

# Diagnosi di Pneumocystis Jirovecii in una persona HIV positiva tramite tecniche tradizionale e metodi di biologia molecolare.

## Diagnosis of Pneumocystis Jirovecii in an HIV positive individual using traditional techniques and molecular biology methods.

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### Riassunto

Pneumocystis jirovecii (PJ) è un microrganismo ubiquitario (micete) trasmesso per via aerea.

Persone immunocompromesse (individui HIV-positivi con conta linfocitaria CD4+ T <200 cell/micoL, disordini linfoproliferativi, trapianto di cellule staminali, malattia di Cushing) sono a rischio di sviluppare polmonite da PJ. Gli autori presentano un caso di polmonite da PJ, la cui diagnosi è stata confermata sia da lavaggio broncoalveolare (BAL) sia dal plasma del paziente con identificazione microscopica.

Il nostro paziente era un uomo caucasico di 35 anni, ricoverato nel reparto di malattie infettive dell'ospedale Sa Luca di Lucca, dal momento che presentava sintomi caratterizzati da febbre, dispnea, e immagine radiologica (tomografia computerizzata torace) compatibile con polmonite interstiziale bilaterale. L'uomo è stato sottoposto a BAL e prelievo di sangue periferico. I campioni sono stati analizzati con tecniche di biologia molecolare per il rilevamento e la quantificazione del DNA di PJ. Il campione BAL è anche stato analizzato per la ricerca microscopica di PJ. I campioni analizzati sono risultati positivi per PJ, sia in RT-PCR che in microscopia ottica.

### Abstract

*Pneumocystis jirovecii (PJ) is a ubiquitous microorganism (mycete) transmitted by air. Immunocompromised individuals (HIV-positive individuals and CD4+ T-cell count < 200/microL, lymphoproliferative disorders, stem cell transplantation, prolonged corticosteroid therapy, Cushing's disease) are at risk of developing PJ pneumonia. The authors present a case of PJ pneumonia, whose diagnosis was confirmed by molecular techniques performed both on bronchoalveolar lavage (BAL) and on the patient's plasma associated with microscopic identification. Our patient was a 35-year-old Caucasian man hospitalized in the infectious diseases department of the San Luca hospital in Lucca, as he presented symptoms characterized by fever, dyspnoea, and radiological picture (chest CT) compatible with bilateral interstitial pneumonia. The patient underwent BAL and peripheral blood sampling. The patient's samples were analysed with molecular biology techniques for the detection and quantification of PJ's DNA. The BAL sample was also analysed to microscopically search for PJ. The analysed samples resulted positive for PJ both in RT - PCR and in optical microscopy.*

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### Introduction

Pneumocystis jirovecii (PJ) is a ubiquitous microorganism (mycete) that does not cause any disease in immunocompetent patients.

Immunocompromised individuals (HIV-positive individuals and CD4+ T-cell count < 200/microL, lymphoproliferative disorders, stem cell transplantation, prolonged corticosteroid therapy, Cushing's

disease) may develop PJ pneumonia (1,2). The typical initial symptoms are fever, non-productive cough, and dyspnoea (difficulty breathing); tachycardia, tachypnoea and sometimes cyanosis are frequently found; sometimes (<3%), extrapulmonary lesions appear involving the lymph nodes, spleen, liver, and bone marrow (3).

Because PJ is extremely difficult to culture in vitro, traditional diagnosis is based on clinical symptoms, radiographic findings, and confirmation by visualization of the organism by staining of lung specimens (bronchoalveolar lavage, lung aspirate, or induced sputum).

The visualization of the microorganism after staining shows a poor sensitivity for the detection of PJ (4) and it is for this reason that the use of molecular biology techniques by PCR can prove particularly useful for the diagnosis of PJ in blood (serum/plasma) or pulmonary samples (5). The report presents the diagnostic sequence of a case of PJ pneumonia, with a diagnosis confirmed both by the classical microscopic identification technique and by molecular biology investigation of blood samples and BAL, highlighting the scope of diagnostic anticipation on the patient.

### Clinical case

A 35-year-old Caucasian man hospitalized in the infectious diseases department of the San Luca hospital in Lucca, with symptoms characterized by fever, dyspnoea, and radiological picture (chest CT) compatible with bilateral interstitial pneumonia.

It was necessary to perform a differential diagnosis to exclude SARS-CoV-2 infection at a time characterized by a critical epidemic situation and a high circulation of the virus which has profoundly changed the epidemiology of pulmonary infectious diseases.

