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Diagnostic performance and clinical decision-making value of three pathogen detection strategies in lung cancer patients with suspected lower respiratory tract infections: a retrospective analysis

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Abstract

Background

For lung cancer patients with suspected lower respiratory tract infections (LRTIs), the clinical value of different pathogen detection strategies remains unclear. This study systematically compared the diagnostic performance and clinical decision-making impacts of bronchoalveolar lavage fluid metagenomic next-generation sequencing (BALF mNGS), BALF conventional microbiological testing (BALF CMT) and non-BALF CMT in this high-risk population.

Methods

This single-centre retrospective study analysed 95 suspected LRTI episodes among lung cancer patients. We systematically evaluated three testing strategies in terms of pathogen characteristics, turnaround time (TAT), diagnostic performance and clinical decision-making impacts.

Results

Non-infectious aetiologies accounted for 44.2% of episodes, with immune checkpoint inhibitor-related pneumonitis (CIP) being the most common. Among 53 confirmed infectious episodes, bacterial, fungal and viral infections accounted for 69.8%, 34.0% and 11.3% (polymicrobial infections included), with monomicrobial infections predominating (66.0%). Major pathogens included *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa* and *Pneumocystis jirovecii.* Most *P. jirovecii* detections indicated true invasive infection, while most *Candida* isolates were colonisation. Across 90 detection events covering 35 pathogens, combined three-assay testing identified 86.7% (78/90) of infectious episodes, while BALF mNGS alone detected 66.7% (60/90) episodes with 28 pathogen species, showing superior detection breadth. The three strategies exhibited comparable median TAT, with BALF mNGS marginally longer. BALF mNGS achieved the highest sensitivity (73.6%) and accuracy (76.8%), whereas BALF CMT yielded the highest specificity (92.9%) and positive predictive value (90.6%). BALF mNGS brought significant beneficial diagnostic impacts (34.7%) and numerical therapeutic improvement (40.0%), with statistical significance observed only for diagnostic impact.

Conclusions

Suspected LRTIs in lung cancer patients often involve non-infectious lesions, with bacterial infections predominant. The three testing strategies are complementary: BALF mNGS delivers broad pathogen coverage and beneficial diagnostic impacts, while conventional testing yields high specificity. These findings support stratified individualised anti-infective management for this vulnerable population.

Introduction

Lung cancer is among the malignant tumours with the highest global incidence and mortality burden. Approximately 2.5 million new lung cancer cases were diagnosed worldwide in 2022, accounting for 12.4% of all cancers, with around 1.8 million deaths (1). Due to tumour progression and impaired host immunity, patients with lung cancer are susceptible to lower respiratory tract infections (LRTIs), which are frequently characterised by complex aetiology and mixed infections (2), markedly increasing challenges for pathogen identification in routine clinical practice. Furthermore, the expanding use of immune checkpoint inhibitors in recent years has led to a rapid rise in the incidence of immune checkpoint inhibitor-related pneumonitis (CIP) (3, 4). Distinguishing infectious pneumonitis from CIP directly guides clinical management. Therefore, rapid and accurate aetiological testing is critical to guide targeted anti-infective therapy and differential diagnosis.

Currently, conventional microbiological testing (CMT) remains the most widely used approach for identifying LRTIs in lung cancer patients (5), yet it has substantial limitations. Microbial culture is time-consuming: bacterial cultures require 48–72 h, fungal cultures up to approximately one week, and *Mycobacterium tuberculosis* cultures as long as four weeks (6). Moreover, the detection yield for fastidious or rare pathogens is low; for example, the positive rate for anaerobes can be as low as 10% (7). In addition, test results are susceptible to pre-test antimicrobial administration and specimen quality (8). Direct microscopy, antigen and antibody assays have suboptimal sensitivity. Although targeted assays such as PCR exhibit high specificity, their narrow pathogen coverage restricts utility in complex infection scenarios (9). Previous studies report that CMT identifies only approximately 40% of pathogens (10), which limits the precision of anti-infective treatment.

Metagenomic next-generation sequencing (mNGS) is an untargeted, high-throughput assay capable of detecting a broad spectrum of pathogens in a single run, offering distinct advantages for the diagnosis of complex, mixed and rare infections (11). Nevertheless, studies evaluating mNGS in patients with lung cancer remain relatively scarce. More importantly, prior studies comparing mNGS with conventional assays often use disparate specimen types (e.g., BALF versus sputum or blood) (12–14). This confounds the respective effects of testing platform and specimen matrix, potentially biasing estimates of the true diagnostic performance of mNGS. Systematic evaluation of different testing methods using well-matched clinical cohorts is therefore essential to define the real-world clinical value of mNGS.

Clinically, conventional microbiological testing of bronchoalveolar lavage fluid (BALF) serves as the cornerstone of aetiological testing but suffers from prolonged turnaround time and low positivity rates. Non-BALF CMT is easier to perform, yet diagnostic accuracy may be compromised by upper respiratory tract contamination. Against this background, using final composite diagnosis (FCD) as the reference standard, this study systematically compared three pathogen detection strategies in lung cancer patients with suspected LRTIs: BALF mNGS, BALF CMT and non-BALF CMT. This study aimed to separate the contributions of testing methodology and specimen type, assess the diagnostic

performance of each strategy and their impacts on clinical decision-making, and provide evidence to optimise testing algorithms and rational antimicrobial stewardship.

Materials and methods

Study population

This is a single-centre retrospective study. A total of 110 testing episodes in lung cancer patients with suspected LRTIs admitted to Peking Union Medical College Hospital between March 2022 and October 2025 were initially screened.

Inclusion criteria: (1) Histologically or cytologically confirmed diagnosis of lung cancer; (2) Clinical suspicion of LRTIs; (3) Completion of all three assays, namely BALF mNGS, BALF CMT and non-BALF CMT. Exclusion criteria: (1) Specimens failing mNGS quality control; (2) Incomplete clinical data; (3) Indeterminate final composite diagnosis; (4) Receipt of only one or two of the three assays (BALF mNGS, BALF CMT and non-BALF CMT). After screening, 95 episodes were ultimately included, and 15 episodes were excluded due to missing clinical data or unclear diagnosis. Characteristics of excluded episodes showed no systematic differences from the included cohort in key clinical variables, including disease severity and follow-up outcomes.

Suspected LRTIs were defined by the following criteria: (1) Age ≥ 18 years; (2) Clinical manifestations consistent with LRTIs including at least one systemic symptom (fever [temperature > 38.0°C] or hypothermia [< 36.0°C], chills, fatigue or general malaise) and at least one respiratory symptom (cough, sputum or purulent sputum, tachypnea or dyspnea [respiratory rate > 20 breaths/min], chest pain, audible moist rales or decreased breath sounds on auscultation); (3) Imaging evidence: chest computed tomography (CT) or chest radiograph showing new or progressive pulmonary infiltration, consolidation or ground-glass opacities, or lung ultrasound findings consistent with pneumonia.

