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# Yield of Metagenomics in Suspected Central Nervous System Infections with Negative Cerebrospinal Fluid Cultures

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**Abstract:** Background: Metagenomic next-generation sequencing (mNGS) represents a promising diagnostic tool for central nervous system infections, and its clinical impact on patient management when the cerebrospinal fluid is culture-negative remains inadequately explored. Methods: We conducted a retrospective cross-sectional study involving patients who underwent culture-negative cerebrospinal fluid mNGS at a tertiary hospital from March 2019 to December 2024, aiming to assess its diagnostic efficacy and clinical implications. Results: A total of 93 culture-negative cerebrospinal fluid samples from 93 patients underwent mNGS. Positive results were observed in 58.1% (54/93) of patients, with 78 microorganisms identified, and 52.6% (41/78) were clinically relevant. Clinically relevant organisms exhibited significantly higher median sequence reads compared with clinically irrelevant microbes (95 vs. 3; $p < 0.0001$). mNGS results positively impacted 65.6% (61/93) of patients by confirming or excluding central nervous system infections. However, among cases with negative clinical impact, 65.6% (21/32) were clinically diagnosed with central nervous system infections. Notably, 56.3% (18/32) of the positive mNGS results were considered non-pathogenic by clinicians, suggesting that mNGS alone may not be sufficient for diagnosing or ruling out central nervous system infections. Additionally, no significant differences were observed in clinical impact between immunocompromised and immunocompetent patients (68% vs. 64.7%, $p = 0.802$). Conclusion: mNGS demonstrates high diagnostic yield and positive clinical impact for patients with culture-negative cerebrospinal fluid. Its clinical applications should take into account factors such as patient demographics, diagnostic performance, and the interpretation of results in conjunction with conventional testing and collaboration within multidisciplinary teams.

**Keywords:** metagenomic next-generation sequencing; cerebrospinal fluid; central nervous system infection; pathogen; clinical impact

## 1. Introduction

Central nervous system (CNS) infections are associated with high mortality, making their diagnosis solely through symptoms, physical examinations, laboratory tests, and imaging examinations challenging. Microbiological testing of cerebrospinal fluid (CSF) is crucial but difficult. The optimal diagnostic approach varies by pathogen, reflecting diverse mechanisms and host immune responses [1]. To navigate these complexities,

clinicians are increasingly adopting advanced detection methods like metagenomic next-generation sequencing (mNGS) for its sensitivity, broad pathogen coverage, and hypothesis-free approach, enabling rapid diagnosis of diverse pathogens [2,3].

In recent years, some studies have demonstrated NGS utility in confirming infections caused by culture-negative or fastidious pathogens, such as *Mycoplasma pneumoniae*, *Rickettsia,* and *Nocardia* [4–6]. However, several factors influence the efficacy of pathogen detection by mNGS, including the patient's immune status and initial suspicion of infection. Additionally, the current operational procedure for culture-negative CSF mNGS testing, including encompassing sampling, bioinformatics analysis, and result interpretation, is intricate and costly. These challenges underscore the need to define optimal timing and patient selection for mNGS.

In our previous study, NGS proved clinically useful for diagnosing culture-negative meningitis and encephalitis [7]. However, its small sample size and short duration were limitations. Since March 2019, over one hundred culture-negative CSF samples underwent mNGS testing, prompting this larger investigation. Therefore, following STROBE metagenomics guidelines [8], this study aimed to evaluate the detection of plausible pathogens, clinical characteristics, and the clinical impact on culture-negative CSF mNGS over a nearly five-year period. Additionally, we also discussed how CSF mNGS findings influenced clinical decisions.

## 2. Methods

### *2.1 Study Design and Participants*

This retrospective observational cross-sectional study was conducted from March 2019 to December 2024. Inclusion criteria encompassed cases with: (1) culture-negative CSF but symptoms unexplained by initial conventional tests; (2) patients in life-threatening situations necessitating urgent, non-targeted pathogen investigation in the absence of specific clinical manifestations. Exclusion criteria included patients with: (1) incomplete clinical data; (2) repeated CSF mNGS detection for a single patient; (3) insufficient CSF sample volume for mNGS detection (Figure 1). Clinical characteristics, radiological findings, laboratory data, and outcomes of the enrolled patients were collected and analyzed. The clinical severity of suspected infection (classified as high or low) was initially assessed by senior clinicians at admission. This classification was then independently reviewed by a multidisciplinary panel of the clinical adjudication committee from the University of Hong Kong—Shenzhen Hospital. The panel's assessment was based on a comprehensive review of the patient's antibiotic and immunosuppressive therapy, immune status, and microbiological and histological results.

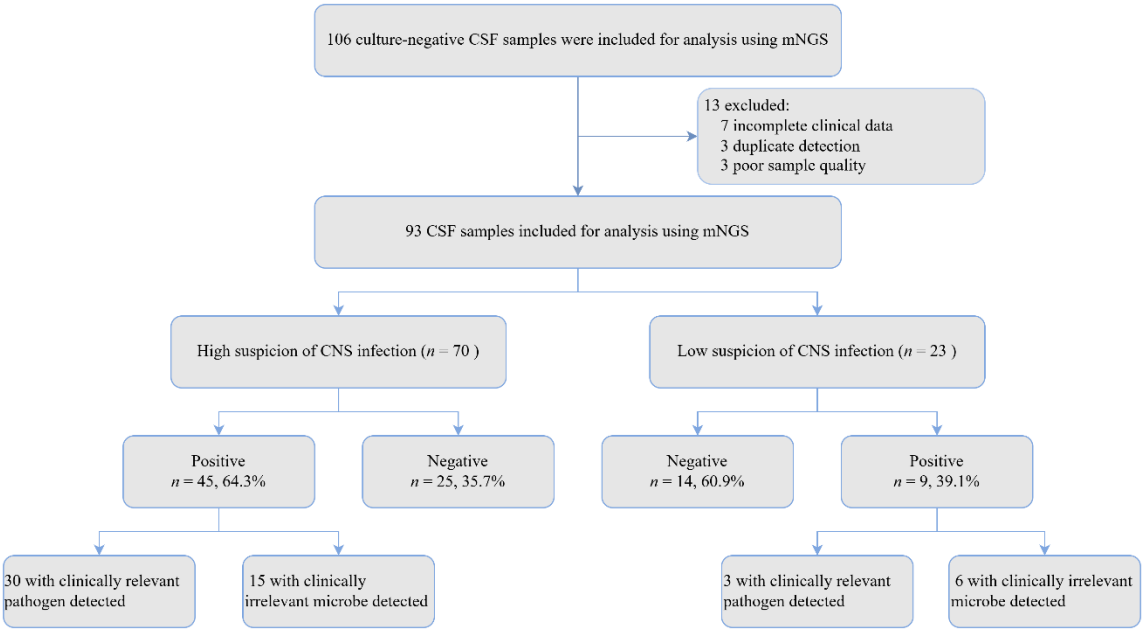


**Figure 1.** Flowchart illustrating the analysis of culture-negative CSF specimens and corresponding patients. CSF specimens were stratified into high-suspicion or low-suspicion categories based on clinical assessment of the corresponding patients before mNGS testing. Detected microorganisms were categorized into clinically relevant pathogens and clinically irrelevant microbes through the treating team (Abbreviations: CSF: cerebrospinal fluid, CNS: central nervous system, mNGS: metagenomic next-generation sequencing).

