Am J Transl Res 2023;15(1):47-62
www.ajtr.org /ISSN:1943-8141/AJTR0143908

Original Article

Clinical application and drug-use-guidance value of metagenomic next-generation sequencing in central nervous system infection

Ke Lin[1*], Hao-Cheng Zhang[1*], Yi Zhang[1*], Yang Zhou[1*], Zhang-Fan Fu[1], Hong-Yu Wang[1], Yuan-Han Zhao[1], Chao Qiu[1,2], Ming-Xiang Fan[3], Jie-Yu Song[1], Jing-Wen Ai[1], Wen-Hong Zhang[1,2,4,5]

[1]Department of Infectious Disease of Huashan Hospital, National Medical Center for Infectious Diseases and Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, Fudan University, Shanghai 200040, China; [2]Key Laboratory of Medical Molecular Virology (MOE/MOH) and Institutes of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai 200032, China; [3]Department of Urology, Tongji Hospital, School of Medicine, Tongji University, Shanghai 200065, China; [4]State Key Laboratory of Genetic Engineering, School of Life Science, Fudan University, Shanghai 200438, China; [5]National Clinical Research Centre for Aging & Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China. [*]Equal contributors.

Received May 4, 2022; Accepted August 11, 2022; Epub January 15, 2023; Published January 30, 2023

Abstract: Objective: Timely and precise etiology diagnosis is crucial for optimized medication regimens and better prognosis in central nervous system infections (CNS infections). We aimed to analyze the impact of mNGS tests on the management of patients with CNS infections. Methods: We conducted a single-center retrospective cohort study to analyze the value of mNGS in clinical applications. Three hundred sixty-nine patients with a CNS infection diagnosis were enrolled, and their clinical data were collected. CDI and DDI were defined in our study to describe the intensity of drug use in different groups. We used LOH and mRS to evaluate if the application of mNGS can benefit CNS infected patients. Results: mNGS reported a 91.67% sensitivity in culture-positive patients and an 88.24% specificity compared with the final diagnoses. Patients who participated with the mNGS test had less drug use, both total (58.77 vs. 81.18) and daily (22.6 vs. 28.12, $P < 0.1$, McNemar) intensity of drug use, and length of hospitalization (23.14 vs. 24.29). Patients with a consciousness grading 1 and 3 had a decrease in CDI (Grade 1, 86.49 vs. 173.37; Grade 3, 48.18 vs. 68.21), DDI (Grade 1, 1.52 vs. 2.72; Grade 3, 2.3 vs. 2.45), and LOH (Grade 1, 32 vs. 40; Grade 3, 21 vs. 23) with the application of mNGS. Patients infected with bacteria in the CNS had a reduced CDI, DDI, and LOH in the mNGS group. This was compared with the TraE group that had 49% of patients altered medication plans, and 24.7% of patients reduced drug intensity four days after mNGS reports. This was because of the reduction of drug types. Conclusion: mNGS showed its high sensitivity and specificity characteristics. mNGS may assist clinicians with more rational medication regimens and reduce the drug intensity for patients. The primary way of achieving this is to reduce the variety of drugs, especially for severe patients and bacterial infections. mNGS has the ability of improving the prognosis of CNS infected patients.

Keywords: Metagenomic next-generation sequencing, central nervous system infection, drug intensity, prognosis, diagnose

Introduction

The central nervous system, or CNS, comprises the brain and the spinal cord. An infection of the CNS can be life-threatening [1-5]. The timely and accurate detection of pathogens is crucial to successfully diagnosing and managing center nervous system infections. Conventional infection diagnosis methods, such as microbial culture, targeted PCR et al., suffer from limited targets and long turn-around time, resulting in urgent recruitment of novel infection diagnosis techniques [6, 7].

Metagenomic next-generation sequencing (mNGS) has been recently used in infection diagnosis practice with satisfactory outcomes. This increases pathogen detection sensitivity in CNS infections [7]. One study showed that mNGS improved the positive rate of pathogen

detection by 13.1% from 55.6% to 68.7% in detecting the pathogens in cerebrospinal fluid (CSF) from patients with bacterial meningitis [8]. In our previous study, mNGS achieved a sensitivity and specificity in CNS infection of 90% and 98.57%, respectively [9], reducing detection turn-around time (unpublished data). Most studies have focused on the diagnostic performance improvement value of mNGS. The lack of standard procedure, biased report criteria, and high economic cost have made it challenging to evaluate the direct benefit acquired by the patients and the public health system [8].

Molecular rapid diagnostic tests provide several opportunities to optimize antimicrobial selections to improve patients' outcomes [9, 10]. These microbiological results are used to guide the choice of antimicrobial drugs. Previous studies have reported the antimicrobial agent adjustments according to mNGS and Filmarray meningitis/encephalitis Panel. mNGS led to a change of treatment in 59 (37.1%) cases, including antibiotics de-escalation in 40 (25.2%) cases in respiratory infections [11]. In pediatric CNS infections, 55.4% patients received antimicrobial de-escalation [12]. Among immunocompromised patients, mNGS played a role in optimizing antibiotic use. Though several studies have made efforts to evaluate avoidance of antibiotic misuse and reduction of hospitalization period after the application of mNGS, there is a lack of comprehensive evaluation of mNGS benefit in CNS infections. A more precise evaluation of antimicrobial use needs to be performed.

In this study, we conducted a retrospective cohort study to evaluate the hospitalization period, antimicrobial drug types, amount after mNGS employment, and the diagnostic yield of mNGS in CNS infection patients. Our aim was to comprehensively estimate the clinical benefits of mNGS and value of universal application in daily clinical practice.

Methods

Setting and data collection

This retrospective cohort study was conducted at Huashan Hospital of Fudan University. All the data for this study were collected from the Electronic Medical Records System of Huashan Hospital. The protocol for the conduct of this study was reviewed and approved by Huashan Hospital ethical committee (Approval number: KY2017-338). Patients or their surrogates signed informed consents for the lumbar puncture. Their information was collected from the Electronic Medical Records System.

Study patients and samples

Patients older than 14 years with an infection of the central nervous system (CNS) were eligible for inclusion if they were admitted to the Department of Infectious Diseases, Huashan Hospital of Fudan University, between March 2014 and December 2018. All the study participants were discharged with confirmed CNS infection as their primary diagnosis by physicians. We excluded patients with a previous diagnosis of CNS infection, results of a positive etiological test, or effective treatments before admission. We reasoned that these patients were treated differently by clinicians based on known or suspected pathogen infections from previous treatment histories. This led to the bias of clinical decision making. Patients without lumbar punctures during the hospitalization were excluded. Enrollment and exclusion criteria were listed in the Table S1. CSF samples were obtained from all the patients and sent for routine and biochemical tests, CSF smear, and the traditional culture of bacteria, fungi, and tuberculosis.

Sample sequencing and data analysis

A volume of 1.5-3 mL CSF samples from each patient was collected according to standard procedures. A 1.5 mL microcentrifuge tube with 0.6 mL sample, enzyme, and 1 g 0.5 mm glass bead was attached to a horizontal platform on a vortex mixer and agitated vigorously at 2800-3200 rpm for 30 min. A 0.3 mL sample was separated into a new 1.5 mL microcentrifuge tube. DNA was extracted using the TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH) in accordance to the manufacturer's recommendation.

