

CASE REPORT

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Pathogenic microorganism DNA high-throughput genetic sequencing to diagnose peritoneal dialysis-associated peritonitis due to *Mycobacterium tuberculosis* infection

Rui-feng Xu¹, Wu-niri Gao¹, Ta-la Hu¹, Xi-feng Wang², Jian-rong Zhao¹ and Yan Meng^{1*}

Abstract

Peritoneal dialysis-associated peritonitis is a serious complication of peritoneal dialysis, and the prevention and treatment of this condition are important for improving the long-term survival and quality of life of patients. However, peritoneal dialysis-associated peritonitis due to *Mycobacterium tuberculosis* infection is relatively rare and not easily diagnosed. Here, we present a case of peritoneal dialysis-associated peritonitis caused by *Mycobacterium tuberculosis* identified by pathogenic microbial DNA high-throughput genetic sequencing. This case demonstrates that pathogenic microbial DNA high-throughput genetic sequencing could be used to improve the detection rate of pathogenic microorganisms in patients with complex conditions, thereby allowing for earlier initiation of treatment.

Keywords Pathogenic microorganism DNA high-throughput genetic sequencing (PMseq-DNA), Peritoneal dialysis, Peritoneal dialysis-associated peritonitis, *Mycobacterium tuberculosis*

Introduction

Peritoneal dialysis-associated peritonitis (PDAD) is a common complication of peritoneal dialysis (PD) and is a major cause of treatment failure in PD patients. Pathogens can be isolated from dialysis effluent in more than 90% of patients with signs and symptoms of peritonitis and increased dialysate neutrophils, with gram-positive bacteria (40–50%) being the most common, fungal peritonitis (2–4%) being uncommon, and *Mycobacterium tuberculosis* (MTB) and other branching mycobacteria being extremely rare [1]. Because of

limitations in traditional pathogen detection and clinical aspects, PDAD patients often have negative results in dialysis effluent cultures, making the identification of the causative pathogen challenging. We report a case of refractory PDAD that was diagnosed as MTB infection via pathogenic microorganism DNA high-throughput genetic sequencing (PMseq-DNA). This detection method can extract all nucleic acids from a variety of infectious samples and perform rapid sequencing on a sequencing platform. A comparison of the sequencing results with a database enables the accurate identification of pathogenic microorganisms within 24 h. This diagnostic method is effective and rapidly produces results.

*Correspondence:

Yan Meng
mbao124@qq.com

¹Department of Nephrology, The Affiliated Hospital of Inner Mongolia Medical University, Hohhot 010050, Inner Mongolia, PR China

²Department of Nephrology, No. 2 Hospital of Hohhot, Hohhot, PR China



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Table 1 Laboratory findings

Sample Type	Project	Day 1	Day 6	Day 11	Day 17	Normal range
Blood	Hemoglobin (g/L)	74	93	72	80	115~150
	RBC ($\times 10^{12}/L$)	2.62	3.31	2.59	2.87	3.8~5.1
	WBC ($\times 10^9/L$)	6.98	7.82	5.93	4.28	3.5~9.5
	Lymphocyte ratio (%)	8	9.5	10	11.8	20~50
	Neutrophil ratio (%)	85.9	83.1	84	84.1	40~75
	Eosinophil ratio (%)	0.1	0.8	0.4	0.5	0.4~8
	K (mmol/L)	3.11	4.62	4.11	2.91	3.5~5.3
	Ca (mmol/L)	1.65	1.83	2.06	1.85	2.15~2.60
	P (mmol/L)	1.74	1.16	1.24	1.08	0.8~1.7
	Na (mmol/L)	133.9	132.2	131	133.6	135~147
	Cl (mmol/L)	87.6	86.6	89	89.7	99~110
	Urea (mmol/L)	16.4	15.2	13.6	10.3	2.2~7.2
	Blood creatinine ($\mu\text{mol}/L$)	594	512	494	431	44~97
	Procalcitonin (ng/ml)	0.857	1.052	1.965	2.36	<0.05
Peritoneal dialysate	Mononuclear cell (%)			44	6	
	Polymorphonuclear cell (%)			56	94	
	Adenosine deaminase (U/L)	0.9				<40