The final composite diagnosis (FCD) was determined by a multidisciplinary team (MDT) through integrated assessment of clinical manifestations, imaging findings, conventional microbiology results, mNGS reports, and treatment response to anti-infective therapy.

Data collection

Demographic and clinical data were collected, including age, sex, tumour type and stage, comorbidities (hypertension, diabetes mellitus), smoking and alcohol use history, anti-tumour treatment (predominantly immunotherapy), and pre-sampling antimicrobial administration. Microbiological data, including FCD, mNGS and CMT results, were recorded. Bronchoscopy was performed for all enrolled patients to obtain bronchoalveolar lavage fluid (BALF); the same BALF aliquot was used for both mNGS and CMT. Non-BALF specimens included blood, drainage fluid, throat swabs, bronchial brushings, pleural effusion, tissue samples and sputum. CMT included microbial culture (aerobic and anaerobic bacterial

culture, fungal culture, mycobacterial culture), direct microscopy, immunological assays and polymerase chain reaction (PCR)-based nucleic acid assays.

Clinical trial number: not applicable. This study was approved by the Ethics Committee of Peking Union Medical College Hospital (Approval No. I-25PJ2109). All participants were waived from the requirement for written informed consent owing to the retrospective nature of the study.

mNGS testing

All samples were processed in the Pathogen Sequencing Laboratory of Peking Union Medical College Hospital following standard operating procedures. The main procedures were as follows: (1) Specimen preprocessing: Host depletion was performed on BALF samples (host depletion was omitted when *Pneumocystis jirovecii* was suspected). Briefly, 0.6 mL BALF was transferred into a grinding tube for homogenisation, and the entire supernatant was collected after homogenisation. (2) Nucleic acid extraction: Genomic DNA was extracted using the TIANamp Micro DNA Kit (DP316, Tiangen Biotech Co., Ltd., Beijing, China). (3) DNA fragmentation: Genomic DNA was fragmented using the DNA Fragmentation Kit (Vision Medicals, Guangzhou, China). (4) Library construction: Libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). PCR conditions were: 72°C for 3 min, 98°C for 30 s; followed by 17 cycles of 98°C for 15 s, 60°C for 30 s and 72°C for 30 s; final extension at 72°C for 5 min. Library quality was assessed using a Qsep1 Bio-Fragment Analyzer. (5) Sequencing: 75 bp single-end sequencing was performed on the Illumina NextSeq CN500 platform, with a minimum yield of 20 million reads per sample. (6) Data processing: Trimmomatic was used to remove low-quality reads, adapter sequences and reads shorter than 40 bp. Microbial sequence alignment and taxonomic classification were subsequently conducted using the Vision Medicals bioinformatics platform and the in-house analytical pipeline of Peking Union Medical College Hospital. (7) Result interpretation: Results were interpreted jointly by a multidisciplinary panel consisting of bioinformaticians, clinical laboratory specialists and infectious disease physicians.

Statistical analysis

All statistical analyses and figure plotting were performed using R software (version 4.5.1). Continuous variables were described as mean ± standard deviation or median (interquartile range) according to their distribution, and categorical variables were presented as counts or percentages. The turnaround time (TAT) of the three testing strategies was compared using the Kruskal–Wallis test. Pairwise comparisons were conducted with Wilcoxon rank-sum tests, and Bonferroni correction was applied for multiple comparisons. Using FCD as the reference standard, the detection rate, sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of each assay were calculated with 95% confidence intervals. Clinical diagnostic impacts of testing were classified as beneficial, neutral, or adverse. Between-group differences were evaluated by Cochran's Q test. If a significant overall difference was identified, pairwise McNemar tests with Bonferroni correction for multiple comparisons were performed. All tests were two-sided, and a P value < 0.05 was considered statistically significant.

To systematically evaluate the impact of different testing strategies on clinical diagnosis and treatment, a previously published classification framework (13) was adopted in this study. Diagnostic impacts were graded D1–D7 and therapeutic impacts T1–T7; detailed definitions are provided in Table 1.

Table 1
Classification criteria for diagnostic and therapeutic impacts of pathogen detection

Grade	Classification definition
Diagnostic impacts	
D1	Pathogens were detected earlier by this strategy than by other strategies
D2	This method uniquely identified the causative pathogen
D3	This method detected co-infection
D4	Negative test result
D5	Concordant results with other methods, with delayed pathogen detection
D6	Detected microorganisms that were not causative pathogens (false positive)
D7	No causative pathogen was detected in patients with infectious symptoms
Therapeutic impact	
T1	Initiation of antimicrobial therapy
T2	Escalation of antimicrobial therapy
T3	De-escalation of antimicrobial therapy
T4	Confirmation of empirical therapy
T5	Positive test result with no change in antimicrobial management
T6	Negative test result with no change in antimicrobial management
T7	Treatment modification driven by a false-positive test result

Results

Basic clinical data

A total of 88 patients with lung cancer were enrolled in this study (Table 2). Each testing episode represented an independent clinical evaluation for suspected LRTIs. Seven patients had repeated testing episodes, all of which were included in the analysis, yielding a total of 95 testing episodes.

Regarding demographic characteristics, the study population was predominantly male (70.5%), with a median age of 65 years (interquartile range [IQR], 59–70 years). For tumour characteristics, non-small

cell lung cancer (NSCLC) accounted for the vast majority (92.1%), mainly including lung adenocarcinoma and lung squamous cell carcinoma. Most patients had advanced-stage disease (stage III–IV, 84.1%). In terms of medical history, smoking was common (61.4%), and approximately one-third of patients had concomitant hypertension.

Among the 95 included episodes, most mNGS assays adopted a DNA-only sequencing workflow (84 episodes, 88.4%), whereas the remaining cases utilised combined DNA and RNA detection strategies. Most tests were performed during hospitalisation (75 episodes, 78.9%). The mean hospital stay was 15.6 days, with a median duration of 12 days (IQR, 8–18 days). The median interval from hospital admission to mNGS testing was 3 days (IQR, 1–7 days).

With respect to anti-tumour immunotherapy, only seven episodes (7.3%) involved patients who had not received immunotherapy, whereas the remaining 88 episodes (92.7%) were from patients receiving immunotherapy. Among these episodes, immune checkpoint inhibitor therapy was administered within 1 month before mNGS testing in 46 episodes (48.4%); 24 episodes (25.3%) at 1–3 months, 7 episodes (7.4%) at 3–6 months, and 11 episodes (11.6%) at > 6 months.

