

Hybrid Capture-based Targeted Next-generation Sequencing for Pathogen Identification in Culture-Negative, Refractory and Relapsing Peritonitis Associated with Peritoneal Dialysis: A Case Series

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Abstract

Background: Culture-negative, refractory and relapsing peritoneal dialysis-associated peritonitis (PDAP) is challenging partly due to limited detection of atypical pathogens by traditional culture. Hybrid capture-based targeted next-generation sequencing (hc-tNGS) can improve pathogen identification in the complicated PDAP, including viral detection.

Methods: A retrospective case series was undertaken to evaluate the clinical significance of hc-tNGS in identifying pathogens in dialysis effluent of patients with culture-negative, refractory, or recurrent PDAP. Dialysis effluent specimens were tested by both traditional culture and hc-tNGS, and pathogen detection rates were compared.

Results: From October 2024 to December 2025, 14 patients were enrolled. The pathogen detection rate of hc-tNGS was higher than that of culture (79% vs 43%). In 6 of 8 culture-negative cases, hc-tNGS identified pathogens. Viral sequences were detected in 7 patients (50%); 6 had clinically relevant viral peritonitis. Virus-positive patients showed significantly lower serum C-reactive protein ($P=0.05$), peripheral white blood cell count ($P=0.02$), and effluent lactate dehydrogenase ($P=0.02$) than virus-negative patients. After treatment was adjusted based on hc-tNGS findings, including antiviral therapy, all 6 patients with clinically relevant viral peritonitis achieved clinical remission. Overall, 9 patients (64%) attained clinical remission, and 5 (35.7%) experienced technical failure.

Conclusion: Hc-tNGS technology has improved the ability to detect pathogens in complicated PDAP, particularly revealing that viral infection is a common yet often overlooked cause of culture-negative, refractory, and recurrent PDAP. Identifying this “low-inflammation” viral phenotype provides a preliminary basis for targeted antiviral treatment, which may further optimize clinical management strategies for high-risk PDAP patients.

Keywords: Peritoneal dialysis-associated peritonitis, hybrid capture-based targeted next-generation sequencing, viral peritonitis

1 Introduction

Peritoneal dialysis-associated peritonitis (PDAP) is a common and serious complication of peritoneal dialysis that compromises treatment efficacy and poses a life-threatening risk to patients [1]. Despite strict adherence to the International Society for Peritoneal Dialysis (ISPD) guidelines for standardized management, a proportion of PDAP cases remain culture-negative, unresponsive to empirical antibacterial therapy (refractory), or recur within a short period. These complex cases often pose significant challenges in diagnosis and treatment and are associated with poorer outcomes [2–3].

Currently, blood culture remains the gold standard for detecting PDAP pathogens, but its

positivity rate is limited, and it is virtually incapable of detecting atypical pathogens such as viruses, mycobacteria, and mycoplasmas [4]. Due to the lack of precise etiological evidence, clinicians typically resort to repeatedly adjusting or escalating antibiotic regimens for patients with culture-negative, refractory, or recurrent PDAP. Such blind antimicrobial therapy not only fails to effectively control nonbacterial infections but also increases the risk of antibiotic-related adverse effects and antimicrobial resistance [5]. As early as 1991, a study suggested that viral infections should be considered in patients with culture-negative peritonitis, particularly those unresponsive to antibiotics or with recurrent episodes [6]. However, due to the limitations of diagnostic methods at the time, this hypothesis long lacked direct support from pathogen evidence. Therefore, there is an urgent need to introduce new diagnostic tools with broader detection capabilities to expand our understanding of the complex pathogen spectrum in PDAP.

In recent years, molecular diagnostic technologies have provided new methods for pathogen detection in infectious diseases. Among these, hybrid capture-based targeted next-generation sequencing (hc-tNGS) technology, which reduces host background interference while balancing detection range and sensitivity, has been progressively applied to the diagnosis of various complex infections in clinical settings [7–12]. However, there is currently a lack of research using this technology as an adjunct diagnostic tool to elucidate the pathogen characteristics of complicated PDAP.

Accordingly, this study employed hc-tNGS to analyze peritoneal dialysis effluent samples from a cohort of patients with culture-negative, refractory and recurrent PDAP, aiming to characterize the pathogen spectrum, with a focus on evaluating viral detection rates and their correlation with clinical manifestations. We also explored the impact of adjusting treatment regimens based on hc-tNGS results on clinical outcomes, with the goal of providing preliminary evidence for the pathogen-based understanding, precision diagnosis and treatment of complicated PDAP, as well as evidence for previous hypotheses regarding the potential involvement of viruses in the pathogenesis of PDAP.

2 Methods

2.1 Study Design and Population

This study was a single-center, retrospective case series. It included adult PD patients hospitalized at the First Affiliated Hospital of Tianjin University of Traditional Chinese Medicine from October 2024 to December 2025.

Inclusion criteria were as follows: (1) Adult patients who were undergoing PD treatment; (2) Met the ISPD criteria for peritonitis, i.e., fulfilled at least two of the following three criteria: abdominal pain and/or cloudy dialysis effluent; dialysis effluent white blood cell (WBC) count $>100/\mu\text{L}$ and polymorphonuclear leukocyte (PMN) $\geq 50\%$ after a dwell time of ≥ 2 h; positive dialysis effluent culture; (3) Met at least one of the following types of peritonitis: culture-negative peritonitis (no microorganisms detected in dialysis effluent culture), refractory peritonitis (persistent cloudy bags or dialysis effluent white blood cell count $>100/\mu\text{L}$ after 5 days of appropriate antibiotic therapy), or recurrent peritonitis (reinfection with the same pathogen or culture-negative peritonitis occurring within 4 weeks of the completion of the previous treatment) [4]; (4) Consented to undergo hc-tNGS testing of peritoneal dialysis effluent. Exclusion criteria were as follows: catheter-associated peritonitis (co-occurring with exit site/tunnel infection and sharing the same pathogen, or culture-negative but clinically consistent with catheter-associated features), enteric peritonitis (caused by enteric factors such as inflammation, perforation, or

ischemia), chemical peritonitis (aseptic, PMN proportion < 50%, or symptoms resolved after dialysis effluent change), and patients who refused informed consent [4].