DNA libraries were constructed through DNA fragmentation, end-repair, adapter-ligation, and PCR amplification. Agilent 2100 was used for quality control of the DNA libraries. Quality qualified libraries were sequenced by the BGISEQ-50/MGISEQ-2000 platform [2].

High-quality sequencing data were generated by removing low-quality reads. This was followed by computational subtraction of human host sequences mapped to the human reference genome (hg19) using the Burrows-Wheeler Alignment [3]. The remaining data by removal of low-complexity reads were classified by simultaneously aligning to four Microbial Genome Databases, consisting of bacteria, fungi, viruses, and parasites. The classification reference databases were downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/). RefSeq contains 4,945 whole-genome sequences of viral taxa, 6,350 bacterial genomes or scaffolds, 1064 fungi related to human infection, and 234 parasites associated with human diseases.

Diagnostic assessment of mNGS

We assessed the diagnostic performance of mNGS through the following steps. We classified the participants into two groups contingent on if they had undergone the mNGS test: Patients who received traditional examinations only (TraE group) and patients who underwent the mNGS test (mNGS group). We calculated mNGS sensitivity compared to culture, mNGS sensitivity compared to culture = mNGS (positive)/culture (positive). The extra detection rate of mNGS compared to culture was statistically evaluated, mNGS extra detection rate = [mNGS (positive) - culture (positive)]/culture (positive). We analyzed the accuracy rate of mNGS, and the accuracy rate = mNGS (positive) & case-consistent/mNGS (positive). Considering the composite criteria of recruiting, we did not discuss the specificity of mNGS here.

We evaluated the consistency between mNGS and clinical diagnosis: mNGS positive/case consistent was determined as the mNGS detected pathogen is inconsistent with the final diagnosis. mNGS positive/case inconsistent represents inconsistent results between mNGS reports and final diagnosis.

Evaluation of clinical outcome

We employed the Medical Research Council (MRC) Grade, Modified Ranking Scales (mRS), and length of hospitalization (LOH) to evaluate patient status. Participants were classified into 3 MRC grades according to their Glasgow Coma Scale (GCS) and their clinical manifestation [13]: Grade 1 (GCS=15), Grade 2 (GCS of 11-14 or GCS of 15 associated with a focal neurological sign), and Grade 3 (GCS $\leq$ 10) (Table S2). As reported, the patient's status can be divided into three categories according to mRS [14]: level 1 (good outcome, grade 0), level 2 (intermediate outcome, grade 1-2), and level 3 (poor prognosis grade 3-5) (Table S3). The LOH represented the length of the patient's hospital stay. We used days as the unit of measurement.

To compare the intensity of antibiotic use between groups and avoid the incomparable defects caused by different types of drugs, we performed the calculation of Defined Daily Dose (DDD) [15]. We introduced the concepts of cumulative drug intensity (CDI) and daily drug intensity (DDI). CDI was defined as the accumulated medication intensity of the patient during the hospital stay. CDI was equal to the sum of the drug intensity of all anti-infective treatments performed during hospitalization. DDI was the average daily medication intensity of the patient, DDI=CDI/LOH.

The primary endpoint was the intensity of drug use and the length of hospital stay. The secondary endpoint was the functional outcome at discharge according to the mRS.

Statistics analysis

For baseline characteristics, blood laboratory tests, and CSF laboratory tests, the Kolmogorov-Smirnov test was used to determine the continuous variants described by medians when not. A Chi-square test was performed to evaluate independent binomial variables. The Mann-Whitney test was used to compare the difference of baseline between TraE group and mNGS group. We performed the Mann-Whitney test to compare the CDI, DDI, and LOH of patients in the TraE group and the mNGS group. We evaluated the difference of CDI, DDI, and LOH of patients in the TraE group and the mNGS group with different mental status or pathogen infection using the Mann-Whitney test. A P value < 0.05 was considered significant. In the process of assessing diagnostic performance, sensitivity, specificity, and the detection rate was calculated according to the definitions above. Statistical analyses and figures were conducted using the SPSS Version 26.0 (IBM Corp., Armonk, NY, USA) and

GraphPad Prism 8.4.0 software (GraphPad Software, San Diego, CA, USA).

Results

General characteristics

In total, 369 patients were enrolled. Patients with confirmed etiological diagnosis before admission or those with a history of effective anti-infection treatment (n=153) were not included. Medication history and etiological examinations interfere with the clinicians' diagnosis and treatment. Five patients without results of CSF laboratory tests were excluded. There were 211 participants who were analyzed (**Figure 1**). Fifty patients were diagnosed with encephalitis, 113 patients were affected with meningitis, and 38 were diagnosed with meningoencephalitis. As for the other ten patients, the exact location of the central nervous system infection was not able to be diagnosed. According to if the patients had undergone mNGS tests, we divide patients into TraE group and mNGS group. The TraE group we recruited included one hundred and thirteen patients who did not undergo the mNGS test. mNGS tests were not used at Huashan hospital until 2017. CSF samples for patients before 2017 came from conventional tests, including CSF routine and biochemical tests, and culture of bacteria, fungi, and tuberculosis. Ninety-eight patients accepted the extra mNGS test according to clinical necessity. They were classified into the mNGS group. All the participants were categorized into bacterial (51 vs. 44), fungal (14 vs. 13), parasitic (1 vs. 3), viral (36 vs. 37), or unclassified infections (11 vs. 1), based on distinguishing if the mNGS was performed. Baseline characteristics of enrolled patients showed no significant difference among groups (**Table 1**).

Overall diagnostic performance of mNGS

We performed an etiology analysis. It showed that, in the mNGS group, the culture reported 24 positive results. The mNGS detection revealed that *Mycobacterium tuberculosis* (n=7) was the most common pathogen. The top three causative pathogens identified were *Mycobacterium tuberculosis*, *Cryptococcus neoformans,* and *Herpes Simplex Virus*-1 (HSV-1) (**Figure 2**).

As shown in **Figure 3** and **Table 2**, mNGS reported a 91.67% (11/12) sensitivity compared to culture in the mNGS group. The specificity compared to clinical diagnosis of mNGS was 88.24% (45/51). For traditional culture, the specificity compared to clinical diagnosis reached 92.31% (12/13). mNGS owned a 39.8% extra detection rate to traditional culture, especially high in virus detection. Compared with traditional culture methods, mNGS detected Cytomegalovirus (CMV), Epstein-Barr virus (EBV), HSV-1, varicella-zoster virus (VZV), Herpes simplex virus 6A (HSV6A), and adenovirus B1 in the mNGS group.

Among 98 patients enrolled in the mNGS group, 45 patients were categorized into mNGS (positive)/case consistent group. Six patients were identified as mNGS (positive)/case inconsistent. This included reports of *Candida parapsilosis* and *Rhodococcus* relatively in 2 patients who were diagnosed as viral infection, both *Prevotella intermedia* and *Streptococcus constellation* in patients diagnosed with *Aspergillus* infection, one report of *Rickettsia* in fungal meningitis, one detection of *Epstein-Barr virus* in bacterial meningitis, and one report of *Oral Streptococcus* in tuberculosis meningoencephalitis patient. In culture-positive cases, mNGS reported one false-negative case of *Mycobacterium tuberculosis* (**Figure 2**).

