行政院國家科學委員會補助專題研究計畫成果報告

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評估利用血清與呼吸道檢體的聚合酵素連鎖反應在早期診斷免疫不全患者的肺囊蟲肺炎和其對於治療反應的角色

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本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

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中文摘要

肺囊蟲肺炎是免疫不全患者，特別是器官、骨髓移植與後天免疫不全病患常見的感染併發症。我們嘗試從免疫不全患者發生肺部感染時所收集的痰液或支氣管內視鏡的刷洗液中，利用聚合酵素連鎖反應，希望能提供較早期的診斷幫忙。除此之外，我們也嘗試從接受骨髓移植的病患中，多次連續隔周所收集的漱口水，利用相同的方法，希望能提供較早期偵測是否發生肺囊蟲的寄生。在一年中，共有 28 位後天免疫不全病患、14 位其他免疫不全患，接受了痰液（37）或支氣管內視鏡刷洗液（5）的聚合酵素連鎖反應檢驗。檢驗前抗肺囊蟲肺炎藥物（trimethoprim-sulfamethoxazole）使用的期間中間值為 6 日。其中只有 2 位病患的痰液呈陽性反應。21 位經細胞學檢驗（2）或臨床上（19）診斷為肺囊蟲肺炎的後天免疫不全病患中，有一位呈陽性反應。而在骨髓移植的病患（15）和 3 位後天免疫不全病患所收集的漱口水中（檢體數，中間值為 3），並無陽性反應。這些患者皆在檢體收集前，使用抗肺囊蟲肺炎藥物初期預防。骨髓移植的病患中，在四個月追蹤中，並沒有患者發生肺囊蟲肺炎。我們的結果顯示，利用聚合酵素連鎖反應在已接受抗肺囊蟲肺炎藥物治療或預防，並且無法取得合適呼吸道檢體的免疫不全患者中，用以診斷肺囊蟲肺炎或偵測有無肺囊蟲的寄生的角色，並不理想。

關鍵詞：肺囊蟲肺炎、免疫不全、後天免疫不全、移植、聚合酵素連鎖反應

Abstract

We utilized polymerase chain reaction (PCR) of the sputum specimens obtained by saline induction, gargling and bronchoalveolar lavage to assess the its role of early diagnosis of PCP in patients with AIDS and transplantation and detection of colonization of *P. carinii* in bone marrow (BM) transplant recipients. Over the past 12 months, 42 immunocompromised hosts, 28 with AIDS and 14 other immunosuppressive diseases, presented with pulmonary infections. 21 AIDS patients were diagnosed with PCP based on cytologic examination (2) and consistent radiographic findings (interstitial pneumonitis) plus favorable responses to trimethoprim-sulfamethexazole (TMP-SMX) monotherapy. 14 other AIDS patients had been diagnosed with pulmonary tuberculosis (7), CMV pneumonitis (2), cryptococcosis (2), non-specific bronchitis (2) and *Mycobacterium avium* complex pneumonia (1). One specimen of the other 14 immunocompromised patients, in whom 3 were diagnosed with PCP by histopathology was positive for PCR. The median interval from initiation of TMP-SMX to collection of airway specimens was 6 days. Serial, weekly sputum specimens collected by gargling from 15 BM transplant recipients with a median number of specimens of 3 were tested with PCR for *P. carinii* colonization. TMP-SMX was routinely administered in those patients before transplantation. The PCR results were negative. After a median follow-up for 4 months, there were no cases of PCP. We concluded that in immunocompromised patients who were taking TMP-SMX for treatment or prophylaxis and could not provided appropriate airway specimens, the yield rates of PCR to detect *P. carinii* was disappointingly low.

Key Words: *Pneumocystis carinii* pneumonia, immunocompromise, AIDS, transplantation, polymerase chain reaction

Background

Pneumocystis carinii pneumonia (PCP) is one of the most common opportunistic pulmonary infections among immunocompromised patients, especially organ or bone marrow (BM) transplant recipients and patients with human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) in Taiwan [1]. Definitive diagnosis of PCP depends on cytologic examination of airway specimens and pathologic examination of the biopsy specimens, although empiric initiation of anti-pneumocystosis has been acceptable in HIV-infected patients who present with consistent clinical and radiologic manifestations (interstitial pneumonitis).

In the care of immunocompromised patients in Taiwan, we have been confronted with the difficulties in obtaining clinical specimens and potential morbidities associated with invasive diagnostic procedures. Bleeding diathesis and respiratory distress of the patients often preclude the performance of bronchoscopy and/or open lung biopsy in non-HIV-infected patients, while in HIV-infected patients, those procedures were considered unnecessary or dangerous to health care workers. Initiation of empiric therapy based on radiographic findings is often the rule than exception. However, clinical and radiographic presentations may be similar in various opportunistic pulmonary infections among immunocompromised patients. Differentiation among pneumonitis due to cytomegalovirus, *P. carinii*, radiation, chemotherapy, or *Crytpococcus neoformans* may be a formidable task based on radiology and clinical presentations.

With the advent of polymerase chain reaction (PCR), diagnosis of PCP without pursuing bronchoscopic or open-lung biopsy has become feasible, although sensitivity and specificity depend upon the methods utilized and clinical specimens studied.