### *2.2. Metagenomic Next-Generation Sequencing (mNGS) Analysis*

CSF samples (1–3 mL) from enrolled patients were collected in sterile containers and preserved during transportation on dry ice at −20 °C. The whole sequencing and pathogen detection pipeline was performed in the laboratory of KingMed Diagnostic (Guangzhou, China). The procedure of filtering, mapping, and alignment is carried out by the widely used pipeline Sequence-Based Ultra-Rapid Pathogen Identification (SURPI) (CA, USA), which is generally considered to be highly accurate. For its clinical database MetagenomicX, KingMed established selection criteria based on NCBI protocols (https://www.ncbi.nlm.nih.gov/nuccore) to curate representative genome assemblies for key pathogenic microorganisms. Reads with multiple locus alignments within the same genus were excluded in secondary analysis. Only reads mapped to the genome within the same species were considered. Sequence reads are defined as a normalized sequence number in 200,000 primary sequences detected and derived from an internal control. To estimate the concentration of the target pathogen, exogenous plasmids of known concentrations were utilized as the internal control. The number of normalized sequence reads for the target pathogen can be calculated using the amplification efficiency ratio of the internal control.

$$\text{Sequence read} = \frac{20 \text{ milions} \times \text{Number of reads only mapped within same taxon}}{\text{Total reads of this sample}}$$

In order to construct the microbial genome database, pathogens and their genomes or assemblies were selected following the Kraken2 criteria for selecting representative assemblies for microorganisms (bacteria, viruses, fungi, protozoa, and other multicellular eukaryotic pathogens) from the Kraken2 database (https://benlangmead.github.io/aws-indexes/k2; accessed on 9 August 2025). Pathogens from Johns Hopkins ABX Guide (https://www.hopkinsguides.com/hopkins/index/Johns_Hopkins_ABX_Guide/Pathogens; accessed on 9 August 2025), Manual of Clinical Microbiology, and clinical case reports or research articles published in current peer-reviewed journals are included in this in-house database. Detailed description of the testing protocol, experimental parameters, quality control, and test result reporting, along with the list of microorganisms detectable by CSF mNGS, are provided in Supplementary Information S1 and S2, respectively. Identified microorganisms and their normalized read counts were reported to the clinical treatment team. mNGS analysis was performed on CSF samples after obtaining informed consent from patients or their legal guardians.

### *2.3. Clinical Relevance of Microorganisms Identified with CSF mNGS Testing*

We determined the clinical relevance of each organism by reviewing the treatment team's responses to it. Relevant organisms were defined as those: (i) consistent with the results of all conventional microbiological tests (culture, serology, and/or PCR) performed within one week of presentation and determined by the treatment team to be a true pathogen; and (ii) not detected by routine testing but whose identification by mNGS prompted targeted treatment based on clinical interpretation. Irrelevant organism refers to: (i) lacking clinical evidence related to its infection and no targeted treatment was administered; and (ii) considered contamination from the environment or normal human flora. All of the above information comes from the review of each patient's electronic medical record and clinical consultation with an ordering clinician or treating team.

### *2.4. Clinical Impact of Culture-Negative CSF mNGS Testing*

In our hospital, every mNGS report was delivered timely to the treatment team (clinicians and infectious disease specialists) and was prospectively used for clinical management. The treating team's interpretation of CSF mNGS results and their subsequent management decisions and actions were considered to assess the real-world impact of CSF mNGS testing. Specifically, whether the mNGS test result has a positive, negative, or no clinical impact was determined primarily based on the following criteria: (i) whether mNGS testing could have changed the clinical reasoning or altered patients' management (e.g., initiation, modification, or discontinuation of antimicrobial therapy), or both; and (ii) the subsequent patients' outcomes (e.g., improved, delayed, worsened, or death). Additionally, descriptive statistical analyses were performed to compare the sequence reads of clinically relevant pathogens versus clinically irrelevant microorganisms and to evaluate the turnaround time for mNGS and conventional tests.

### *2.5. Statistical Analysis*

Demographic data were summarized using descriptive statistics. The statistical significance of differences in sequence reads between causative or non-pathogenic microbes. The *Chi-square* test was employed for categorical variables, while either the unpaired Student's *t*-test or the Mann-Whitney U test was used for continuous variables, depending on the data distribution. Statistical tests were performed using the SPSS software (version 26.0; SPSS

Inc., Chicago, IL, USA) and GraphPad software (version 9.3.1; GraphPad Software, San Diego, CA, USA) with a $p$-value < 0.05 as the significance threshold.

### *2.7. Clinical Adjudication Committee*

Cases were independently reviewed by a multidisciplinary adjudication panel of three specialists: a neurologist, an infectious disease specialist, and a clinical microbiologist. None of the adjudicators was involved in the patients' diagnosis or treatment, ensuring an objective evaluation of the mNGS reports. Initial disagreements were resolved through structured panel discussion until a full consensus was reached for every case.

### *2.8. Ethics Statement*

This study was carried out according to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of The University of Hong Kong—Shenzhen Hospital ([2022]-120).

## 3. Results

### *3.1. Patient Characteristics*

Of 106 CSF samples, 13 were excluded due to incomplete clinical data ($n$ = 7), duplicate detection ($n$ = 3), and poor quality ($n$ = 3), leaving 93 samples from 93 patients for analysis (Figure 1). Median age was 38 years (range: 1–83), with 75.3% (70/93) being adults and 60.2% (56/93) male. 53.8% (50/93) patients were previously healthy, while 26.9% (25/93) were immunocompromised, including diabetes (10/93, 10.7%), hematological diseases (5/93, 5.4%) and solid malignancies (5/93, 5.4%) (Table 1). Empirical antimicrobial therapy preceded mNGS in 31.2% (29/93) of patients. Suspected community-acquired CNS infection was the most common indication for CSF mNGS testing (39.8%, 37/93), followed by ruling out CNS infection (28.0%, 26/93) and sepsis (16.1%, 15/93).