The diagnostic criteria of COVID-19 pneumonia required a chest CT scan and a genomic test, RT-PCR. A nasopharyngeal swab was performed for SARS-CoV-2 virus with negative result.

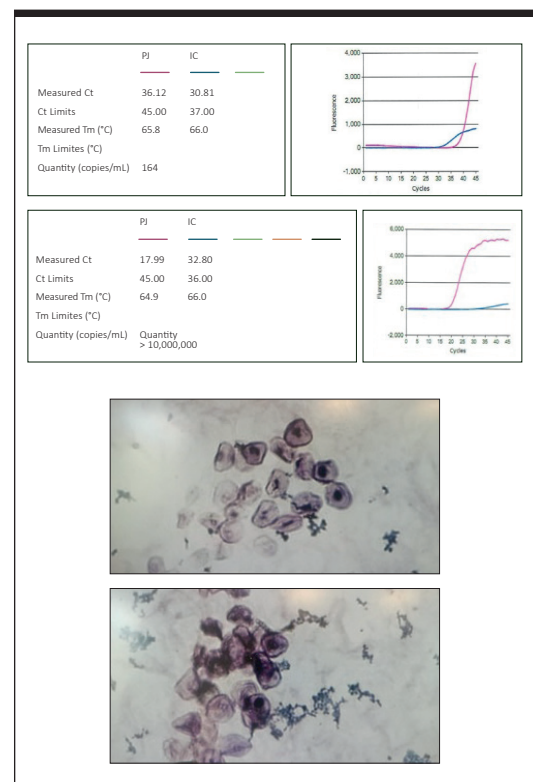
The patient referred to risky behaviours and had conducted the HIV test a few days earlier, with positive results.

With the exclusion of the SARS-CoV-2 virus as the causative agent of bilateral interstitial pneumonia, the patient's symptoms supported the diagnostic hypothesis of PJ pneumonia. The first haematological findings showed an impairment of the immune

system (CD4: 26 cells/uL, 6%) and an HIV RNA equal to 557,000 copies/mL. The overall clinical picture led to the diagnostic hypothesis of PJ pneumonia and the patient was treated with co-trimoxazole (administered intravenously 80 mg/kg) and cortisone therapy (Prednisone).

The patient was then subjected to alveolar bronchial lavage and a blood sample to confirm the diagnostic hypothesis.

Both samples were analysed by a quantitative PCR nucleic acid amplification assay for the detection and quantification of PJ DNA on ELITe InGenius® instrumentation with dedicated kit (Pneumocystis ELITE MGB® Kit). Both BAL and plasma were positive for PJ (10,000,000 copies/mL on BAL and 164 copies/mL on plasma) (**Figure 1**); positivity for cytomegalovirus on BAL is also highlighted (3469 IU/mL with reference range 178-10,000,000); hepatitis B virus (with viraemia of 89,800 copies) and delta virus co-infection (anti-delta antibodies positive, HDV-



**Figure 1.** RT-PCR Amplification Curves of BAL and Patient Plasma in the Presence of Pneumocystis Jirovecii DNA and Microscope image after Grocott-Gomori (GMS) staining of Pneumocystis Jirovecii.

RNA not detected). The BAL sample, subjected to microscopic examination of the PJ confirmed the initial diagnostic hypothesis of PJ pneumonia. About two weeks after the start of the antiviral therapy, a clear clinical improvement was observed with normalization of the pulmonary radiological picture. The patient was discharged after three weeks with oral therapy: co-trimoxazole two tablets 160mg/800 mg 3 times/days and valganciclovir 900 mg two tablets 2 times/days, Methylprednisolone 40 mg for 7 days as maintenance therapy and antiviral treatment for HIV and hepatitis B with three oral antiviral medicines (Bictegravir, Tenofovir, Emtricitabina). Prior to discharge from the hospital, the patient signed a specific informed consensus to use of the results obtained from the study of his clinical case.

## Discussion

The anticipation of an appropriate pharmacological therapy is essential in HIV-positive individuals, since the initiation of antibiotic treatment 24 hours before appears to be associated with a significant reduction in mortality (6). A rapid confirmation of the diagnostic question is therefore crucial for the choice of the therapeutic approach. The "traditional" analytical methods such as the microscopic visualization of PJ on BAL or material of pulmonary origin present some significant criticalities, which lead to a lengthening of the execution times of the investigations and make it objectively complex.

