

## CASE REPORT

# Unusual coexistence of opportunistic lung infections in a human immunodeficiency virus positive patient suffering from persistent *Pneumocystis jirovecii* pneumonia: A case report<sup>☆</sup>

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Received 30 May 2012; accepted 14 January 2013

### KEYWORDS

Human immunodeficiency virus (HIV);  
*Pneumocystis jirovecii* Pneumonia (PcP);  
Opportunistic infections;  
Lungs

### PALAVRAS-CHAVE

Vírus da imunodeficiência humana (VIH);  
Pneumonia por *Pneumocystis jirovecii* (PPc);  
Infeções oportunistas;  
Pulmões

**Abstract** It is well-established that HIV patients are at high risk of opportunistic infections (OI), like the ones caused by *Pneumocystis jirovecii*, a worldwide pathogen implicated in interstitial pneumonia (PcP). We present a case of a newly diagnosed HIV-1 patient with multiple OI, including a persistent form of PcP, an invasive aspergillosis (IA), cytomegalovirus and *Mycobacterium xenopi* lung infection. We describe the combination of laboratorial screening, surgery and antimicrobial therapy which were crucial for patient recovery.

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**Coexistência de infeções oportunistas pulmonares num doente com infeção por vírus da imunodeficiência humana e uma forma persistente de pneumonia por *Pneumocystis jirovecii*: caso clínico**

**Resumo** Como é sabido, nos doentes com infeção por vírus da imunodeficiência humana (VIH) existe um alto risco de ocorrência de infeções oportunistas (IO), tais como as infeções por *Pneumocystis jirovecii*, um agente patogénico com distribuição mundial, que provoca pneumonia intersticial (PPc). Apresentamos um caso de um doente recém-diagnosticado com infeção por VIH-1 e múltiplas IO pulmonares, incluindo uma forma persistente de PPc, aspergilose invasiva (AI), e infeções por citomegalovírus e por *Mycobacterium xenopi*. Descrevemos

<sup>☆</sup> Please cite this article as: Ponces Bento D, et al. Coexistência de infeções oportunistas pulmonares num doente com infeção por VIH e uma forma persistente de pneumonia por *Pneumocystis jirovecii*: caso clínico. Rev Port Pneumol. 2013. <http://dx.doi.org/10.1016/j.rppneu.2013.01.005>.

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a combinação de fatores cruciais para a recuperação do doente, que incluíram a obtenção de dados laboratoriais, intervenção cirúrgica e múltipla terapêutica antimicrobiana.

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## Clinical case

A 59-year-old previously healthy man presented with a 59-month history of progressive weakness, dysphagia, loss of appetite, weight loss and occasional cough with purulent sputum. In November 2007 he was admitted with acute dyspnea. He was febrile (38.0°C), exhibited an oropharyngeal thrush, and had inspiratory crackles in both hemithoraxes. Blood analysis displayed a partial respiratory failure (pO<sub>2</sub> 66 mmHg, pCO<sub>2</sub> 34 mmHg); the chest radiography revealed a marked diffuse bilateral interstitial nodular infiltrate. Search for HIV antibodies (ELISA) was positive. A clinical diagnosis of PcP, oral and esophageal candidiasis were assumed. Cotrimoxazole (1960 mg q.i.d.), prednisolone (40 mg b.i.d. i.v.) and fluconazole (200 mg q.d. i.v.) were started.

Upper gastrointestinal endoscopy confirmed esophageal candidiasis. Fluconazole was stopped after two weeks of treatment, and cotrimoxazole dose was reduced to 960 mg q.d., after 21 days. Prednisolone was tapered to 10 mg q.d. The remaining analysis presented a peripheral blood TCD4<sup>+</sup> of 21 cell/μl (5%) and an HIV-1 viral load of 307,285 copies/ml.

