

A qualitative, enzyme-linked immunospot assay is a promising diagnostic method for tuberculous peritonitis among continuous ambulatory peritoneal dialysis patients: A single-center experience.

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Abstract

The prevalence of Tuberculous Peritonitis (TBP) among Continuous Ambulatory Peritoneal Dialysis (CAPD) patients in China is unknown. A qualitative Enzyme-Linked Immunospot (ELISPOT) assay has been proven effective for Tuberculosis (TB) diagnosis. The effectiveness of ELISPOT for TBP diagnosis is unknown. We report our single-center experience of TBP management among CAPD patients. This is a single-center retrospective study of 178 CAPD patients from August 1997 to December 2015. The ELISPOT assay was used for detection of interferon (IFN)- γ , IFN- γ (PB+P8.10), and IFN- γ (P8.10) in blood and peritoneal effluent fluid in suspected cases of TBP. Antituberculous therapy combined with CAPD was administered to confirmed TBP patients. Clinical and laboratory data were reassessed. The prevalence of TBP in this study was only 0.56%, accounting for 0.9% of all peritonitis cases. The incidence of TBP was 1/806 person-months. Levels of IFN- γ , IFN- γ (PB+P8.10), and IFN- γ (P8.10) in blood and dialysate effluent were more than 10 times higher than the reference range at the onset of TBP and gradually returned to normal with antituberculous treatment. Tenckhoff catheters functioned well. The prevalence of TBP among CAPD patients was low in our center. TBP should be considered in patients with neutrophilic sterile peritonitis and no response to broad-spectrum antibacterial medication. ELISPOT is a promising diagnostic method for TBP among CAPD patients. The peritoneal catheter can be retained with early diagnosis and timely antituberculous treatment.

Keywords: CAPD, Qualitative enzyme-linked immunospot assay, Tuberculous peritonitis.

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Introduction

More than three million annual deaths worldwide are attributable to *Mycobacterium tuberculosis* [1]. The incidence of Tuberculosis (TB) in end-stage renal disease is at least 5-15 times higher than that in the general population [2-4]. The attributable mortality rate is 15% in Tuberculous Peritonitis (TBP) patients undergoing Continuous Ambulatory Peritoneal Dialysis (CAPD) [5]. Nearly 200 cases of TBP complicating CAPD were reported as of 2012 [6,7], but the TBP prevalence in China has not been reported in the English-language literature.

The gold-standard for TBP diagnosis is culture growth of *Mycobacterium* from ascites fluid or a peritoneal biopsy. Many methods can be used for TBP diagnosis. As a positive culture typically takes 4-6 w to return, delay in diagnosis and initiation of appropriate antituberculous treatment is not uncommon. The use of laparoscopy-guided peritoneal biopsy [8,9] or exploratory laparotomy [6,10] for early diagnosis has been

suggested. However, operative risks, with the possibility of postoperative peritoneal dialysis leakage and the need to interrupt CAPD require careful evaluation. Thus, effective and early diagnostic methods are urgently needed. Recent research suggests that a T-cell-based assay for interferon gamma, the Enzyme-Linked Immunosorbent Spot Test (ELISPOT), holds promise in the diagnosis of *M. tuberculosis* infection [11-13]. However, the effectiveness of ELISPOT for TBP diagnosis remains unclear.

Whether the Tenckhoff catheter must be removed in TBP patients remains controversial. The aim of this study was to determine efficacy of the ELISPOT assay for the diagnosis of TBP in patients undergoing CAPD. To the best of our knowledge, this is the first report of TBP in patients on CAPD who were diagnosed with the ELISPOT method in China.

Subjects and Methods

Patient selection

Dialysis records of 178 CAPD patients followed in our center from 1997 to 2015 were retrospectively reviewed. All patients used Baxter DIANEAL CAPD solution. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Shenzhen University. Written informed consent was obtained from all participants.

Data collection and treatment protocol

A Tenckhoff straight catheter with two cuffs was surgically implanted. CAPD patients were instructed to report to the institute with any infectious or mechanical complication. Microscopy and culture were performed for bacteriological and fungal isolates. TBP was routinely considered in all patients who had culture-negative peritonitis unresponsive to conventional antibiotic therapy.

A qualitative Enzyme-Linked Immunospot (ELISPOT) assay [14] has been used for Interferon (IFN)- γ , IFN- γ (PB+P8.10), and IFN- γ (P8.10) (ELISPOT) detection in blood and peritoneal effluent fluid to confirm the diagnosis in patients with suspected TBP. The diagnosis of TBP was based on levels of IFN- γ , IFN- γ (PB+P8.10), and IFN- γ (P8.10) three times higher than the reference range.

Clinical and laboratory data were evaluated at study inception, and at 3, 6, and 12 months of follow-up. CAPD was not interrupted during antituberculosis therapy.

Definitive diagnosis and antituberculosis therapy were performed according to the International Society of Peritoneal Dialysis recommendations [15].

Response to treatment was defined as the resolution of clinical signs and symptoms of peritonitis and normalization of the peritoneal dialysis fluid white blood cell count.

