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The Application Value of Droplet Digital PCR Technology in the Diagnosis of Bacterial Infections in Febrile Patients

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Abstract: Droplet digital PCR (ddPCR), as the third-generation PCR technology, demonstrates significant advantages in the etiological diagnosis of infectious diseases due to its absolute quantification, ultra-high sensitivity, and multiplex detection capabilities. This article reports a case of a patient with fever of unknown origin, where ddPCR rapidly confirmed a drug-resistant bacterial infection and dynamically monitored treatment efficacy. Combining literature evidence, this paper systematically elaborates on the technical principles, clinical performance, and practical value of ddPCR in febrile patients.

Keywords: Droplet digital PCR; Bacterial infection; Nucleic acid detection; Drug resistance gene

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1. Introduction

Fever poses a significant diagnostic challenge in clinical practice. Traditional methods like blood culture are time-consuming and lack sensitivity, while metagenomic next-generation sequencing (mNGS) is costly and may miss low-biomass infections. Droplet digital PCR (ddPCR), as a third-generation PCR technology, offers absolute quantification and exceptional sensitivity, enabling the detection of rare pathogens. This article reports a case of fever of unknown origin where ddPCR rapidly identified a drug-resistant bacterial infection and guided treatment, highlighting its clinical utility.

2. Case information

The patient, a 78-year-old female, was admitted to the hospital on December 28, 2024, due to "persistent cough and expectoration for over three months, exacerbated by dyspnea for 12 days".

2.1. Past medical history

She was diagnosed with "gouty arthritis and Alzheimer's disease" in 2020, suffered a "cerebral infarction" in October 2023, resulting in impaired limb movement, as well as speech and swallowing difficulties; she was diagnosed with "sepsis, severe pneumonia, type II respiratory failure, and severe malnutrition" in January 2024, and the infection was controlled after treatment. Physical examination revealed a temperature of 39.0 ℃, blood pressure of 102/56 mmHg (maintained with norepinephrine), indwelling gastric tube and nasotracheal intubation, anemic appearance, coarse breath sounds in both lungs, a small number of crackles in the upper lungs, and a few moist rales in the lower lungs, as well as mild pitting edema in both lower extremities. Blood tests indicated a WBC count of 37.4×10^9/L, with 84.2% neutrophils, CRP of 183 mg/L, and PCT of 2.5 ng/mL.

2.2. Preliminary examinations

Chest CT showed pneumonia in both lungs, fibrotic lesions in the right upper lung, progression of inflammation in the left lower lobe compared to previous scans, segmental atelectasis in the dorsal and posterior basal segments of the left lower lobe, and bronchial mucus plugging in the left lower lobe; blood culture (negative at 48 hours). Multidrug-resistant *Acinetobacter baumannii* was cultured from bronchoalveolar lavage fluid. Echocardiography revealed mild mitral and tricuspid regurgitation, decreased left ventricular compliance, and left ventricular systolic function below the normal range. Treatment with broad-spectrum antibiotics (piperacillin-tazobactam combined with levofloxacin sodium chloride injection) for 7 days was ineffective.

3. Detection methods

3.1. ddPCR detection process

3.1.1. Sample processing

Collect 5 mL of lung lavage fluid from patients and utilize the fully automated nucleic acid detection reaction system construction system AP10 and the droplet digital PCR system D3207 produced by Pioneer Genomics Technology Co., Ltd. to complete nucleic acid extraction, amplification, and analysis through digital PCR microdroplet chips.

3.1.2. Technical parameters

The amplification time is 0.5 hours, and the lower limit of detection is 1 copy/μL.

3.2. Comparison with traditional methods

Lung lavage fluid culture and blood culture (using the BACTEC 9120 system).

4. Results

4.1. Etiological diagnosis

4.1.1. Lung lavage fluid ddPCR results

Pseudomonas aeruginosa, 309,315.00 copies/μL; *Acinetobacter baumannii*, 21,680.00 copies/μL; *Streptococcus* spp., 161,335.00 copies/μL; *Stenotrophomonas maltophilia*, > 1,000,000 copies/μL.

4.1.2. Lung lavage fluid and blood culture

Still remain negative after 72 hours.

4.2. Treatment adjustment and monitoring

Based on the ddPCR results, imipenem/cilastatin (0.5 g q6h) was switched to in combination with tigecycline (initial dose of 100 mg, followed by 50 mg q12h starting from the second dose). On the 7th day of treatment, the body temperature dropped to 37.8 ℃. On the 14th day, the body temperature returned to normal, vasoactive drugs were withdrawn, and the patient was weaned off ventilator-assisted breathing. Repeated ddPCR tests showed *Pseudomonas aeruginosa* at 19,940.00 copies/μL, *Acinetobacter baumannii* at 0 copies/μL, *Streptococcus* spp. at 12,790.00 copies/μL, and *Stenotrophomonas maltophilia* at 120,880.00 copies/μL. Imaging revealed scattered pneumonia in both lungs, with fibrotic lesions in the right upper lobe. Repeated examination showed that the inflammation in the left lower lobe had been absorbed compared to before, and the dorsal segment and posterior basal segment of the left lower lobe were no longer present, indicating that the lung tissue had re-expanded.