Partial clinical improvement was observed with the above therapy, and the patient remained febrile. At the fourth week of hospitalization, a computed tomography (CT) of the thorax revealed a bilateral alveolar condensation and effusion, and a cavity with a *halo* sign in the left lung (Fig. 1). A bronchoalveolar lavage (BAL 1) was collected during a bronchoscopy. A high burden of *P. jirovecii* cysts (4–30 cysts in one field at ×1000 magnification) was found using indirect immunofluorescence with monoclonal antibodies (IF) (MonoFluo kit *P. jirovecii*; Bio-Rad, France). The presence of *P. jirovecii* organisms was confirmed by amplification of the mitochondrial large-subunit rRNA (*mtLSU rRNA*) gene using nested-PCR. Likewise, *Aspergillus fumigatus* was revealed in BAL culture. Therapy was modified to clindamycin (600 mg q.i.d.), primaquine (30 mg q.d.) and voriconazole (200 mg b.i.d. after a loading dose).

As the patient was febrile, we also searched for pp6 cytomegalovirus (CMV) antigenaemia that was positive (248 infected cells/50,000 leucocytes). Fundoscopy, cerebrospinal fluid analysis, upper and lower gastrointestinal endoscopy did not suggest CMV disease. However, DNA of CMV was found in PCR of BAL 1. Ganciclovir (325 mg b.i.d.) was started.

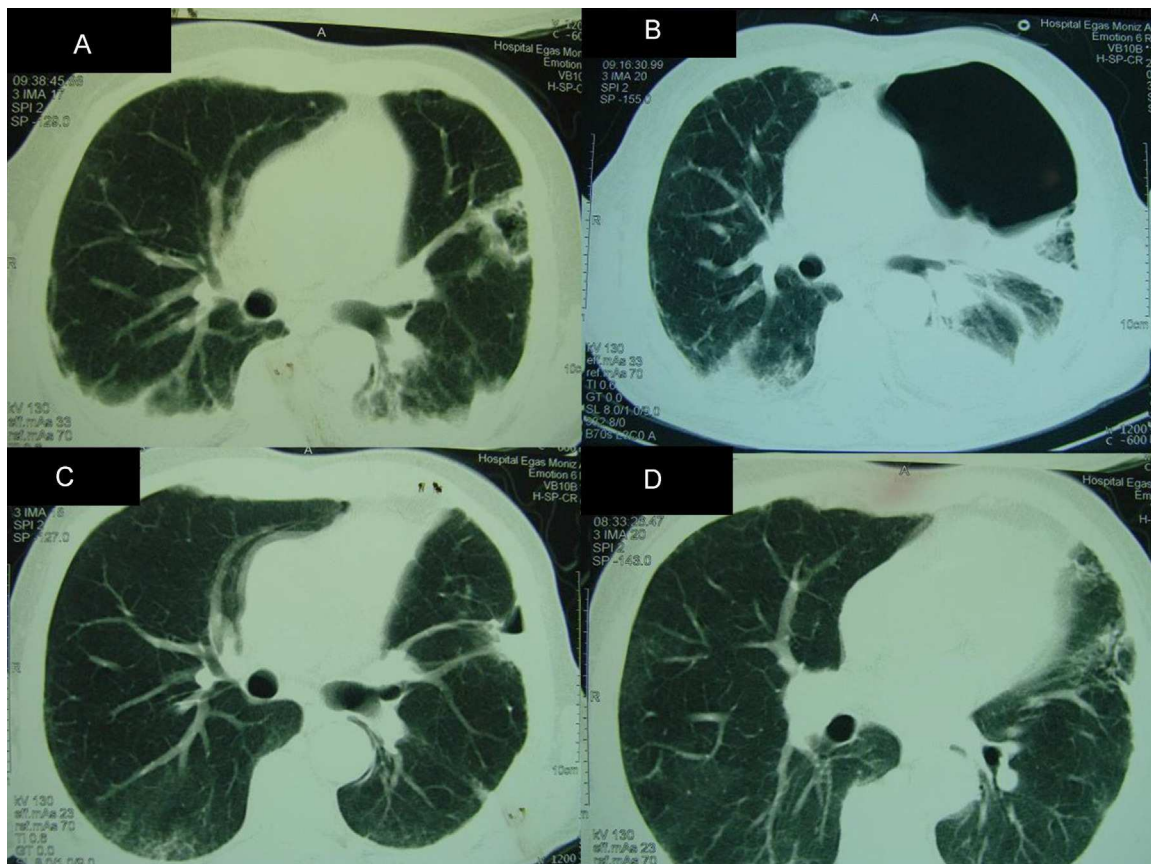
The patient finished the second anti-*Pneumocystis* treatment (26 days of clindamycin and primaquine) and ganciclovir (after 21 days). Prophylaxis with atovaquone (1500 mg q.d.), pyrimethamine (50 mg q.d.), and valganciclovir (900 mg q.d.) was begun, and voriconazole was maintained.

