Quantitative Polymerase Chain Reaction in Diagnosis of Pneumocystis Pneumonia

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Abstract

Introduction: Pneumocystis pneumonia (PCP) remains a serious cause of sickness and death in immunocompromised patients. Lung injury and respiratory impairment during pneumocystis pneumonia are mediated by marked inflammatory responses in the host to the organism. Quantitative PCR (qPCR) is more sensitive than microscopy for detecting Pj in BAL. The relevant threshold remains to be determined and may vary according to the underlying disease.

Methods: All BAL samples were obtained from immunocompromised and non-immunocompromised patients presenting with respiratory symptoms referred to the Department of infectious diseases of Alzahra Hospital, Isfahan, Iran. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) version 22.

Results: 2 patients (12%) of immunocompromised patients were positive for Pj. Among non-immunocompromised patients, 3 (8%) were positive for Pj. Fisher exact test demonstrated that Pj positivity was not significantly different between the two groups. The overall frequency of Pj positivity was estimated as 10%.

Conclusion: 12% of immunocompromised patients and 8% of non-immunocompromised were colonized by Pj which may progress to PJP or contaminate susceptible individuals.

Key words: Pneumocystis pneumonia (PCP), Quantitative Polymerase Chain Reaction, immunocompromised patients

Please cite this article as: Pozveh M. et al. Quantitative Polymerase Chain Reaction in Diagnosis of Pneumocystis Pneumonia. World Family Medicine. 2018; 16(1):88-91
DOI: 10.5742/MEWFM.2018.93203

Introduction

Pneumocystis pneumonia (PCP) remains a serious cause of sickness and death in immunocompromised patients (1). Respiratory impairment and lung injury during pneumocystis pneumonia are mediated by marked inflammatory responses in the host to the organism (2,3). The incidence of PCP in human immunodeficiency virus (HIV)-infected patients has decreased since the introduction of chemoprophylaxis and antiretroviral therapy (4-6). In fact, various opportunistic infections consisting of PCP, fungi and viruses may involve immunocompromised and transplanted patients (7-10). Meanwhile, the incidence of PCP in non-HIV immunocompromised patients is increasing (2-6). The importance of PCP diagnosis in non-HIV immunocompromised patients includes: 1- its non-specific symptoms. 2- It has been reported in almost all of immunocompromised and drug-dependent immunodeficiency patients, 3- high risk of fulminant respiratory failure and high fatality rate among these patients and 4- PCP is a preventable disorder. Therefore, rapid diagnosis would be valuable in clinical practice.

Studies highlighted the low burden of Pneumocystis jirovecii (Pj) in non-HIV immunocompromised patients and the lack of sensitivity of microscopic methods.(11) The use of the polymerase chain reaction (PCR) to detect pneumocystis nucleic acids has been an active area of research. PCR has been shown to have great sensitivity and specificity for the diagnosis of pneumocystis pneumonia from specimens of induced sputum and bronchoalveolar-lavage fluid (BAL). (12) Quantitative PCR (qPCR) is more sensitive than microscopy for detecting Pj in BAL. The relevant threshold remains to be determined and may vary according to the underlying disease (13).

The detection of Pj in individuals presenting without pneumonia or with pneumonia from another etiology has been defined as colonization or "carriage" (14). Conventional Pj PCR is qualitative and very sensitive, but does not differentiate between active PCP and Pj colonization. The aim of this study was to use Pj qPCR in order to detect and evaluate Pj colonization in immunocompromised and non-immunocompromised patients.

Methods and materials

Patients and clinical samples

All BAL samples obtained from immunocompromised and non-immunocompromised patients presenting with respiratory symptoms referred to Department of infectious diseases of Alzahra Hospital, Isfahan, Iran. The data were collected between January 2016 to January 2017.

Staining methods

The bronchoalveolar lavage (BAL) and samples were stained and examined by a qualified microscopist at the Laboratory of Cyto-pathology, with 400 µl of fluid analyzed by Fast Giemsa and 400 µl of Papanicolaou staining.

Real-time qPCR

A DNA extract solution from each sample was tested with a Pj qPCR targeting the Serine Endopeptidase KEXI gene of Pj as previously described.(15) From the portion of BAL fluid wash 2 remaining after microscopy and microbiological cultures, 1.5 ml were centrifuged at 10,000 × g for ten min. Supernatant was aspirated, and the cell pellet was stored in 200 µl of the washing solution at −40°C until further processing. After thawing, DNA was extracted using a QIAamp DNA minikit (Ribopreb) according to the manufacturer's instructions, except that total DNA was eluted from the spin columns with 50 µl of elution buffer in order to increase the DNA concentration. 5 µl of this DNA extract was used for qPCRs.