### *3.2. Microorganism Identification Using mNGS*

Microorganisms were detected in 58.1% (54/93, 95%CI: 48–68.1%) of the CSF mNGS samples. Among these, 61.1% (33/54) contained clinically relevant pathogens, all from the confirmed CNS infection group (Figure 2). A total of 78 organisms were identified, including 48.7% (38/78) bacteria, 41% (32/78) DNA viruses, 7.7% (6/78) RNA viruses, 1.3% (1/78) fungi, and 1.3% (1/78) parasites. Of these, 52.6% (41/78, 95%CI: 41.5–63.6%) were ultimately deemed clinically relevant. Notably, 100% (6/6) of the detected RNA viruses were classified as clinically relevant, a proportion significantly higher than that of DNA viruses (59.4%, 19/32) and bacteria (39.5%, 15/38).

Among the bacteria, 23 bacteria were considered clinically irrelevant microbes, predominantly *Escherichia coli*, *Pseudomonas* species, and *Staphylococcus* species. Thirteen clinically irrelevant viruses were identified, primarily EBV and CMV. *Pneumocystis jirovecii* was detected in one CSF sample, accompanied by 2 sequence reads of CMV. However, clinicians ultimately disregarded the mNGS findings, and the patient was subsequently diagnosed with bacterial meningitis (pathogen not found).

**Table 1.** Demographic and clinical characteristics of patients included in this study.

| The Study Cohort | Number of Patients, *n* (%) | Diagnosis of CNS Infection, *n* (%) | Diagnosis of Non-CNS Infection, *n* (%) | CSF mNGS Testing with a Positive Result, *n* (%) | CSF mNGS Tests with Positive Clinical Impact, *n* (%) |
|---|---|---|---|---|---|
| No. of patients | 93 (100.0) | 54 (58.1) | 39 (41.9) | 54 (58.1) | 61 (65.6) |
| Age | | | | | |
| Age, years | 38 (1–83) | | | | |
| Children (≤15 years) | 23 (24.7) | 16 (69.6) | 7 (30.4) | 12 (52.2) | 16 (69.6) |
| Adults (>15 years) | 70 (75.3) | 38 (52.9) | 32 (45.7) | 42 (60) | 45 (64.3) |
| Gender | | | | | |
| Male | 56 (60.2) | 36 (64.3) | 20 (35.7) | 34 (60.7) | 35 (62.5) |
| Female | 37 (39.8) | 18 (48.6) | 19 (51.4) | 20 (54.1) | 26 (70.3) |
| Primary medical condition | | | | | |
| Cardiovascular diseases | 23 (24.7) | 11 (47.8) | 12 (52.2) | 16 (69.6) | 14 (60.9) |
| Diabetes | 10 (10.7) | 2 (20) | 8 (80) | 5 (50) | 5 (50) |
| Hematological diseases | 5 (5.4) | 1 (20) | 4 (80) | 0 | 4 (80) |
| Autoimmune diseases | 3 (3.2) | 1 (33.3) | 2 (66.7) | 2 (66.7) | 3 (100) |
| Solid neoplasm | 5 (5.4) | 3 (60) | 2 (40) | 3 (60) | 4 (80) |
| Solid organ transplant | 1 (1.1) | 1 (100) | 0 | 1 (100) | 1 (100) |
| HIV | 1 (1.1) | 1 (100) | 0 | 1 (100) | 1 (100) |
| AIGA | 2 (2.2) | 2 (100) | 0 | 2 (100) | 1 (50) |
| Chronic otitis media | 4 (4.3) | 2 (50) | 2 (50) | 0 | 1 (25) |
| Premature infant | 2 (2.2) | 0 | 2 (100) | 0 | 2 (100) |
| Health | 50 (53.8) | 32 (64) | 18 (36) | 28 (56) | 34 (68) |
| Immunocompromised | 25 (26.9) | 11 (44) | 14 (56) | 14 (56) | 17 (68) |
| Symptoms on presentation | | | | | |
| Fever | 73 (78.5) | 47 (64.4) | 26 (35.6) | 47 (64.4) | 45 (61.6) |
| Stiff-neck | 16 (17.2) | 11 (68.8) | 5 (31.2) | 12 (75) | 10 (63.5) |
| Headache | 52 (55.9) | 37 (71.2) | 15 (28.8) | 37 (71.2) | 32 (61.5) |
| Vomit | 25 (26.9) | 15 (60) | 10 (40) | 18 (72) | 20 (80) |
| Unconsciousness | 49 (52.7) | 27 (55.1) | 22 (44.9) | 25 (51) | 32 (65.3) |
| Hemiplegia | 11 (11.8) | 5 (45.5) | 6 (54.5) | 6 (54.5) | 8 (72.3) |
| Seizures | 12 (12.9) | 6 (50) | 6 (50) | 6 (50) | 7 (58.3) |
| CSF cytology | | | | | |
| Nucleated cell ($\times 10^6$/L) | 42 (3, 192) | 102.5 (9, 369.5) | 7 (2, 47) | 77 (7.8, 297.5) | 49 (2.5, 135) |
| Protein (mg/L) | 737 (302, 1272) | 793 (375.5, 1481) | 475 (243, 1186) | 610.5 (314, 1151) | 617 (306.5, 1202) |
| Glucose (mmol/L) | 3.31 (2.78, 3.84) | 3.03 (2.41, 3.50) | 3.73 (3.15, 4.95) | 3.23 (2.54, 3.71) | 3.01 (3.33, 3.84) |
| Antibiotics prior to mNGS | | | | | |
| Yes | 30 (31.2) | 14 (46.7) | 16 (53.3) | 15 (50) | 16 (53.3) |
| No | 63 (68.8) | 40 (63.5) | 23 (36.5) | 39 (61.9) | 45 (71.4) |

AIGA: Adult-onset immunodeficiency due to interferon gamma autoantibodies; CNS: Central nervous system; mNGS: Metagenomic next-generation sequencing. Data are median (IQR) or *n* (%).

