be confirmed by identification of the causative organism, by analysis of the sputum, a bronchoalveolar lavage or a tissue biopsy. In both histologic and cytologic specimens, the cysts are contained within frothy exudates, which form aggregated clumps. The cysts often collapse forming crescent-shaped bodies that resemble ping-pong balls. We recently diagnosed nine cases of PCP using an immunohistochemical stain for *Pneumocystis*. The patients consisted of five human immunodeficiency virus positive individuals, two renal transplant recipients, and two patients with a malignant disease. All nine patients were infected with *P. jirovecii*, which was positive for monoclonal antibody 3F6. In conclusion, the immunohistochemical stain used in this report is a new technique for the detection of *P. jirovecii* infection.

Key Words: *Pneumocystis jirovecii*; Bronchoalveolar lavage; Immunohistochemistry

Pneumocystis jirovecii pneumonia is an important cause of morbidity and mortality in immunocompromised patients, such as human immunodeficiency virus (HIV)-infected individuals, solid organ transplant recipients, patients with various malignant conditions, or individuals immunosuppressed by prolonged steroid use. *Pneumocystis pneumonia* (PCP) has been a major cause of death since the 1980's, especially in acquired immune deficiency syndrome (AIDS) patients.¹

The incidence rate of PCP has declined since the introduction of prophylactic antibiotics and effective antiretroviral therapy; furthermore, the mortality rate has decreased to approximately 5% as a result of effective treatment and wider recognition of the disease. However, the number of HIV-positive individuals has increased continuously in the same time-period;² there have also been reports indicating an increased risk of *Pneumocystis* infection with the use of new immunosuppressive drugs and the presence of preceding immunomodulating infections, such as cytomegalovirus (CMV).³ Thus, the need for rapid detection of PCP infection is important for effective treatment.

For many years, the detection of *Pneumocystis* organisms for diagnostic purposes has been dependent upon the histological

or cytological identification of the organisms from respiratory samples, supplemented with various special stains such as the Giemsa stain, Grocott-Gomori methenamine silver (GMS) stain or by direct fluorescence assay.⁴ Recently, DNA amplification procedures have proved to be a successful, sensitive and specific method of detecting *P. jirovecii*. However, this method is not yet standardized nor is it frequently used in diagnostic services.⁵

A few recent studies have suggested that a serological test can be used for detecting IgM antibodies of major surface glycoproteins of *P. jirovecii*.⁶⁻⁸ Linder *et al.*⁹ developed monoclonal antibodies against *Pneumocystis carinii*, using a urea extract of PC-infected lung tissue as the antigen in an enzyme-linked immunosorbent assay. The monoclonal antibodies 3F6 and 2E3 reacted with antigenic determinants of PC parasites, and were useful in the identification of both cysts and trophozoites from fixed smears of infected lung tissue and bronchoalveolar lavage (BAL) fluid.

Here we report the usefulness of immunohistochemistry in detection of *P. jirovecii*, and suggest a rapid and convenient method as a routine test for the diagnosis of PCP.

CASE REPORT

Clinical findings of the patients

Nine patients, admitted to Korea University Anam Hospital between 2008 and 2010, were diagnosed with PCP; the patients consisted of five HIV-seropositive individuals, two renal transplant recipients, and two patients being treated for cancer.

All the patients exhibited classic radiological features of PCP, bilateral infiltrates extending from the perihilar regions. High-resolution computed tomography also presented standard features of PCP; patchy, ground-glass opacities with interlobular septal thickening. Some patients had small amount of pleural effusion.

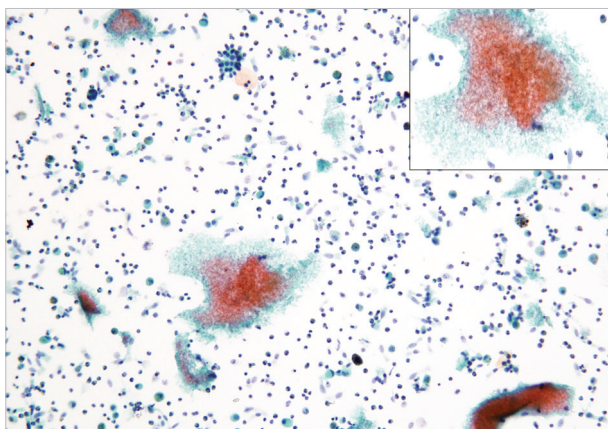


Fig. 1. The liquid-based cytology preparation of a bronchoalveolar lavage. Numerous inflammatory cells and alveolar macrophages can be seen. There are several proteinaceous casts containing tiny vacuolated, bubbly frothy exudates (Papanicolaou stain).

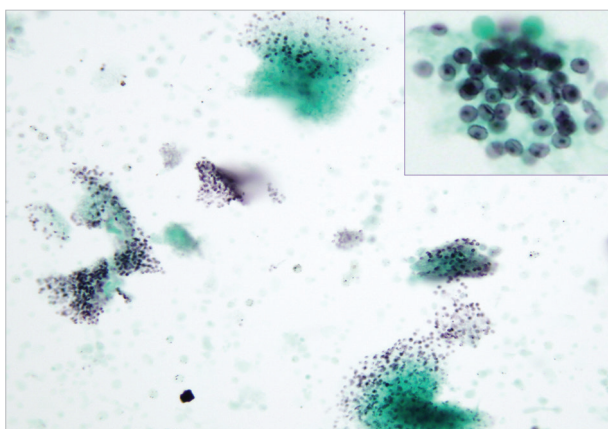


Fig. 2. Clusters of *Pneumocystis jirovecii* cysts (Grocott-Gomori methenamine silver stain). Inset shows round or crescent-shaped cysts containing a nucleolus.