The comparison of drug use intensity between TraE group and mNGS group

Comparing the mNGS group and the TraE group, we found a significant difference in the CDI between the two groups during hospitalization (**Figure 4**). The cumulative drug intensity during the hospitalization of the mNGS group was lower than that of the TraE group (81.18 vs. 58.77, respectively), with a decrease of 27.6%. We found that the average DDI was lower in the mNGS group (28.12 vs. 22.6 P=0.04), which meant the intensity of medication decreased by 19.6%.

We used the MRC grade (the definition of MRC grade as explained above) to classify patients according to their state of consciousness when they were admitted to the hospital. It was observed that the patients in Grade 1 were in a worse state of consciousness and a more

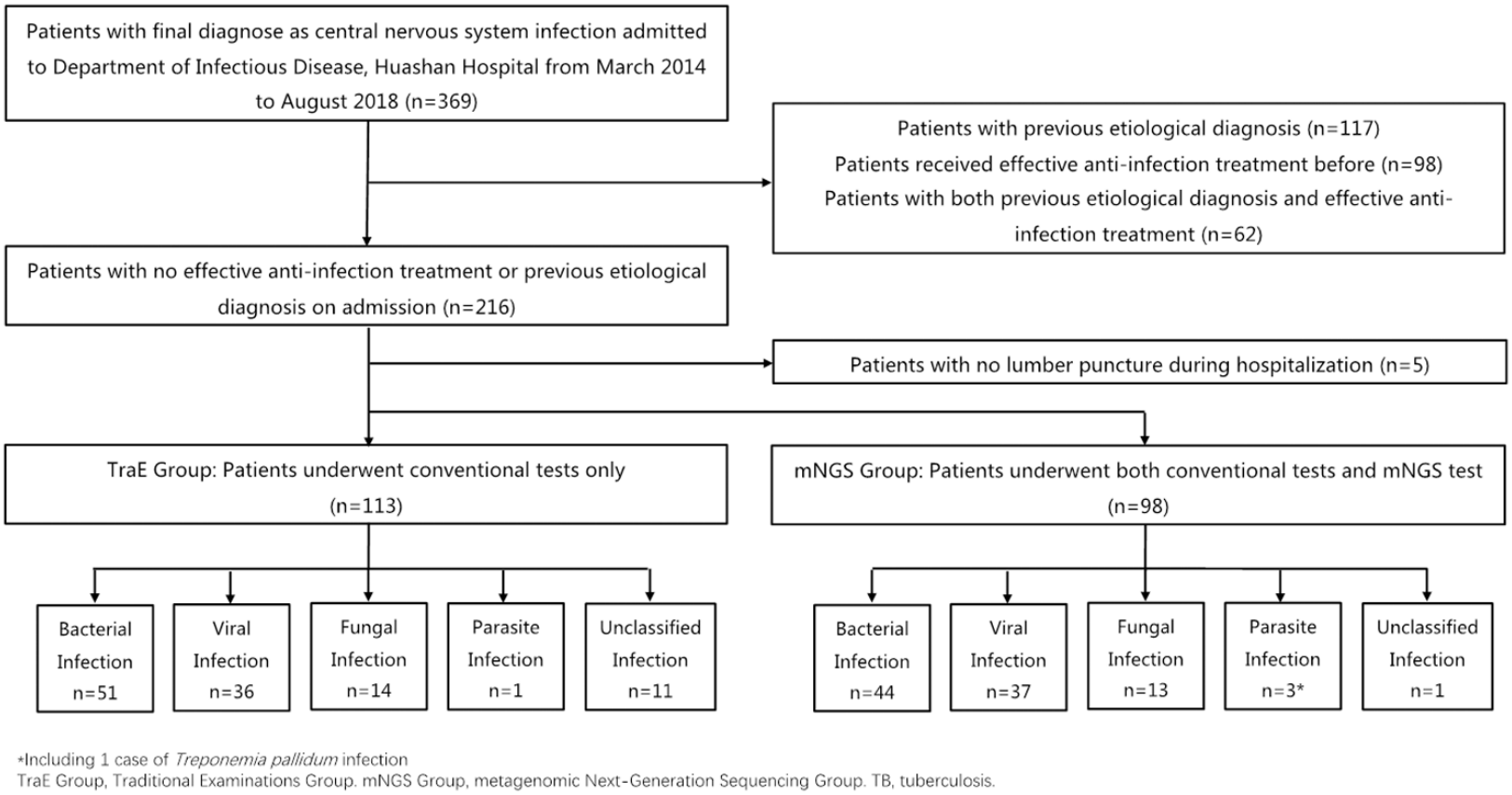

Figure 1. Flowchart of enrollment and classification.

Table 1. Demographical characteristics of enrolled patients

Characteristic	TraE Group	mNGS Group	*p* value
Age			
Median (IQR) - yr	46 (31-57.5)	44 (29-57)	0.505
Distribution - no. (%)			0.911
13-18 yr	5 (4.4)	6 (6.1)	
19-25 yr	13 (11.5)	11 (11.2)	
26-40 yr	30 (26.6)	23 (23.5)	
41-60 yr	41 (36.3)	40 (40.8)	
> 60 yr	24 (21.2)	18 (18.4)	
Male sex - no. (%)	68 (60.2)	66 (67.3)	0.281
Syndrome - no. (%)			0.132
Meningitis only	67 (59.3)	46 (46.9)	
Encephalitis only	22 (19.5)	28 (25.6)	
Meningitis with encephalitis	17 (15.0)	21 (21.4)	
Unclassified	7 (6.2)	3 (3.1)	
CNS infection - no. (%)			0.089
Bacterial infection	51 (45.1)	44 (44.9)	
Viral infection	36 (31.9)	37 (37.7)	
Fungal infection	14 (12.4)	13 (13.3)	
Parasitic infection	1 (0.9)	3 (3.1)	
Unclassified	11 (9.7)	1 (1.0)	
Immunosuppression - no. (%)	21 (18.6)	10 (10.2)	0.086
MRC Grade^{+} - no. (%)			0.103
Grade 1	86 (76.1)	72 (73.4)	
Grade 2	16 (14.2)	8 (8.2)	
Grade 3	11 (9.7)	18 (18.4)	
Body temperature - median (IQR), °C	37.0 (36.7-37.6)	37.0 (36.5-38.0)	0.819
Blood laboratory examination - median (IQR)			
WBC, *10^9/L	7.62 (5.72-10.22)	7.33 (5.79-9.47)	0.743
Neutrophil, %	74.1 (62.8-80.8)	72.2 (64.3-79.3)	0.937
C-reaction protein, mg/L	6.6 (3.0-20.1)	5.4 (3.0-25.2)	0.851
Procalcitonin, ng/mL	0.06 (0.04-0.12)	0.06 (0.04-0.11)	0.721
CSF laboratory examination - median (IQR)			
WBC, *10^6/L	75.0 (9.0-160.5)	100.0 (23.0-250.0)	0.104
Protein, mg/L	1287.0 (743.5-2310.5)	1359.0 (730.0-2425.0)	0.617
Multinuclear cell, %	20.0 (10.0-32.8)	16.0 (10.0-35.0)	0.638
Chlorides in CSF, mmol/L	116 (110-119)	116 (107-120)	0.988
Glucose in CSF, mmol/L	2.4 (1.9-2.8)	2.30 (1.80-2.83)	0.796

IQR, interquartile range. TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group. WBC, white blood cell. CSF, cerebrospinal fluid. MRC Grade^{+}: Medical Research Council (MRC) grade 1 indicates a Glasgow coma score of 15 (on a scale of 3 to 15, with lower scores indicating reduced levels of consciousness) with no neurologic signs, grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less.

severe disease situation. The application of mNGS decreased the median of DDI (4.57 vs. 2.24 P=0.0494) and CDI (73.25 vs. 48.06) during the hospital stay compared to the TraE group (**Figure 5**; **Table 3**).