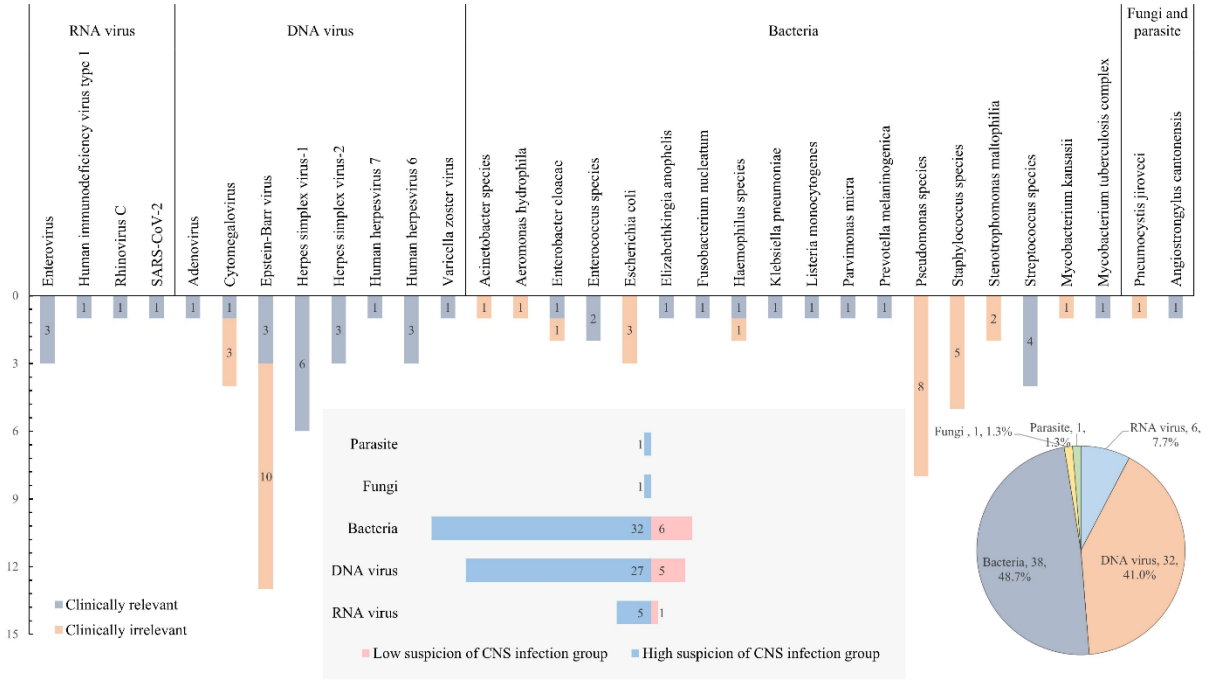


**Figure 2.** Summary of the proportions of organisms identified that either resulted in clinical impact or were deemed clinically irrelevant. The upper column chart illustrates all identified organisms, while the lower shaded bar chart provides a comparative summary of organisms from the two groups. The lower pie chart in the bottom right represents the proportional distribution of organisms across distinct taxonomic groups.

### *3.3. Patients Confirmed CNS Infection by mNGS*

Among the 33 patients with confirmed CNS infections identified by mNGS, 26 were immunocompetent individuals (Table 2). The etiologies included viral (19 patients), bacterial (12 patients), coinfection (1 patient), and parasitic (1 patient). We evaluated the relationship between sequence reads and the identification of clinically relevant organisms. The median sequence reads for clinically relevant organisms (95 [Interquartile Range (IQR): 8–798]) were significantly higher than those for irrelevant microbes (3 [IQR: 1–31], $p < 0.0001$) (Figure 3A). mNGS also provided rapid results (median turnaround time of 29 hours, IQR: 26–32.5) (Figure 3B).

Common bacterial pathogens identified: *Streptococcus pneumoniae* ($n$ = 2), *Streptococcus agalactiae*, *Enterococcus cecorum*, *Enterococcus faecium*, *Enterobacter cloacae*, *Elizabethkingia anopheles*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, and *Listeria monocytogenes* (all $n$ = 1). In a brain abscess case caused by odontogenic infection, mNGS detected *Fusobacterium nucleatum*, *Prevotella melaninogenica*, and *Parvimonas micra* in the patient's CSF. Additionally, mNGS diagnosed uncommon infections like zoonotic *Streptococcus suis* in an immunocompetent patient exposed to raw pork (Patient 6).

mNGS identified 24 clinically relevant viruses, comprising 19 DNA viruses and 5 RNA viruses. This capability was exemplified by the diagnosis of rare CNS viral infections: a 26-year-old male with acute eosinophilic meningitis was found to have early HIV infection with latent syphilis and HSV-2 meningitis. CSF mNGS detected HSV-2 (912 sequence reads) and HIV-1 (4648 sequence reads) (Patient 3) [9]. Additionally, an immunocompetent patient with SARS-CoV-2 pneumonia and encephalitis had SARS-CoV-2 (25 sequence reads) and Epstein-Barr virus (3 sequence reads) detected in the CSF by mNGS (Patient 8).

*Angiostrongylus cantonensis* was uniquely detected with 159 sequence reads, and is significantly critical for clinical management. The patient had *Achatina fulica* exposure and presented with eosinophilic meningitis. Brain imaging revealed bilateral paraventricular ischemic lesions and parietal microhemorrhages. Furthermore, *Angiostrongylus cantonensis* antibodies were detected in the patient's serum by enzyme-linked immunosorbent assay (Patient 81).

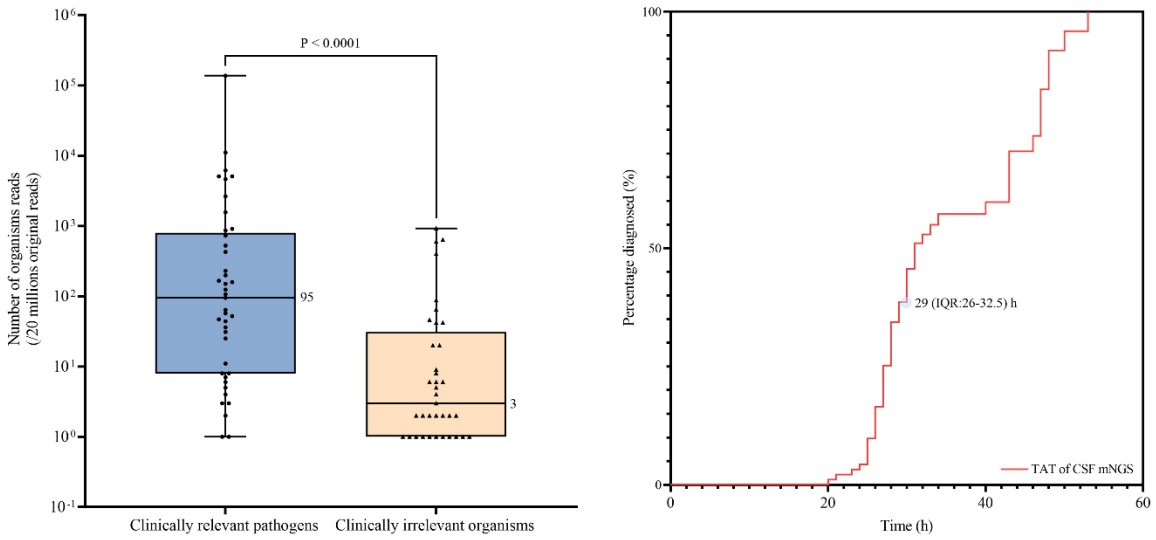


**Figure 3.** Clinical effect of mNGS testing. (**A**) The median sequence reads of clinically relevant pathogens were 95 (IQR: 8–798) vs. 3 (IQR: 1–31) for clinically irrelevant microbes ($p < 0.0001$); (**B**) Turnaround time (TAT) for 93 culture-negative mNGS tests from sample collection to result report. The red line in the figure represents the distribution of TAT for the 93 tests, with the median TAT being 29 (indicated by the light gray circles in the figure, IQR: 26–32.5) hours.