Table 2
Baseline characteristics of enrolled patient

Characteristic	Patients (n = 88)
Sex	
Male	62 (70.5%)
Female	26 (29.5%)
Age, years	
18–65	46 (52.3%)
66–79	41 (46.6%)
>80	1 (1.1%)
Lung cancer subtype	
Non-small cell lung cancer	81 (92.0%)
Lung adenocarcinoma	44 (50.0%)
Lung squamous cell carcinoma	33 (37.5%)
Others	4 (4.5%)
Small cell lung cancer	7 (8.0%)
TNM stage	
III-IV	74 (84.1%)
I-II	6 (6.8%)
Unknown stage	8 (9.1%)
Medical history	
Smoking	54 (61.4%)
Alcohol consumption	43 (48.9%)
Hypertension	29 (33.0%)
Diabetes	14 (15.9%)
Hyperlipidaemia	9 (10.2%)

Note: Percentages were calculated from the total of 88 patients. Subsequent diagnostic and microbiological analyses were performed at the testing-episode level (95 episodes in total; seven patients contributed multiple episodes).

Etiological characteristics of LRTIs among patients with lung cancer

Among the 95 testing episodes of suspected LRTIs in lung cancer patients, non-infectious aetiologies accounted for 44.2% (42/95) of all cases. Within the non-infectious subgroup, CIP represented the leading cause (47.6%), followed by tumour progression (28.6%) and targeted therapy-related pneumonitis (14.3%), whereas radiation pneumonitis and other undefined pulmonary injuries occurred less frequently (Fig. 1A).

Of the 53 microbiologically confirmed infectious episodes, bacterial infections predominated (37/53, 69.8%), followed by fungal infections (18/53, 34.0%), while viral infections were relatively rare (6/53, 11.3%). Notably, multiple pathogen categories could be detected in a single episode due to polymicrobial co-infections. Monomicrobial infection constituted the dominant infectious pattern (35/53, 66.0%), and the remaining 18 episodes were defined as polymicrobial infections. Mixed infections included dual-pathogen and triple-pathogen combinations, among which six episodes presented bacterial–fungal–viral triple infections (Fig. 1B).

Pathogen spectrum analysis identified a total of 35 microbial species across 90 independent detection events (Fig. 1C). Bacteria were the predominant pathogens, with 63 detection events (66.3%) across 25 species. The three most frequently detected bacterial pathogens were *Klebsiella pneumoniae* (10 events, 10.5%), *Staphylococcus aureus* (6 events, 6.3%), and *Pseudomonas aeruginosa* (5 events, 5.2%). Six fungal species were detected in 19 events, dominated by *Pneumocystis jirovecii* (10 events, 10.5%) and *Aspergillus* spp. (6 events, 6.3%). Viral detections were relatively rare, comprising 8 events (8.4%) from four species: SARS-CoV-2, influenza A virus, Epstein–Barr virus, and cytomegalovirus.

Pathogen detection performance of the three testing strategies

Among microbiologically confirmed LRTI episodes in patients with lung cancer, detection of each individual pathogen was counted as one pathogen detection event, yielding a total of 90 events. Seventy-eight events (86.7%) were detected by at least one of the three testing strategies (BALF mNGS, BALF CMT, non-BALF CMT) (Fig. 2A). BALF mNGS, BALF CMT and non-BALF CMT identified 60 (66.7%), 34 (37.8%) and 33 (36.7%) events, respectively. BALF mNGS uniquely detected 29 events (37.2%), a proportion markedly higher than those for BALF CMT (6 events, 7.69%) and non-BALF CMT (7 events, 8.97%). Paired co-detection events were as follows: BALF mNGS + BALF CMT (10 events, 12.8%), BALF mNGS + non-BALF CMT (8 events, 10.3%), BALF CMT + non-BALF CMT (5 events, 6.41%). Thirteen events (16.7%) were concurrently detected by all three assays. The remaining 12 events (13.3%) were not detected by any of the three assays but were confirmed by integrated clinical assessment. Among these, six events yielded sequence signals via BALF mNGS but were not reported, involving *Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Burkholderia cenocepacia, Acinetobacter baumannii*, and *Actinomyces* spp. The other six missed pathogens included *Aspergillus* spp., *Mycobacterium tuberculosis*, and cytomegalovirus.

Regarding the distribution of clinically concordant pathogens (Fig. 2B), BALF mNGS detected 28 pathogen species (20 bacterial, four fungal and four viral), more than BALF CMT (20 species) and non-

BALF CMT (17 species). *Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most frequently identified bacterial pathogens. Total detection frequencies of fungi and viruses were comparable across the three assays. Analysis of clinically irrelevant microbial detections (Fig. 2C) demonstrated that BALF mNGS detected more species and higher frequencies of irrelevant bacteria and viruses than conventional testing, whereas conventional assays identified more irrelevant fungi. Comparison of pathogens uniquely or firstly detected (Fig. 2D) showed that BALF mNGS identified 41 events involving 20 bacterial species, outperforming conventional tests, while fungal and viral detection yields were similar among the three strategies. Furthermore, in episodes positive by conventional testing but negative by BALF mNGS (Fig. 2E), opportunistic pathogens predominated, including *Actinomyces* spp., *Klebsiella pneumoniae, Pneumocystis jirovecii*, and *Candida albicans.* All six detected *Pneumocystis jirovecii* isolates were clinically confirmed as causative pathogens, whereas 13 out of 14 *Candida albicans* detections lacked clinical relevance.

Turnaround time (TAT) of the three testing strategies

Turnaround time (TAT) distributions differed among the three testing strategies (Fig. 3). The median TAT of BALF mNGS was 53.27 h (IQR: 49.31–71.89 h), 50.23 h (IQR: 45.92–71.77 h) for BALF CMT, and 50.43 h (IQR: 24.23–72.51 h) for non-BALF CMT. A Friedman test was used for overall comparison ($P < 0.001$). Pairwise comparisons demonstrated a statistically significant difference only between BALF CMT and non-BALF CMT (Bonferroni-adjusted $P = 0.039$), while no significant differences were observed in the remaining pairwise comparisons.

Impact of the three detection strategies on the diagnosis and treatment of suspected LRTIs in lung cancer

A total of 95 testing episodes of suspected LRTIs were enrolled, and the final composite diagnosis (FCD) was used as the reference standard to evaluate diagnostic performance (Fig. 4A). BALF mNGS exhibited higher sensitivity (73.6%, 95% CI: 59.7%-84.7%) and accuracy (76.8%, 95% CI: 67.1%-84.9%) than BALF CMT (sensitivity 54.7%, accuracy 71.6%) and non-BALF CMT (sensitivity 47.2%, accuracy 63.2%). BALF CMT achieved the highest specificity (92.9%, 95% CI: 80.5%-98.5%). Paired methodological comparison revealed discordant results between BALF mNGS and BALF CMT. McNemar's test indicated a trend toward higher positive detection rate for BALF mNGS relative to BALF CMT, although the difference did not reach statistical significance ($P = 0.055$, Fig. 4C). The diagnostic yield of conventional microbiological testing from BALF and non-BALF specimens was comparable, with no statistically significant difference ($P = 0.424$).