Demographic data, clinical presentations, laboratory findings, microbiological results, treatment regimens, and follow-up data were collected from electronic medical records. This study adhered to the principles of the Declaration of Helsinki, and was approved by the hospital's institutional review board (TYLL2026[Z]-020).

2.2 Culture Methods and Clinical Management

Upon admission, dialysis effluent cultures were performed by trained PD nurses in accordance with ISPD recommendations: 50 mL of dialysis effluent was aseptically collected after remaining in the peritoneal cavity for ≥ 2 hours; following centrifugation and concentration, the dialysis effluent was inoculated into separate aerobic and anaerobic culture bottles; the culture was considered negative if no growth was observed after 72 hours [13–14].

Treatment of PDAP followed ISPD guidelines, with antibiotics primarily administered intraperitoneally [4]. The empirical regimen for patients at their first visit typically consisted of first-generation cephalosporins or vancomycin combined with third-generation cephalosporins or aminoglycosides; patients transferred from other hospitals after treatment failure received an escalated antibiotic regimen based on the original protocol. The antibiotic regimen was dynamically adjusted based on clinical response, culture results, and hc-tNGS findings. Daily monitoring of WBC count and PMN proportion in the dialysis effluent was performed to assess resolution. If the WBC count in the dialysis effluent had decreased but remained above 100/ μ L on day 5, and the patient strongly wished to retain the catheter, the treatment course could be appropriately extended; if there was no resolution after escalated the antibiotics for 5 days, the catheter was removed and the patient was switched to hemodialysis. The duration of the antibiotic regimen was generally 2 or 3 weeks, depending on the type of pathogen.

2.3 Hc-tNGS Methods

After the dialysis effluent had remained in the peritoneal cavity for ≥ 2 hours, 20 mL of dialysis effluent was aseptically collected, stored at 4°C, and sent to a third-party clinical laboratory (Tianjin KingMed Medical Testing Laboratory, Tianjin, China) within 24 hours of collection. Hc-tNGS technology was used to perform nucleic acid testing on the samples. This technology provided broad-spectrum coverage of over 20,000 microorganisms and could specifically enrich more than 3,000 clinically common pathogens. The testing process included sample preparation, nucleic acid extraction, library construction, high-throughput sequencing, and bioinformatics analysis. All operations were strictly performed in accordance with standardized laboratory procedures. Sequencing results were comprehensively interpreted and analyzed by professionals in conjunction with negative controls and the patient's clinical background.

2.4 Definitions of Clinically Relevant Viral Peritonitis

Detection did not equate to infection. The definitive identification of the pathogen causing PDAP required consideration of clinical manifestations, microbial characteristics, and disease outcomes [15]. Therefore, this study predefined the criteria for diagnosing clinically relevant viral peritonitis as requiring the simultaneous fulfillment of the following four criteria:

- (1) Met the ISPD diagnostic criteria for PDAP;
- (2) Was unexplained by routine bacteriology/mycology: negative results from standard bacterial and fungal cultures, or poor clinical/cytological response following empirical antibiotic regimen (including refractory and recurrent peritonitis);

(3) Detection of viruses using hc-tNGS in peritoneal dialysis effluent, and meeting any one of the following interpretation criteria: (a) Pure viral infection: Only viruses were detected in the sample, and their sequence count or relative abundance was significantly higher than the background threshold. (b) Mixed infection: Both bacteria/fungi and viruses were detected simultaneously, but the virus accounted for the dominant proportion of the pathogen population in terms of sequence count and relative abundance. (4) Clinical symptom improvement and/or a decrease in white blood cell count in the dialysis effluent occurred following antiviral treatment.

2.5 Definition of Outcomes

The primary outcome was clinical remission, defined as resolution of abdominal pain, clarification of the dialysis effluent, and the peritoneal dialysis effluent WBC count < 100/μL. Secondary outcomes included technical failure (removal of the catheter and transition to hemodialysis due to inadequate control of the current peritonitis), recurrence/relapse (reoccurrence of peritonitis within 4 weeks after the end of treatment), and peritonitis-related death (death within 30 days after peritonitis or during hospitalization) [4].

2.6 Statistical Analysis

Statistical analysis was performed using SPSS 26.0 (IBM Corp, Armonk, NY, USA). Given the small sample size and the exploratory nature of this case series, the analysis was primarily descriptive. Continuous variables were expressed as median (IQR), and categorical variables as frequency (%). For comparisons between groups, the Mann–Whitney U test was used for continuous variables, and Fisher's exact test for categorical variables. All tests were two-sided, and exact P-values were reported. Due to the limited sample size, results were used only to generate hypotheses, and no correction for multiple comparisons was performed.

3 Results

3.1 Patients and Clinical Characteristics

The study ultimately included 14 patients with complicated peritonitis. The cohort consisted of 6 males (43%), with a median age of 56 years (IQR, 52.25–65.75) and a median dialysis duration of 34 months (IQR, 22.5–67.25), and all patients had concomitant hypertension. Further clinical characteristics are summarized in Table 1. The timing of hc-tNGS submission varied across patient subgroups. The overall principle was to submit samples on the first day of diagnosis at our hospital for culture-negative, refractory, or recurrent PDAP (Table 2). Prior to hc-tNGS testing, with the exception of recurrent patients, the median duration of antibiotic use in this cohort had reached 4.5 days (IQR, 4–7). However, WBC count in the peritoneal dialysis effluent had not yet returned to normal.

