

Evaluation of direct immunofluorescent method in the detection of *Pneumocystis jiroveci* among patients with lower respiratory tract infections

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ABSTRACT

Objective: Evaluate the use of direct immunofluorescent method in the detection of *Pneumocystis jiroveci* from induced sputum of patients with LRT infections.

Patients and methods: Thirty patients with LRT infections were included (24 patients were immunocompromised and 6 immunocompetent). The present study conducted for 6 months from January-June, 2011. Smears were prepared from their induced sputa and stained with direct fluorescent antibody stain, then examined under fluorescent microscope.

Results: Sputum of 5(16.7%) patients revealed positive results for the presence of *P. jiroveci* and all of them were immunocompromised (two acute myeloid leukemia, one acute lymphoid leukemia, one non-Hodgkin's lymphoma, and one asthma under long course of corticosteroid therapy).

Conclusion: Direct fluorescent antibody technique is more sensitive than the conventional methods for the detection of *P. jiroveci* in LRT infections.

Keywords: *Pneumocystis pneumoniae*, *Pneumocystis jiroveci*, immunocompromised patients.

ملخص البحث

هدف الدراسة: تقييم استخدام طريقة التالف الضوئي المناعي المباشر في تشخيص الفطر *Pneumocystis jiroveci* من المرضى المصابين بجمع الجهاز التنفسي السفلي.

المرضى وطرائق العمل: تضمنت هذه الدراسة 30 مريضاً يعانون من جمع الجهاز التنفسي السفلي، 24 مريضاً من ذوي المناعة المنقوصة و 6 مرضى من ذوي المناعة الكفوءة. اجريت الدراسة خلال 6 اشهر للفترة من كانون الثاني- حزيران-2011. حضرت المسحات من القشع المستخرج من المرضى ثم صبغت بالصبغة المباشرة للتالف المناعي الضوئي وفحصت بمجهر التالف الضوئي.

النتائج: تم تمييز *P. jiroveci* من قشع 5 (16.7%) مرضى من ذوي المناعة المنقوصة (اثنان مصابين بمرض الدم النخاعي ، واحد مرض الدم للمفاوي الحاد، واحد ورم غدد هوجكن غير اللمفاوية، وواحد مصاب بالربو وتحت علاج طويل بالكورتزون).

الاستنتاج: ان طريقة التالف الضوئي المناعي المباشر حساسة اكثر من الطرق التقليدية لاستكشاف فطر *P. jiroveci* في جمع الجهاز التنفسي السفلي.

Pneumocystis jiroveci (formly carinii), remains an important cause of pneumonia¹. Immunocompromised patients with HIV infection, hematologic malignancies, prolonged corticosteroid therapy, or intensive immunosuppressive therapy are under risk of infection with *P. jiroveci*^{2,3}.

Microscopic identification of the organism be achieved from clinical samples of sputum, bronchoalveolar large (BAL), or lung biopsy⁴. The conventional methods for the detection of *P. jiroveci* is generally established by morphologically demonstrating pneumocystis organism by different methods. These methods were Giemsa or Gram-Weigert stain for trophozoites and Gomori methenamine silver stain, toluidine blue or calcofluor white for cysts^{5,6}. A more sensitive and rapid diagnostic method is the immunofluorescent stains (direct and indirect fluorescent antibody). The direct fluorescent antibody (DFA) has a higher sensitivity and specificity for the detection of pneumocystis⁷, while the indirect antibody technique gives a wider range of detection⁸. Furthermore, PCR-based amplification assays has an increased sensitivity for the detection of *P. jiroveci* compared to other tests⁹.

The main aim of the present study is to evaluate the use of DFA technique in the detection of *P. jiroveci* from induced sputum of the patients with lower respiratory tract (LRT) infections.