Eight weeks after admission, 57 days of anti-*Pneumocystis* treatment and 30 days of voriconazole, were provided. A new BAL fluid was collected (BAL 2), revealing the persistence of a high parasite burden of *P. jirovecii* (4–30 cysts in one field at ×1000 magnification) and *A. fumigatus*. CMV DNA was still positive in BAL 2. As the patient was afebrile and CMV antigenaemia became negative, we did not reinstitute anti-CMV therapy in full dose. However, the patient maintained significant cough and dyspnea. A new CT of the thorax still revealed alveolar condensation and the aspergilloma cavity with the previous dimensions. We opted to start a third course of PcP therapy (clindamycin and primaquine); and to add caspofungin (50 mg q.d. after a loading dose) to voriconazole. Meanwhile, the laboratory noticed the growth of *Mycobacterium xenopi* in BAL 1. Ethambutol (1200 mg q.d.), azithromycin (500 mg, q.d.) and levofloxacin (500 mg, q.d.) were started. Given the patient's profound immunosuppression, antiretroviral (ARV) therapy was also begun. After excluding HLA-B57 01, and to avoid interactions, we chose abacavir (ABC, 600 mg q.d.), lamivudine (3TC, 300 mg q.d.) and indinavir (IDV, 800 mg t.i.d.).

The two BALs collected during the episode were genetically characterized at five distinct *P. jirovecii* loci encoding the mitochondrial large-subunit rRNA (*mtLSU rRNA*), the cytochrome *b* (*CYB*), the superoxide dismutase (*SOD*), the dihydrofolate reductase (*DHFR*), and the dihydropteroate synthase (*DHPS*) using PCR with DNA sequencing analysis. Both samples revealed identical multiple genotypes combinations (Table 1).

One week later, the patient developed a left hydropneumothorax. He was submitted to thoracotomy, pleural decortication and aspergilloma removing (Fig. 1). In the removed tissue, *P. jirovecii* and *A. fumigatus* were identified. Two mutations were detected at codons 75 and 78 of the *DHPS* gene for the first time (GenBank: accession numbers GU479992, GU479993), in which a threonine was present instead of an isoleucine and a proline was present instead of a leucine, respectively. *P. jirovecii* genotyping results in the pleura tissue and in the aspergilloma sections are summarized in Table 1.

Since surgery, the patient has had a gradual and sustained improvement. He was discharged 16 weeks after admission. Overall, he completed 77 days of PcP therapy (21 days of cotrimoxazole and 56 days course of clindamycin and primaquine); 22 days of ganciclovir; 9 months of voriconazole (with 71 days of caspofungin); and 15 months of antibiologic treatment. ARV was later simplified, with indinavir substituted by efavirenz. Twenty months after discharge, the patient regained his lifestyle, had undetectable HIV-1 viral load and displayed 350 TCD4<sup>+</sup> lymphocytes/μl (11%).



**Figure 1** Radiologic findings during hospital admission. Computed tomographies (CT) of the thorax demonstrating: (A) (4th week after admission) bilateral pleural effusion and condensation, with an aspergilloma on left hemithorax; (B) (9th week after admission) hydropneumothorax; (C and D) (6th month after admission) improvement of pleural effusion and hydropneumothorax. Aspergilloma was removed during the surgery.