We divided patients into viral-, bacterial-, and fungal-infections subgroups based on the etiology diagnosis. We found that compared to the TraE group, the median values of DDI (3.50 vs. 2.81) and CDI (70.33 vs. 53.00) of the mNGS group in the subgroup of bacterial infections were lower. The application of mNGS did not bring the consistent decrease of DDI (viral-infection subgroup: 1.54 vs. 1.50; fungal-infection subgroup: 1.78 vs. 2.37) and CDI (viral-

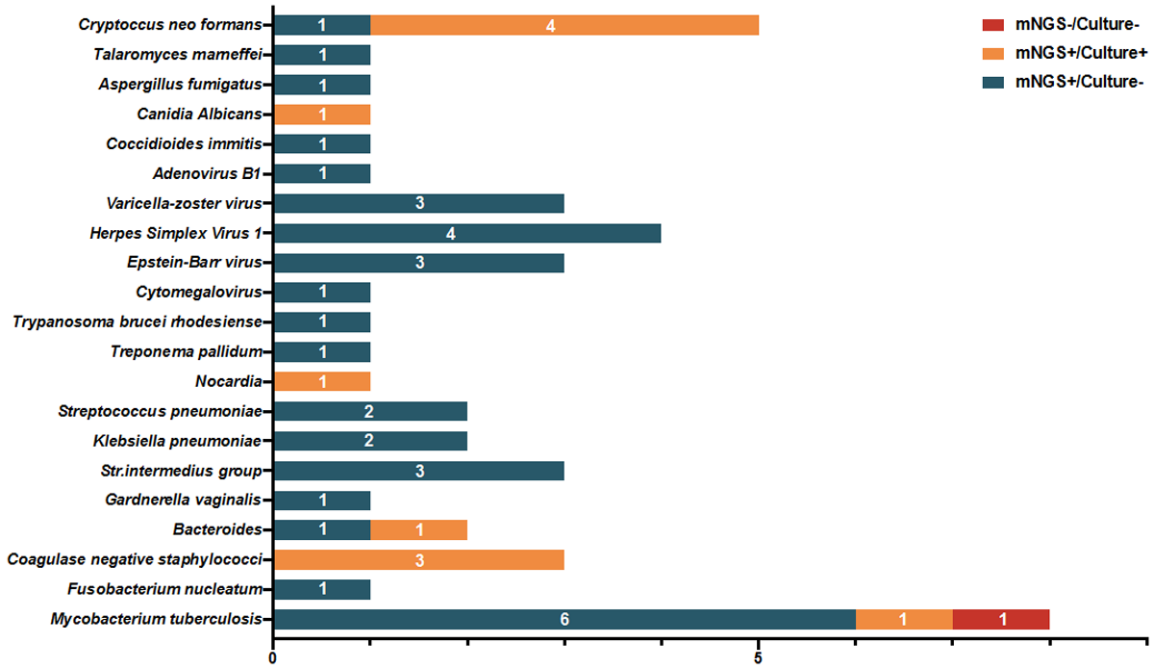

Figure 2. Distribution of detected pathogen by mNGS and culture.

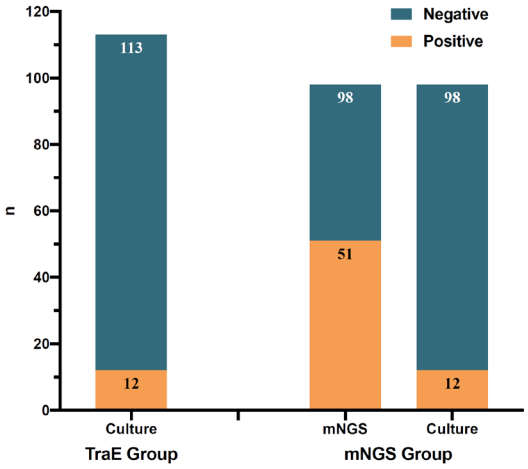

Figure 3. Diagnostic performance of mNGS. The sensitivity and detection rate of mNGS compared to culture and specificity compared to clinical diagnosis. TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group.

infection subgroup: 21.19 vs. 26.83; fungal-infection subgroup: 36.60 vs. 47.00) in both viral-infection and fungal-infection subgroups (**Figure 6**; **Table 4**).

Co-infection, such as respiratory and urinary tract infections, was a confounding factor that was not be ignored because it influenced the choice of a medication regime. We believe that the DDI value of patients without co-infection can better reflect the advantage of mNGS in medication guidance since the length of hospitalization was excluded as an interference factor. We regrouped patients into "co-infection" and "non-co-infection" groups, and the differences between the TraE group and mNGS group were analyzed (**Figure 7**; Table S4). We observed that the average of DDI value of the mNGS group was lower than that of the TraE group, which counted 2.25 and 2.72, respectively. We excluded people with co-infection in different pathogenic subgroups and compared the DDI value with or without mNGS. The DDI value showed a decrease with the performance of the mNGS test in viral and bacterial infection subgroups. There was no significant difference among fungal infections (Table S5).

The positive impact of mNGS on patient prognosis

The intensity of medication in the mNGS group helped make the LOH of patients shorter and showed that there was a decrease of 4.7% in the mNGS group when compared with the TraE group. The average of LOH counted 23.14 (days) and 24.29 (days), respectively (**Figure 4**). Among those patients with worse status on

Table 2. Clinical diagnose efficiency of mNGS in CNS infection

Methods	Sensitivity compared to culture	Concordance rate compared with final diagnosis	Additional detection rate
mNGS	91.67% (11/12)	88.24% (45/51)	39.80% (39/98)
Culture	-	92.31% (12/13)	-

mNGS, metagenomic next-generation sequencing. CNS, central nervous system.

admission (MRC Grade 1), the application of mNGS brought to a significant shortening of LOH. The median of LOH was 23.50 (days) and 40.00 (days) in the mNGS group and TraE group, respectively (**Figure 5B**).

The mRS was evaluated to reflect the patients' nervous system function and self-care after discharge. The scores helped to classify patients into three groups. The range was level 1 to level 3. Level 1 showed the best prognosis and level 3 represented the worst. Using the analysis results, we concluded that the ratio of level 3 was less in the mNGS group than that in the TraE group (26.5% vs. 17.3%, respectively). It showed that the use of mNGS contributed to the improvement of patient's prognosis (**Figure 8**).

Clinical decision making influenced by the application of mNGS

We analyzed the impact of mNGS on doctors' clinical decision-making. We found that among 98 people in the mNGS group, 48 (49%) patients' medication were altered. Among them, 15 (15.3%) patients' medication regimen was changed. There were 24 (24.5%) patients who experienced drug de-escalation (the clinicians replaced high-escalation antibiotics with low-escalation antibiotics in two patients who had an increase in DDI for other reasons) because of mNGS applications. The other nine people were discharged after the mNGS results feedback (**Figure 9A**).