3.2 Hc-tNGS Results

Hc-tNGS significantly improved the pathogen detection rate (79% vs 43% for ascites culture). Among the 6 culture-positive patients, hc-tNGS results were highly consistent with culture in 5 cases. More importantly, among the 8 culture-negative patients, hc-tNGS successfully identified pathogens in 6 cases (75%). Detailed peritoneal dialysis effluent culture and hc-tNGS results are shown in Table 2.

Across the entire cohort, 7 cases (50%) tested positive for at least one virus, including CMV, EBV, HHV-6B, and HHV-7 (Figure S1, Figure 1A). Based on the predefined criteria, 6 cases (86%) were classified as clinically relevant viral peritonitis, while 1 case was classified as a non-clinically relevant infection due to the absolute predominance of bacteria (Table S1). All detected viral sequences in this study had a depth of ≥16, ruling out the possibility of sequencing

false positives.

3.3 Phenotype Stratification

3.3.1 Comparison between the Virus-positive Group and the Virus-negative Group

After grouping patients based on whether viruses were detected by hc-tNGS, the demographic and baseline clinical characteristics of the virus-positive group (n = 7) and the virus-negative group (n = 7) were generally similar (Table S2). Compared with the virus-negative group, the virus-positive group had a lower systemic inflammatory burden, as evidenced by lower serum CRP [19.7 (IQR, 16.12–43.82) vs 173.51 (IQR, 38.57–185.48) mg/L, P = 0.05] and peripheral blood white blood cell count [6.0 (IQR, 4.76–6.79) vs 9.72 (IQR, 8.20–15.04) $\times 10^9/L$, P = 0.02]. Additionally, LDH levels in peritoneal dialysis effluent were lower in the virus-positive group than in the virus-negative group [32.2 (IQR, 27.75–33.05) vs 110.4 (IQR, 35.45–226.4) $\times 10^9/L$, P = 0.02], suggesting that the extent of local peritoneal inflammation or tissue damage may have been milder. Regarding clinical outcomes, 7 (100%) patients in the virus-positive group all achieved clinical remission, whereas only 2 (29%) in the virus-negative group did so. Overall, the virus-positive group exhibited a phenotype characterized by “low systemic inflammatory burden and high remission rate”, though this may be related to the fact that the negative group included more high-risk pathogens (such as fungi and *Pseudomonas aeruginosa*).

3.3.2 Comparison between the Pure Viral Infection Group and the Bacterial-viral Mixed Infection Group

Among the 7 virus-positive patients, 4 had pure viral infections, and 3 had mixed bacterial-viral infections. Due to the extremely small sample sizes in both groups, the comparisons were limited to descriptive analysis, and no formal statistical inferences were made. Patients in the pure viral infection group were younger, had a shorter duration of dialysis, and had a lower prevalence of a history of PDAP (Table S3). Compared with the mixed infection group, the pure viral infection group exhibited milder local inflammation, as evidenced by lower peritoneal dialysis effluent WBC count [1,245 (IQR, 406.5–2,104) vs 10,190 (IQR, 5205–11946.5) cells/ μL] and a lower PMN proportion [0.55 (IQR, 0.29–0.83) vs 0.85 (IQR, 0.75–0.88)]. However, while both groups achieved 100% clinical remission, the time to negative peritoneal dialysis effluent in the pure viral infection group was longer [12 (IQR, 10.5–13) vs 5 (IQR, 5.0–6.5) days], suggesting that viral-associated inflammation may resolve more slowly or respond only partially to conventional antibiotic regimens.

3.4 Treatment and Follow-up

Based on the hc-tNGS results, we adjusted the patients' treatment regimens to provide precision-targeted therapy (Table S4). 6 patients (43%) newly diagnosed with clinically relevant viral infections all achieved clinical remission after the addition of antiviral treatment (Figure 2A). The remaining patients received antifungal treatment, escalated antibacterial regimens, or continued maintenance treatment based on the pathogen profile, with prophylactic antifungal agents added for some refractory patients in accordance with ISPD guidelines [4].

Overall, 9 patients (64%) in the cohort achieved clinical remission, 5 patients (35.7%) experienced technical failure, and there were no deaths (Figure 2B). The median time to negative peritoneal dialysis effluent WBC count in patients achieving clinical remission was 8 days (IQR, 5–11). To date, only one patient with *Staphylococcus aureus* infection repeated after 4 months; the remaining patients have had a favorable prognosis.

4 Discussion

In this study, we applied hc-tNGS technology to thoroughly investigate the etiology of this “complicated and challenging subgroup”, constituted by culture-negative, refractory, and recurrent PDAP. The results demonstrated that hc-tNGS significantly increased the pathogen detection rate from 43% with traditional culture to 79%. Notably, viral genomic sequences were detected in 50% of patients in this high-risk cohort, and 86% of these cases met our predefined “CRVP” diagnostic criteria. To our knowledge, this is the first case series to systematically elucidate the clinical phenotypes and treatment outcomes of viral PDAP using next-generation sequencing, suggesting that the role of viral etiology in PDAP has long been underestimated.

Viral-associated peritonitis is not a new concept. In 1986, Stnijk et al. first detected echovirus in a patient with culture-negative refractory peritonitis, and in 1991, Lewis et al. identified CMV in a patient with six relapsing episodes within six months, urging vigilance for viral infection in antibiotic-unresponsive and culture-negative PDAP [6, 16]. However, over the past 40 years, related reports have remained limited to sporadic cases (Table S5) [6, 16–21]. These historical cases shared several key characteristics: they predominantly involved female patients, were dominated by DNA viruses in the pathogen spectrum (Figure 1B), showed low PMN proportions in peritoneal dialysis effluent, responded poorly to antibiotic treatment, and improved with antiviral treatment or spontaneous resolution, which were largely consistent with our findings.