### *3.4. Factors Affecting Culture-Negative CSF mNGS Clinical Impact*

Multivariable modified Poisson regression analysis revealed no significant association with patient age or sex (Figure 4). The overall CSF mNGS detection rate was lower in immunocompromised patients (28%, 7/25) than in immunocompetent individuals (38.2%, 26/68); however, no statistically significant difference was observed within the immunocompromised subgroup about clinical positive impact ($p = 0.802$). Similarly, no significant difference in clinical positive impact was found between patients with high versus low suspicion of CNS infection (62.9% vs. 73.9%; PR: 1.17, 95%CI: 0.82–1.70; $p = 0.403$). Although a higher rate of positive clinical impact was observed in patients who had not received antibiotics before mNGS testing compared to those who had (71.4% vs. 53.3%), this difference was not statistically significant (PR: 1.34, 95%CI: 0.87–2.06, $p = 0.185$). Furthermore, neither presenting symptoms nor CSF cytology categories were significantly associated with the clinical positive impact of CSF mNGS (all $p > 0.05$). Notably, the clinical positive impact did not differ significantly between those with positive versus negative CSF mNGS results (66.7% vs. 64.1%; PR: 0.96, 95%CI: 0.68–1.35; $p = 0.814$). These findings suggest that in the context of mNGS testing, clinical interpretation play a more critical role in guiding patient diagnosis and management than the test result alone.

**Table 2.** Clinical and laboratory findings of 33 patients confirmed CNS infection by mNGS.

| Patient ID | Patient History | Immunocompromised | CSF Analysis | | | | Brain Imaging Indicated Infection | Antibiotics before mNGS Report | Antibiotics after mNGS Report | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pathogen Detected by mNGS (Sequence Reads) | Opening Pressure ($mmH_2O$) | Nucleated Cell ($\times10^6$/L) | Protein | | | | |
| P1 | 1 yo baby | No | Enterovirus (1), CMV (3) | NP | 3 | 353 | No | Ceftriaxone | None | Recovered |
| P2 | 13 yo adolescent | No | Enterovirus (5084), EBV (2) | 220 | 79 | 326 | Yes | Ceftriaxone, acyclovir | None | Recovered |
| P3 | 26 yo male living with HIV | Yes | HIV-1 (4648), HSV-2 (912) | 220 | 737 | 1718 | No | Ceftriaxone | Acyclovir, penicillin | Recovered |
| P4 | 39 yo male | No | HSV-1 (124) | 142 | 90 | 737 | No | Acyclovir | Acyclovir | Recovered |
| P5 | 41 yo male with a kidney transplant | Yes | *Streptococcus pneumoniae* (230) | 300 | 8072 | 6607 | Yes | Ceftriaxone, linezolid | Ceftriaxone | Recovered |
| P6 | 58 yo male | No | *Streptococcus suis* (1564) | 180 | 157 | 801 | Yes | Meropenem, linezolid | Ceftriaxone | Recovered |
| P7 | 60 yo male | No | HSV-1 (44) | 270 | 118 | 288 | Yes | Ceftriaxone, doxycycline, acyclovir | Acyclovir | Recovered |
| P8 | 68 yo male | No | EBV (3), SARS-CoV-2 (25) | 215 | 118 | 1152 | Yes | Ceftriaxone, vancomycin, acyclovir | Acyclovir | Recovered |
| P9 | 68 yo male | No | *Enterobacter cloacae* (52) | 300 | 264 | 553 | Yes | Meropenem, vancomycin | Meropenem | Recovered |
| P10 | 82 yo female | No | *Enterococcus faecium* (64), EBV (6) | 65 | 42 | 16151 | No | Meropenem | Levofloxacin | Recovered |
| P24 | 39 yo female | No | Rhinovirus C (5091) | 170 | 3 | 317 | Yes | None | None | Recovered |
| P44 | 57 yo female with cancer | Yes | *Fusobacterium nucleatum* (36), *Prevotella melaninogenica* (31), *Parvimonas micra* (11) | >320 | 1742 | 3156 | Yes | Ceftriaxone, vancomycin | Meropenem, vancomycin | Recovered |
| P49 | 27 yo female | No | HSV-1 (11092) | 190 | 183 | 272 | Yes | Ceftriaxone, acyclovir | Acyclovir | Recovered |
| P55 | 80 yo male with diabetes | Yes | *Klebsiella pneumoniae* (6166) | 170 | 647 | 7892 | Yes | Meropenem | Ceftriaxone | Recovered |
| P57 | 1 yo baby | No | *Enterococcus cecorum* (526) | NP | 8 | 604 | No | Meropenem | Meropenem | Recovered |
| P58 | 1 yo baby | No | *Streptococcus agalactiae* (95) | NP | 55 | 579 | Yes | Ceftriaxone | Ceftriaxone | Recovered |
| P64 | 14 yo adolescent | No | HHV-7 (198) | 250 | 1 | 296 | Yes | Ceftriaxone | Acyclovir | Recovered |
| P67 | 8 yo child | No | EBV (150) | 120 | 49 | 245 | No | Ceftriaxone | Acyclovir | Recovered |
| P70 | 1 yo baby | No | HHV-6 (47) | NP | 53 | 523 | No | Ceftriaxone | Acyclovir | Recovered |
| P72 | 5 yo child | No | Enterovirus (8) | 100 | 115 | 222 | No | Ceftriaxone | None | Recovered |
| P74 | 10 yo child | No | HHV-6 (57) | 191 | 2 | 263 | No | None | Acyclovir | Recovered |
| P75 | 1 yo baby | No | *Elizabethkingia anophelis* (166) | NP | 70 | 776 | No | Ceftriaxone | Ceftriaxone | Recovered |