5. Discussion

Traditional methods for detecting pneumonia pathogens (such as culture and serological testing) have limitations such as time-consuming processes and low sensitivity (approximately 20–30%). Isolating and culturing pathogens requires at least 1 to 3 days to obtain results, with a low detection rate [1,2]. ddPCR offers the following three technological advantages: Firstly, ddPCR boasts advantages of ultra-high sensitivity, high specificity, and rapid detection, along with technical characteristics such as high tolerance, absolute quantification, and independence from standard curves. It can reduce the limit of detection to 1 copy/μL, which is a 100-fold improvement over traditional PCR, making it particularly suitable for screening pathogens with low viral loads [3,4]. For instance, in the studies conducted by Shen Jiang and Zhao Dongyang, ddPCR was able to detect rare mutations or pathogens with low viral loads as low as 0.001%, achieving a sensitivity of 88.89% and a specificity of 55.61%. It is applicable for detecting latent infections in elderly patients under immunosuppressive conditions, with a reporting time of 3–4 hours, significantly shortening the diagnostic time [5]. Secondly, ddPCR enables multi-target joint detection, allowing for the simultaneous detection and accurate identification of multiple pathogens and drug-resistant genes, including 12 types of bacteria and 1 type of fungus, namely *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus* spp., *Candida* spp., *Streptococcus* spp., *Stenotrophomonas maltophilia*, *Enterobacter cloacae*, *Proteus mirabilis*, coagulase-negative *staphylococci*, and *Serratia marcescens*. The five drug-resistance genes include KPC, mecA, OXA-48, NDM/IMP, and vanA/vanM, covering common drug-resistance genes for three major classes of antibiotics: carbapenems, methicillin, and vancomycin. In contrast, internationally leading technologies such as the BioFire® Pneumonia Panel can simultaneously detect 33 pathogens and drug-resistance genes [6]. Thirdly, ddPCR possesses dynamic monitoring capabilities, enabling the construction of a pathogen load-efficacy model through absolute quantification (such as the correlation between a 50% reduction in pathogen load after 72 hours of treatment and 28-day survival rates [7]). By integrating artificial intelligence and big data analysis techniques, it can deeply mine vast amounts of pathogenic bacteria genetic data, identify new drug-resistant genes and mutant strains of pathogens, and provide support for precision medicine. The pathogens causing pneumonia in elderly patients are complex, with mixed infections of bacteria (such as Klebsiella pneumoniae), viruses (such as influenza viruses),

and fungi being common. The repeated use of multiple antibiotics results in a low detection rate of infections by traditional culture methods, with only 15%. In contrast, ddPCR can simultaneously identify bacteria, fungi, and viruses, increasing the detection rate to over 40%. The detection sensitivity of the digital PCR platform reaches at least 0.1%, enabling precise detection of ultra-trace amounts of pathogens and drug-resistant gene information, thereby improving the detection rate of mixed infections. Research conducted at Nanjing Drum Tower Hospital shows that ddPCR achieves a mixed pathogen detection rate of 23.3% in bloodstream infections, significantly outperforming blood culture (9.1%) [2]. Based on the advantage of ddPCR technology in detecting drug-resistant genes, it can rapidly screen for the types and load information of drug-resistant genes, guiding early targeted treatment with carbapenems or vancomycin. The team led by Qu Hongping verified that ddPCR can accurately detect the sensitivity and specificity of carbapenem-resistant genes (such as blaKPC and blaNDM) at 84.9% and 92.5%, respectively, and methicillin-resistant genes (mecA), providing results 3–5 days earlier than phenotypic drug susceptibility testing [8]. Not only blood samples can be used for detection; companies like Linghang Gene Technology are compatible with cerebrospinal fluid, bronchoalveolar lavage fluid, pleural fluid, and ascites, offering operability and accuracy in detecting pathogens in elderly patients with respiratory diseases. Due to prolonged hospitalization and antibiotic exposure, elderly patients have a high infection rate with drug-resistant bacteria, reaching 35–50%. ddPCR completes detection within 3–4 hours, reducing the time by 90% compared to traditional culture methods. Moreover, ddPCR can simultaneously detect carbapenemase genes (such as blaKPC and blaNDM-1) and methicillin-resistant genes (mecA), clarifying the resistance mechanism within 2–3 hours and securing a critical time window for anti-infective treatment. Research at Ruijin Hospital confirmed that for infections caused by Klebsiella pneumoniae carrying blaKPC, adjusting the dosage of carbapenems based on ddPCR results increased the treatment effectiveness rate from 52% to 82%, shortened the antibiotic adjustment time to within 6 hours, and reduced mortality by 27% [9]. Additionally, the multiplex detection kit can cover six common types of drug-resistant genes, including beta-lactams and fluoroquinolones, providing a molecular basis for the selection of narrow-spectrum antibiotics. Leveraging the absolute quantification capability of ddPCR, the overuse of broad-spectrum antibiotics can be avoided, and hospital stays can be shortened. In this case, the treatment cycle was reduced from an estimated 28 days to 14 days, offering a quantitative basis for evaluating treatment efficacy. However, ddPCR also has certain limitations. For instance, the qualification rate of sputum sample collection is only 58%, leading to an increased false-positive rate of nucleic acid detection, which reaches 12% [10]. The absence of clinical guidelines and recommendations results in variations in the elements and criteria for performance validation among different laboratories, leading to discrepancies in clinical usage outcomes. Therefore, it is necessary to compare the detection results among digital PCR platforms with different liquid dispensing principles. The consistency of detection results between droplet digital PCR methods and traditional detection methods also requires extensive comparative and validation experiments [11].