The biological materials used for the optical microscopy investigation such as BAL, induced sputum or pulmonary aspiration are collected with invasive procedures that are poorly tolerated by patients and require adequate programming, thus lengthening the times of analysis and of response. Since there are no in vitro culture systems suitable for the growth of this microorganism, the optical microscope reading with specific Grocott-Gomori staining (GMS) of the lung samples constitutes, therefore, the gold standard for the diagnosis of PJ pneumonia. With GMS the cell wall of PJ is outlined by a brown to black spot that forms from the reduction of silver ions. GMS staining has a positive and negative predictive value greater than 90% when performed on BAL, while the sensitivity of the method varies from 31 to 97% depending on the quality of the lung sample used. Although

Grocott-Gomori staining is specific for the presence of *Pneumocystis jirovecii*, if negative, it does not exclude the presence of the microorganism (7). Even if the microscopic investigation appears to be a relatively simple method, it is closely related to the ability and experience of the operator in charge of the investigation. The difficulties encountered in diagnosing PJ pneumonia in a short time has favoured the use of alternative, equally accurate, and more easily implemented diagnostic strategies, in emergency laboratories. The use of molecular biology techniques such as real-time PCR allows the detection of PJ DNA by amplification of specific genetic targets thus highlighting the presence of PJ even in the absence of findings in optical microscopy after staining (8). RT-PCR is a method with high sensitivity and specificity (98-99%) for the search for PJ in both HIV positive and HIV negative patients. Because sensitivity is high, a false negative test result is rare, therefore, a negative PCR on BAL means that the presence of PJ is unlikely and therefore other diagnostic hypotheses should be considered to understand the aetiology of the patient's symptoms. Conversely, the high specificity means that a positive BAL PCR is highly indicative of the presence of *Pneumocystis jirovecii* (9-11). In the described clinical case of PJ's pneumonia, the search for PJ's DNA also proved useful on a sample of the patient's blood, an easily and quickly accessible biological material, which can allow a considerable diagnostic anticipation.

The search for PJ using RT-PCR both on BAL and on the patient's plasma demonstrated how the massive fungal infection in the lungs, evidenced by the high quantity of DNA of respiratory origin detected in the samples, originated a passage of the genetic material in the bloodstream (haematogenous spread). It is known that PJ reaches and replicates in the pulmonary alveoli as an extracellular parasite and that the involvement of the pulmonary interstitium leads to a reduction in gaseous exchanges with a consequent reduction in the availability of oxygen for the body (12). In the clinical case analysed, the search for the microorganism with RT-PCR in a blood sample, an easily accessible material, made it possible to anticipate and confirm the diagnostic question with respect to the processing of more complex samples of pulmonary origin to find, with a lengthening of response times of even 5 days.

## Conclusions

The rapid confirmation of the diagnostic hypothesis, that allows the start of a targeted therapy, is a crucial point for the reduction of the hospitalization and patient mortality rate. The clinical case presented confirms the usefulness of using RT-PCR in the diagnosis and monitoring of patients with PJ pneumonia. The answers obtained from laboratory investigations on the various biological matrices analysed proved to be consistent and making possible both to anticipate the patient's diagnostic classification and to monitor the course of the disease. The clinical course of a complex case confirms the assumption of the importance of a rapid definition of the diagnostic question, even in the case of subjects affected by PJ pneumonia. The high sensitivity of RT-PCR allows the detection of PJ significantly earlier (several weeks earlier) than traditional staining methods. While postponing the definitive diagnostic confirmation with the result of the investigation of material of pulmonary origin in optical microscopy, the RT-

PCR investigation of the PJ in plasma and on lung material proved to be decisive in directing drug therapy with significant benefits for the assisted subject. The analytical method currently considered as a diagnostic "gold standard" (reading under an optical microscope after staining of material of lung origin) is not optimal, both in terms of timing and sensitivity and should probably be re-evaluated in the light of the use of the molecular methods. The exclusive use of traditional method use could, therefore, induce a significant diagnostic delay with consequent deferral of the treatment of the active infection. The need for differential diagnosis between fungal infections and other respiratory infections has been answered by the use of molecular biology techniques. RT-PCR makes it possible to analyse, simultaneously and in a short time, different biological matrices with significantly faster and easier to implement procedure in hospital laboratories, helping to correctly direct the most suitable clinical and therapeutic pathway. ■

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