We compared the DDI at the mNGS examination day and four days after the mNGS results were reported among the mNGS group. Nine patients were discharged within four days after the mNGS report. Of the remaining 89 participants, 22 (24.7%) patients showed a decrease in DDI. We analyzed the detailed reasons for medication de-escalation: reduction of drug types (57.14%), reduction in medication dose (10.71%), drug replacement by lower-escalation ones (7.41%), and drug adjustments (25.00%) for the treatment of different pathogen types (**Figure 9B**).

Discussion

Our retrospective study, which assessed the difference of the drug intensity and functional outcome of patients with CNS infection between groups performed with or without mNGS tests, identified a better prognosis and lower intensity of medication in patients who underwent mNGS tests. The improvement of medication intensity and prognosis due to the application of mNGS was significant in individuals with worse consciousness on admission or in people with CNS bacterial infection.

CNS infection is a life-threatening disease responsible for severe disability or death. CNS infections depend on therapeutic resources, including timely access to anti-infection therapy, and antibiotic or antiviral drug usage [5, 7, 16, 17]. Several studies reported the superiority of mNGS for diagnosing pathogens in infectious diseases when compared to conventional methods. Our data confirmed the high sensitivity and specificity of mNGS in the detection of pathogens. This was consistent with previous studies of mNGS [7, 18-20]. We found the sensitivity of the mNGS reached 91.67% compared to culture. The specificity was 88.24% in the final diagnosis. The extradetection rate of mNGS was as high as 39.8% compared to culture. This study revealed that mNGS was of excellent application value in the diagnosis of CNS infections.

Previous studies have confirmed the guidance role of mNGS in clinical treatment. Hu et al. declared a considerable modification of infection diagnoses based on mNGS [21]. Another research showed that, among patients with suspected infection undergoing immunosuppressive corticosteroid therapy, mNGS played a role in optimizing antibiotic regimes [22]. These studies did not analyze populations with CNS infections. Instead, these studies explored the role of mNGS in guiding anti-infection treatment. Our study evaluated the intensity of drug usage and its adjustment based on each mNGS tests among patients with CNS infec-

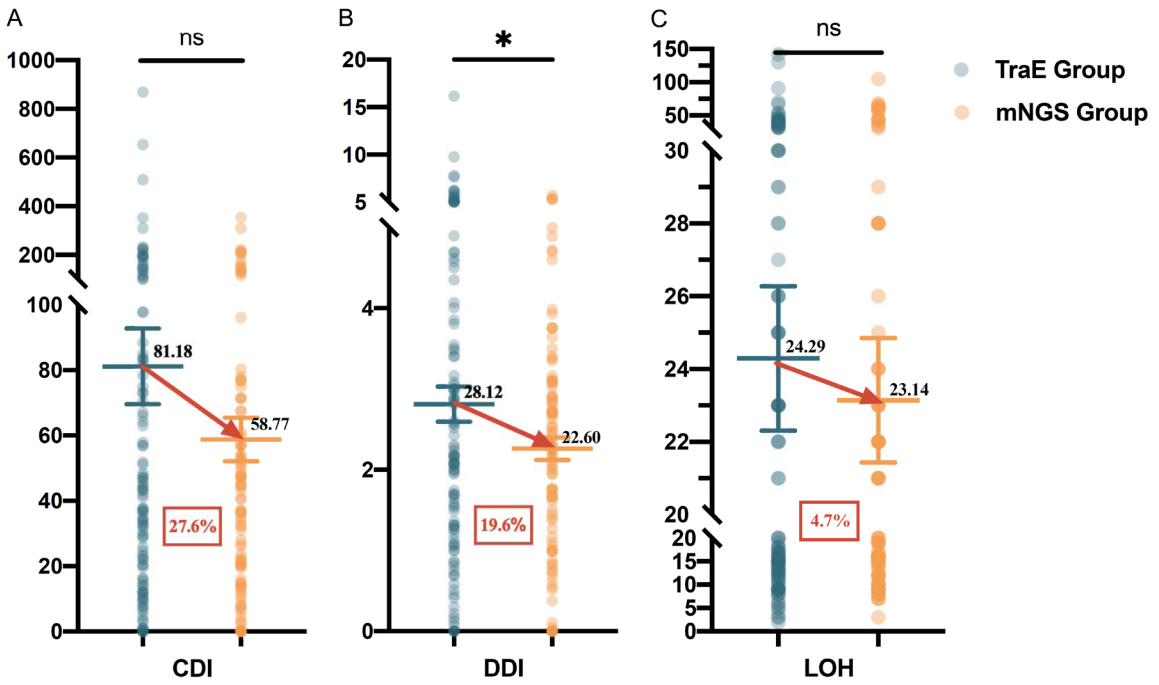

Figure 4. Comparison of CDI, DDI, and LOH between TraE group and mNGS group. The number in the red box showed the decline percentage of CDI (A), DDI (B), and LOH (C) between the two groups. TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

tions. We revealed a difference in the intensity of anti-infection drugs used between participants associated with mNGS and those who were not. This confirmed that the application of mNGS reduced the medication intensity. We evaluated the patients without co-infection and compared them with the TraE group. The CDI and DDI value of the mNGS group showed a noticeable reduction. We excluded the confounding factors of co-infection. The benefit of mNGS on reducing drug intensity was confirmed.

We found that the application of mNGS had a more significant impact on patients with bacterial CNS infection. Data showed that in the bacterial and fungal infection subgroups, the CDI value of the mNGS group was lower than that of the TraE group. The patients involved with mNGS tests in the viral and bacterial infection subgroups had lower DDI values. The reasons were as follows: In the treatment of bacterial infections, the de-escalation of broad-spectrum antibiotics brought more remarkable changes to the intensity of medication. The clinical manifestations were typical. The clinician's empirical judgments were more accurate. Less fluctuation of the medication intensity was observed. A diagnosis of fungal infections would not be easily made when the etiological evidence or other approval tests was absent. Most of them were performed with antibiotics empirically. The clinician changed the medication to fungal drugs after obtaining the mNGS reports. The intensity of the drug tended to increase.

Patients with the worst (Grade 3) and mildest (Grade 1) neurological function were inclined to benefit more from the mNGS examination, since it reduced the length of hospitalization and the amount of drug used. It was explained that the reports of the mNGS helped clinicians to optimize the anti-infection regimens. It sped up the recovery of critically ill patients and helped to avoid antibiotic overuse. Targeted medical therapy relying on the mNGS reports reduced unnecessary hospitalizations, especially for those with relatively mild symptoms. We concluded that, for critical patients with CNS infection, the application of mNGS was more valuable and instructive in diagnosis and treatment.