In order to facilitate early initiation of anti-pneumocystosis and avoid potential morbidities associated with invasive diagnostic procedures, we initiated this study with aims to assess the role of PCR in the detection of *P. carinii* in a variety of airway specimens in immunocompromised patients who presented with pneumonia. We also assessed the role of PCR in the detection of *P. carinii* colonization in patients who were undergoing bone marrow transplantation (BMT). Decisions to continue or discontinue primary prophylaxis for PCP may thus be made and to reduce risks of toxicities of anti-penumocystosis medications during the period of marrow grafting.

The study subjects were enrolled from patients who were admitted to AIDS unit and other wards at the National Taiwan University Hospital because of pulmonary lesions and from patients who were admitted to BMT unit from July 2000 to June 2001. Patients provided sputum specimens after giving their verbal informed consent. For those who underwent bronchoscopy, bronchoalveolar lavage (BAL) was performed. Those specimens were

submitted for cytologic examination using Pappanicolou stains. For those who underwent lung biopsy, the specimens were routinely stained with Gomori-methenamine silver (GMS) stain. PCR detection of *P. carinii* was performed in clinical specimens by following the protocol described previously [2]. In brief, after lysis of *P. carinii* from airway specimens, the whole genomic DNA was extracted and precipitated. Purification of DNA was performed with Qiagen purification kit and the product was subjected to PCR using primers designed by Lu JJ, et al. [2]. Two pairs of primers were used for internal transcribed spacer (ITS) region of the *P. carinii* DNA. For the colonization study in BMT patients, specimens were collected after their performance of oral gargling with saline for 30 seconds to one minute and subjected to PCR.

Results and Discussions

Over the past 12 months, 42 immunocompromised hosts, 28 with AIDS and 14 other immunosuppressive diseases, presented with pulmonary infections. PCP was diagnosed in 21 AIDS patients based on cytologic examinations (3 patients) and consistent radiographic findings plus favorable clinical and radiologic responses to trimethoprim-sulfamethexazole (TMP-SMX) monotherapy (18). Only one specimen was tested with a positive reaction by PCR. Fourteen other AIDS patients had been diagnosed with pulmonary tuberculosis (7), CMV pneumonitis (2), cryptococcosis (2), non-specific bronchitis (2), and MAC pneumonia (1). Dual infections were detected in 7 patients whose PCR were all negative.

One PCR of 14 other immunocompromised patients was positive for PCR detection of *P. carinii*. Of the 14 patients, 2 transplant recipients and 1 patient with lymphoid malignancy were diagnosed with PCP by histopathology. All study patients except for the two renal transplant recipients had received TMP-SMX treatment prior to collection of airway specimens. The median interval from initiation of TMP-SMX to collection of airway specimens was 6 days (range, 1 to 10 days).

The two PCR products were sequenced and nucleotide sequence was compared with those sequence data published by Lu JJ, et al. [3]. The two positive samples were classified as two major types: A and B. The two differed in sequence at position 6,14,76, and 77. Type A had a C residue at position 6 and a T residue at position 14, and there was a 2-bp deletion at positions 76 and 77. Type B had a T residue at position 6 and A residue at position 76, and G residue at position 77 and a base was missing at position 14.

In the colonization study, serial, weekly sputum specimens were collected by gargling to be tested with PCR for *P. carinii* colonization from 15 BMT patients who provided a median number of specimens of 3 (range, 2-5). In this study group, TMP-SMX was routinely administered in those patients before transplantation and was discontinued when donor marrow was transfused because of fear of bone marrow suppression from continuing use of TMP-SMX. The median duration from the last day TMP-SMX was taken to the first day serial gargling

specimens were collected was 10 days (range, 3 to 21 days). All of the PCR results were negative. After a median follow-up for 4 months, there were no cases of PCP.

We concluded that in immunocompromised patients who were taking TMP-SMX for treatment or prophylaxis, the yield rate of PCR of PCP was disappointingly low. The low diagnostic yield might have been due to difficulties in obtaining appropriate airway specimens and preceding use of TMP-SMX or other anti-pneumocystosis therapy.

The results of this study fell far short of the goals which our original proposal aimed to achieve. The study was limited in many ways. First, clinical specimens of good quality were difficult to obtain, despite use of saline induction. On most of the occasions, patients provided saliva because of the fact patients with PCP most often than not did not produce sputum.

Second, empirical use of TMP-SMX was a common practice, either prophylactically or therapeutically, in patients undergoing transplantation or with HIV infection who presented with interstitial pneumonitis. Invasive diagnostic procedures are often delayed until patients fail to respond to empiric therapy. Therefore, diagnostic sensitivity for PCP, even with invasive procedures, may be reduced. Use of TMP-SMX prior to performance may also reduce the detectability of PCR for *P. carinii*, although the extent to which it may cause remains to be studied. Therefore, a very small case number of definitive PCP precluded us from estimating the sensitivity of PCR to diagnose PCP in immunocompromised patients receiving TMP-SMX. Had bronchoscopy been performed earlier and more PCP cases been definitively diagnosed, we might have been able to make reasonable estimates of sensitivity and specificity of this new diagnostic modality. This may be improved if attending physicians agree to accept the opinions of infectious disease consultation that invasive diagnostic procedures be performed earlier and if, regardless of HIV status, bronchoscopy can be expedited.

Third, use of PCR for detection of *P. carinii* colonization during the early post-transplantation phase may not be feasible or practical because it would be difficult or impossible to discontinue TMP-SMX prophylaxis in transplant recipients.

Our plan in the near future is to continue this study and enroll a larger case number but with an effort to expedite the invasive diagnostic procedures in order to obtain appropriate clinical specimens for definitive diagnosis to be made and assessment of PCR.

References

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