Evaluation of real-time PCR for diagnosing *Pneumocystis jirovecii* pneumonia in non-HIV immunocompromised patients

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Objectives: *Pneumocystis jirovecii* pneumonia (PJP) is an important cause of morbidity and mortality in immunocompromised patients. Microscopic diagnosis relies predominantly on immunofluorescence staining of a lower respiratory tract specimens which has limitations. Real-time PCR may assist in diagnosis. The main objectives of the study were to evaluate prospectively two methods for the detection of *P. jirovecii* in clinical specimens and compared a real-time PCR methods (MycAssay *Pneumocystis*) with our standard indirect immunofluorescence assay (IFA).

Methods: In this study, results of the respiratory fluid samples of patients with clinical suspicion of PJP (n=135) that were sent to Mycology Reference Laboratory, Public Health Institution of Turkey between August 2013 and September 2014 from the transplant, oncology unit and tertiary care teaching and university hospitals in Ankara were evaluated. Respiratory fluid samples were examined for the presence of *Pneumocystis* DNA by the MycAssay *Pneumocystis* that targets the *P. jirovecii* mitochondrial large subunit and compared to microscopic diagnosis using an IFA test (Cellabs Pneumo CEL). The sensitivity, specificity, and positive and negative predictive values were calculated by standard methods.

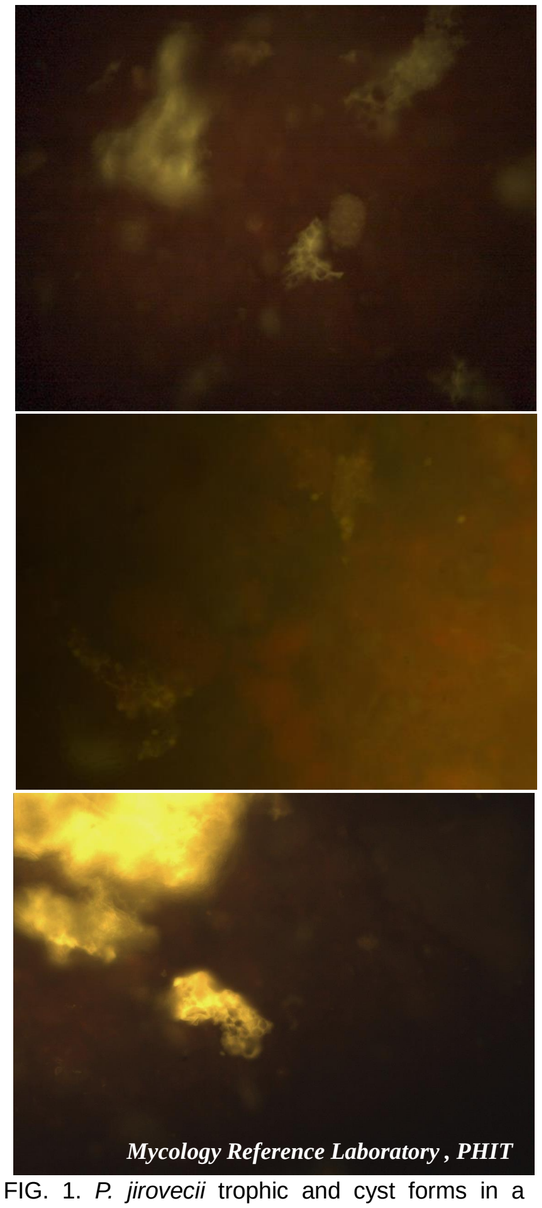

FIG. 1. *P. jirovecii* trophic and cyst forms in a smear of bronchoalveolar lavage material.

Results: The median age of the patients was 41.5 and their sex ratio (M/F) 1.2. PCR results were compared with *P. jirovecii*-IFA test results. All patients (n=135) were HIV negative. A total of 135 respiratory fluid samples were received from patients with a clinical suspicion of pneumocystosis. The specimens consisted of 83 bronchoalveolar lavage specimens, 26 tracheal-bronchial aspirate, 22 induced sputa, 2 fine needle aspiration lung biopsies, and 2 pleural fluid specimens. Microscopical examination was positive (Figure 1) for 11.8% (16/135) of the patients and real-time PCR was positive for 13.3% (18/135) of the patients. Out of 135, 15 were positive and 116 were negative by both detection methods. The remaining, 3 were positive by PCR-negative by microscopy and 1 was positive by microscopy-negative by PCR (Table 1). Three positive PCR results may indicate *P. jirovecii* colonization in patients with chronic lung disease and no clinical suspicion of PJP. However, all our patients had acute pulmonary infiltrates and severe immunodeficiency and most of them were under treatment for active malignancy. Positive PCR in this setting should be taken as possibly indicating infection. The performance of PCR for diagnosing PJP, with IFA as the reference standard, PCR sensitivity, specificity, and positive and negative predictive values were 93.8%, 97.5%, 83.3%, and 99.1% respectively.

TABLE 1.Comparison of detection of *P. jirovecii* in respiratory samples by the MycAssay *Pneumocystis* real-time PCR assay and a laboratory standard of IFA[a].

	IFA					
	Positive		Negative		Total	
	n	%	n	%	n	%
Real-time PCR						
Positive	15	(83,3)	3	(16,7)	18	(13,3)
Negative	1	(0,9)	116	(99,1)	117	(86,7)
Total	16	(11,9)	119	(88,1)	135	(100,0)

[a] A total of 135 respiratory fluid samples of patients with clinical suspicion of PJP were tested.

Conclusion: The detection of *Pneumocystis* DNA in clinical specimens by using the MycAssay *Pneumocystis* – real-time PCR may be important for HIV-negative immunocompromised patients, who develop often PJP with many fewer organisms than HIV-infected patients.

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References:
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