| Patient ID | Patient History | Immunocompromised | CSF Analysis | | | | Brain Imaging Indicated Infection | Antibiotics before mNGS Report | Antibiotics after mNGS Report | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pathogen Detected by mNGS (Sequence Reads) | Opening Pressure ($mmH_2O$) | Nucleated Cell ($\times10^6$/L) | Protein | | | | |
| P78 | 54 yo female with autoimmune disease | Yes | EBV (2637) | 200 | 60 | 1530 | Yes | None | Acyclovir | Recovered |
| P81 | 63 yo female | No | *Angiostrongylus cantonensis* (159) | 300 | 274 | 617 | Yes | Doxycycline, acyclovir | Albendazole | Recovered |
| P82 | 32 yo male | No | *Mycobacterium tuberculosis complex* (2) | >320 | 68 | 1014 | No | Cefuroxime, acyclovir, doxycycline, ceftriaxone | Isoniazid, rifampin, ethambutol, pyrazinamide | Recovered |
| P83 | 69 yo male with cancer | Yes | VZV (137,150) | 155 | 699 | 8632 | Yes | Ceftriaxone, acyclovir | Acyclovir | Recovered |
| P85 | 38 yo male | No | Adenovirus (1) | 320 | 817 | 985 | Yes | Acyclovir, doxycycline, ceftriaxone | None | Recovered |
| P86 | 68 yo male | No | HHV-6 (428) | 120 | 1 | 816 | Yes | Amoxicillin/clavulanate, doxycycline | None | Recovered |
| P87 | 72 yo female | No | HSV-1 (730), HSV-2 (5) | 150 | 49 | 432 | Yes | None | Acyclovir | Recovered |
| P89 | 31 yo male | No | HSV-1 (866), HSV-2 (6), *Streptococcus pneumoniae* (4) | 330 | 121 | 785 | Yes | Amoxicillin/clavulanate, acyclovir | Ceftriaxone, vancomycin, Meropenem, acyclovir | Recovered |
| P90 | 33 yo female | No | *Haemophilus influenzae* (8) | 202 | 1010 | 1151 | No | Acyclovir, Vancomycin, ceftriaxone | Ceftriaxone | Recovered |
| P91 | 59 yo male with AIGA | Yes | *Listeria monocytogenes* (7) | 160 | 387 | 383 | Yes | Piperacillin/tazobactam | Ampicillin | Recovered |
| P93 | 39 yo male | No | HSV-1 (107) | 142 | 125 | 737 | No | None | Acyclovir | Recovered |

AIGA: Adult-onset immunodeficiency due to interferon gamma autoantibodies; CNS: Central nervous system; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; HSV: Herpes simplex virus; HHV: Human herpesvirus; VZV: Varicella zoster virus; NP: Not performed.

| Group | Patients, n (%) | CSF mNGS tests with positive clinical impact, n (%) | PR ((95%CI) | *P* value |
|---|---|---|---|---|
| Age (years) | | | | |
| ≤15 | 23 (24.7) | 16 (69.6) | Ref | |
| >15 | 70 (75.3) | 45 (64.3) | 0.92 (0.63-1.35) | 0.685 |
| Sex | | | | |
| Male | 56 (60.2) | 35 (62.5) | Ref | |
| Female | 37 (39.8) | 26 (70.3) | 1.12 (0.82-1.54) | 0.473 |
| Immune status | | | | |
| Immunocompetent | 68 (73.1) | 44 (64.7) | Ref | |
| Immunocompromised | 25 (26.9) | 17 (68) | 1.05 (0.72-1.53) | 0.802 |
| Suspicion CNS infection | | | | |
| High | 70 (75.3) | 44 (62.9) | Ref | |
| Low | 23 (24.7) | 17 (73.9) | 1.17(0.82-1.7) | 0.403 |
| Antibiotics prior to mNGS | | | | |
| Yes | 30 (31.2) | 16 (53.3) | Ref | |
| No | 63 (68.8) | 45 (71.4) | 1.34 (0.87-2.06) | 0.185 |
| Symptoms on presentation | | | | |
| Fever | 73 (78.5) | 45 (61.6) | Ref | |
| Stiff-neck | 16 (17.2) | 10 (63.5) | 1.01 (0.64-1.61) | 0.956 |
| Headache | 52 (55.9) | 32 (61.5) | 1.00 (0.73-1.36) | 0.985 |
| Vomit | 25 (26.9) | 20 (80) | 1.30 (0.97-1.74) | 0.081 |
| Unconsciousness | 49 (52.7) | 32 (65.3) | 1.06 (0.78-1.44) | 0.706 |
| Hemiplegia | 11 (11.8) | 8 (72.3) | 1.18 (0.74-1.88) | 0.489 |
| CSF cytology | | | | |
| Nucleated cell elevated | 66 (71) | 44 (66.7) | Ref | |
| Protein elevated | 58 (62.4) | 38 (65.5) | 0.98 (0.75-1.29) | 0.899 |
| Glucose decreased | 10 (10.8) | 6 (60) | 0.90 (0.49-1.65) | 0.734 |
| CSF mNGS result | | | | |
| Positive | 54 (58.1) | 36 (66.7) | Ref | |
| Negative | 39 (41.9) | 25 (64.1) | 0.96 (0.68-1.35) | 0.814 |
| Total | 93 (100) | 61 (65.6) | | |

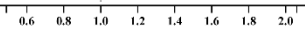


**Figure 4.** Factors affecting culture-negative CSF mNGS clinical impact. The association between various factors (age, sex, immune status, suspicion tier, antibiotic prior, symptoms on presentation, CSF cytology, and CSF result) and positive clinical impact was assessed using a modified Poisson regression approach to calculate prevalence ratios (PRs). Only *p*-values less than 0.05 were considered statistically significant.

### *3.5. Clinical Impact of Culture-Negative CSF mNGS Testing on Patient Management*

Culture-negative CSF mNGS results yielded positive clinical impact in 65.6% (61/93, 95%CI: 55.5–74.7%) of patients (Table 3), diagnosing CNS infection and initiating targeted therapy (33/93, 35.5%), ruling out CNS infection (5/93, 5.4%), and ruling out infection to start non-antibiotic therapy (23/93, 24.7%). Among the 33 cases that were diagnosed as CNS infection diagnoses, mNGS enabled earlier diagnoses in 21.2% (7/33) cases, facilitated the diagnosis and escalation of therapy in 12.1% (4/33) cases, facilitated the diagnosis and maintenance of therapy in 15.2% (5/33) cases, and facilitated the diagnosis and de-escalation of therapy in 51.5% (17/33) cases.

**Table 3.** Clinical impact of culture-negative CSF mNGS testing ($n$ = 93).

| Categories of Clinical Impact | No. (%) [95%CI] of CSF mNGS Tests |
|---|---|
| Positive impact | 61/93 (65.6%) [55.5–74.7%] |
| Enabled diagnosis of CNS infection and initiation of targeted therapy | 33/93 (35.5%) [26.5–45.6%] |
| Facilitated the diagnosis earlier than conventional methods | 7/33 (21.2%) |
| Facilitated the diagnosis and escalation of therapy | 4/33 (12.1%) |
| Facilitated the diagnosis and maintained therapy | 5/33 (15.2%) |
| Facilitated the diagnosis and de-escalation of therapy | 17/33 (51.5%) |
| Enabled ruling out of CNS infection | 5/93 (5.4%) [2.3–12.0%] |
| Enabled ruling out of infection and initiation of noninfectious therapy | 23/93 (24.7%) [17.1–34.4%] |
| No impact | 32/93 (34.4%) [25.5–44.5%] |
| Microbes deemed non-pathogenic by the treatment team | 12/93 (12.9%) [7.5–21.2%] |
| Redundant information, antibiotics, and the clinical plan were not changed | 6/93 (6.5%) [3.0–13.4%] |
| A negative result with no clinical significance | 14/93 (15.1%) [9.2–23.7%] |

CNS: central nervous system; mNGS: Metagenomic next-generation sequencing.