## Discussion

PcP remains a frequent AIDS-defining opportunistic infection (OI) in Europe and in the United States.<sup>1</sup> It is considered a complex pathophysiological phenomenon that may involve other fungal, bacterial or viral coinfections. However, few publications described coinfections involving *P. jirovecii*, *Aspergillus*, mycobacteria or CMV. Globally, coinfections in PcP are related to a worst outcome of PcP.<sup>2-5</sup>

Invasive Aspergillosis (IA) is a rare OI in HIV patients, being more frequently identified in individuals with cancer

and neutropenia. The subacute presentation of IA presented here is called Chronic Necrotizing Aspergillosis. It characteristically appears in patients moderately immunosuppressed (i.e. diabetes and/or low-dose corticosteroids), with underlying structural lung disease.<sup>6</sup>

Two of the more important factors in *Aspergillus* pathogenesis are based on the dysfunction of host lung epithelia (a physical and biochemical immune barrier to *Aspergillus*), and the presence of sialic acid residues in conidia surface, that will bind with high affinity to fibrinogen, to effectively attach to the epithelium.<sup>8</sup> In PcP, damaging of the alveolar

**Table 1** *Pneumocystis jirovecii* genotypes detected in the samples analyzed.

Sample ID	Locus				
	<i>mtLSU rRNA</i>	<i>CYB</i>	<i>SOD</i>	<i>DHFR</i>	<i>DHPS</i>
BAL 1	Genotype 2	CYB 1/2 <sup>a</sup>	SOD 1	DHFR Wt	DHPS Wt
BAL 2	Genotype 2	CYB 1/2 <sup>a</sup>	SOD 1	DHFR Wt	DHPS Wt
Aspergilloma	Genotype 3	CYB 1/2 <sup>a</sup>	SOD 1	DHFR Wt	DHPS 75
Adjacent Pleura	Genotype 1/3 <sup>a</sup>	CYB 1/2 <sup>a</sup>	SOD 1	DHFR Wt	DHPS 75/78 <sup>a</sup>

Genotypes nomenclature based on Esteves et al. 2012.<sup>12</sup>

Wt: wildtype.

<sup>a</sup> Isolates containing more than one nucleotide sequence type.

epithelium, accumulation of surfactant-like material, fibrinogen expression and fibrin deposition are characteristic.<sup>7</sup> The combination of HIV infection, corticosteroid therapy and lung epithelial damage produced by *P. jirovecii*, may have contributed to the development of IA. In fact, the association of these two diseases in HIV-patients was previously suggested in other case reports and in a small retrospective study.<sup>3</sup>

Risk factors for treatment or prophylaxis failure in PcP are TCD4<sup>+</sup> less than 50 cell/ $\mu$ l, prior PcP episode and use a cotrimoxazole-sparing regimen.<sup>9</sup> Several polymorphisms in *P. jirovecii* genetic loci are potentially linked with antimicrobial failure, specifically the previously recognized mutations at *DHPS* codons 55 and 57, associated with sulpha resistance. In our case, those mutations were not noticed; however, new point mutations at codons 75 and 78 were found. Persistence of *P. jirovecii* after cotrimoxazole therapy suggests, but does not prove, that the change in these two residues may have, somehow, affected *DHPS* enzymatic activity.<sup>10,11</sup> Further studies are required to investigate this relationship.

A substitution of a phenylalanine by a leucine at *CYB* codon 280 was also detected. *CYB* mutations are associated with atovaquone exposure; and it has been suggested that point mutations found in the Qo region (one of two coenzyme Q binding sites on cytochrome *b*) may be related with atovaquone prophylaxis failure. Since the amino acid 280 is not involved in Coenzyme Q or atovaquone binding, this mutation is probably due to genotype variation, and not related to failure of prophylaxis with atovaquone.<sup>12,13</sup>

Specific *P. jirovecii* genotypes may be associated with distinctive outcomes of PcP episodes. In previous studies, high parasite burden was more frequently detected in samples with *mtLSU rRNA* genotype 2/3, *SOD 1* and *DHFR* wildtype; and samples with *mtLSU rRNA* genotype 2/3 and *SOD 1* were more common in patients with the poorest outcome.<sup>14,15</sup> The genotypes found in this case may suggest a PcP with more virulent variants (*mtLSU rRNA* genotype 2/3, *SOD 1* and *DHFR* wildtype).

