Case Report

Recurrent *pneumocystosis* pneumonia/chronic obstructive pulmonary disease and mild immunodeficiency in a human immunodeficiency virus-negative subject

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Introduction: *Pneumocystis jiroveci* is the most common opportunistic infection in human immunodeficiency virus (HIV)-infected patients. However, the infection is increasing in other immunosuppressive-associated conditions, such as haematological malignancies, organ transplantation, connective tissues diseases, primary immunodeficiency and long-term corticosteroid therapy. Here, we report a HIV-negative subject.

Case presentation: Here, we report a human immunodeficiency virus (HIV)-negative subject with chronic obstructive pulmonary disease (COPD), and mild hypogammaglobulinaemia, low levels of total CD19⁺ B-cells, and a transitory decrease in IgG and IgG subclasses who evolved to recurrent *Pneumocystis* pneumonia (PCP). In addition, the patient was co-infected with *Candida* spp., and developed oral and oesophageal candidiasis. He was treated with sulfamethoxazole/trimethoprim/pentamidine and improved significantly. **Conclusion:** These results suggest that, in HIV-negative subjects, clinicians should be aware of the possible association between mild immunosuppressive illness and COPD in inducing PCP, even with normal levels of T-cell subsets.

Keywords: *Pneumocystis* pneumonia; chronic obstructive pulmonary disease; CD19⁺ B-cells; sulfamethoxazole-trimethoprim.

Received 20 January 2014

Accepted 29 April 2014

Introduction

Pneumocystis jiroveci is one of the most frequent opportunistic pathogens in human immunodeficiency virus (HIV)-infected individuals and other immunosuppressive-associated conditions, such as haematological malignancies, organ transplantation, connective tissues diseases, primary immunodeficiency (PID) and long-term corticosteroid therapy. After the introduction of highly active antiretroviral therapy (HAART), *Pneumocystis* pneumonia (PCP) in HIV patients decreased significantly; conversely, both the incidence, and mortality rates of PCP in non-HIV patients are increasing. Furthermore, the severity of the disease in non-HIV patients is higher than in HIV-positive ones (Overgaard & Helweg-Larsen, 2007; Kelly & Shellito, 2010). Recently, an association between *P. jiroveci* colonization and chronic obstructive pulmonary disease (COPD) in HIV-positive and HIV-negative individuals was reported (Kling

et al., 2010). Many questions about PCP epidemiology and transmission remain unanswered, and the routes of transmission among humans are unclear. Defects and low levels of CD4⁺ T-lymphocytes are primary risk factors for developing PCP, mainly in HIV-positive patients. Nonetheless, the immune response to *Pneumocystis* infection is complex (Nüesch *et al.*, 1999). Defects in B-cells and antibody production may also predispose to PCP, and in patients without predisposing factors, the disease is an extremely rare condition (Norris & Morris, 2011). In HIV-infected patients, *P. jiroveci* is becoming a health problem in Latin American countries, with rates of PCP ranging from 6 to 55 % depending on the free access of HAART. Few data are available on PCP in HIV-negative subjects in the region (Calderón *et al.*, 2013). The current case presents an HIV-negative patient with COPD and mild immunodeficiency that evolved to recurrent PCP.

Case report

Written consent from the patient was obtained for publication of this case report. In June 2012, a 51-year-old man, native from and residing in Sao Paulo, SP, Brazil, was admitted to the semi-intensive care unit of Ipiranga Hospital, Sao Paulo. He presented a 7-day history of dyspnea, productive cough with yellow sputum, fever, occasional chills, dysphagia and odynophagia due to several lesions in his oral mucosa and tongue. His reported habits included 35 pack-years of smoking and no alcohol or current illicit drug use. No environmental or occupational exposures, or corticosteroid or other immunosuppressive therapies were characterized. The patient reported a 7 kg weight loss in 1 month and previous diagnosis of COPD. His medical history revealed hospitalization in a private hospital in Sao Paulo in 2000, with an acute respiratory syndrome, requiring the intensive care unit with invasive mechanical ventilation, that developed to cardiorespiratory failure. He was diagnosed and treated for PCP and oral candidiasis. At that occasion, the search for HIV and other common infectious agents was negative. In an attempt to proceed with the investigation, he was referred to the PID ambulatory department of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP), and was followed from 2000 to 2006. Lymphocyte immunophenotyping and peripheral blood mononuclear cell proliferative assays were performed from 2000 to 2006 (Table 1, Fig. 1).