No clinical impact occurred in 34.4% (32/93) of patients. The lack of clinical impact in the positive CSF mNGS results fell into two categories: (i) the organisms were not considered to be the true pathogens by the treating team, as the corresponding patients displayed no related clinical signs of infection (i.e., considered contamination from the environment or normal human flora) (12/93, 12.9%); (ii) identification of a new organism without a change in treatment strategy due to the effectiveness of empirical therapy (6/93, 6.5%) (Figure 5). The remainder (14/93, 15.1%) had no microbes detected.

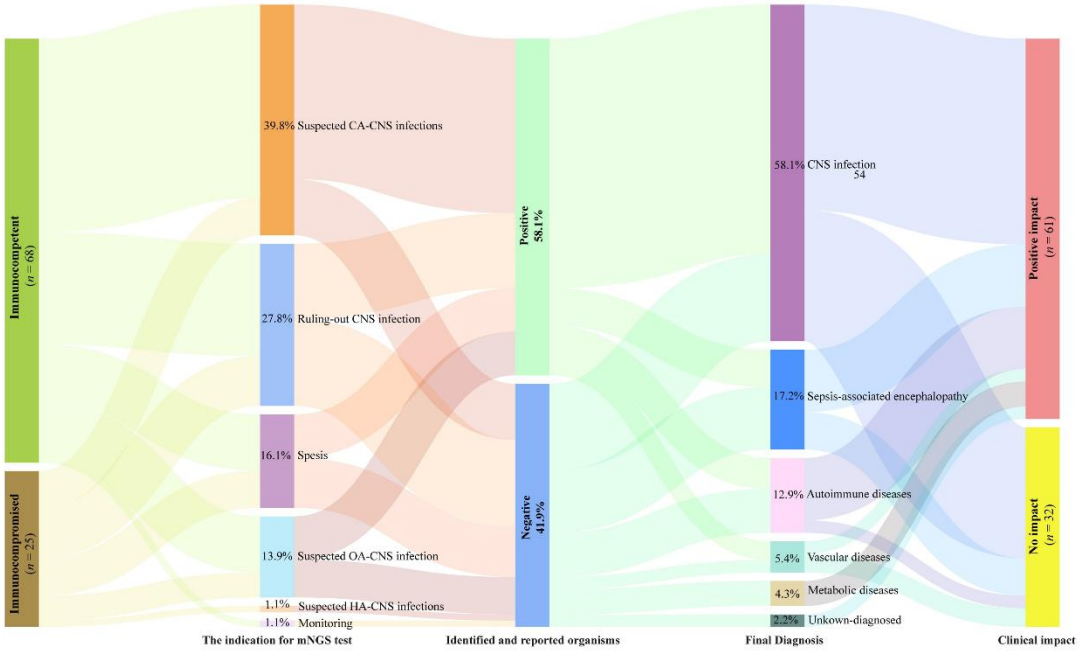


**Figure 5.** Summary of the clinical impact of culture-negative CSF mNGS testing on patient management. The figure illustrates outcomes stratified by subgroup, with percentages within each column representing the proportion of tests demonstrating clinical impact (Abbreviations: CA-CNS: Community-acquired central nervous system, OA-CNS: Operation-associated central nervous system, HA-CNS: Hospital-acquired central nervous system).

## 4. Discussion

Clinical mNGS provides a comprehensive analysis of microbes and host genetic material (DNA and RNA), revolutionizing the field of diagnostic microbiology. This emerging approach is transforming the diagnosis and treatment of infectious diseases, with applications extending to areas such as antimicrobial resistance and microbiome research [3,10]. However, most studies focused on its diagnostic performance versus conventional tests, and few have addressed its practical impact in CNS infectious diseases [11,12]. Our study found that mNGS effectively identified pathogens in culture-negative CSF samples, with 61.1% (33/54) containing clinically relevant pathogens. Furthermore, 65.6% (61/93) of mNGS tests showed positively impacted patient management, as determined through clinical assessment. This suggests that mNGS could beneficially reshape CNS infection

treatment frameworks. Collaboration among clinicians, microbiologists, and infectious disease specialists facilitates rapid pathogen identification despite variations in false positives and negatives across different samples analyzed by NGS [7,9]. This enables earlier targeted therapy, ultimately improving patients' outcomes, which is consistent with other research in the field [13,14]. Consequently, it may be prudent to recommend mNGS as a routine complement to first-line diagnostic tests for culture-negative CNS infections.

Although CSF samples are obtained from sterile sites, contamination by non-pathogenic organisms can occur due to the detection of cell-free microbial nucleic acid fragments through mNGS [15]. A key challenge in CSF mNGS analysis is to distinguish whether detected microbes represent true infections or not, particularly in immunocompromised patients or those with compromised mucosal barrier function. While clinically relevant organisms had significantly higher median sequence reads than irrelevant microbes (95 vs. 3), establishing universal diagnostic thresholds based solely on read counts is nearly impossible due to sample handling, pathogen variability, and patient factors. For example, Enterovirus, Adenovirus, and *Mycobacterium tuberculosis* were clinically confirmed pathogens despite reads below reporting thresholds (sequence reads = 3). It is important to note that reporting sub-threshold results based on clinical chart reviews is not a standardized approach and is often not feasible. In most cases, clinicians primarily rely on clinical signs and empirical knowledge. Therefore, integrating mNGS with conventional tests is essential for comprehensive diagnosis [16]. Furthermore, mNGS currently lacks the capability to reliably predict antimicrobial resistance, necessitating a skilled interpretation team for the results, especially experts with expertise in microbiology and infectious disease. Over the past 12 years, our department has developed a collaborative model that enables accurate mNGS interpretation and tailored management, potentially explaining the higher clinical relevance rate (52.6%) in this study compared to some reports [17]. Given these findings, we recommend that for microorganisms with low sequence reads but clinical relevance, mNGS results should be validated using additional conventional microbiological tests to enhance the reliability of clinical predictions. Based on these research findings, we have developed a diagnostic flowchart to guide the clinical management of patients with suspected CNS infections when CSF cultures yield negative results at our hospital (Figure 6).