In recent years, metagenomic next-generation sequencing (mNGS) has been increasingly applied to PDAP diagnosis, offering higher detection rates and shorter turnaround times than traditional culture [5]. Four previous mNGS studies reported viral detection rates ranging from 5.9% to 35.5% (Table S6, Figure 1C), lower than the 50% observed here [13, 22–24]. This discrepancy likely stems from two factors: first, our inclusion criteria specifically enriched culture-negative, refractory, or relapsing cases, which are more likely to harbor non-bacterial pathogens; second, hc-tNGS employs probe-based capture and simultaneous DNA/RNA extraction, providing superior sensitivity for low-load and RNA viruses compared to conventional mNGS [25]. These findings indicate that viruses are not extremely rare pathogens in PDAP; a significant proportion of refractory or culture-negative cases may be driven by occult viral infections, with historically low detection rates reflecting limitations of traditional methods rather than true rarity.

Combining our data with previous literature, the Herpesviridae family dominates the viral spectrum associated with PDAP (86.3%), with CMV accounting for 61.6% (Figure 1D). This distribution reflects the unique biology of herpesviruses: CMV, EBV, HHV-6/7, and HSV establish lifelong latency in immune cells and readily reactivate in the setting of end-stage renal disease (ESRD)-related immune dysfunction [26–27]. We propose a “double-hit” mechanism for virus-associated PDAP: uremia-induced immunosenescence (including T-cell senescence, decreased T-cell receptor diversity, and B-cell apoptosis) impairs immune surveillance of latent viruses, lowering the reactivation threshold; concurrently, chronic peritoneal dialysis creates a pro-inflammatory peritoneal microenvironment that promotes intraperitoneal viral replication via infected leukocyte migration or endothelial cell tropism [28–30]. In contrast, enteric viruses likely result from compromised intestinal mucosal barriers, while Human pegivirus C and Hepatitis B virus may represent blood-borne bystander shedding. Detection of β/γ papillomaviruses warrants consideration of skin colonization or low-biomass sample contamination.

Importantly, viral “detection” does not equate to “pathogenicity.” Viral sequences may merely reflect latent infection, harmless shedding, or bystander effects. To maximize pathogen attribution

reliability, we developed the stricter CRVP framework: patients meeting ISPD peritonitis criteria with negative routine bacteriology/mycology and clear virological evidence in the peritoneal dialysis effluent require observation for clinical improvement following antiviral treatment. This framework emphasizes time-series interpretation and treatment response, enhancing clinical credibility. In our cohort, all patients who received antiviral treatment achieved remission without relapse, supporting hc-tNGS utility in guiding treatment adjustments. For refractory, culture-negative, or recurrent PDAP, diagnostic strategies should incorporate molecular viral screening rather than repeated traditional cultures alone, with prompt antiviral consideration to preserve catheter function. However, these findings remain at the "hypothesis-generating" level, as concurrent antibiotic adjustments or natural disease fluctuations may have influenced outcomes, requiring prospective validation with dynamic viral load monitoring.

We observed lower systemic and local inflammatory burdens at enrollment and higher remission rates in the virus-positive group, but this difference cannot be solely attributed to viral etiology, as the virus-negative group included high-risk pathogens (fungi, *Pseudomonas aeruginosa*) associated with worse outcomes. Notably, pure viral infections differed significantly from mixed viral-bacterial infections: pure viral cases were younger, had shorter dialysis duration and no prior peritonitis, and exhibited low PDE WBC/PMN counts. Despite milder initial inflammation, their time to recovery was significantly longer, consistent with viral pathophysiology—viruses primarily induce lymphocyte/monocyte responses and cytopathic effects, which resolve more slowly than bacterial endotoxin-triggered neutrophil storms [31–33]. Mixed infections occurred predominantly in older patients with longer dialysis duration and prior PDAP history, potentially reflecting bacterial inflammation triggering viral reactivation or chronic viral latency impairing peritoneal immune defenses and increasing bacterial susceptibility [34–35].

Based on these findings, we recommend including viral infection in the differential diagnosis of PDAP and considering molecular sequencing in three scenarios: (1) negative traditional cultures; (2) persistent PDE leukocytosis after 5 days of empirical antibiotics; (3) relapsing peritonitis within 4 weeks of antibiotic discontinuation. If hc-tNGS/mNGS detects viral sequences without alternative microbiological explanation, experimental antiviral treatment may be initiated under close monitoring, with WBC count in the effluent as a short-term efficacy indicator. Sequence detection should serve as a supplement to standard culture rather than a substitute, and clinical application must integrate microbiological results with overall clinical judgment (Figure 3).

This study has several limitations. First, as a single-center, retrospective, small-sample case series, it is primarily intended to generate hypotheses and cannot accurately estimate the true prevalence of virus-associated peritonitis in the general PDAP population, and the statistical power for subgroup comparisons is also limited. Second, the lack of concurrent peripheral blood viral load data restricts comparative analysis of systemic versus local viral replication. Third, there is currently a lack of universally accepted molecular diagnostic gold standards for pathogen identification, such as specific thresholds for metrics like the number of sequenced reads, genome coverage, or microbial abundance. Fourth, antiviral therapy was not administered randomly, and its efficacy may have been influenced by concurrent antibiotic adjustments and temporal trends. Finally, testing costs, turnaround time, and cost-effectiveness have not been evaluated, limiting the widespread adoption of hc-tNGS. Future multicenter prospective studies are needed to determine whether hc-tNGS guided antiviral treatment can improve technical survival and reduce the risk of

recurrence, using predefined trigger algorithms combined with dynamic quantitative PCR validation, control strategies, and cost-effectiveness analyses.