Dialysis efficiency was assessed with the peritoneal equilibrium test as described by Twardowski et al. [16]. Nutritional status was assessed with the Subjective Global Assessment (SGA) [17] method. Assessment criteria: C: severely malnourished; B: moderately malnourished or suspected malnourished; A: well-nourished.

Results

Baseline characteristics and TBP prevalence

Of 178 patients, 106 were 106 men and 72 were women, with a mean age of 48 ± 11.8 y (range, 23 to 83 y). The main underlying causes of renal failure were chronic glomerulonephritis (43%), diabetes mellitus (29%), hypertension (11%), and others (17%). Only one woman had TBP among the 178 patients. The TBP incidence was 0.56%, accounting for 0.9% of all peritonitis episodes. The incidence of TBP was 1/806 person-months.

Clinical and laboratory outcomes

One patient developed TBP after three months on peritoneal dialysis. TBP diagnosis was confirmed eight days after peritonitis symptom onset. The peritoneal dialysis catheter functioned well during follow-up. The patient is currently in good health. The main clinical and laboratory data during follow-up are shown in Table 1.

Discussion

The incidence of active TB has been increasing worldwide, and dialysis patients are at much higher risk for developing TB than individuals with normal renal function [1]. TB in dialysis populations is many times more common than in the general population, e.g., 12 times higher in San Francisco [18], 10 times higher in Brooklyn, New York [19], and 6-16 times higher in Japan [20]. The susceptibility of the dialysis population to TB is multifactorial, and involves impaired cellular immunity [21], suppressed lymphocytic mitogenic response [22], and defects in leukocyte function following exposure to dialysis membranes [23].

CAPD patients are more susceptible to TB than hemodialysis patients. *In vitro* studies suggested that alterations in pH, osmolality, and peritoneal fluid volume that accompany CAPD may hinder both phagocytic and lymphocytic activities in peritoneal fluid, and thus permit infection with a smaller microbial inoculum [24]. As of 2012, nearly 200 cases of TBP complicating CAPD had been reported in the English-language literature, with the initial case report in 1980 [6,7]. The prevalence of TBP in the CAPD population ranges from 1.7%-3.2% according to most authors [7,25]. In this study, the TBP prevalence was only 0.56%, which is lower than that in the literatures. We believe that the relatively low incidence of TBP among our CAPD patients is related to relatively high living standards in our city. However, Hong Kong, a neighboring city, has a more than three times higher TBP prevalence of 1.8% [25]. The explanation for the difference remains unclear.

Unfortunately, the presentation of TBP in CAPD patients is nonspecific. Abdominal pain, fever, cloudy effluent dialysate, and polymorphonuclear cell-predominant dialysate in patients who are undergoing CAPD can be caused by infection with either mycobacteria or other, more common pathogens. Nevertheless, a triad of abdominal pain, abdomen distension, and fever has been reported to occur in less than 60% of CAPD patients [26]. In our patient, abdominal pain, high fever (sustained temperature $>38.5^{\circ}\text{C}$), and cloudy fluid were the main symptoms, and peritonitis did not resolve despite one week of conventional broad-spectrum antimicrobial treatment. Subsequently, an ELISPOT assay was used to confirm TBP diagnosis. Thus, TBP should be considered in patients with neutrophilic sterile peritonitis, with no response to broad-spectrum antibacterial medication.

The exact portal of entry of *M. tuberculosis* into the peritoneum remains unclear. Some studies proposed that infection with *M. tuberculosis* is acquired by direct

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contamination *via* peritoneal dialysate, whereas others suggested that infection is spread from another focus of TB in the body [25]. Extrapulmonary TB has been noted in more than 40%-50% of CAPD patients with TB in several studies [26]. Extrapertoneal TB was present in 8%-28% of CAPD patients [27]. In the present case, no extraperitoneal TB was observed.

The interval between the onset of symptoms and diagnosis of TB among CAPD patients averages 4 w [24]. The treatment delay is a significant factor for mortality in TBP patients [5]. About 80% of patients died within 6 w of presentation, often before the result of mycobacterial culture was available [27]. Early diagnosis and timely antituberculous treatment are pivotal to the survival and prognosis in TBP patients. Unfortunately, because the onset can be insidious, the early diagnosis of TBP among CAPD patients remains a great challenge [28]. The gold standard for diagnosis is culture growth of *Mycobacterium* on ascites fluid or a peritoneal biopsy specimen [29]. Many methods [5,7] have been used to confirm TBP diagnosis among CAPD patients, including culture (54%), peritoneal biopsy (19%), smear (16%), acid-fast bacilli staining (6%), and Polymerase Chain Reaction (PCR) (5%). Each diagnostic test has disadvantages, either lacking sensitivity or specificity, or being very time-consuming [26]. Radiologic imaging techniques including computed tomography and magnetic resonance imaging are not considered sensitive or specific for diagnostic purposes in TB. Peritoneal biopsy can cause severe complications including bowel perforation, intraperitoneal bleeding, subcutaneous hematoma, and interruption of peritoneal catheter function [30].