Physical examination showed a bad health status with cyanosis in extremities, 86 % O₂ saturation, fever (38.5 °C), tachypnea, tachycardia and emaciation.

A chest X-ray showed a mild bilateral perihilar infiltrate (Fig. 2). Bronchoscopy with bronchoalveolar lavage and a transbronchial biopsy was obtained. An acid-fast stain in both sputum and the bronchoalveolar lavage were negative, but a Giemsa stain identified *P. jiroveci*. An oesophagogastroduodenoscopy was carried out, and an erosive distal oesophagitis (grade B of the Los Angeles classification) due to *Candida* spp. was diagnosed in the biopsies.

In the admission, according to the clinical history, physical examination and chest X-ray, the patient was diagnosed with community-acquired pneumonia, and exacerbated COPD. He was started with an intravenous (IV) regimen of ceftriaxone 1000 mg and clarithromycin 500 mg, both twice a day. On the fourth day after admission, because of symptom permanence and *P. jiroveci* identification, ceftriaxone, and clarithromycin were cancelled, and sulfamethoxazole-trimethoprim IV 100 mg kg⁻¹ (cotrimoxazole) was administered four times daily, plus prednisone 50 mg twice daily. Nine days later, he developed pruritus, and a severe cutaneous rash on his torso/chest and limbs. A drug reaction to cotrimoxazole was suspected, and he was switched to pentamidine 4 mg kg⁻¹ once a day plus prednisone 0.5 mg/kg⁻¹ for 14 days. For oral and oesophageal candidiasis, the patient was treated with nystatin 500 000 U of oral suspension four times a day, and fluconazole 200 mg on the first day, followed by 100 mg once daily for 2 weeks. His oral symptoms improved in 7 days (Fig. 1).

Laboratory parameters in the admission showed leukocytosis of $13.9 \times 10^9 \text{ l}^{-1}$ with neutrophilia ($13.30 \times 10^9 \text{ l}^{-1}$).

Table 1. Follow-up of the immunological status of the patient, from 2000 to 2014, conducted in Hospital das Clínicas and Ipiranga Hospital, Sao Paulo, Brazil

Test	Year					Normal levels
	2000	2002	2012	2013	2014	
Leukocytes ($\times 10^9 \text{ l}^{-1}$)	11.9	9.1	13.9	14.8	8.3	4.5–11
Lymphocytes ($\times 10^9 \text{ l}^{-1}$)	3.7	3.6	0.33	0.74	2.41	1–3.8
Neutrophils ($\times 10^9 \text{ l}^{-1}$)	7.4	7.9	13.3	13.4	5.10	1.8–7.7
Serum immunoglobulins						
Total IgG (g l ⁻¹)	8.05	8.61	3.42	12.10	11.2	9.52–15.38
IgG1 (g l ⁻¹)	ND	ND	2.21	3.02	2.94	2.39–10.83
IgG2 (g l ⁻¹)	ND	ND	1.15	1.71	1.79	1.23–5.48
IgG3 (g l ⁻¹)	ND	ND	0.25	0.34	0.28	0.27–1.4
IgG4 (g l ⁻¹)	ND	ND	0.18	0.22	0.20	0.11–6.20
IgA (g l ⁻¹)	2.86	2.43	3.68	2.49	ND	0.68–4.23
IgM (g l ⁻¹)	0.64	0.42	0.84	0.59	ND	0.50–1.96
IgE (μg l ⁻¹)	ND	ND	142	210	ND	24–430
Lymphocyte proliferation (5th–95th percentiles)						
Phytohemagglutinin Stimulation index	50.89	105.91	ND	ND	ND	18.28–343
Pokeweed stimulation index	67.86	204.82	ND	ND	ND	8.42–107.4
OKT3 stimulation index	27.29	79.30	ND	ND	ND	15.62–219.3
Albumin (g l ⁻¹)	ND	32	22	32	34	35–55
Gammaglobulins (g l ⁻¹)	ND	6	3	6	5	7–15

ND, Not determined.