All the HIV-seropositive patients were male; four were in their forties and one in his seventies. Oral candidiasis and CMV infection were concurrently associated in two patients, respectively, and two of them also had tuberculosis. The two renal transplant recipients had been treated with immunosuppressant drugs; one of them was CMV-positive. The last two patients had malignant lesions. Of which, the 58-year-old male patient had been treated for prostatic cancer, and also suffered from chronic renal dysfunction, infectious spondylitis, neutropenic fever, and spine compression. The 71-year-old female patient had been treated for stage IIa, diffuse large B cell lymphoma. All patients were receiving treatment of either Bactrim or Cotrim, but one HIV-seropositive patient and the two patients with malignant lesions died of multiple organ failure and sepsis.

Cytologic and special stain findings

The specimens analyzed in this report were obtained from bronchoalveolar lavage, and prepared with liquid-based cytology (SurePath™, BD Diagnostics-Tripath, Burlington, VT, USA). Papanicolaou (Pap) stain and the immunohistochemical stain for *P. jirovecii* were used in all specimens. GMS stain was done in only seven patients. The Pap stain revealed typical frothy, bubbly clusters with tiny vacuoles (Fig. 1); although, in one case, this typical exudate was not evident. The GMS stain revealed crescent-shaped bodies, with a nucleus, that resembled crushed ping-pong balls, and found in clusters or isolated trophozoites (Fig. 2). The immunohistochemical stain presented typical clusters of cysts with a smooth, homogeneously granular pattern (Fig. 3). Although GMS stain provided more easily rec-

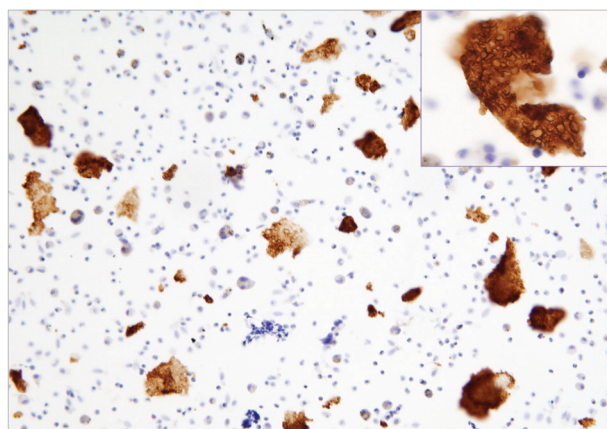


Fig. 3. The exudate is positively stained with immunohistochemical stain, using an antibody of *Pneumocystis jirovecii*. Large or small clusters of cysts measuring about 5 μ m in diameter are easily recognizable (labeled streptavidin-biotin method).

ognizable cysts than the Pap smear, the immunohistochemical stain presented the most distinct and diagnostic characteristics of the three stains used.

DISCUSSION

The clinical presentation of PCP is highly variable and not specific to any underlying immunocompromised status.⁴ Radiological findings in most patients, especially with high resolution computed tomography scanning, typically present patchy, ground-glass opacities with interlobular septal thickening; upon which further examination for PCP is suggested.¹⁰ Although PCP can be effectively prevented or treated, the lesions associated with the disease present a serious site of opportunistic infection in those who are undiagnosed or noncompliant with prophylactic medications, and new cases should be managed during the early phase of the treatment for PCP.¹¹

The gold standard test for the diagnosis for PCP is discovery of *P. jirovecii* in the BAL fluid.⁴ However, *Pneumocystis* cannot be cultured in laboratory unlike many other fungal pathogens. Thus various methods have been tested for rapid and definite detection of this organism.

The characteristic *Pneumocystis* cysts and clusters could be found in Pap stain, which was sufficient for diagnosis because it presented readily recognizable bubbly frothy casts. The diagnostic power of BAL increased when combined with GMS stain. GMS stain shows readily recognizable, crescent-shaped bodies with nuclei, in clusters or isolated trophozoites. However, diagnosis can be confused with other yeast-like pathogens such as *Candida* and *Cryptococcus*.¹² Filaux *et al.*¹³ recently introduced a real-time polymerase chain reaction assay test, in which a negative result allows the diagnostic exclusion of PCP.

In this study, we successfully identified the presence of *P. jirovecii* in all nine patients, using an immunohistochemical method that utilized the monoclonal antibody 3F6. The monoclonal antibody 3F6 was intended for use in the immunohistochemistry of paraffin-embedded bronchoalveolar cells and lung tissues.⁹ However, because this antibody does not show cross reactivity with *Aspergillus*, *Candida*, *Entamoeba*, *Giardia*, *Leishmania*, *Plasmodium*, *Toxoplasma*, *Trypanosome*, and *Cryptosporidium*, it provides a very specific method for the detection of *P. jirovecii*.¹⁴

Daly *et al.*⁶ reported that major surface glycoprotein can be used when determining PCP infection in HIV infected patients. Immunofluorescent detection using a monoclonal antibody has previously been introduced to improve the sensitivity

and specificity of PCP diagnosis.^{10,15,16} However, the immunofluorescent technique requires an immunofluorescent microscope, the photographs taken from which provide the result. The immunohistochemical stain method is easy to perform and the slides can be stored for a long time. Recently, a liquid-based cytology technique has been widely employed within cytology laboratories; with this technique a thin layered smear can provide an effective sample for the immunohistochemical stain and diagnosis. Even in the early stage of infection, with small numbers of the organism, the immunohistochemical stain method is capable of detecting the organism.

From the results of this study, we suggest the use of an immunohistochemical stain in a liquid-based cytology as a definitive method, to be routinely applied in the diagnosis of PCP.

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