Patients and Methods

Thirty patients with LRT infections were included. Twenty four patients were presumptively immunocompromised (with underlying diseases) and 6 were immunocompetent (without underlying diseases). The present study conducted for 6 months from January-June, 2011. The studied patients were hospitalized in Ibn-Sina

Teaching Hospital, and Oncology and Nuclear Medicine Hospital, Mosul Iraq. The immunocompromised patients had different underlying diseases including various types of leukemia¹¹, non Hodgkin's lymphoma⁶, multiple myeloma², bicyclic neutropenia², bronchogenic carcinoma¹, and asthmatic patients with prolonged corticosteroid therapy². Patients with malignancies were under chemo and / or radiation therapy. The duration of the underlying causes of immunosuppression in these patients ranged from 6 months-16 years. However, the duration of LRT infection at the time of sampling of the patients were ranged from 5 days to 3 months. Most patients have low grade fever, dry cough with minimal frothy sputum and shortness of breath.

Sampling and preparation of the specimens

The induced sputa were collected at the first morning in steril containers after induction by hypertonic saline (3%) and sent to the laboratory within 1 hour. The samples were shaken by vortex for 1-2 minutes for homogenization. Smears were prepared and fixed in acetone for 10 minutes. The DFA staining was performed, using a commercial kit for the detection of *P. jiroveci* purchased from Meri-Fluor- pneumocystis catalog No. 222030, Meridian, Bioscience, Inc, Ohio USA. Fluorescein isothiocyanate (FITC) labeled monoclonal antibody (McA) was applied for the detection of *P. jiroveci* (cysts, sporozoites, and trophozoites), and the staining procedure was conducted according to the manufacturer's instructions.

Results

The age of the 30 studied patients ranged from 18-71 (mean \pm SD = 42.03 \pm 17.1) years. The males were 11 and females were 19.

Sputum of 5 (16.7%) out of 30 patients with LRT infections showed positive results for *P. jiroveci*, and all of them were immunocompromised. Under high power magnification of the fluorescent microscope, either the trophozoites, cysts or both were detected in the positive samples (Fig. I).

The clinical data of the 5 patients with positive results for *P. jiroveci* are summarized in Table I. The patients were 3 females, and 2 males, and their ages ranged between 35-71 years. Two of the patients were with acute myeloid leukemia, one with acute lymphoid leukemia, one with non-Hodgkin's lymphoma, and one with asthma under long coarse of corticosteroid therapy.