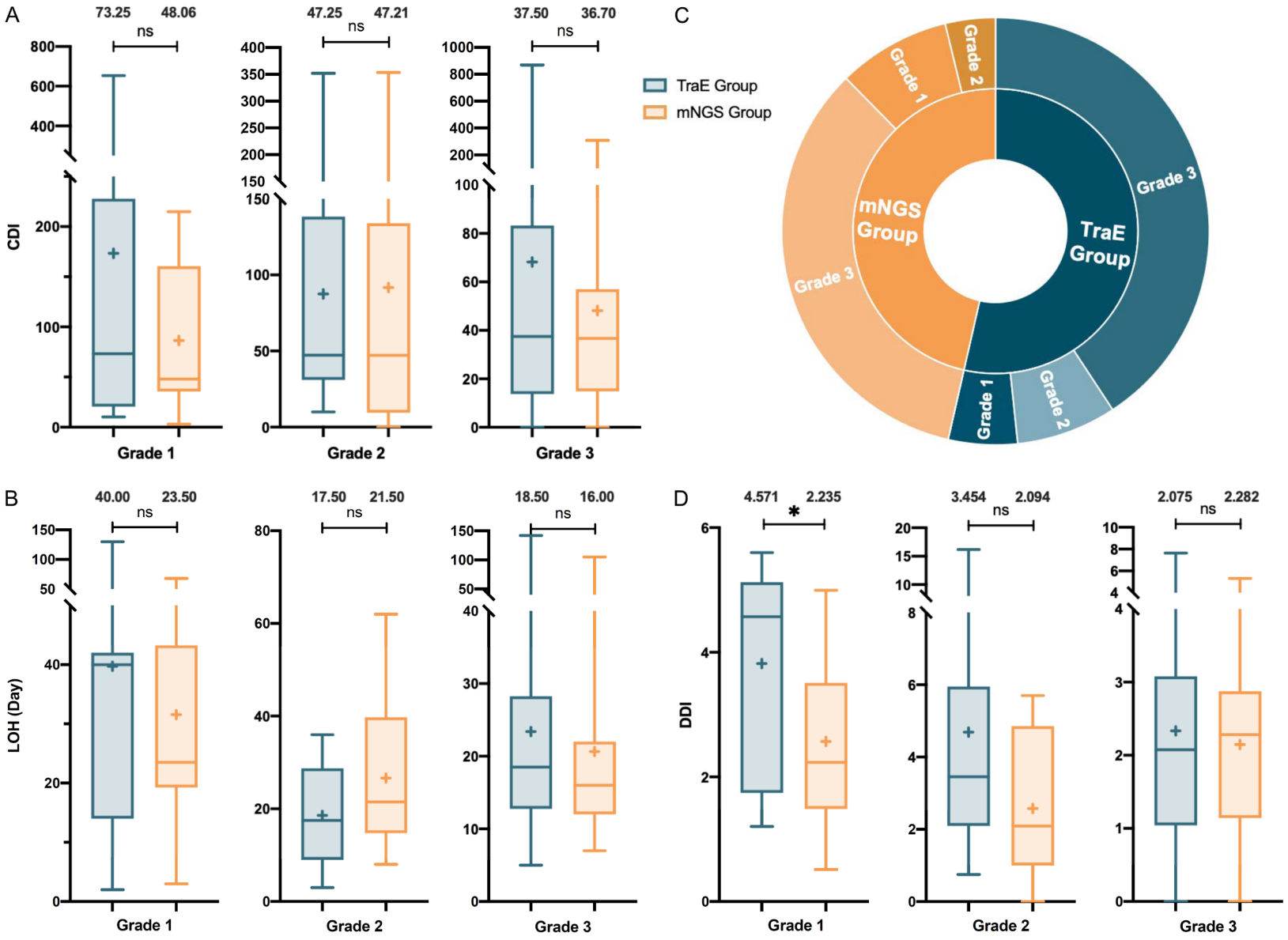

Figure 5. Intensity of drug use and length of hospital stay in patients with different GCS at admission. A. Comparison of the median CDI between TraE group and the mNGS group in Grade 1, 2, and 3. B. Comparison of the median LOH between TraE group and the mNGS group in Grade 1, 2, and 3. C. The composition of Grade 1,

2, and 3 in the TraE group and the mNGS group respectively. D. Comparison of the median DDI between TraE group and the mNGS group in Grade 1, 2, and 3. Medical Research Council (MRC) grade 1 indicates a Glasgow coma score of 15 (on a scale of 3 to 15, with lower scores indicating reduced levels of consciousness) with no neurologic signs, grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less. TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

Table 3. Comparison of CDI, DDI, and LOH between TraE group and mNGS group with different MRC Grade

	MRC Grade	TraE Group	mNGS Group	*P* Value
CDI (IQR)	Grade 1	73.25 (20.50-227.88)	48.06 (35.67-160.48)	0.7739
	Grade 2	47.25 (30.98-138.32)	47.21 (9.50-134.05)	0.6633
	Grade 3	37.50 (13.70-83.27)	36.70 (14.81-57.00)	0.3842
DDI (IQR)	Grade 1	4.57 (1.74-5.13)	2.24 (1.49-3.51)	0.0494
	Grade 2	3.45 (2.10-5.95)	2.09 (1.00-4.86)	0.1677
	Grade 3	2.08 (1.04-3.08)	2.28 (1.14-2.88)	0.9965
LOH (Day, IQR)	Grade 1	40.00 (14.00-42.00)	23.50 (19.25-43.25)	0.6986
	Grade 2	17.50 (9.00-28.75)	21.50 (14.75-39.75)	0.4430
	Grade 3	18.50 (12.75-28.25)	16.00 (12.00-22.00)	0.2061

TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group. Medical Research Council (MRC) grade 1 indicates a Glasgow coma score of 15 (on a scale of 3 to 15, with lower scores indicating reduced levels of consciousness) with no neurologic signs, grade 2 a score of 11 to 14 (or 15 with focal neurologic signs), and grade 3 a score of 10 or less. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

In total, we reported 49% of altered medication and 24.7% of decreased drug usage. This revealedthe value of mNGS in guiding the clinical medication plan and benefited the precise use of antibiotics. We analyzed the reasons for decreased drug intensity. The reduction of drugs counted for the most, counting 57.14%. It was explained that the patients were treated with multiple drugs simultaneously on admission due to the unclear etiology diagnosis. The clinician identified the targeted pathogen with mNGS tests and excluded suspected pathogens, reducing the types of drugs. There were other reasons for the decrease of drug intensity such as drug adjustment, drug downgrade, and reduction of drug dose. With the report of mNGS, clinicians found the empirical medication did not fully cover the actual pathogens patients were infected with. The anti-infection regimes were able to be adjusted. Narrow-spectrum anti-infection drugs were used precisely according to the target pathogen reported by mNGS. The previous study of Filmarray meningitis/encephalitis Panel has shown that it improved the antibiotic regimens on CNS infection patients [10, 23, 24]. The research focused on the patients who had bacterial meningitis, without an overall population of CNS infected patients restricted by the limited scope of its pathogen examination and the study design itself.