Fig. 1. Immunophenotyping follow-up of peripheral blood leukocytes of the patient, from 2000 to 2014, conducted in Hospital das Clínicas and Ipiranga Hospital, Sao Paulo, Brazil.

Immunophenotyping of T-cell subsets showed CD3 levels of $1009 \text{ cells } \mu\text{L}^{-1}$, CD4 levels of $689 \text{ cells } \mu\text{L}^{-1}$ and CD8 levels $320 \text{ cells } \mu\text{L}^{-1}$ (CD4:CD8 ratio=2.12). Serum electrophoresis showed low levels of total protein of 51 g l^{-1} , albumin 22 g l^{-1} and gamma-globulin 3 g l^{-1} . Immunoglobulin levels at this time are shown in Table 1 (2012 column). C-reactive protein in the serum was followed routinely and found to be at normal levels. It is well known that malignancies and metabolic, autoimmune and viral/bacterial chronic infections predispose individuals to immunosuppression and are linked to PCP. To exclude secondary hypogammaglobulinaemia, laboratory and image diagnosis screening were carried out, and these diseases were excluded. The patient remained in the internal medicine ward and improved significantly. He was discharged 34 days after admission.

One month after leaving the hospital, normal levels of CD3/CD4/CD8 T-lymphocytes were obtained, but mild hypogammaglobulinaemia still remained (Table 1, 2013 column). A reduced number of CD19⁺ B-cells was detected in his follow-up (2000, $92 \text{ cells } \mu\text{L}^{-1}$; 2002, $70 \text{ cells } \mu\text{L}^{-1}$;

Fig. 2. Chest X-ray showing a mild bilateral parahilar infiltrate.

2013, $67 \text{ cells } \mu\text{L}^{-1}$; 2014, $35 \text{ cells } \mu\text{L}^{-1}$). Normal ranges determined in our laboratory from an adult Brazilian population were $200\text{--}400 \text{ cells } \mu\text{L}^{-1}$. He was followed in ambulatory care in the pulmonary and infectious diseases department of Ipiranga Hospital. In July 2013, the patient was hospitalized again with acute respiratory distress. At present, he remains under close follow-up with intermittent home oxygen therapy.

Fig. 3. Candidiasis and hairy leukoplakia in the tongue of the patient, Ipiranga Hospital, Sao Paulo, Brazil, June, 2012.

Discussion

In HIV-negative subjects, the morbidity and mortality rates of PCP are higher than in HIV-infected subjects, reaching 30–50 % (Overgaard & Helweg-Larsen, 2007). In our patient, the first PCP event occurred in 2000, 12 years before the actual illness. On that occasion, he reported a 23 pack-years tobacco use and a diagnosis of COPD. An investigation conducted at the PID ambulatory department of HC-FMUSP from 2000 to 2006 showed mild and transient alterations in his humoral immune response (Table 1, Fig. 1). From 2000 to 2014, he presented a persistent decrease in peripheral CD19⁺ B-cells with transient low levels of total IgG and IgG isotypes. In protein electrophoresis, gammaglobulins were under or at the lower limit of normality (Table 1).