5 Conclusion

In conclusion, the etiological role of viruses has been long underestimated in culture-negative, refractory, and recurrent PDAP. Hc-tNGS provides incremental diagnostic information beyond traditional culture and, within a framework integrating alternative etiology exclusion and treatment response assessment, helps identify patients who may benefit from antiviral therapy. These findings provide preliminary evidence for etiological stratification and precision therapy in complex PDAP. However, their causality and clinical benefits still require further validation through prospective, multicenter studies.

References

- [1] Kraft L, Oppold J, Kitterer D, Kimmel M, Twardowski L, Biegger D, et al. Changing microbiology and outcomes of PD-associated peritonitis over four decades. *Clin Kidney J*. 2025;19(1):sfaf387.
- [2] Chienwichai K, Sangaew S, Chuachanpipat L, Chang A. Comparison of clinical outcomes between culture-negative and positive peritonitis in patients undergoing maintenance peritoneal dialysis: a prospective cohort study. *BMC Nephrol*. 2023;24(1):340.
- [3] Wang AH, Sawyer K, Shah AD. Persistent peritonitis in peritoneal dialysis: a comprehensive review of recurrent, relapsing, refractory, and repeat peritonitis. *Int Urol Nephrol*. 2024;56(2):583-595.
- [4] Li PK, Chow KM, Cho Y, Fan S, Figueiredo AE, Harris T, et al. ISPD peritonitis guideline recommendations: 2022 update on prevention and treatment. *Perit Dial Int*. 2022;42(2):110-153.
- [5] Dong X, Xie C, Zhang Z, Ye H, Liu R, Chen W, et al. Diagnostic challenges in peritoneal dialysis-associated peritonitis with atypical and rare pathogens: A new era of metagenomic next-generation sequencing precision diagnosis. *Perit Dial Int*. 2025;May 19:8968608251333879.
- [6] Lewis SL. Recurrent peritonitis: evidence for possible viral etiology. *Am J Kidney Dis*. 1991;17(3):343-345.
- [7] Diao Z, Han D, Zhang R, Li J. Metagenomics next-generation sequencing tests take the stage in the diagnosis of lower respiratory tract infections. *J Adv Res*. 2021;38:201-212.
- [8] Li C, Zhong X, Guo S, Liu F, Song Y, Zhao Z, et al. A case report of rapid diagnosis of *Sporothrix globosa* infection using MetaCAP. *Front Med (Lausanne)*. 2025;12:1644400.
- [9] Ma X, Wang Y, Wu Q, Ma X, Wang Q, Guo Q. Brucellosis infection complicated with myelitis: a case report and literature review. *Front Cell Infect Microbiol*. 2024;14:1378331.
- [10] Cai S, Yuan J, Li Y, Guo F, Lin Z, Li H, et al. Etiological diagnostic performance of probe capture-based targeted next-generation sequencing in bloodstream infection. *J Thorac Dis*. 2024;16(4):2539-2549.
- [11] Zeng H, Miao W, Liang S, Wang W, Zhang J. Recurrent septic arthritis caused by *Gemella morbillorum*: a case report and literature review. *BMC Infect Dis*. 2024;24(1):1332.
- [12] Long BQ, Long Q, Lai MY, Yang L, You FR, Guo HW. *Mycobacterium marinum* cutaneous infection misdiagnosed as sporotrichosis in a patient with systemic lupus erythematosus: A case report. *Heliyon*. 2024;10(14):e34444.
- [13] Ye P, Xie C, Wu C, Yu C, Chen Y, Liang Z, et al. The application of metagenomic next-generation sequencing for detection of pathogens from dialysis effluent in peritoneal

- dialysis-associated peritonitis. *Perit Dial Int.* 2022;42(6):585-590.
- [14] Piraino B, Bailie GR, Bernardini J, Boeschoten E, Gupta A, Holmes C, et al. Peritoneal dialysis-related infections recommendations: 2005 update. *Perit Dial Int.* 2005;25(2):107-131.
- [15] Mancini A, Todd L. Inconsistencies in ISPD peritonitis recommendations: 2016 Update on prevention and treatment and The ISPD catheter-related infection recommendations: 2017 update. *Perit Dial Int.* 2018;38(4):309–10.
- [16] Struijk DG, van Ketel RJ, Krediet RT, Boeschoten EW, Arisz L. Viral peritonitis in a continuous ambulatory peritoneal dialysis patient. *Nephron.* 1986;44(4):384.
- [17] Yakulis R, Babinchak TJ. Herpes simplex peritonitis: case report. *Clin Infect Dis.* 1999;28(6):1212-1215.
- [18] Liesker J, van Elsacker-Niele AM, Blanken R, Halma C. A cloudy bag and genital ulcers. *Clin Nephrol.* 2006;65(5):378-379.
- [19] Pauwels S, De Moor B, Stas K, Magerman K, Gyssens IC, Van Ranst M, et al. Cocksackievirus B1 peritonitis in a patient treated with continuous ambulatory peritoneal dialysis: a case report and brief review of the literature. *Clin Microbiol Infect.* 2012;18(10):E431-E434.
- [20] Dhoot A, Auguste B, Ng J, Genis H, Andany N, Tanna G. A Case Report of Herpes Simplex Virus Associated Peritoneal Dialysis Peritonitis With Novel Use of Intraperitoneal Acyclovir. *Kidney Med.* 2025;7(4):100968.
- [21] Deng C, Chen J, Liu J, Zhang W, Zhang H, Li Z. Peritoneal Dialysis-Associated Peritonitis Caused by *Lautropia Mirabilis* and Concurrent Viral Infection: A Case Report. *Case Rep Nephrol Dial.* 2025;15(1):269-277.
- [22] Burnham P, Chen F, Cheng AP, Srivatana V, Zhang LT, Edusei E, et al. Peritoneal Effluent Cell-Free DNA Sequencing in Peritoneal Dialysis Patients With and Without Peritonitis. *Kidney Med.* 2021;4(1):100383.
- [23] Guo SS, Fu G, Hu YW, Liu J, Wang YZ. Application of metagenomic next-generation sequencing technology in the etiological diagnosis of peritoneal dialysis-associated peritonitis. *Open Life Sci.* 2024;19(1):20220865.
- [24] Li A, Chen Z, Deng Q, Zheng C, Hu H, Ye Z. Clinical Value of Metagenomic next-generation Sequencing in Early Diagnosis of Peritoneal Dialysis-Associated Peritonitis: A Randomised Controlled Observational Trial. *Nephrology (Carlton).* 2025;30(8):e70094.
- [25] Yin Y, Zhu P, Guo Y, Li Y, Chen H, Liu J, et al. Enhancing lower respiratory tract infection diagnosis: implementation and clinical assessment of multiplex PCR-based and hybrid capture-based targeted next-generation sequencing. *EBioMedicine.* 2024;107:105307.
- [26] Jordan MC, Jordan GW, Stevens JG, et al. Latent herpesviruses of humans. *Ann Intern Med.* 1984;100(6):866-880.
- [27] Winkelspecht B, Mueller-Lantzsch N, Köhler H. Serological evidence for reactivation of EBV infection due to uraemic immunodeficiency. *Nephrol Dial Transplant.* 1997;12(10):2099-2104.
- [28] Wu W, Song A, Xie K, et al. Characteristics of T cell premature senescence in maintenance hemodialysis patients. *Inflamm Res.* 2024;73(8):1299-1309.
- [29] Peroumal D, Jawale CV, Choi W, et al. The survival of B cells is compromised in kidney disease. *Nat Commun.* 2024;15(1):10842.
- [30] Adler B, Sattler C, Adler H. Herpesviruses and Their Host Cells: A Successful Liaison. *Trends Microbiol.* 2017;25(3):229-241.