6. Conclusion

This case demonstrates that ddPCR offers core advantages of rapidity, precision, and quantifiability in diagnosing bacterial infections in elderly patients with fever, particularly in cases with negative blood cultures or complicated and severe infections. With the promotion of domestic equipment (such as the Xinyi D50, Linghang AD3207, and AD9600) and the advancement of multicenter studies, ddPCR is expected to become a first-line tool for the etiological diagnosis of fever, propelling the field of infectious diseases into the era of precision medicine.

Disclosure statement

The author declares no conflict of interest.

References

[1] Respiratory Disease Branch of the Chinese Medical Association, 2016, Guidelines for the Diagnosis and Treatment of Community-Acquired Pneumonia in Chinese Adults (2016 Edition). Chinese Journal of Tuberculosis and Respiratory Diseases, 39(4): 253–279.

[2] Li M, Zhao L, Zhu Y, et al., 2023, Clinical Value of Droplet Digital PCR in the Diagnosis and Dynamic Monitoring of Suspected Bacterial Bloodstream Infections. Clinica Chimica Acta; International Journal of Clinical Chemistry, 550: 117566.

[3] Xu W, Song D, Chen D, et al., 2021, Research Progress on Pathogen Detection Technology Based on the Biosensing Principle of CRISPR/Cas. Chinese Journal of Biotechnology, 41(8): 67–74.

[4] Liu J, Yan S, 2021, Next-Generation Pathogen Nucleic Acid Detection Technology Based on the CRISPR-Cas System. International Journal of Biologicals, 44(5): 241–247.

[5] Jiang S, Zhao D, Wang C, et al., 2024, Clinical Evaluation of Droplet Digital PCR in the Early Identification of Suspected Sepsis Patients in the Emergency Department: A Prospective Observational Study. Frontiers in Cellular and Infection Microbiology, 14: 1358801.

[6] Ning Y, Bai Z, Wang H, 2021, Etiological Examination and Pathogen Spectrum of Adult Pneumonia. Chinese Journal of Laboratory Medicine, 44(2): 175–178.

[7] Shao Z, Zhu J, Wei Y, et al., 2022, Pathogen Load and Species Monitored by Droplet Digital PCR in Patients with Bloodstream Infections: A Prospective Case Series Study. BMC Infectious Diseases, 22(1): 771.

[8] Qu H, Tan R, 2022, Clinical Application of Prevention and Control Mode for Drug-Resistant Bacterial Infections in the Intensive Care Unit. Chinese Journal of Infection Control, 21(12): 1161–1163.

[9] Hu B, Tao Y, Shao Z, et al., 2021, A Comparison of Blood Pathogen Detection Among Droplet Digital PCR, Metagenomic Next-Generation Sequencing, and Blood Culture in Critically Ill Patients with Suspected Bloodstream Infections. Frontiers in Microbiology, 12: 641202.

[10] Mei H, Tang Y, Yang H, et al., 2019, Analysis of Clinical Characteristics, Pathogen Distribution, and Risk Factors Affecting Mortality in Elderly Patients with Severe Pneumonia. Practical Preventive Medicine, 26(3): 352–354.

[11] Guan M, Guo W, Liu W, et al., 2019, Clinical Applications and Challenges of Digital PCR. International Journal of Laboratory Medicine, 40(14): 1665–1669 + 1673.