After nine weeks of anti-*Pneumocystis* therapy, we detected a high load of *P. jirovecii* cysts inside the aspergilloma. Local pH, temperature and surrounding fibrosis in aspergilloma cavity may have affected antibiotic penetration, possibly leading to the emergence of resistant mutations. Factors produced by *A. fumigatus* such as gliotoxin, a potent local immunosuppressor, and elastases, that break the surrounding tissues,<sup>8</sup> may have also contributed to the adherence and persistence of *P. jirovecii*. Although we observed a clinical response to voriconazole, surgery was more strikingly associated with clinical improvement, maybe by diminishing the burden of infection, and perhaps, by eradicating a biological sanctuary for *P. jirovecii* and *Aspergillus* multiplication and cooperation.

The clinical significance and pathogenicity of *M. xenopi* and CMV in the lungs are still controversial. The American Thoracic Society (ATS) states that *Mycobacterium xenopi* can act as a pathogen, especially in people with lung alterations. It presents more often as a pulmonary cavity, but in HIV patients, disseminated disease is also common. This agent is sometimes implied as a colonizer, or as a water-associated contaminator of medical devices and laboratory equipment, being responsible for "pseudo" outbreaks. ATS criteria for atypical mycobacterial imply exclusion of other causes,

pulmonary symptoms and a reliable microbiological finding, like the growth in BAL culture.<sup>16</sup> We believed that our patient had significant risk factors and fulfilled criteria for infection. Interestingly, a case of a *M. xenopi* and *Aspergillus* pulmonary infection, in a HIV-positive patient with a previous *P. jirovecii* infection was already previously reported.<sup>3</sup> The clinical significance of CMV detection in respiratory specimens remains questionable in HIV patients, compared with transplanted patients where its role is much more established.<sup>17</sup> Important data regarding this issue come from autopsy studies. In 75 subjects that died of HIV infection, half of them from respiratory failure, 44 patients (59%) had histologic evidence of CMV infection. In over half of these patients, the CMV was thought to have caused little or no pulmonary dysfunction, but in contrast, CMV was thought to have had some role in 21 of the 44 patients.<sup>18</sup> CMV is often identified in the lungs of patients with severe pneumonia, and in HIV setting, especially with PcP. In 111 patients with AIDS and a first episode of PCP, 48% had CMV demonstrated on BAL. There was no difference between CMV positive and negative patients in terms of initial presentation, long-term survival, hospital mortality, or length of hospital stay.<sup>19</sup>

While the role of these two microorganisms in this setting, and the best approach is still debatable, we opted to start anti-mycobacterial and anti-viral therapy, given his poor evolution and symptoms.

In conclusion, we present a case of a newly diagnosed HIV-1 patient suffering from a persistent PcP, IA and probable lung infections caused by *M. xenopi* and CMV. We detected genotype profiles associated with more virulent *P. jirovecii*, and two new point mutations in *P. jirovecii DHPS* gene were described for the first time. Genetic variation of *P. jirovecii* in the same host and in different parts of the lung tissue confirmed that this infection could be polyclonal. Despite the current availability of multiple antibacterial, antifungal and antiviral drugs, we wish to signal this combination of factors that can challenge the management of HIV-associated OI.

## Ethical disclosures

**Protection of human and animal subjects.** The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

**Confidentiality of data.** The authors declare that they have followed the protocols of their work center on the publication of patient data and that all the patients included in the study received sufficient information and gave their written informed consent to participate in the study.

**Right to privacy and informed consent.** The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

## Conflicts of interest

The authors have no conflicts of interest to declare.



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