Based on the predefined D1–D7 criteria, the impacts of testing strategies on clinical diagnostic decisions were categorized as beneficial, neutral, or adverse (Fig. 4B). BALF mNGS yielded the highest proportion of beneficial diagnostic impacts (33/95, 34.7%), which was significantly higher than BALF CMT (14/95, 14.7%) and non-BALF CMT (16/95, 16.8%). BALF CMT had the largest proportion of neutral results (73/95, 76.8%), whereas non-BALF CMT showed the highest rate of adverse diagnostic impacts

(16/95, 16.8%). Overall comparison demonstrated significant intergroup differences in the distribution of beneficial and neutral impacts (P < 0.001), while no significant difference was observed for adverse impacts (P = 0.093). Pairwise comparisons showed that beneficial diagnostic impacts of BALF mNGS were significantly greater than those of the two conventional assays (adjusted P = 0.008 and 0.023, respectively). The proportion of neutral results for BALF mNGS was significantly lower than that for BALF CMT (adjusted P < 0.001); no other pairwise comparisons reached statistical significance.

The predefined T1–T7 criteria were applied to assess the impacts of the three strategies on anti-infective treatment decisions, which were similarly classified as beneficial, neutral, or adverse. BALF mNGS was associated with the highest rate of beneficial treatment modifications (38/95, 40.0%), exceeding BALF CMT (27/95, 28.4%) and non-BALF CMT (25/95, 26.3%, Fig. 4D). Significant intergroup differences were identified in the distribution of beneficial treatment impacts (P = 0.028) and neutral impacts (P = 0.031). Adverse treatment impacts were rare overall, with only one case (1.1%) observed in the BALF CMT group, and no between-group difference (P = 0.368). Although BALF mNGS was more likely to guide optimisation of anti-infective regimens, none of the pairwise comparisons reached statistical significance.

Discussion

In this study of lung cancer patients with suspected LRTIs, non-infectious aetiologies were prevalent, while confirmed infections were predominantly bacterial. Detection of *Pneumocystis jiroveciiwas* highly predictive of clinically confirmed invasive infection. BALF mNGS provided the broadest pathogen spectrum, the highest sensitivity and accuracy, and yielded more beneficial impacts on diagnostic decision-making. Nonetheless, mNGS-positive results require careful interpretation to distinguish colonisation and clinically irrelevant microbes from true pathogens. BALF CMT exhibited high specificity and reliable performance for pathogen verification. Non-BALF CMT was convenient to obtain but a negative result could not exclude infection.

In patients with lung cancer receiving immunotherapy or targeted therapy, treatment-related lung injury, most commonly CIP, represents a key cause of pulmonary opacities and respiratory symptoms (4, 15, 16). The overlapping clinical and radiographic manifestations of infectious and non-infectious lung lesions substantially complicate differential diagnosis. Therefore, aetiological testing contributes not only to pathogen identification but also to excluding infectious aetiologies and supporting alternative clinical diagnoses, thereby facilitating clinical decision-making (17–19). In terms of pathogen characteristics, bacterial infections remained predominant (69.8%), with *Klebsiella pneumoniae, Staphylococcus aureus*, and *Pseudomonas aeruginosa* being the most common pathogens. Previous studies have also reported that bacteria predominate, including *K. pneumoniae, Acinetobacter baumannii*, *P. aeruginosa*, and *Haemophilus influenzae* (20). Impaired immune function, repeated hospitalisations, and broad-spectrum antibiotic exposure in patients with lung cancer predispose them to colonisation and secondary infection by opportunistic pathogens (21). Empirical antimicrobial therapy should therefore prioritise coverage of common hospital-associated Gram-negative opportunistic pathogens.

Fungal infections accounted for 34.0% of all confirmed LRTIs, higher than the 12% reported in a Chinese meta-analysis based on conventional pathogen detection (2), but lower than the 50% reported in similar studies using mNGS (22). Fungal pathogens were dominated by *P. jirovecii* and *Aspergillus* spp., consistent with previous reports that *Pneumocystis pneumonia* and invasive pulmonary aspergillosis (IPA) are more common in elderly and lymphopenic patients (21). Although other studies have reported *Candida* and *Aspergillus* as the main fungi (20, 22), most *Candida* detections in the present study were considered colonisation and excluded from confirmed infections. By contrast, *P.* jirovecii detections were mostly clinically pathogenic; only one case was judged non-infectious due to low mNGS abundance, highlighting its high clinical predictive value for true infection in lung cancer populations. Previous studies have noted that in populations with post-immunotherapy infection or high-sensitivity detection strategies, fungal and viral detection rates are often elevated, accompanied by a higher proportion of polymicrobial infections (23). However, monomicrobial infection predominated in this study (66.0%), with limited mixed infections. This discrepancy is attributable to differences in study populations and diagnostic criteria: prior studies focused on severely immunosuppressed patients at high risk for polymicrobial infections (24), whereas the present study adopted a strict composite diagnostic framework, excluding non-pathogenic colonisers and low-abundance non-causative microbial signals, better reflecting real-world infectious patterns in lung cancer patients. Overall, under a strict clinical diagnostic framework, bacterial infections remained predominant, fungi were important supplements, and the infection pattern tended to be monomicrobial.

Regarding pathogen detection across the three strategies, combined testing identified 86.7% of all valid pathogen detection events, whereas single-assay detection was suboptimal. BALF mNGS achieved the highest single-method detection rate (66.7%), followed by BALF CMT (37.8%) and non-BALF CMT (36.7%). These findings indicate that a single test cannot fully identify the complex pathogen spectrum in patients with lung cancer, and combined testing can broaden pathogen coverage. Previous studies have also confirmed that multi-method combinations can improve pathogen detection rates and diagnostic accuracy (25). However, although combined testing improved pathogen coverage, given the high cost of mNGS, only one anaerobic infection was detected, and no clear rare or special pathogens were found, most pathogens could be covered by routine empirical antimicrobial therapy. Therefore, the clinical benefit of routine combined mNGS testing for lung cancer patients with suspected LRTIs may be limited, and it is not the preferred strategy for all patients. In addition, 13.3% of pathogen detection events were missed by all three strategies, with two major underlying causes. First, six specimens had pathogen loads below the technical detection threshold, including four *Aspergillus* episodes, one *Mycobacterium tuberculosis* episode, and one cytomegalovirus infection. The rigid cell wall structure of these pathogens may reduce lysis efficiency, highlighting the necessity of optimising mNGS pre-analytical protocols. Second, six infectious episodes generated valid mNGS sequencing signals but were not reported clinically, mostly corresponding to opportunistic pathogens. Such unreported signals may result from difficulties in distinguishing colonisation from true infection or low pathogen abundance. Previous studies have indicated that mNGS results are affected by background filtering algorithms and reporting thresholds; low-abundance or clinically equivocal pathogens are often excluded, reducing

report-level sensitivity (26). In addition, differences in data processing and interpretation standards among centers may lead to insufficient consistency of mNGS results (27, 28). Mechanistically, this “detected but not reported” phenomenon suggests a gap between the detection capability and the reporting system of mNGS. Furthermore, these unreported low-abundance pathogens have important methodological implications, suggesting that after optimising pre-analytical processes (e.g., DNA extraction) or adjusting reporting thresholds (29), some signals may become reportable, thereby improving detection sensitivity. In summary, although combined testing can improve pathogen detection rates, it may not be the optimal decision for such patients; pre-analytical processing and reporting/analysis systems still have substantial scope for optimisation.