All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) version 22.

Results

53 patients enrolled in this study (16 immunocompromised and 37 non-immunocompromised). Chi-square test did not reveal any statistical difference in the sex of two groups. (p=0.41) Independent t-test showed that the mean age of immunocompromised patients was significantly lower.

2 patients (12%) of immunocompromised patients were positive for Pj. Among non-immunocompromised patients, 3 (8%) were positive for Pj. Fisher exact test demonstrated that Pj positivity was not significantly different between two groups. The overall frequency of Pj positivity was estimated as 10%. Detailed data of patients is demonstrated in Table 1.

Discussion

The most important diagnostic tool for pneumocystis infection is a high clinical suspicion. In the right clinical setting, an immunosuppressed patient with new onset of dyspnea or new symptoms of pneumonia, with or without radiological findings, should prompt further assessment, particularly if they are not receiving chemoprophylaxis. Since Pneumocystis cannot be cultured, the gold standard for detection is microscopic visualization of the organism (16).

The diagnostic performance of the microscopic visualization of Pj depends on the quality and type of sample, the number of organisms, and the experience of the microscopist. (17)The higher sensitivity of PCR for Pj detection has been demonstrated previously.(11) Pneumocystis colonization that is, detection of the organism or its DNA, without symptoms or signs of pneumonia, has recently been described, and accumulating evidence suggests that it may be an important clinical phenomenon. Sensitive molecular techniques such as PCR are frequently used to identify Pneumocystis colonization. (18)

In this study, we observed that qPCR results in the immunocompromised group did not have significant differences with those in the non-immunocompromised

Table 1: patients' characteristics in immunocompromised (A) and non-immunocompromised group (B).

A

Chemotherapy/radiotherapy	Immunosuppressive	Bone marrow transplantation	Corticosteroids
6	5	1	4

B

Chronic lung disease	Cardiovascular disease	Tuberculosis	Interstitial lung disease	No underlying disease
9	4	1	1	22

group. qPCR assay has the ability to detect P. carinii in lower respiratory tract specimens or oral washes. qPCR with application of cutoff values could improve the specificity of previously described qualitative PCR, by distinguishing between infection and colonization. It can help discriminate PCP from colonization and is in concordance with the hypothesis that the burden of Pj is lower in colonized people than in patients with PCP. (19)

A study conducted in 2004 showed that qPCR is useful for diagnosing non-HIV-infected immunocompromised patients, who often present with PCP with a low burden of Pj organisms and may not be diagnosed using microscopic examination.(1) PCR technique has advantages of being sensitive and noninvasive. Although PCR can detect colonization and still produce a false positive result, doctors should take account of clinical factors and be able to make the correct diagnosis. Early diagnosis and treatment are important for the survival of PJP patients without HIV infection, suggesting that a rapid and accurate PJP diagnosis method such as qPCR should be used in these patients with a low burden of Pj. (20)

A meta-analysis on 10 studies showed that PCR has good diagnosis accuracy and may be a useful tool for the diagnosis of PJP in immunocompromised patients. (21). Other studies highlighted the usefulness of qPCR in discriminating colonization from PCP (14, 15). It is known that colonization can sometimes lead to the development of PCP. (14)

We need to consider limitations when interpreting our results: this is a single-center study, and the number of patients is relatively small. Without a test capable of confirming or excluding the diagnosis of PJP, the classification of patients into definite or non-definite PCP is uncertain. However, one positive point of our study is that patients were classified by a multidisciplinary group of experts, ruling by consensus in view of all the clinical and complementary data. The fact that the experts did not know the qPCR results avoids classification bias. Our study had a small number of patients and the observed differences between the groups was not statistically significant. We can note that none of the enrolled patients in our study did not receive anti-pneumocystis prophylaxis. There is no current recommendation for anti-PCP prophylaxis or empirical therapy for non-HIV immunocompromised patients (21-23).

Conclusion

In this study we observe that 12% of immunocompromised patients and 8% of non-immunocompromised were colonized by Pj which may progress to PJP or contaminate susceptible individuals. More studies with larger sample size are needed to differentiate Pj colonization from PJP and to assess cut off for Pj gene expression.

Limitations of the study

Our study was conducted on a limited proportion of patients. We suggest further investigation on this aspect of infection.

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