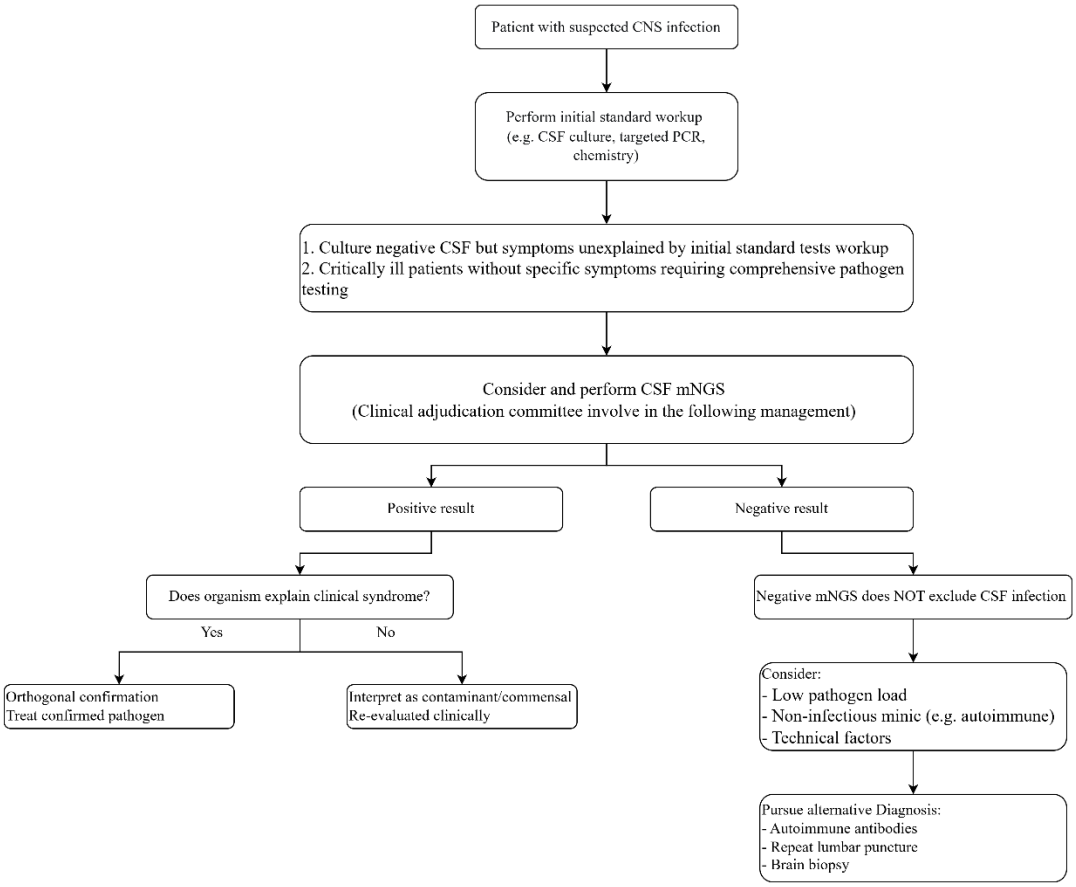


**Figure 6.** A practical diagnostic algorithm for a patient with suspected CNS infection (Abbreviations: CNS: central nervous system; CSF: cerebrospinal fluid; mNGS: Metagenomic next-generation sequencing).

Since this study focused on culture-negative CSF samples, mNGS yielded a positive clinical impact in 65.6% of cases, higher than a recent retrospective study [18]. These findings align with reports on plasma mNGS by Han et al. and Rossoff et al., suggesting that patient selection contributes to higher utility [19,20]. In this study, mNGS provided rapid results, expediting intervention. Notably, 64.1% (25/39) of *negative* mNGS tests still positively impacted management. However, among the subgroups of 32 cases with negative clinical impact, 65.6% (21/32) of patients were clinically diagnosed with CNS infections. Of these, 56.3% (18/32) mNGS tested positive but were considered non-pathogenic. For instance, in a patient with anti-interferon-gamma autoantibody syndrome diagnosed with disseminated *Mycobacterium abscessus* infection, contrast brain MRI revealed multiple high-signal lesions consistent with meningitis. Despite these clinical and imaging findings, mNGS failed to detect the pathogen (Patient 31). 35.8% (14/39) of negative mNGS did not alter antibiotics, indicating mNGS cannot reliably exclude infection. In combination with other studies, we recommend that the mNGS test be used in patients who have a high probability of being positive and clinical impact [13,21]. Additionally, clinical impact did not differ between immunocompromised and immunocompetent patients ($p > 0.05$) (Figure 4). Further research is needed to confirm this observation.

This study also explored the application of CSF mNGS in nosocomial infection control and RNA virus detection. In one neonate with meningitis, *Elizabethkingia anophelis* (sequence reads:166) was rapidly identified, enabling targeted treatment and immediate outbreak prevention measures (Patient 75). Separately, an immunocompetent woman who developed unconsciousness following an acute upper respiratory infection, and mNGS detected Rhinovirus C (sequence reads: 5091) in her CSF, which is not a routinely identified pathogen in our center (Patient 24). However, as most CNS infections involve DNA pathogens, routine RNA testing warrants careful consideration due to added costs. Given the substantial expense of mNGS testing (approximately $2000–3000 in the US and 3000–4000 RMB in China) and nearly one-third of results lacking diagnostic impact, we recommend prioritizing it for cases with high clinical suspicion of infection but negative conventional testing [13]. Prior to testing, consultation with infectious disease or microbiology specialists is also advisable.

There are some limitations to this study. First, due to the clinical complexity of patients, establishing a fair gold standard for defining clinical impact is challenging, as seen in previous studies [22–24]. This assessment may over or underestimate how test results influenced management and outcomes. Second, we did not compare mNGS directly with conventional microbiological methods, although we noted pathogens missed by mNGS, highlighting the need for complementary approaches. Third, due to limited sensitivity, we did not evaluate the ability of mNGS to detect resistance genes or predict microbial resistance phenotypes. Fourth, we did not assess how the turnaround time of each CSF mNGS test influenced patient outcomes. Finally, due to the limited number of cases, we did not perform a stratified analysis for every infection type.

In summary, our study confirms the clinical utility of CSF mNGS, demonstrating both a high diagnostic yield and a positive impact on patient management. However, accurate interpretation of complex mNGS datasets requires close collaboration between laboratory specialists and clinicians. Further research and clinical practice are needed to optimize patient selection, testing timing, and refinement of interpretive frameworks to maximize diagnostic yield and therapeutic guidance.

## Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/250923141241411 2/eMicrobe-2508000258-Supplementary-Materials.zip. References [25–36] are cited in the supplementary materials.

1. Supplementary Material S1. Supplementary Information:
   - Information 1–3. CSF Procedure and quality control of mNGS in this study.
   - Information 4. Pathogens detected using CSF mNGS.
   - Information 5. Conventional microbiological tests for CSF.
   - Information 6. List of ≤5 sequence reads organisms.
2. Supplementary Material S2. Clinical data of enrolled patients in the study.

## Author Contributions

C.D. planned the study and wrote the manuscript and provided graphical support; J.H., Y.Y., and R.L. provided test support; J.L. provided hospital infection control; L.L. and Q.Y. provided clinical management of the patient; C.D. and F.X. reviewed, edited, and approved the draft. All authors have read and agreed to the published version of the manuscript.

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## Data Availability Statement

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

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## Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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