Each testing strategy exhibited distinct strengths across pathogen categories. Previous studies have demonstrated that mNGS provides broader bacterial and viral pathogen coverage, especially in polymicrobial infections (11, 30). Our study replicated this advantage for bacteria but not for viruses, and mNGS showed no clear superiority for fungal detection. Conventional fungal diagnostics, including culture, antigen testing and microscopy, target analytes that do not fully overlap with mNGS nucleic acid targets, which may contribute to false-negative mNGS results for fungi. Therefore, if fungal infection is clinically suspected, particularly for P. *jirovecii* or *Aspergillus*, negative mNGS results should be supplemented with conventional or pathogen-specific assays (11, 31). We observed divergent patterns of non-clinically relevant detections across platforms: mNGS identified more irrelevant bacterial and viral sequences, whereas conventional assays generated more non-clinically meaningful fungal signals, predominantly *Candida* albicans, which were classified as colonisation in most cases. This pattern reflects inherent technological biases: mNGS is vulnerable to microecological background noise and contamination (32, 33), while conventional testing is affected by culture conditions, environmental contaminants and subjective interpretation (8, 34, 35). Future work should establish lung cancer-specific pre-analytical standards and interpretive frameworks, rather than applying simplistic rules equating “detection” with “pathogenicity” or “positive conventional result” with true infection.

This study further evaluated the turnaround time (TAT) profiles of the three strategies. Contrary to previous reports that mNGS enables earlier pathogen identification, especially for difficult-to-culture pathogens and complex infections (12, 36), mNGS offered no time benefit in our cohort. the median TAT of the three strategies was generally similar, with BALF mNGS being the longest (53.27 h), while non-BALF CMT and BALF CMT were shorter. This phenomenon may be related to the predominance of common opportunistic pathogens and the particularity of the lung cancer population. Notably, although the median TAT was similar, the TAT distribution of mNGS was more concentrated with smaller dispersion, suggesting a more standardised and reproducible workflow. In contrast, conventional microbiological tests showed greater variability in TAT, possibly related to culture cycles, test combinations, laboratory processing, and result interpretation differences (6, 9). In addition, some non-BALF CMT had very short TAT, possibly due to convenient specimen acquisition and relatively simple pre-processing, also suggesting that rapid conventional methods such as smears, antigen detection, or targeted molecular tests still have practical time advantages in specific clinical scenarios (37). In summary, the TAT of pathogen detection is determined by multiple factors including specimen type,

testing workflow, and laboratory operational pathways, rather than simply depending on detection technology. Therefore, the key bottleneck restricting testing efficiency is the integrated clinical-laboratory pathway, rather than detection technique itself.

In terms of diagnostic performance, BALF mNGS achieved the highest sensitivity (73.6%) and accuracy (76.8%), demonstrating superior comprehensive pathogen identification capability. Although pairwise McNemar's testing showed only a marginal trend toward higher detection rates compared with BALF CMT (P = 0.055), this finding indicates that mNGS improves overall diagnostic yield rather than simply replacing conventional assays. In previous studies in oncology populations, the overall sensitivity of mNGS reached 95.5% (38). The relatively lower sensitivity in this study suggests heterogeneity in diagnostic performance across studies. This difference may be related to study design and control strategies: this study used the same BALF specimen for parallel comparison of two methods, whereas some previous studies used sputum or different specimen types as comparators (39), whose lower sensitivity may amplify the advantage of mNGS; in addition, differences in population characteristics, nucleic acid extraction efficiency, host sequence depletion, and interpretation thresholds may also affect results (40). In contrast, BALF CMT showed better specificity (92.9%) and positive predictive value (90.6%), consistent with previous studies (14, 23), suggesting that conventional methods, due to stricter positive criteria, still have value in result stability and false-positive control. Notably, BALF CMT and non-BALF CMT showed no statistical difference, suggesting that for conventional testing, the specimen source has limited benefit in improving overall detection capability, and its clinical value may depend more on the detection technology itself. Previous studies have mostly focused on comparing the sensitivity of mNGS and conventional methods (14); this study simultaneously included BALF and non-BALF specimens, placing detection method and sampling source in the same framework, which distinguishes it from previous studies (30). Our results suggest that mNGS contributes more substantially to incremental clinical diagnostic information than specimen type selection. Clinically, detection yield and result reliability should be balanced appropriately. BALF mNGS serves as a valuable adjunct to conventional testing, while conventional assays remain essential for pathogen confirmation; the two modalities are complementary rather than mutually exclusive.

Most importantly, this study objectively evaluated the real-world clinical impact of each testing strategy on diagnostic and therapeutic decisions based on standardised D1–D7 diagnostic and T1–T7 therapeutic grading criteria. BALF mNGS was significantly superior to BALF CMT and non-BALF CMT in positive diagnostic impact and reduced "no impact" results. This indicates that the true value of mNGS lies not only in "detecting more" but also in "providing more meaningful information." This is particularly important in patients with lung cancer, who often have multiple factors such as tumor progression, drug-induced lung injury, immunosuppression, and opportunistic infections; what clinicians need most is not generalized microbial detection but pathogen evidence that can truly support or modify diagnostic judgment. From this perspective, this study goes further than previous studies that simply reported sensitivity or detection rates (41), answering the more valuable question of whether "detecting more with mNGS" actually changes clinical judgment. At the same time, this study found that the diagnostic advantage of mNGS did not fully translate into a therapeutic advantage. Although BALF mNGS had the

highest proportion of positive therapeutic impact, pairwise comparisons did not reach significance. This result does not weaken the value of mNGS but more realistically reflects the complexity of clinical decision-making. Therapeutic adjustment depends not only on pathogen results but also on patient baseline status, imaging severity, prior medication history, tumor treatment stage, and the decision preferences of clinicians and patients (42). These results emphasise that diagnostic benefits cannot be fully equivalent to therapeutic benefits, which is a critical consideration for translational research of molecular diagnostics.

This study has several limitations. First, the single-centre retrospective design and limited sample size may introduce selection bias. Second, conventional testing was performed at the clinician's discretion rather than uniformly implemented across all episodes, potentially causing additional selection bias. Third, final diagnostic attribution and mNGS interpretation relied on multidisciplinary consensus judgment, which inevitably contained subjective components. Fourth, the study focused exclusively on lung cancer patients; generalisability to other immunosuppressed populations requires further validation. Future multicentre prospective studies are warranted to standardise pre-analytical processing, unify interpretation and reporting criteria, validate cost-effectiveness, and explore the clinical value of targeted pathogen panels for this vulnerable population.