- [31] Jukema BN, de Hond TAP, Kroon M, Maranus AE, Koenderman L, Kaasjager KAH. Point-of-care neutrophil and monocyte surface markers differentiate bacterial from viral infections at the emergency department within 30 min. *J Leukoc Biol.* 2024;115(4):714-722.
- [32] Liu X, Liu C, Zhang T. The Immunoregulatory Mechanisms of Human Cytomegalovirus from Primary Infection to Reactivation. *Pathogens.* 2025;14(10):998.
- [33] Kiers D, Koch RM, Hamers L, Gerretsen J, Thijs EJ, van Ede L, et al. Characterization of a model of systemic inflammation in humans in vivo elicited by continuous infusion of endotoxin. *Sci Rep.* 2017;7:40149.

Table 1. Baseline characteristics of the complicated PDAP patients (n = 14).

Characteristic	Value
Age, years	56 [52.25-65.75]
Sex, male	6 (43%)
Duration of dialysis, months	34 [22.5-67.25]
Comorbid	
Hypertension	14 (100%)
Diabetes mellitus	5 (36%)
Cardiovascular disease	4 (29%)
Previous immunosuppressants using	3 (21%)
Previous peritonitis, times	0 [0-1]
Laboratory tests, serum	
CRP, mg/L	38.57 [18.65-159.45]
WBC count, 10 ⁹ /L	7.3 [5.53-9.46]
Neutrophil count, 10 ⁹ /L	5.31 [4.53-7.31]
Lymphocyte count, 10 ⁹ /L	0.79 [0.53-1.44]
Monocyte count, 10 ⁹ /L	0.46 [0.35-0.64]
Creatinine, mg/dL	786.64 [672.55-901.05]
GFR, mL/min/1.73 m ²	5 [4-6]
Albumin, g/dL	26.9 [25.7-30.75]
Laboratory tests, effluent	
Day 1 effluent WBC count, 10 ⁶ /L	1750 [332.25-5818.75]
Day 1 effluent PMN proportion	0.83 [0.66-0.90]
Day 1 effluent GLU, mmol/L	20.38 [16.45-33.05]
Day 1 effluent LDH, U/L	34.15 [31.3-93.43]
Day 1 effluent ADA, U/L	2 [1.25-2.75]

Values for continuous variables given as median [IQR], and for categorical variables as count (percentage). Abbreviation: CRP, C-reactive protein; GFR, glomerular filtration rate; GLU, glucose; LDH, lactate dehydrogenase; ADA, adenosine deaminase.

P.S: In this study, patients with negative culture results, refractory, or relapsing PDAP are collectively referred to as patients with complicated PDAP.

Table 2. Peritoneal dialysis effluent culture results, hc-tNGS submission status, test results, treatment regimens and outcomes for the complicated PDAP patients (n = 14).

