Malaysian J Pathol 2018; 40(1) : 69 – 72

CASE REPORT

The crucial role of molecular testing to facilitate the diagnosis of pneumocystis pneumonia during pregnancy

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Abstract

Pneumocystis pneumonia is an important human immunodeficiency virus (HIV)-associated opportunistic infection, and especially so in pregnant HIV-positive patients. We report a case of a 40-year-old woman in her first trimester of pregnancy who initially presented with acute gastroenteritis symptoms but due to a history of high-risk behaviour and the observation of oral thrush, she was worked up for HIV infection. Her retroviral status was positive and her $CD4^+$ T cell count was only 8 cells/μL. She was also worked up for pneumocystis pneumonia due to the presence of mild resting tachypnoea and a notable drop in oxygen saturation (from 100% to 88%) following brief ambulation. Her chest radiograph revealed bilaterally symmetrical lower zone reticular opacities and Giemsa staining of her bronchoalveolar lavage (BAL) was negative for *Pneumocystis jirovecii* cysts. However, real-time *P. jirovecii* polymerase chain reaction (PCR) testing on the same BAL specimen revealed the presence of the organism. A course of oral co-trimoxazole plus prednisolone was commenced and her clinical condition improved.

Keywords: Co-trimoxazole, *Pneumocystis jirovecii*, pregnancy, real-time PCR

INTRODUCTION

Initially named *Pneumocystis carinii*, the fungal organism which is known today as *Pneumocystis jirovecii* causes pneumocystis pneumonia (PCP) in immunosuppressed patients, namely those infected with HIV or those who are on immunosuppressive therapy for transplantation, malignancies, or connective tissue diseases.[1] Prior to the widespread prescription of combination antiretroviral therapy, PCP was one of the most frequent AIDS-defining opportunistic infections in HIV-infected patients, and it was mostly seen when $CD4^+$ T cell counts have declined to below 200 cells/mL.[2] Making a definitive diagnosis of PCP is not merely of academic interest because the infection is treatable using a specific antimicrobial agent (i.e. co-trimoxazole) which has limited efficacy to treat pulmonary infections caused by other organisms. Also, although co-trimoxazole is the most effective agent to treat PCP, adverse effects are common and patients with known sulfa drug allergies cannot tolerate this drug.[2] The US Food and Drug Administration classifies co-trimoxazole as a pregnancy category D drug, which essentially means that there is positive evidence of risk (congenital anomalies) to the human fetus.[3] We report a case of PCP during pregnancy which had a subtle presentation and the crucial role played by a molecular investigation in clinching the diagnosis.

CASE REPORT

A 40-year-old gravida 2, para 1 Filipino woman who was in her 13th week of pregnancy presented to the emergency department of UKM Medical Centre with a one-week history of diarrhoea and vomiting. There was also a one-month history of productive cough but with no haemoptysis, pleuritic chest pains or fever. She admitted to being sexually promiscuous. On examination, she was neither visibly pale, cyanosed nor

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jaundiced, but had oral thrush. Her blood pressure on presentation was 120/68 mmHg, heart rate was 90 beats/minute, respiratory rate was 20 breaths/minute, temperature was 37°C and her oxygen saturation (SpO_2) on room air was 100%. Due to the resting tachypnoea, the patient was asked to ambulate and her SpO_2 dropped to 88% after walking for two minutes. Cardiovascular, respiratory and abdominal examinations were otherwise unremarkable. The initial working diagnosis was acute gastroenteritis. Blood, stool and sputum samples were sent for bacteriological culture. In view of her high-risk behaviour and oral thrush, a retroviral screening was also ordered. A chest X-ray revealed bilateral lower zone interstitial opacities (Fig. 1).