Conclusions

In patients with lung cancer and suspected LRTIs, non-infectious aetiologies accounted for a high proportion, with CIP being the most common; infections were predominantly bacterial, with fungi as important supplements. Common pathogens included *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Pneumocystis jirovecii*, among which *P. jirovecii* mostly had definite pathogenic significance, whereas *Candida* was mostly colonisation. Each of the three detection strategies exhibits distinct strengths: BALF CMT had the highest specificity and positive predictive value; non-BALF CMT was convenient to obtain and could achieve a shorter minimum TAT; BALF mNGS had the broadest pathogen spectrum, the highest sensitivity and accuracy, and a more positive impact on diagnostic decision-making. However, mNGS did not show a TAT advantage, its diagnostic benefit did not fully translate into therapeutic decision-making benefit, and the clinical benefit of routine combined mNGS may be limited. Clinical practice should deploy these testing strategies in a stratified, scenario-based combined approach, emphasising the distinction between colonisation and true infection, to optimise pathogen identification and antimicrobial stewardship.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Peking Union Medical College Hospital (decision no. I-25PJ2109, dated 04 September 2025) and was conducted in accordance with the Declaration of

Helsinki. Owing to its observational retrospective design, the requirement for informed consent was waived by the ethics committee.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable. No identifying information or images of any participant are included in this article.

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Author Contribution

SZ and JD made the conception and design of the work; MLZ and XFC guided the methods; SZ, XFC, HTS, JYG and MYL collected the clinical data; MDZ, DZ and MLZ performed the diagnostic report interpretation; XFC and DZ interpreted the data; SZ drafted the manuscript; JD reviewed the manuscript; DZ administrated the project; QWY and SZ acquired the fund of the study.

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Data Availability

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

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Figures

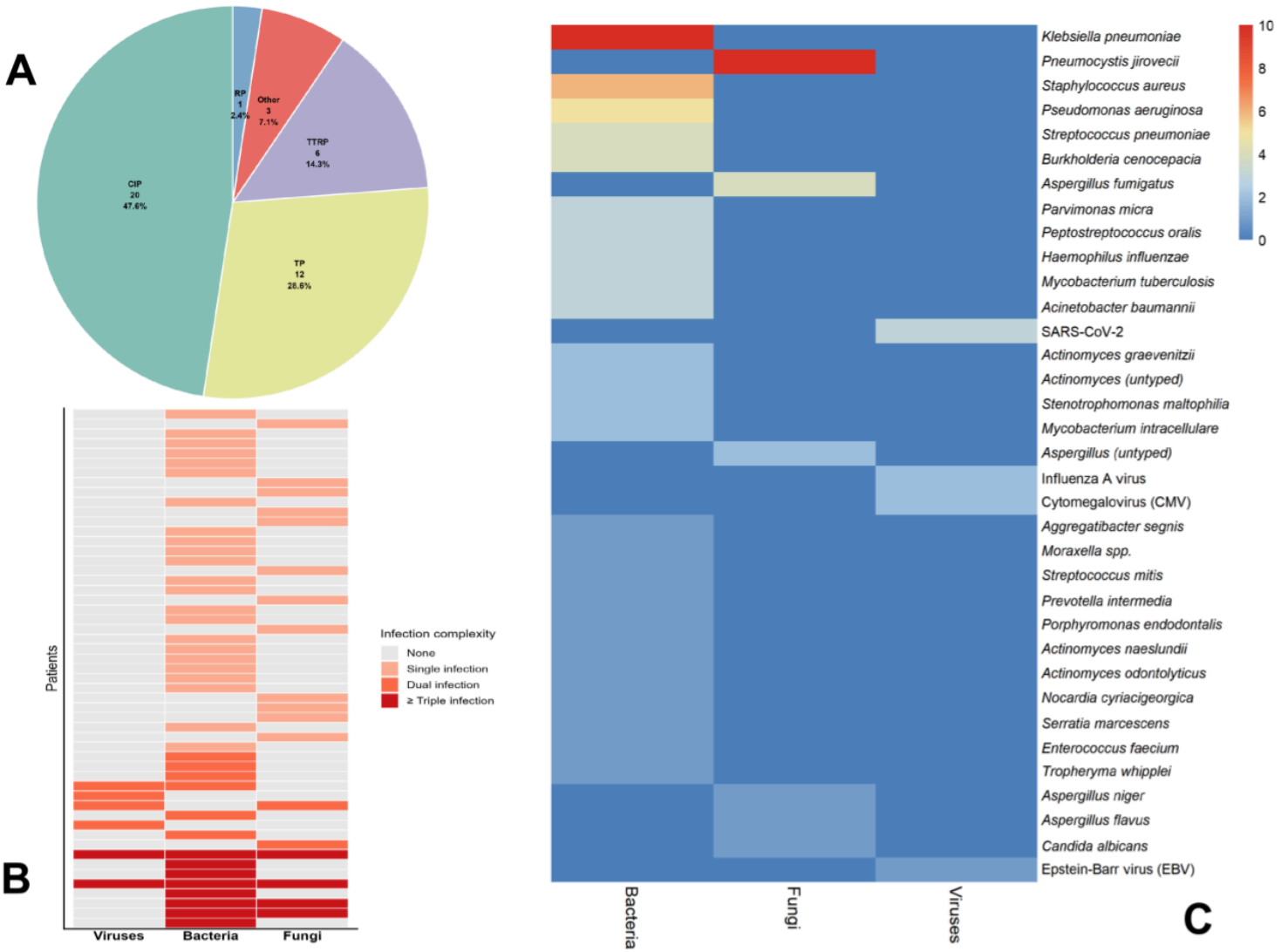


Figure 1

Aetiology and pathogen distribution among lung cancer patients with suspected LRTIs**.** (A) Composition of non-infectious aetiologies (n = 42). Immune checkpoint inhibitor pneumonitis (CIP, 47.6%, 20/42), tumour progression (TP, 28.6%, 12/42), targeted therapy-related pneumonitis (TTRP, 14.3%, 6/42), radiation pneumonitis (RP, 2.4%, 1/42), and other undefined pulmonary injuries (7.1%, 3/42). (B) Distribution and infection complexity of infectious pathogens (n = 53). Each row represents one testing episode; colours indicate infection complexity, and the horizontal axis denotes pathogen type. (C) Heatmap showing pathogen detection frequency (90 detection events). Rows correspond to individual pathogens and columns to pathogen categories. The colour gradient from blue to red represents rising detection frequency. Two columns for untyped strains were included for *Actinomyces* spp and *Aspergillus* spp, respectively.

