Clinical performance of real-time PCR in the diagnosis of *Pneumocystis jirovecii* pneumonia compared with immunofluorescence assay

Veintimilla C[1]., Alvarez-Uria A[1,2]., Martín-Rabadán P.[1,3], Alcalá L. [1,3], Muñoz P.[1,2,3,4], Marín M.[1,2,3].

[1]Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Marañón; [2]Instituto de Investigación Sanitaria Gregorio Marañón; [3]CIBER Enfermedades Infecciosas-CIBERES (CB06/06/0058), Madrid; [4]Universidad Complutense de Madrid, Spain

Contact: cristina.veintimilla@gmail.com

Background

The laboratory diagnosis of *Pneumocystis jirovecii* pneumonia (PJP) has been traditionally based on microscopy techniques, which have suboptimal sensitivity and depends on the experience and skills of the microbiologist. Molecular detection assays based in PCR (Polymerase chain reaction) could improve sensitivity.

We evaluated the utility of a real-time PCR assay in the routine diagnosis of PJP compared with IFA (Immunofluorescence assay) performed in respiratory samples of patients with PJP suspicion.

Methods

From September 2015 to April 2018

302 respiratory samples were studied by a RT-PCR and IFA

PCR targeting the large subunit of rRNA gene of *P. jirovecii* (PJIR kit Progenie Molecular) and Immunofluorescence assay (MONOFLUO *P. carinii* IFA BioRad

Definite PJP clinical diagnosis was established by infectious diseases consultants considering symptoms, radiological and laboratory findings.

Results

Fig. 1. PJP microbiological diagnosis

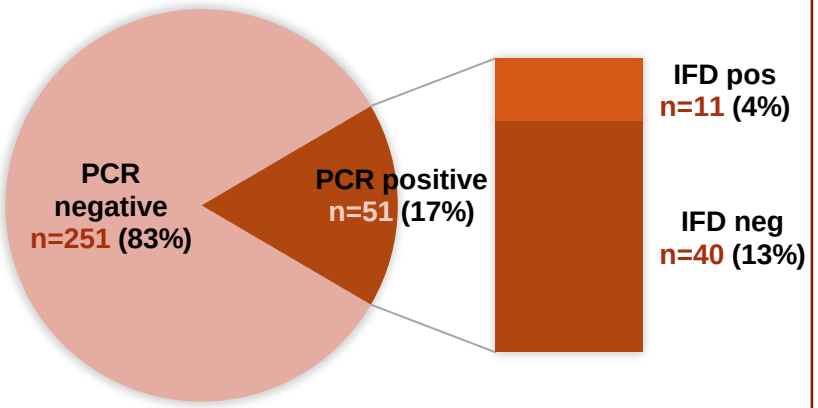

Table 2. Validation test IFA vs PCR assay.

	Sensitivity	Specificity	PPV	NPV
IFA	26%, 95% CI (15.9-39.6)	100%, 95% CI (98.5-100)	100%, 95% CI (77.2-100)	87.2%, 95% CI (82.9-90.6)
PCR	92%, 95% CI (81.2-96.8)	98%, 95% CI (95.4-99.2)	90.2, 95% CI (79.0-95.7)	98.4%, 95% IC (96.0-99.4)

Abb: IC, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 2. Validation of PCR assay by type of respiratory samples

	Sensitivity	Specificity	PPV	NPV
BALF (n = 182)	96.2%	99.4%	96.2%	99.4%
Tracheal aspirate (n = 53)	100%	100%	100%	100%
Sputum (n = 67)	83.3%	91.8%	79%	93.8%

Abb: BALF, bronchoalveolar lavage fluid.

Table 3. Comparison among diagnosis methods stratified by immunocompromised profile.

	Positive patients/ number tested (%)	Statistical significance (Chi-Square)
HIV-cohort, n= 56		
IFA	5/56 (9)	p <0.001
PCR	11/56 (20)	
Non-HIV cohort, n = 246		
IFA	8/246 (3.3)	p <0.001
PCR	32/246 (13)	

A definitive diagnosis of PJP was considered in 50 (16.6%) patients, including 4 (1.3%) cases with negative PJ-PCR. Five cases (9.8%) with positive PJ-PCR were considered as colonization.

Conclusion

P. jirovecii PCR improves the diagnostic sensitivity and NPV of PJP diagnosis respecting to IFA, regardless of respiratory sample type. PCR methods increase 4-fold detection in non-HIV patients. Our results suggest that Clinical Microbiology laboratories should use PCR techniques to diagnose PJP better than IFA.