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Letter to the Editor

Third-generation nanopore sequencing for rapid diagnosis of *Pneumocystis jirovecii* pneumonia and co-pathogens

Dear Editor,

We read with great interest the article by Szvalb et al., which examined the correlation between serum (1,3)-β-D-glucan (BDG) and quantitative PCR (qPCR) results in diagnosing *Pneumocystis jirovecii pneumonia* (PJP).[1] The study analyzed respiratory samples obtained via bronchoscopy from non-HIV/AIDS patients, with serum BDG levels classified according to standard diagnostic criteria. It found a low correlation between BDG and qPCR results, with BDG's diagnostic performance varying across different clinical scenarios, ultimately failing short of meeting the needs for reliable clinical diagnosis or confirmation. While BDG has certain limitations, the study suggests that negative BDG results may help exclude PJP, and it emphasizes the need for future research to develop more effective clinical prediction models or diagnostic methods.

PJP is a severe respiratory infection caused by *Pneumocystis jirovecii* (PJ), primarily affecting immunosuppressed individuals, such as those with AIDS, organ transplant recipients, and cancer patients.[2] The rising use of immunosuppressants has led to an increased incidence of PJP in HIV-negative populations, leading to significant morbidity and mortality.[3,4] Early diagnosis is crucial for improving patient outcomes. While PJ is the main pathogen responsible for this disease, its early symptoms, such as fever, dry cough, and dyspnea, are non-specific, making diagnosis particularly challenging.[5] In recent years, advancements in molecular biology techniques have provided new avenues for early diagnosis. Among these, third-generation nanopore sequencing has shown great potential for diagnosing pulmonary infections and identifying pathogen due to its high throughput, long read length, real-time analysis, and portability.[6] This technology directly reads DNA or RNA sequences by detecting changes in electrical current as nucleic acid molecules pass through a nanopore, enabling rapid and accurate pathogen identification.[7]

Our study included 83 patients suspected of having PJP based on clinical symptoms or physical examination results from August 2022 to July 2024. The objective was to evaluate the diagnostic value of third-generation nanopore sequencing for PJP and to explore its ability to detect co-pathogens. The study was approved by the Human Research Ethics Committee of the Fourth People's Hospital of Nanning. Given its retrospective nature and the absence of identifiable personal information, patient consent was waived. Using clinical comprehensive diagnosis as the reference standard, patient samples (sputum, bronchoalveolar lavage fluid, etc.) were collected and analyzed via nanopore sequencing, Gomori methenamine silver (GMS) staining, and BDG testing. The diagnostic performance of each method was compared using metrics including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), Kappa coefficient, and the area under the receiver operating characteristic curve (AUC). McNemar's chi-square test was applied, with statistically significant defined as $P < 0.05$.

Patients were divided into two groups, including the PJP group (n=27) and the non-PJP group (n=56). Basic clinical characteristics and laboratory test are detailed in Supplementary Tables 1 and 2. A comparison of the diagnostic performance between nanopore sequencing and traditional detection methods showed that nanopore sequencing had the highest sensitivity (96.30%), specificity (100.00%), PPV (100.00%), NPV (98.20%), and Kappa coefficient (0.972) (Table 1).

Based on previous extensive studies,[8,9] patients were further classified into two groups according to their lactic dehydrogenase (LDH) levels, including High-LDH (> 250 U/L) and Low-LDH (< 250 U/L). In the high-LDH level group, nanopore sequencing accurately detected or ruled out all PJP cases. In the low-LDH level group, it demonstrated a sensitivity of 93.80%, specificity of 100.00%, positive predictive value (PPV) of 100.00%, negative predictive value (NPV) of 97.90%, and a Kappa coefficient of 0.957 (Supplementary Table 3). These results suggest that LDH levels may serve as an effective auxiliary diagnostic tool to assist nanopore sequencing in PJP diagnosis, but further validation with larger sample sizes and multicenter studies is necessary. Supplementary Table 4 shows the detection of PJP in 9 HIV-positive patients. Clinical diagnosis confirmed 8 of these patients as having PJP, while 1 patient was determined not to have PJP. Among these, GMS staining detected only 1 positive Bronchoalveolar Lavage Fluid (BALF) sample, while BDG testing identified 5 positive and 4 negative patients. Notably, nanopore sequencing correctly identified all 8 confirmed cases of PJP and accurately ruled out the 1 negative case. These results indicate that nanopore sequencing has high sensitivity and specificity for PJP detection, but further validation using large-scale samples is required. Fig. 1 illustrates the mixed infection scenarios detected by nanopore sequencing. In non-PJP patients, the most common finding was single bacterial infection, accounting for 30.36% (17 cases), followed by bacterial and viral co-infections (21.43%, 12 cases) and multiple pathogen infections (19.64%, 11 cases). Cases with no detected pathogens accounted for only 5.36% (3 cases). In PJP patients, the highest proportion was bacterial and viral co-infections, at 37.04% (10 cases), followed by bacterial and fungal co-infections (22.22%, 6 cases), with cases showing no pathogen detected also at 5.36% (3 cases). As shown in Fig. 2, non-PJP patients were mainly infected with *Mycobacterium tuberculosis*, while PJP patients were primarily infected with human herpesvirus 4 and other pathogens.