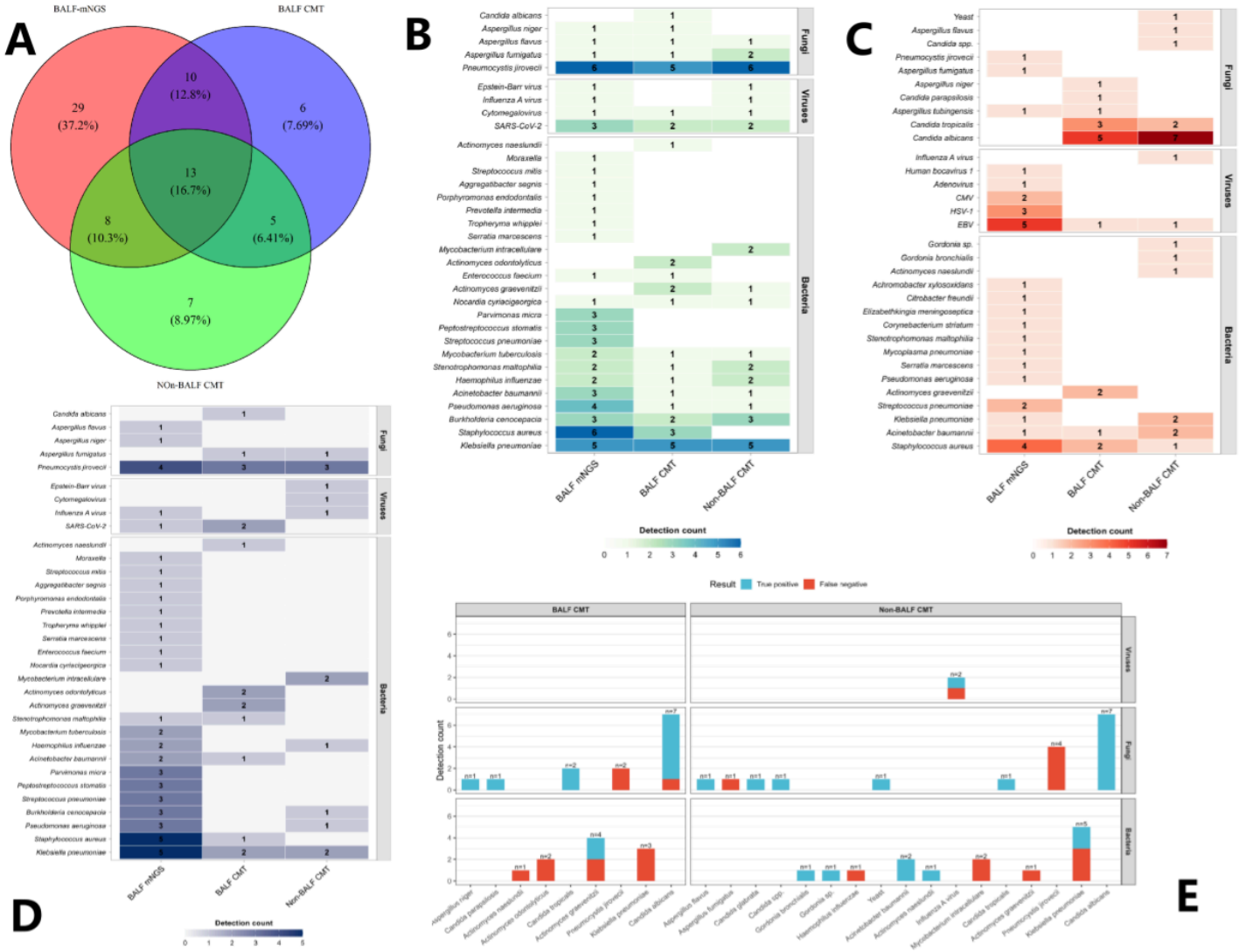


Figure 2

Comparison of pathogen detection profiles among the three testing strategies. (A) Overlap of pathogen detections across the three strategies (n = 78). Unique detections by BALF mNGS, BALF CMT, and non-BALF CMT were 29, 6, and 7 detection events, respectively. (B) Frequency distribution of clinically concordant pathogens. BALF mNGS showed superiority in the number and frequency of detected bacteria and viruses, while fungal detection frequencies were comparable among the three strategies. (C) Distribution of clinically irrelevant microbial detections. BALF mNGS yielded more irrelevant bacterial and viral detections, whereas conventional testing identified more irrelevant fungi. (D) Pathogens uniquely or firstly detected by each assay. BALF mNGS exhibited substantial advantages in bacterial detection, with no differences in fungal and viral detection compared with the other methods. (E) Distribution of pathogens missed by BALF mNGS. Most detections of *Candida albicans* were not clinically causative, whereas all detections of *Pneumocystis jirovecii* represented true infections.