Patient no.	Effluent culture results	Submission for hc-tNGS		Hc-tNGS findings			
		Date of submission	Timing for submission	Name of pathogen	Sequence number	Relative abundance	Coverage
1	-	Day 4	Culture negative	Aspergillus niger	1647	99.95%	0.24%
2	-	Day 4	Culture negative	-	-	-	-
3	-	Day 4	Culture negative	CMV	65	100%	0.85%
4	-	Day 1	Relapsing (20 days after stopping the medication) Refractory (The 10-day course of antibiotic treatment at the previous hospital was ineffective.)	-	-	-	-
5	-	Day 1	Relapsing (4 days after stopping the medication) Refractory (The 9-day course of antibiotic treatment at the previous hospital was ineffective.)	EBV	244	100%	3.98%
6	Staphylococcus aureus	Day 1	Relapsing (4 days after stopping the medication) Refractory (The 9-day course of antibiotic treatment at the previous hospital was ineffective.)	Staphylococcus aureus	7512	99.21%	2.29%
7	Staphylococcus capitis	Day 1	Relapsing (4 days after stopping the medication) Refractory (The 9-day course of antibiotic treatment at the previous hospital was ineffective.)	-	-	-	-
8	Pseudomonas aeruginosa	Day 1	Relapsing (4 days after stopping the medication) Refractory (The 9-day course of antibiotic treatment at the previous hospital was ineffective.)	Pseudomonas aeruginosa	3462	98.42%	2.74%
9	-	Day 4	Culture negative	EBV	510	98.01%	11.85%
10	-	Day 1	Relapsing (3 days after stopping the medication) Refractory (The 7-day course of antibiotic treatment at the previous hospital was ineffective.)	HHV-7	375	89.27%	9.11%
11	Escherichia coli	Day 1	Relapsing (3 days after stopping the medication)	Escherichia coli	401	30.98%	0.27%
				HHV-6B	67707	99%	57.50%
				Corynebacterium	21686	80.89%	5.59%
12	-	Day 4	Culture negative	lipophiloflavum			
				EBV	20	53%	1.81%
				CMV	16	42%	1.03%
				CMV	111	93%	1.75%
13	Staphylococcus epidermidis	Day 1	Relapsing (12 days after stopping the medication) Refractory (The 5-day course of antibiotic treatment after admission was ineffective.)	Staphylococcus epidermidis	101	34%	0.17%
14	Pseudomonas aeruginosa	Day 5	Relapsing (12 days after stopping the medication) Refractory (The 5-day course of antibiotic treatment after admission was ineffective.)	Pseudomonas aeruginosa	37966	100%	5.34%

Abbreviation: CMV, Cytomegalo virus; EBV, Epstein-Barr virus; HHV-7, Human herpes virus-7; HHV-6B, Human herpes virus-6B; IP, intraperitoneal; Qd, once a day; Bid, twice a day; PO, oral administration.

Figure 1. Viral spectra detected in PDAP patients. (a) Viral spectrum of PDAP patients in this case series; (b) Viral spectrum of PDAP patients with confirmed or suspected virus-associated peritonitis from previous reports; (c) Viral spectrum detected in PDAP patients using mNGS in recent years; (d) Viral spectrum detected in PDAP patients across all studies.

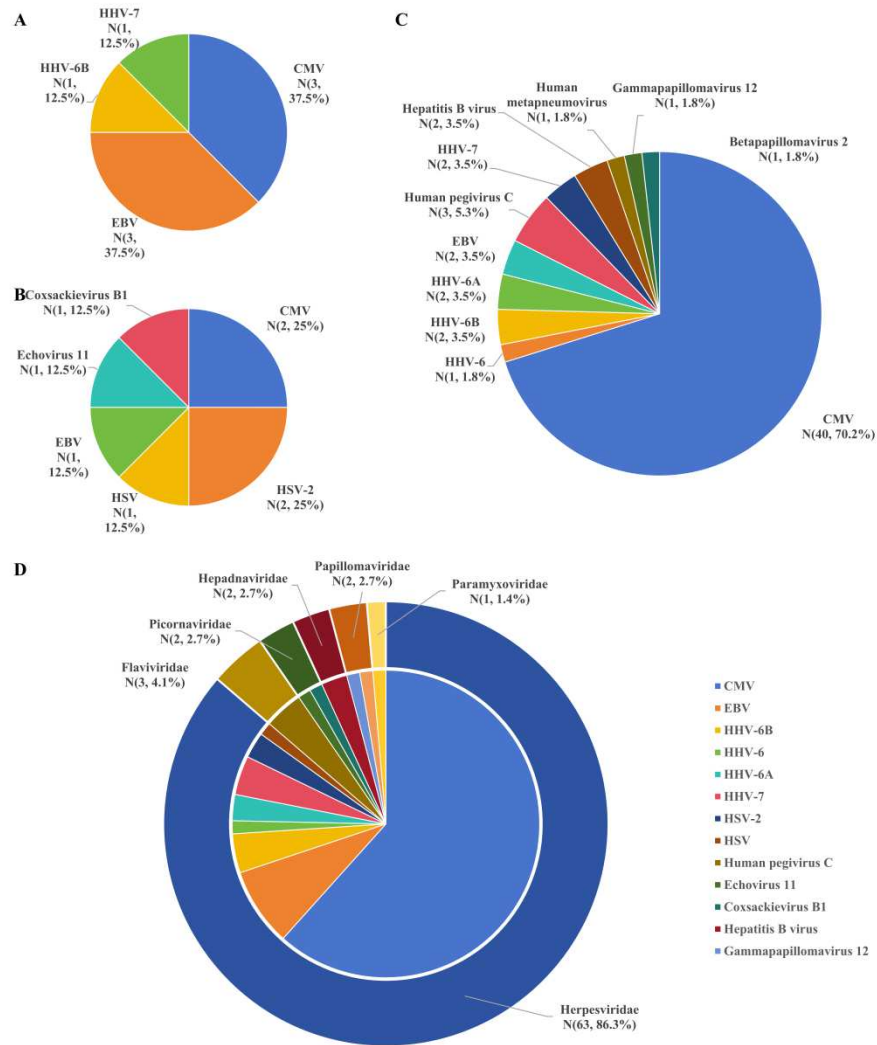


Figure 2. Clinical decision points and prognosis in the complicated PDAP patients (n = 14). (A) Changes in white blood cell counts in peritoneal dialysis effluent before and after antiviral treatment in patients with CRVP (n=6). (B) Swimlane diagram of decision points and prognosis in the complicated PDAP patients (n = 14).