The sensitivity of direct microscopic smear detection of acid-fast bacilli in ascites fluid is reported to range from 0% to 6% [31].

Although culture is the most widely used diagnostic method for TBP in CAPD patients, the dialysate effluent culture is positive in only about 25% of patients, due to low mycobacterial numbers [32]. Moreover, the utility of cultures is even more questionable when considering the delay of 4 to 6 w before a result is obtained.

PCR assays, which amplify mycobacterial 16S ribosomal RNA, show promise for rapid detection of mycobacteria. However, the utility of ascites fluid for PCR assays in detecting TBP has not been well established. Moreover, PCR is expensive and has a high rate of false-positive and false-negative results [33].

Adenosine Deaminase Activity (ADA) in ascites fluid has been proposed as a useful noninvasive diagnostic method for TBP [34]. However, the sensitivity is extremely low in cirrhosis patients (about 30%) [35]. Moreover, there are few reports about the utility of ADA in CAPD patients.

The ELISPOT immunological assay measures IFN- γ -producing T-cell responses to early secreted antigenic targets of *M. tuberculosis*, and has been shown to be promising for TBP diagnosis among CAPD patients. The sensitivity and specificity of ELISPOT in the diagnosis of extrapulmonary TB

were 94% and 88%, respectively. Another study even demonstrated a positive ELISPOT test on ascites fluid in 6 of 6 patients with confirmed abdominopelvic TB [36]. Moreover, immunosuppression does not affect the diagnostic sensitivity of the ELISPOT assay for extrapulmonary TB [37]. Nevertheless, because of its noninvasive and timely features, the ELISPOT assay on peripheral blood or ascites fluid may prove to be a promising diagnostic method for the diagnosis of active TBP [38]. In the present study, the qualitative ELISPOT assay was used for IFN- γ , IFN- γ (PB+P8.10), and IFN- γ (P8.10) detection in blood and effluent dialysate to confirm the diagnosis in suspected TBP patients. The IFN- γ , IFN- γ (PB+P8.10), and IFN- γ (P8.10) values remarkably increased at the onset of presentation and gradually declined to the reference range with antituberculous treatment.

Whether the Tenckhoff catheter must be removed in TBP patients remains controversial.

About 53% of TBP patients accepted peritoneal dialysis catheter removal before completion of therapy for various reasons [5]. A 2010 update of recommendations for peritoneal dialysis-related infections [39] suggested that patients with cloudy effluent on appropriate antibiotics should have the catheter removed after 5 d, but growing data show that peritoneal catheter removal in TBP patients has no apparent benefit and does not increase the efficacy of treatment [40]. Some authors have advocated removal of the catheter for a short period of time, with subsequent reimplantation [41]. Available data indicate that there is no significant difference in patient survival between TBP patients with or without peritoneal catheter removal [7]. Because our patient refused hemodialysis, the peritoneal catheter was retained. The catheter functioned well, and the dialysis-related parameters steadily improved during follow-up (Table 1).

In conclusion, the prevalence of TBP among CAPD patients was low in this single-center study in China. TBP should be considered in patients with neutrophilic sterile peritonitis, with no response to broad-spectrum antibacterial medications. ELISPOT is a promising diagnostic method for TBP among CAPD patients and its utility requires further observation. The peritoneal catheter may be retained with early diagnosis and timely antituberculous treatment.

Table 1. Clinical and laboratory data at different follow-up time.

Clinical and laboratory data	0 m	3 m	6 m	12 m
BMI (kg/m ²)	15.6	17.2	17.6	18.3
SGA	C	C	B	B
Scr (μ mol/L)	675	490	643	481
UA(μ mol/L)	563	417	411	398
HGB (g/L)	51	61	83	99
Effluent leukocyte count	520	36	43	56
hs-CRP (mg/L)	54	49	11	9
PPD skin test	(-)	(-)	(-)	(-)

Serum albumin (g/L)	21	26	29	36
Cholesterol (mmol/L)	4.3	3.6	3.9	4.6
LDL (mmol/L)	2.4	1.7	1	2.3
Kt/v	1.43	1.56	1.61	1.61
Ccr (L/w)	56	51	58	56
Dialysate/plasma ratios of Scr at 4 h	0.79	0.71	0.68	0.69
Net ultrafiltration (ml/d)	400 ± 36	432 ± 66	466 ± 73	499 ± 59
Blood				
IFN1	391	116	30	20
IFN2	387	136	20	16
IFN3	387	136	20	16
Ascites				
IFN1	391	221	20	20
IFN2	387	139	30	20
IFN3	387	139	30	20

Note: BMI: Body Mass Index; SGA: Subjective Global Assessment; HGB: Hemoglobin; hs-CRP: hypersensitive C-Reactive Protein; LDL: Low-Density Lipoprotein; Ccr: Creatinine Clearance; PPD: Purified Protein Derivative; Scr: Serum Creatinine; UA: Uric Acid. IFN1: (reference range: 0-40); IFN2: (reference range: 0-30); IFN3: (reference range: 0-30).

Conflicts of Interest Statement

The authors have no conflicts of interest to disclose.

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