Since the hallmark results of Jacobs *et al.* (1991) for PCP in adult patients without predisposing illness, a significant number of publications have arisen, addressing different aspects and peculiarities of both the infectious agent and the host. It has been demonstrated that *P. jiroveci* can chronically colonize patients with underlying diseases associated with mild or moderate immunodeficiency (Nevez *et al.*, 1999). Our patient showed normal levels of peripheral lymphocytes and T-cell subsets CD3, CD4 and CD8. A lymphocyte proliferation test with phytoagglutinin, pokeweed and OKT3 mitogens also showed normal levels, thus suggesting that his cellular immune response was preserved. Supporting our results, using a simian–human immunodeficiency virus model of infection, Kling *et al.* (2010) demonstrated that no differences were found in the number of T-cell subsets in HIV-infected monkeys harbouring *P. jiroveci* and PCP, and in HIV-infected monkeys free of *P. jiroveci* infection. They concluded that, although immunological control of *Pneumocystis* infection is strongly correlated to CD4⁺ T-cell responses, B-cells and antibodies play a key role in preventing the spreading and severity of the disease. There is increasing evidence underscoring the importance of the *Pneumocystis*-specific humoral response in humans and animal models, highlighted in this patient due to his low levels of peripheral CD19⁺ B-cells, as well as both IgG antibodies and gammaglobulins. Reduced levels of circulating IgG antibodies have been found in monkeys with PCP, and mice deficient in B-cells are highly susceptible to PCP. It was demonstrated that antibodies are crucial for the clearance of primary infection by *P. jiroveci* (Lund *et al.*, 2003; Kling *et al.*, 2010). An important co-morbidity of the patient that certainly fuelled his recurrent disease was the COPD diagnosis. Individuals with COPD are highly susceptible to infections by *P. jiroveci*, with increased production of pro-inflammatory cytokines and progression of the disease. We suggest that COPD alone is not sufficient to trigger recurrent and severe PCP in an HIV-negative subject. However, a possible synergism between COPD and the mild deficient humoral response is an important predisposing factor for the development of PCP. The mechanisms underlying the immunodeficiency of our patient – lower levels of CD19⁺

B-cells, mild hypogammaglobulinaemia and a transient decrease in IgG levels and IgG isotypes – were not determined. Patients with hypogammaglobulinaemia who do not fulfil the diagnosis criteria for common variable immunodeficiency are classified as having idiopathic primary hypogammaglobulinaemia. The management of these patients becomes a dilemma, since clinical, immunological, therapeutic, and diagnostic characteristics are not well understood. Idiopathic primary hypogammaglobulinaemia subjects have a moderate decrease of immunoglobulin levels and less severe hypogammaglobulinaemia compared with common variable immunodeficiency patients. The majority show mild or no abnormalities in peripheral B-cell subsets (transitional B-cells, naive mature B-cells and memory B-cell subsets), suggesting that they suffer from a plasma cell survival and/or homing defect. *Candida* spp. co-infection was diagnosed in the oral mucosa of both hospitalizations, highlighting the immunosuppressive status of the patient. The association of mild immunodeficiency and COPD in triggering PCP has worldwide implications. COPD is an important cause of morbidity and mortality worldwide, and its global prevalence in adults aged >40 years is approximately 9–10 % (Calderón *et al.*, 2013). Concomitantly, the numbers of immunocompromised individuals with primary immunodeficiency, underlying malignancies or organ transplantation or on immunosuppressive drugs are increasing worldwide (Overgaard & Helweg-Larsen, 2007). These are among the risk factors for the development of acute and chronic pulmonary diseases. Taken together, they may become an important predisposing factor for the development of PCP. The majority show mild or no abnormalities in peripheral B-cell subsets (transitional B-cells, naive mature B-cells and memory B-cell subsets), suggesting that they suffer from a plasma cell survival and/or homing defect (Driessen *et al.* 2013)

In conclusion, we suggest that, in our patient, a possible synergism between COPD and mild immunodeficiency was an important predisposing factor for the development of severe and recurrent PCP. To our knowledge, no description of such an association has been published previously in the literature. In HIV-negative subjects, clinicians should be aware of the possible association between mild immunosuppressive illness and COPD for the development of PCP, even with normal levels of T-cell subsets. Careful immunological testing and follow-up must be done in individuals with recurrent pulmonary diseases.

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