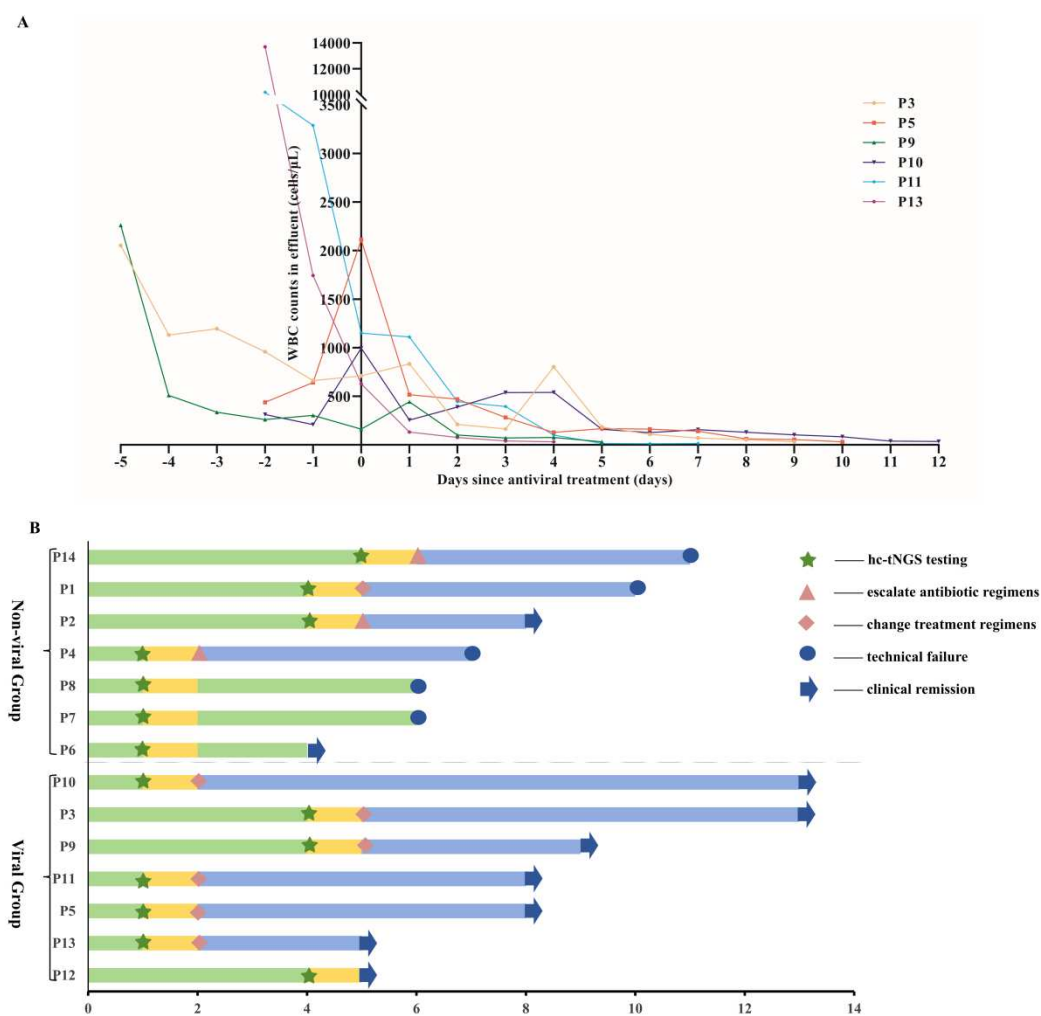
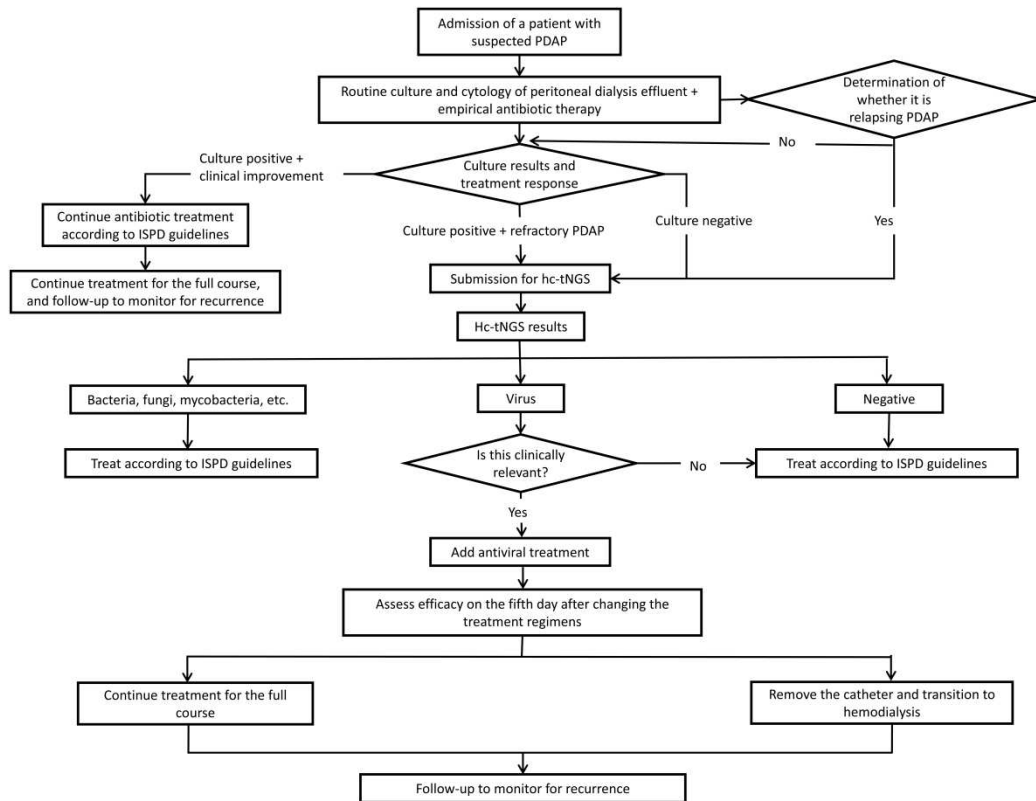


Figure 3. Clinical management flowchart for PDAP patients.



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