Non-typhoidal *Salmonella* sp. was cultured from her stool specimen and the isolate was susceptible to all antibiotics tested, including ampicillin. A decision was then made to commence intravenous ceftriaxone 2 g daily for 5 days. Blood culture yielded no growth after 5 days while a mixed growth of normal mouth flora was obtained from the sputum specimen. The retroviral screening was reactive for HIV and subsequent investigations revealed a $CD4^+$ T cell count of 8 cells/μL, a HIV viral load of 197,399 copies/mL and a serum lactate dehydrogenase level of 136 U/L (normal range: 125-220 U/L). A bronchoscopy was performed, and her BAL fluid was sent for real-time qualitative *P. jirovecii* PCR (LightMix® Modular *Pneumocystis jirovecii* Kit, TIB MOLBIOL, Germany) and real-time *Mycobacterium* PCR (LyteStar™ TB/NTM PCR Kit 3.0, ADT Biotech Sdn Bhd, Malaysia) tests. *Mycobacterium tuberculosis* and non-tuberculous mycobacteria DNA were not detected but the BAL was positive for *P. jirovecii* DNA (Table 1). Her same BAL specimen was also stained with Giemsa and subjected to a microscopic examination for *Pneumocystis jirovecii* cysts but none were visualised.

A diagnosis of pneumocystis pneumonia was made and a 3-week course of oral co-trimoxazole was commenced with a total daily dose of 10 tablets (each tablet containing 80 mg of trimethoprim and 400 mg of sulfamethoxazole). A 3-week tapering course of oral prednisolone was also started with an initial twice daily dose of 40 mg. Her resting tachypnoea and cough resolved after being on co-trimoxazole for a week and her SpO_2 became stable on mild exertion. Antiretroviral therapy consisting of tenofovir 300 mg plus emtricitabine 200 mg (dispensed as a fixed dose combination known as Tenvir-Em) and nevirapine 2000 mg was started at 16 weeks of gestation, just prior to her discharge.

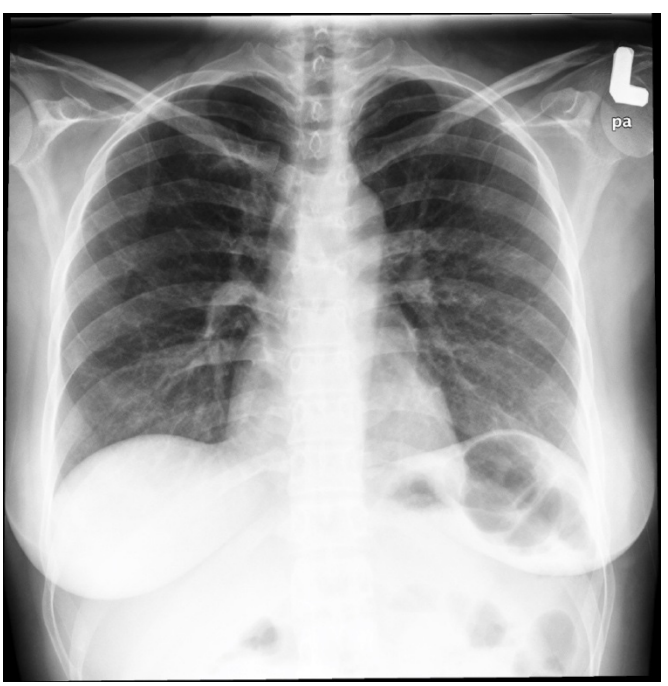

FIG. 1: Chest X-Ray showing bilaterally symmetrical reticular opacities in both lower lung fields.

TABLE 1: Real-time *P. jirovecii* PCR results

	Patient's specimen	Positive control
Cycle threshold value	25.9	22.2
Interpretation	*P. jirovecii* DNA detected	*P. jirovecii* DNA detected

DISCUSSION

P. jirovecii was once thought to be a protozoon when it was first described in the early 1900s,[4] and it was not until the late 1980s that the organism was proven to be a fungus through DNA analysis.[5] It had morphological features of a protozoon and responded to anti-protozoal rather than anti-fungal drugs.[5] Although *Pneumocystis* infects many mammals, human-derived *Pneumocystis* was only found in humans and it was distinct at the molecular level from those infecting other mammals.[4] Thus, animals are highly unlikely reservoirs of infection and there is evidence supporting airborne transmission between humans.[1] The HIV-associated PCP rates differ throughout the world, with Europe reporting a rate of 16% and studies from Africa reporting a rate of up to 39%.[6] In Malaysia, a study conducted in University Malaya Medical Centre found that nearly 63% of HIV/AIDS patients had PCP.[7]

The common clinical presentations of PCP in a HIV-positive patient are progressive dyspnoea, non-productive cough and low-grade fever.[2] While our patient did complain of cough, it was productive in nature and although she was slightly tachypnoeic, there was no actual complaint of breathlessness. Moreover, even though the presence of bilaterally symmetrical reticular opacities on her chest radiograph are suggestive of PCP,[6] there are no radiological findings which are specific to PCP.[8] The serum lactate dehydrogenase level may be elevated in patients with PCP but its accuracy for the diagnosis of PCP in HIV-positive patients is only 58% and is even lower in HIV-negative individuals.[9] Our patient's LDH level was within the normal reference range and thus was of no assistance in diagnosis.