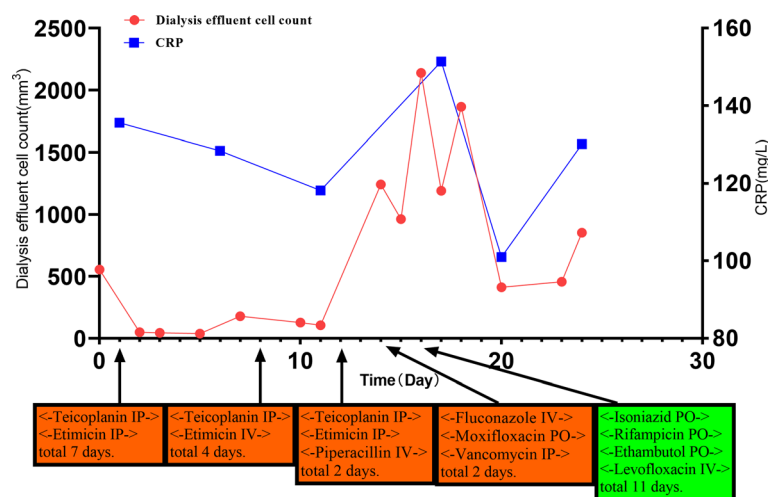


Fig. 1 Changes in the dialysis effluent cell count, CRP level, and medication used during treatment. IP, intraperitoneal; IV, intravenous injection; PO, taken orally; CRP, C-reactive protein

Case introduction

Chief complaint and medical history

A 52-year-old woman with end-stage kidney disease (ESKD) secondary to essential hypertension started PD on September 10, 2021. On June 15, 2022, the patient was admitted to the hospital due to abdominal pain. The color of the dialysis effluent was light yellow and slightly turbid, with a cell count of $1051/\text{mm}^3$. The patient, who was diagnosed with PDAD, was treated with Etimicin in the dialysis solution once a day via peritoneal perfusion for 12 days and then discharged. Antifungals were not administered during this period for treatment or prevention. On July 20, 2022, her abdominal pain worsened, with flocculent material observed in the dialysis effluent. She was admitted to the emergency department. Her body temperature was $36.7\text{ }^\circ\text{C}$, with tenderness upon

abdominal palpation, and rebound tenderness was provoked. The patient had no known past history of tuberculosis or tuberculosis exposure.

Laboratory and ancillary tests

Upon hospital admission, the patient’s initial dialysis effluent cell count was recorded at $555/\text{mm}^3$, with polymorphonuclear cells accounting for 77.1% and mononuclear cells accounting for 22.9%. A routine blood examination revealed a white blood cell count of $6.98 \times 10^9/L$. Comprehensive details of the remaining laboratory test results are provided in Table 1. The dynamic changes in the cell count in the dialysis effluent and C-reactive protein (CRP) concentration during the second treatment period are depicted in Fig. 1. Dialysis effluent culture revealed no pathogenic bacteria

on the 13th day. On the 14th day, the result of the fungal 1,3- β -D-glucan test (G test) was 56.1 pg/mL (normal value < 100.5), and the *Aspergillus* galactomannan antigen test (GM test) result was 0.35 S/CO (normal value 0–0.5). On the same day, PMseq-DNA was conducted on the patient's dialysis effluent. One day later, 3052 sequences were detected for the *Mycobacterium tuberculosis complex*, whereas 3 sequences were detected for MTB. Because the extraction of MTB DNA is difficult and the detection rate of MTB in mNGS is low, specific sequences of the MTB complex are usually detected to increase the detection rate. The MTB complex refers to a group of mycobacteria with highly homologous genomes that can cause tuberculosis, including *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, and *Mycobacterium microtus*. If the specific DNA sequences of the MTB complex are detected, the possibility of pathogenic Mycobacterium infection is very high. If some specific sequences of MTB are detected, the possibility of MTB infection is very high, even if only one specific sequence is detected.

Main treatment

There were multiple changes in antimicrobials used in the management of this patient's care (Fig. 1). Initial dialysis effluent testing confirmed PDAD, and intraperitoneal Teicoplanin and Etimicin were used. Constipation was successfully treated with Mosapride and a glycerin enema. Intraperitoneal antibiotics were continued for a total of 7 days, and improvements in abdominal pain and dialysis effluent cell counts were noted. On the 8th day, the patient's abdominal pain was significantly relieved, and the dialysis effluent was slightly turbid. Antibiotics were reinitiated with intraperitoneal Teicoplanin and intravenous Etimicin. On the 9th day after admission, the patient developed a fever with a body temperature of 38.5 °C, which was subsequently reduced following the administration of physical cooling measures. On the 12th day, as the patient's abdominal pain intensified, the anti-infection treatment regimen was modified to include intraperitoneal Teicoplanin and Etimicin, along with intravenous Piperacillin. However, the patient's abdominal pain worsened on Day 14 with an increasing dialysis effluent cell count. Intraperitoneal Vancomycin, oral Moxifloxacin, and intravenous Fluconazole were added. On the 15th day, PMseq-DNA revealed that the number of MTB sequences was high, indicating tuberculous peritonitis. Considering the patient's symptoms of nausea and loss of appetite as well as the significant gastrointestinal side effects that Pyrazinamide may cause, the antituberculosis treatment plan was changed to oral Isoniazid, Rifampicin, and Ethambutol, supplemented with intravenous Levofloxacin. To reduce the potential liver damage caused by antituberculosis drugs, we routinely

take preventive measures by administering Bicyclol to patients. After the 20th day, the patient's abdominal pain was significantly relieved, the cell count of the dialysis effluent decreased significantly, and systematic standardized antituberculosis treatment was continued. After the 26th day, the patient's symptoms improved significantly, and the patient only sometimes experienced nausea, which was considered related to oral antituberculosis drugs. Vitamin B6 was administered to ameliorate the side effects of the antituberculosis drugs. The patient underwent three months of antituberculous treatment after being discharged because of severe gastrointestinal reactions.