However, it is important to note that in clinical practice, while nanopore sequencing can detect multiple pathogens, there is no standardized interpretation of pathogen abundance across different laboratories and research institutions. The lack of a consensus threshold to clearly distinguish between colonization and infection

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Table 1
Comparison of Diagnostic Accuracy Among Three Detection Methods.

Methods	Clinical Diagnosis		Sensitivity	Specificity	PPV	NPV	kappa
	Negative	Positive					
Nanopore sequencing							
Negative	56	1	96.30%	100.00%	100.00%	98.20%	0.972
Positive	0	26					
GMS							
Negative	56	17	37.00%	100.00%	100.00%	76.70%	0.443
Positive	0	10					
Serum BDG							
Negative	49	20	25.90%	87.50%	50.00%	71.00%	0.153

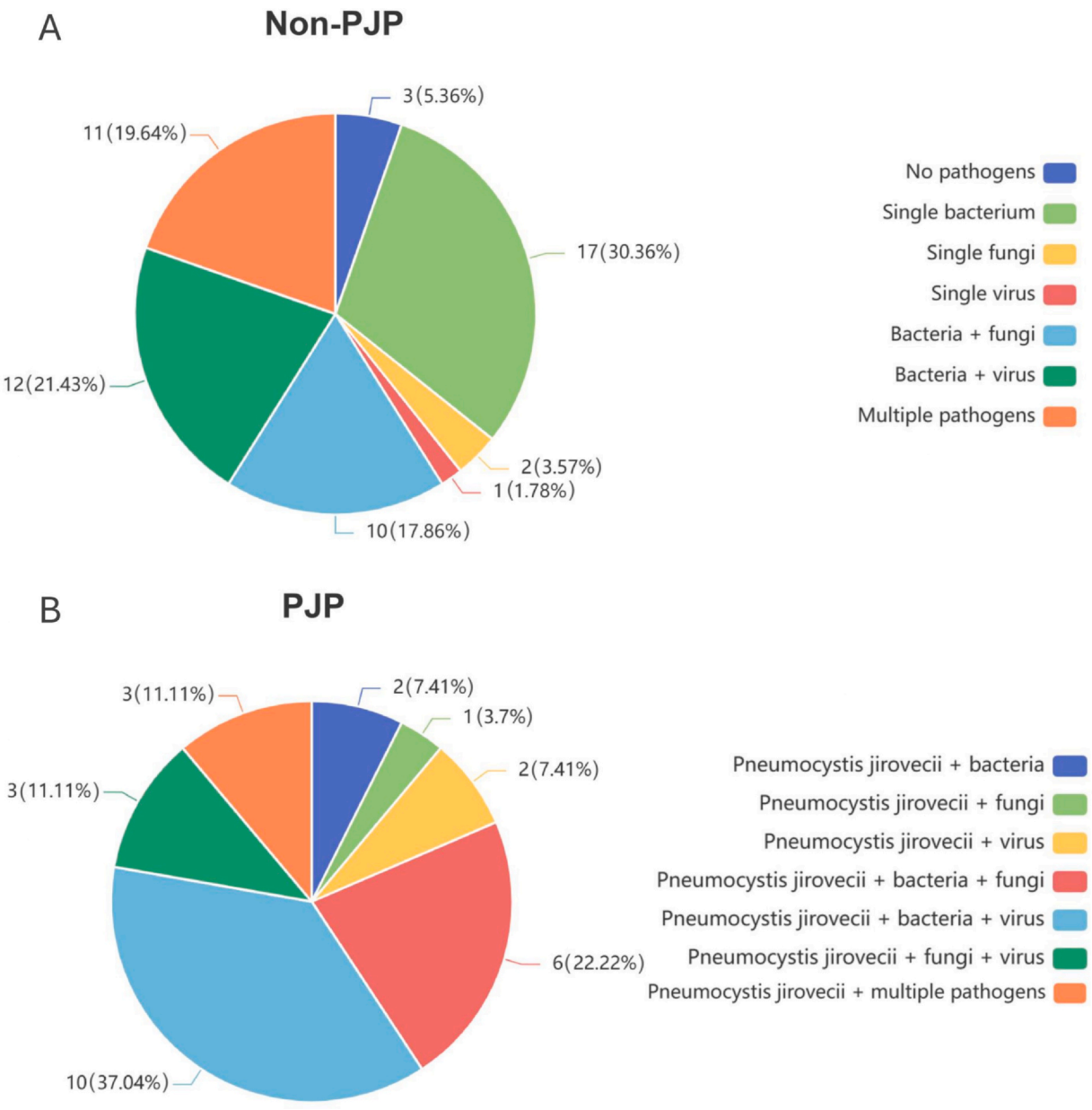

Fig. 1. Proportions of Mixed Pathogens Detected by Nanopore Sequencing (A. Non-PJP Patients; B. PJP Patients).

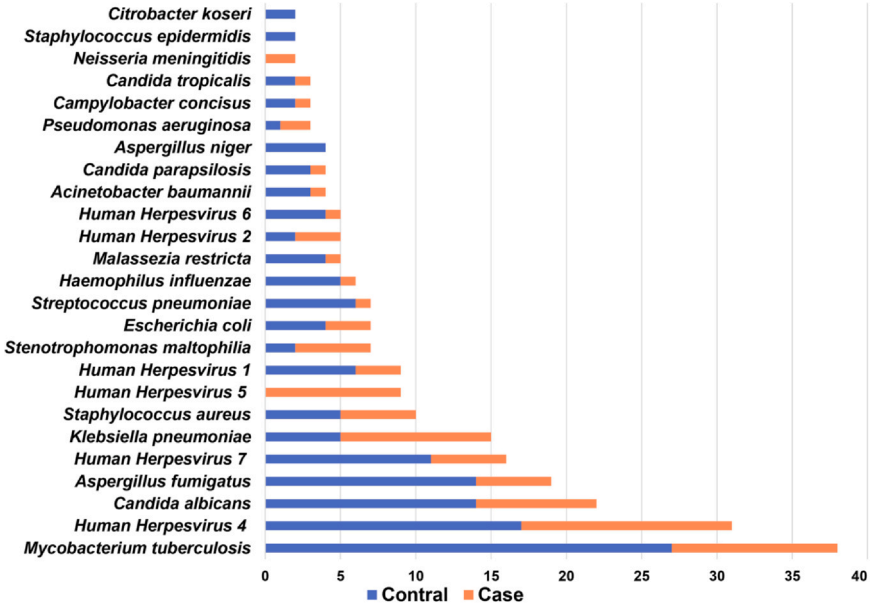

Fig. 2. Common Mixed Pathogens Detected by Nanopore Sequencing.

can lead to diagnostic uncertainty. Thus, nanopore sequencing alone may not provide a comprehensive evaluation of a patient's infection status. To address this, it is recommended that nanopore sequencing be combined with other diagnostic methods, such as microscopic examination, serological testing, and histopathology, for a more thorough assessment of pathogen colonization and infection.

Conclusion

In conclusion, nanopore sequencing technology demonstrates significant potential in the diagnosis of PJP, offering high sensitivity and specificity that enable the accurate detection of PJP cases. This provides a robust foundation for clinical diagnosis and decision-making. Additionally, its ability to identify mixed infections involving multiple pathogens is crucial for gaining a comprehensive understanding of the patient's condition and optimizing treatment strategies.

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2025.106420.

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