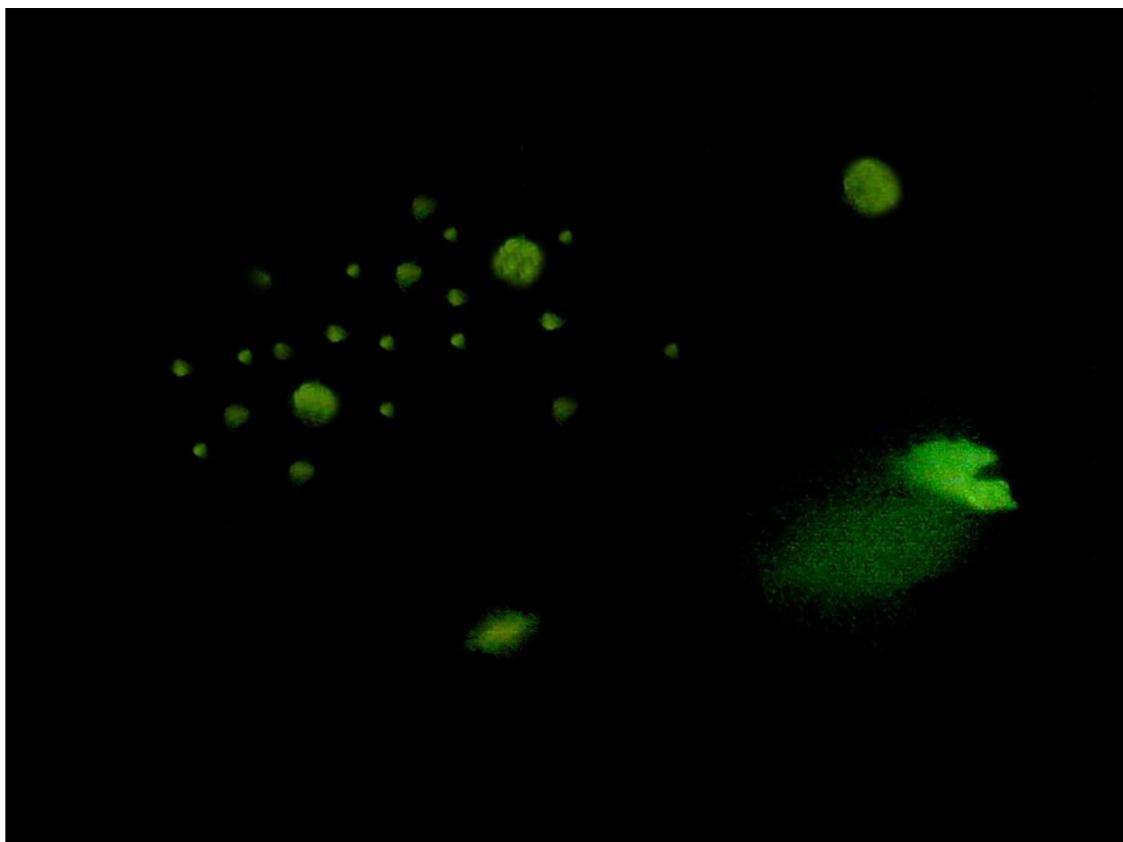


Figure 1. *Pneumocystis jiroveci* (cysts, trophozoites) in induced sputum stained with direct fluorescent antibody stain, under fluorescent microscope (40X).

Table 1. Clinical data of patients with positive results for *P. jiroveci*

| Items | No. |
|------------------------------------|------------------|
| 1. No. of cases | 5 |
| 2. Gender | |
| males | 2 |
| females | 3 |
| 3. Age | 35-71 |
| 4. Immunosuppressive diseases | |
| • acute myloid leukemia. | 2 |
| • acute lymphoid leukemia. | 1 |
| • non-Hodgkin's lymphoma | 1 |
| • asthma + corticosteroid | 1 |
| 5. Immunosuppressive therapy | |
| • chemotherapy | 4 |
| • corticosteroid | 1 |
| 6. Duration of the disease | 6 month-16 Years |
| 7. Symptom and signs | |
| • fever | 4 |
| • dry cough | 4 |
| 8. Croase appearance of the sputum | |
| • forthy white | 4 |
| • thick white | 1 |

Discussion

Pneumocystis jiroveci is emerging as an important pathogen in immunocompromised patients¹⁰. The disease probably occurs either by new acquisition of infection or reactivation of latent infection. Moreover, case clustering among immuosuppressed patients suggest that pneumocystis spread is usually by air borne route¹¹. Five immunocompromised individuals out of 30 patients studied revealed the presence of pneumocystis in their seputa when examined by the DFA technique. Fluorescent antibody staining technique is now frequently used for this purpose with high efficiency¹². The 5 patients positive for *P. jiroveci* were presumptively immunocompromised for reasons other than HIV (Table I). These results were in keeping with the finding of

Saksasithon and co workers (2003) who reported that the patients who were immunocompromised for reasons such as haemtological malignancies or immunosuppressive therapy for autoimmune disease are also at risk for pneumocystis pneumonia (PCP). Furthermore, the predominant presenting symptoms of the *P. jiroveci* positive patients were dry cough (4/5) and fever (4/5). Other investigators reported that fever was the main presenting symptom in PCP¹².

The first report from Mosul –Iraq in 2009 about the identification of *p. carinii* in LRT infections was based on direct identification using Giemsa, toludin blue and calcofluor staining¹³. The present study (from the same geographical area) applied IF staining using monoclonal antibody for the identification of *P. jiroveci*. There was a higher frequency of *P. jiroveci*

identification between the first and the current report (8.6% Vs 16.7% respectively). Flood and coworkers (1991) mentioned that DFA was more sensitive than the conventional method (48% Vs 39%) in the detection of *P. jiroveci*. Other workers reported that the frequency of *P. jiroveci* detection in clinical respiratory specimens by Merifluor pneumocystic stain was 90.8%¹⁵. The DFA has also the advantage of being less time consuming, although more expensive than the conventional methods.

In conclusion, the method of DFA technique has a superior diagnostic utility than the conventional methods for the detection of *P. jiroveci* among immunocompromised patients with LRT infections.

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