Discussion

Continuous improvements in peritoneal dialysis equipment and technology have increased the survival rate of PD patients. However, the occurrence of complications during treatment cannot be ignored, the most common of which is PDAD. This condition is the direct or main cause of death in more than 15% of PD patients [2]. A diagnosis of tuberculous peritonitis is extremely rare among PD patients and is characterized by a low incidence and nonspecific symptoms. A systematic review of 216 cases of tuberculous peritonitis in PD patients revealed that the predominant clinical manifestations are abdominal pain (81.8%), fever (67.2%), and cloudy dialysis effluent (38%). Although distinct from the usual bacterial peritonitis presentation, specific symptoms include hypercalcemia (3.6%), weight loss (27.7%), extraperitoneal tuberculosis (30.1%), and a lymphocyte predominant peritoneal dialysate (31.6%) [3]. In this case, the patient did not present with distinctive symptoms of tuberculous peritonitis that differ from those of typical bacterial infections.

Tuberculous peritonitis is often associated with a significant time delay in diagnosis from the onset of symptoms to confirmation, with an average duration of 6.1 weeks [3]. Tuberculous peritonitis is often diagnosed in patients undergoing PD with a culture and smear of the dialysis effluent. Other rapid diagnostic techniques, such as dialysis effluent polymerase chain reaction (PCR) and peritoneal biopsy, are less commonly used, which may be related to their cost-effectiveness in clinical practice. The advantages and disadvantages of various diagnostic methods are shown in Table 2. Dialysis effluent culture plays a crucial role in diagnosing peritonitis because of its high specificity and ability to conduct antibiotic sensitivity testing. However, a major limitation of this method is the relatively long time required for culture, with MTB culture potentially needing up to 40 days of culture. Although dialysis effluent smears are less expensive and provide preliminary results more quickly, they have limitations in terms of detection sensitivity and specificity.

Table 2 Comparison of methods for diagnosing tuberculous peritonitis

Item	Advantage	Disadvantage	Consume time	Cost (USD)
Dialysis effluent smear	Simple, rapid, inexpensive.	Low sensitivity.	Approximately 2 h	8~12
Dialysis effluent culture	Definitive standard for diagnose, guides antibiotic selection.	Long incubation period, antibiotic exposure may affect sensitivity	6 weeks	83~98
Dialysis effluent neutrophil-to-lymphocyte ratio	Easily obtainable, especially in countries with moderate tuberculosis burden, acceptable sensitivity and specificity	Only <i>staphylococcus aureus</i> was used as a control and was not extended to other bacteria and fungi.	Approximately 3 h	1~5
Ascitic fluid adenosine deaminase (ADA)	Relatively fast, low cost, good sensitivity and specificity.	Elevated ADA in liver diseases and malignancies, may be misdiagnosed as tuberculosis	Approximately 3 h	1~2
Laparoscopy and tissue biopsy	Pathological examination possible, high accuracy	Invasive procedure, may cause bleeding and intra-abdominal adhesions.	Approximately 7 days	Approximately 280
Xpert MTB/RIF	Rifampicin resistance testing possible, provides reliable results quickly.	Requires specialized equipment and reagents.	Approximately 2 h	Approximately 17
PMseq-DNA	Wide detection range, including viruses, bacteria, fungi, and parasites, unaffected by antibiotic use.	High cost	24 h	463