Due to the lack of typical/specific clinical, radiological or biochemical features, we resorted to microbiological investigations to rule in (or rule out) PCP. The microbiological detection of *P. jirovecii* from respiratory specimens can be achieved by using either Giemsa or immunofluorescent staining methods to visualize the cysts or by utilising molecular methods (i.e. PCR) to detect fungal DNA. We acknowledge that in low-resource settings or in developing countries, molecular tests may not be readily available due to the high financial costs generally associated with PCR. The preferred respiratory specimen for the laboratory diagnosis of PCP is a bronchoalveolar lavage (sensitivity of 98% or higher), although induced sputum (sensitivity of 50-90%) is also acceptable.[10,11] Among these three methods, PCR has been reported to have the highest detection rate and Giemsa staining the lowest.[12] Therefore, it was not surprising that the fungus was detected in the real-time PCR but not in the Giemsa stain. Culture on artificial media or in cell lines is not used because the fungus cannot be cultured.[10]

Suitable molecular targets for *P. jirovecii* PCR include the dihydrofolate reductase gene, heat shock protein 70 gene, mitochondrial ribosomal large-subunit gene and the multicopy surface glycoprotein (MSG) gene.[10,11] The kit used for our patient targeted the MSG gene, with a detection limit of 10 genome equivalent copies per reaction, which corresponds to a Ct value of between 36 and 38. Such is the reliability of real-time PCR in diagnosing PCP that a negative result virtually excludes the diagnosis.[10] However, due to the highly sensitive nature of PCR, it is vital to differentiate *P. jirovecii* colonisation from true infection to guide therapy. The Ct value obtained by real-time PCR allows us to estimate the fungal burden. A lower Ct value correlates with a higher fungal burden and increased likelihood of pneumonia. In HIV-positive patients, a Ct value of <27 has been reported to reliably differentiate colonisation from pneumonia with 100% specificity.[13] Our patient's Ct value was 25.9 and this sealed the diagnosis and paved the way for co-trimoxazole therapy. Her clinical response to the drug also validated our diagnosis retrospectively.

It is imperative to correctly diagnose and manage PCP in pregnant HIV-positive patients because the infection has a more aggressive course and a higher mortality rate (50% vs. <16% in non-pregnant HIV patients), possibility due to the physiological waning of cell-mediated immunity associated with pregnancy itself.[14] To compound matters, the drugs commonly

employed to treat PCP (e.g. co-trimoxazole and pentamidine) can potentially adversely affect the developing foetus. Among the various treatment strategies for PCP, regimes containing co-trimoxazole have been reported to have the best outcomes.[14] The antimicrobial regime for our patient consisted of trimethoprim at a dose of 15 mg/kg and sulfamethoxazole at a dose of 75 mg/kg body weight, which are the minimum recommended doses for treating PCP in HIV-positive patients in general.[2] Since our patient weighed 53 kg, this translated to 10 tablets of single-strength co-trimoxazole daily.

In conclusion, a high index of suspicion is needed to make a diagnosis of PCP in pregnant HIV-positive patients. They may not necessarily present with the classical symptom triad of dyspnoea, fever and dry cough. When the clinical presentation and results of radiological, biochemical and/or conventional microbiological investigations are not sufficiently convincing to make a definitive diagnosis of PCP, the threshold to secure a molecular diagnosis should be low to support the administration of co-trimoxazole to pregnant patients.

ACKNOWLEDGEMENT

We would like to thank Universiti Kebangsaan Malaysia for providing a research grant (code: GGPM-2016-058) which enabled us to offer molecular *P. jirovecii* testing for the patient described in this article. We are also grateful to the Dean of Universiti Kebangsaan Malaysia's Faculty of Medicine for her permission to publish this case report.

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