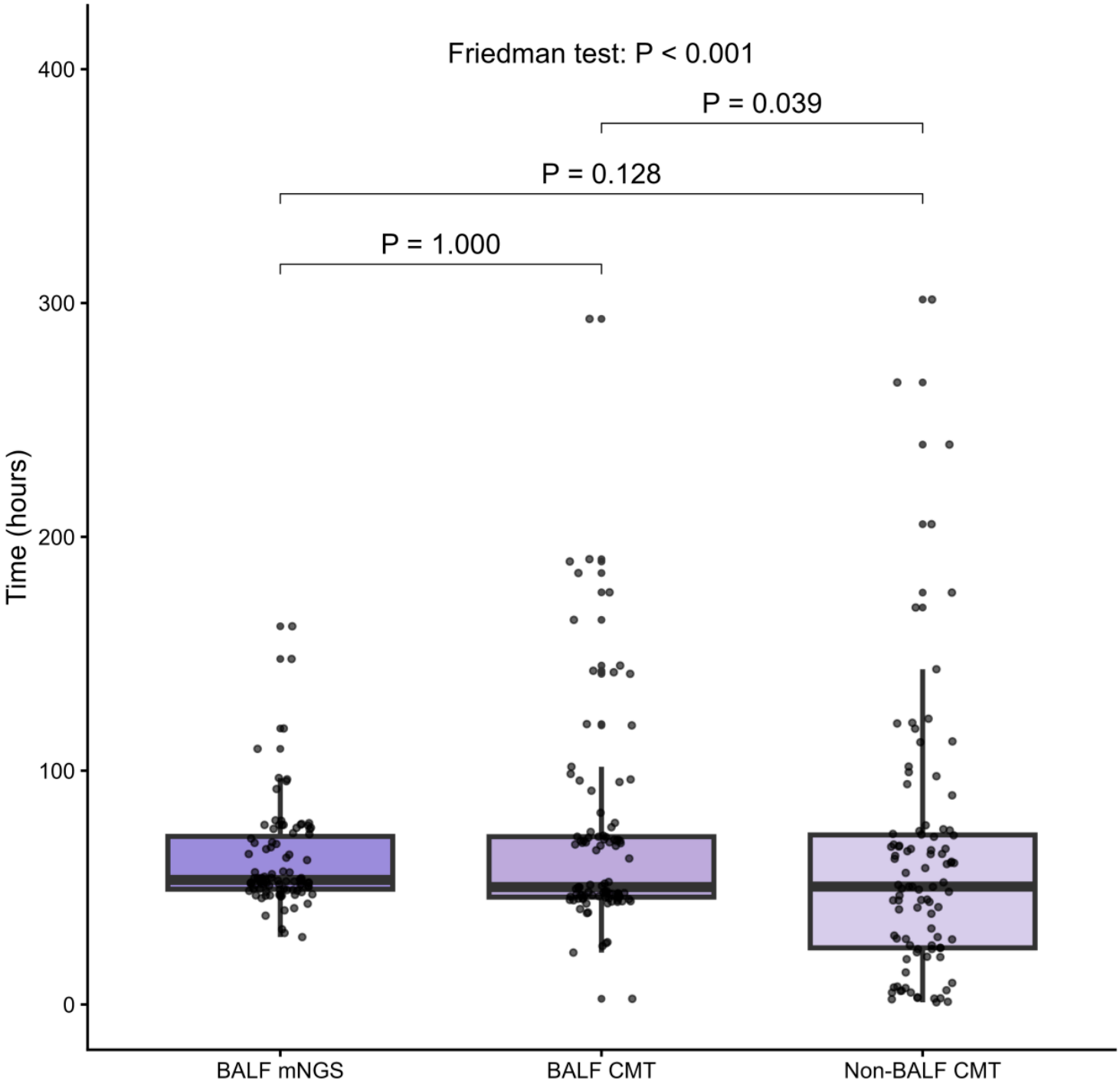


Figure 3

Comparison of turnaround time (TAT) among the different testing strategies. Box plots show the distribution of TAT for BALF mNGS, BALF CMT, and non-BALF CMT; each point represents an individual testing episode.

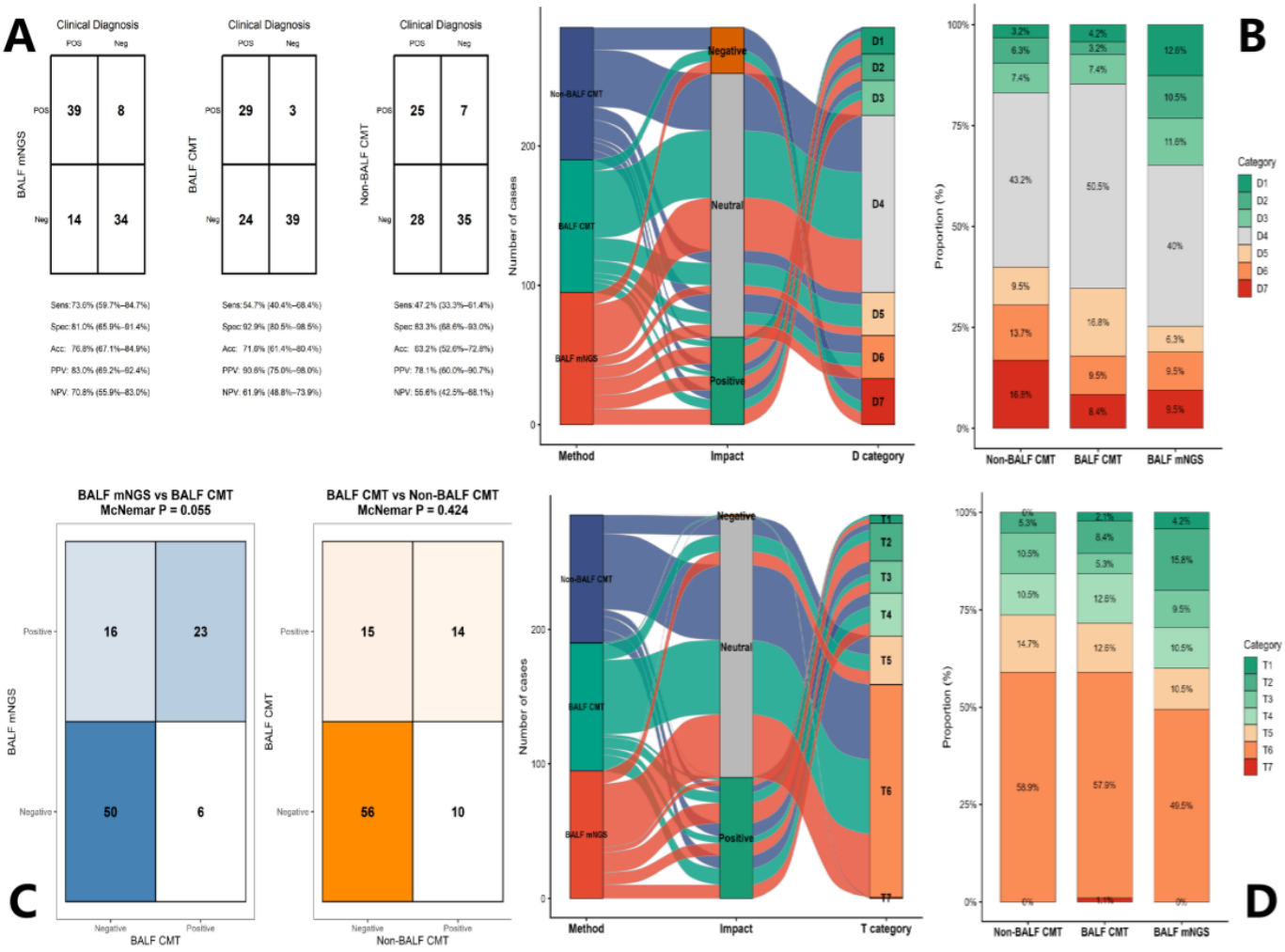


Figure 4

Comparison of diagnostic performance and clinical impacts across the three testing strategies. (A) 2×2 contingency tables for each assay against the final composite diagnosis, with sensitivity (Sens), specificity (Spec), accuracy (Acc), positive predictive value (PPV), and negative predictive value (NPV) ((all with 95% CIs). (B) Distribution of diagnostic impacts. Left: Sankey diagrams illustrating the flow of diagnostic impacts (D1–D7) for each testing strategy. Right: proportional composition of D1–D7 categories; beneficial impacts correspond to D1–D3, neutral impacts to D4–D6, and adverse impacts to D7. (C) Paired comparisons of detection results using McNemar's test. Left: BALF mNGS versus BALF CMT; Right: BALF CMT versus non-BALF CMT. (D) Distribution of therapeutic impacts. Left: Sankey diagrams illustrating the flow of therapeutic impacts (T1–T7) for each testing strategy. Right: proportional composition of T1–T7 categories; beneficial impacts correspond to T1–T4, neutral impacts to T5–T6, and adverse impacts to T7.