The results of the smear examination are influenced by the volume of the sample: at least 5000 bacteria/mL are needed to detect mycobacteria. In contrast, culture positivity has a lower threshold for the number of bacteria, with a diagnosis possible with as few as 10 bacteria [4]. In addition, adenosine deaminase (ADA) and neutrophil-to-lymphocyte ratio (NLR) can also assist in the diagnosis of tuberculous peritonitis. Liu et al. evaluated 191 patients with suspected tuberculous peritonitis and reported that ADA in peritoneal effusion, with a cut-off value of 31.5 U/l, exhibited a sensitivity of 89.6% and specificity of 92.1%. Additionally, the ADA concentration in peritoneal effusion samples from tuberculous peritonitis patients was significantly greater than that in those from nontuberculous peritonitis patients and was positively correlated with the mycobacterial load [5]. Winston et al. demonstrated that an NLR < 15 in the dialysis effluent was the optimal cutoff value for differentiating tuberculous/nontuberculous peritonitis from bacterial peritonitis, with a sensitivity of 81% and a specificity of 70% [6]. Dialysis effluent PCR requires the design of specific short DNA segments (primers), which are tailored according to the unique gene sequences of the target microorganisms. Therefore, its limitation lies in the need to assume the type of pathogenic microorganism in advance. PCR can be used to detect low-concentration microorganisms, and this process is typically completed within a few hours. Xpert MTB/RIF, which uses heminested real-time PCR, enables the rapid detection of MTB and resistance to Rifampin. Vishal et al. conducted a systematic review of the diagnostic accuracy of Xpert MTB/RIF in abdominal tuberculosis and reported that Xpert

MTB/RIF has moderate sensitivity and good specificity for diagnosing peritoneal tuberculosis [7]. When ascitic fluid culture is used as a test indicator, the pooled sensitivity and specificity of Xpert MTB/RIF are 64% and 97%, respectively. Although laparoscopy and tissue biopsy are invasive methods for diagnosing tuberculous peritonitis, they have the advantage of a high diagnostic accuracy. Division et al. conducted a systematic review of tuberculous peritonitis and reported that when the macroscopic appearance of laparoscopy is combined with histological results, the sensitivity and specificity are 93% and 98%, respectively [4].

PMseq-DNA is a method based on metagenomic next-generation sequencing (mNGS) technology. mNGS is a powerful tool for analyzing the entire genomes of both human and microbial organisms in clinical samples. It allows the unbiased detection of various pathogenic microorganisms, including viruses, bacteria, fungi, and parasites [8]. PMseq-DNA offers a broad detection range, including 6350 bacterial species (including 133 Mycobacterium species and 122 Mycoplasma/Chlamydia/Rickettsia species), 1798 DNA viruses, 1064 fungi, and 234 parasites. PMseq-DNA testing, costing approximately 463 USD, enabled a diagnosis confirmation within just one day from the time the sample was submitted. It can be applied to various samples, such as cerebrospinal fluid, bronchoalveolar lavage fluid, bone marrow, and blood. The short turnaround time and wide detection range of PMseq-DNA make clinical diagnosis more convenient and reliable, especially for patients with complex conditions. Since its emergence in 2004, the cost of high-throughput or next-generation sequencing has decreased

by several orders of magnitude [9]. It has become a key technological platform for detecting microorganisms in clinical samples from patients. For some difficult to treat and critically ill patients, the use of high-throughput genomic sequencing of pathogenic microorganisms is advisable. In a comparative study of the diagnostic performance between mNGS and traditional culture methods, mNGS demonstrated greater sensitivity (50.7% vs. 35.2%), whereas the difference in specificity between the two methods was not significant (85.7% vs. 89.1%). Additionally, mNGS has a clear advantage in detecting MTB, viruses, anaerobic bacteria, and fungi and maintains a relatively high positive rate in samples exposed to antibiotics (52.7% vs. 34.4%), which further demonstrates the potential and value of mNGS in clinical diagnostics [10]. In light of the excellent performance of mNGS in detecting MTB, we hope that some foundations can support this testing to reduce its cost and make it a routine detection method in high-MTB burden countries.

PMseq-DNA has been applied in various clinical fields, including central nervous system infections, bloodstream infections, respiratory infections and gastrointestinal infections, demonstrating significant advantages over traditional detection methods. PMseq-DNA can be used to analyze a variety of sample types and is not affected by the patient's prior antibiotic use. Its extensive detection range eliminates the need to presume the type of pathogenic microorganism, and it can even detect new types of pathogens. The time from sample submission to result generation is only 24 h. Although the cost of detection is high at approximately 460 dollars, with the advancement of technology, mNGS is expected to become a routine detection method in the future.

Abbreviations

PDAD	Peritoneal dialysis-associated peritonitis
PD	Peritoneal dialysis
MTB	Mycobacterium tuberculosis
PMseq-DNA	Pathogenic microorganism DNA high-throughput genetic sequencing
ESKD	End-stage kidney disease
CRP	C-reactive protein
G test	1, 3-β-D-glucan test
GM test	Galactomannan antigen test
mNGS	Metagenomic next-generation sequencing
PCR	polymerase chain reaction

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Author contributions

R.X. conducted a literature search on the topic and drafted and revised the paper. W.G., T.H. and X.W. conducted a literature search on the topic and revised the paper. J.Z. gives guidance and revises the paper. Y.M. supervised the work and revised the paper.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Informed consent was obtained from the patient in both written and verbal formats to publish this case report and any accompanying images.

Competing interests

The authors declare no competing interests.

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