We used the mRS to assess the patient's self-care ability and neurological impairment at the time of discharge. We found that the patients' ability of being included in the poor prognosis (level 3) would decrease from 26.5% to 17.3% result to mNGS application. This showed the ability of mNGS in improving the results of patients with CNS infections. The conclusion showed that the application of mNGS in CNS-infected populations improved patients' prognosis and optimized the drug intensity of patients during hospitalization. The previous study reported a better improvement rate among patients who adjusted medication according to mNGS than those performed with medication empirically [22]. We speculated that the mNGS' rapid, accurate, and broad pathogen detection characteristics precisely identified pathogen types, accelerating the workup and treatment. CNS infection is a severe and dangerous infectious disease, demanding therapy. CSF culture has difficulty in timely feedback because of its low positive rate and time-consuming characteristics. The application of the mNGS test promotes the implementation of the optimal treatment. This

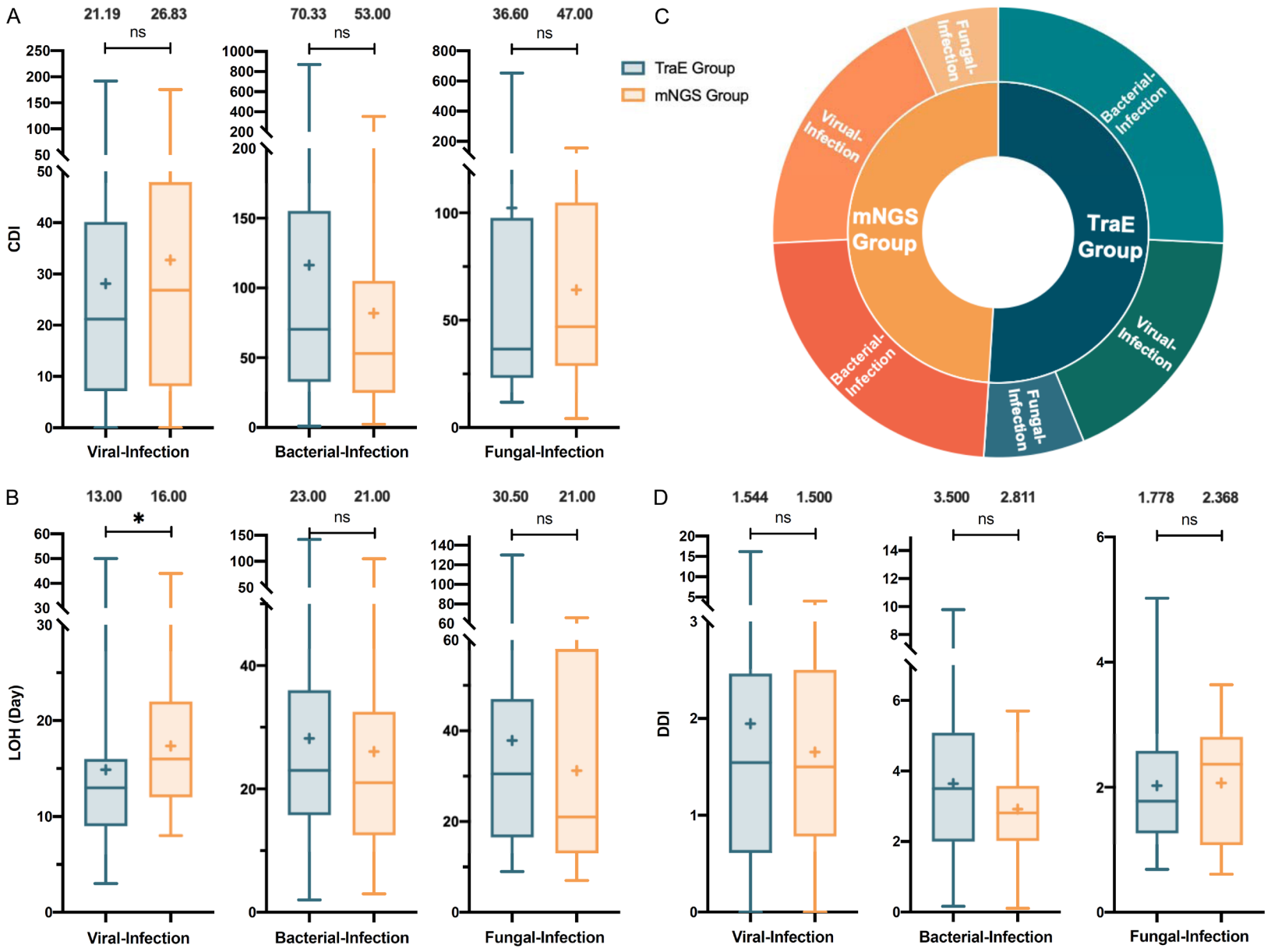
A
21.19
26.83
ns
CDI
Viral-Infection
70.33
53.00
ns
Bacterial-Infection
36.60
47.00
ns
Fungal-Infection
C
TraE Group
mNGS Group
Fungal-Infection
Bacterial-Infection
Virual-Infection
mNGS Group
TraE Group
Virual-Infection
Fungal-Infection
Bacterial-Infection
B
13.00
16.00
*
LOH (Day)
Viral-Infection
23.00
21.00
ns
Bacterial-Infection
30.50
21.00
ns
Fungal-Infection
D
1.544
1.500
ns
DDI
Viral-Infection
3.500
2.811
ns
Bacterial-Infection
1.778
2.368
ns
Fungal-Infection

Figure 6. Intensity of drug use and length of hospital stay in patients with different pathogens infections. A. Comparison of the median CDI between TraE group and the mNGS group in viral-, bacterial- and fungal-infection. B. Comparison of the median LOH between TraE group and the mNGS group in in viral-, bacterial- and fungal-infection. C. The composition of different kind of infections in the TraE group and the mNGS group respectively. D. Comparison of the median DDI between TraE group and the mNGS group in viral-, bacterial- and fungal-infection. TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

Table 4. Comparison of CDI, DDI, and LOH between TraE group and mNGS group with different pathogen infections

	Infection	TraE Group	mNGS Group	*P* Value
CDI (IQR)	Viral infection	21.19 (7.15-40.13)	26.83 (8.13-47.90)	0.3807
	Bacterial Infection	70.33 (32.67-155.17)	53.00 (24.83-105.10)	0.2893
	Fungal Infection	36.60 (23.13-97.68)	47.00 (28.70-104.83)	0.9149
DDI (IQR)	Viral infection	1.54 (0.61-2.46)	1.50 (0.78-2.50)	0.8113
	Bacterial Infection	3.50 (2.00-5.08)	2.81 (2.02-3.58)	0.1143
	Fungal Infection	1.78 (1.26-2.58)	2.37 (1.08-2.80)	0.7109
LOH (Day, IQR)	Viral infection	13.00 (9.00-16.00)	16.00 (12.00-22.00)	0.0208
	Bacterial Infection	23.00 (16.00-36.00)	21.00 (12.50-32.50)	0.4321
	Fungal Infection	30.50 (16.50-47.00)	21.00 (13.00-58.00)	0.5105

TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

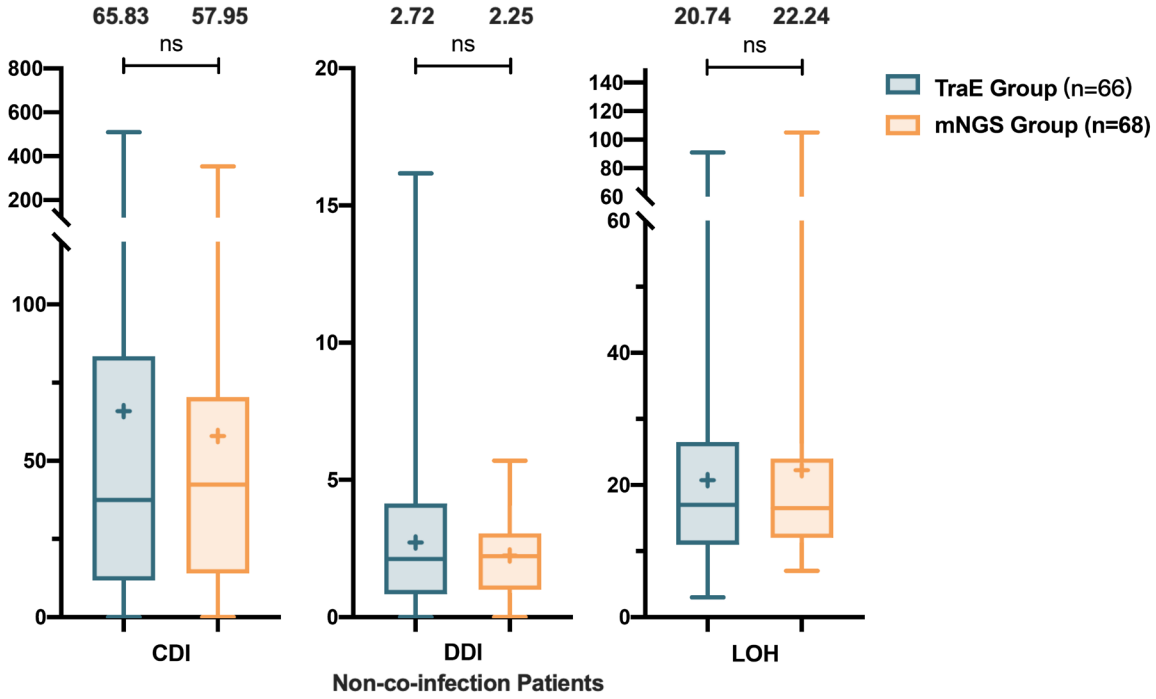

Figure 7. Comparison between TraE group and mNGS group of non-co-infection patients in average of CDI, DDI, and LOH. In total, 66 patients in the TraE group were non-co-infection, and 68 patients in the mNGS group were non-co-infection. mNGS group, metagenomic next-generation sequencing group. CDI, cumulative drug intensity. DDI, daily drug intensity. LOH, length of hospitalization.

leads to rapid remissions of the disease and a better prognosis.

Our study had several strengths. Our retrospective research analyzed a group of patients

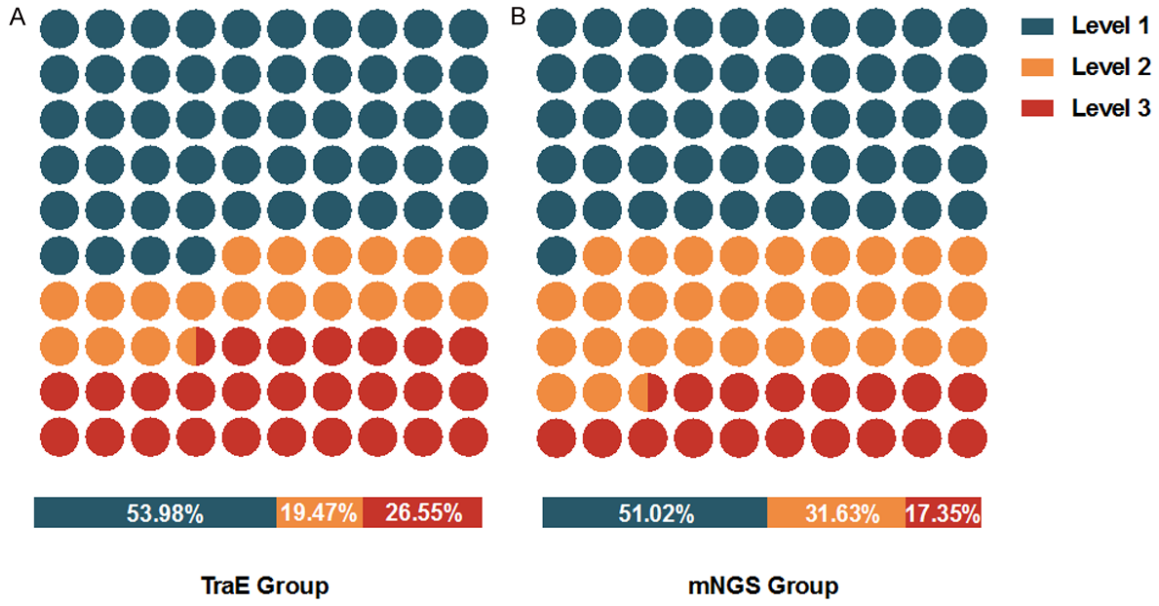

Figure 8. mRS Grades of patients in the TraE group and the mNGS group at discharge. Patients were divided into different levels according to their mRS Grades at discharge. We compared the composition of level 1, 2, and 3 patients in the TraE group and mNGS group, and the percentages were listed above. TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group. mRS, modified Rankin Scale. Level 1: Good outcome. Level 2: Intermediate outcome. Level 3: Poor outcome.

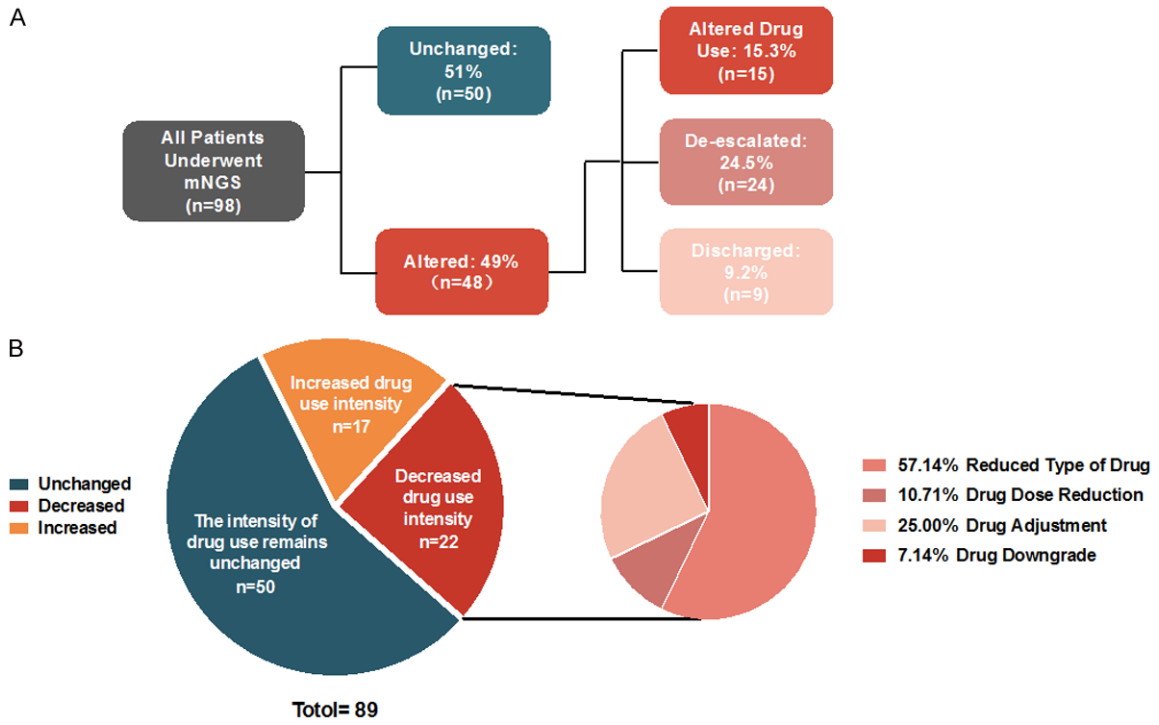

Figure 9. Changes of drug use intensity in the mNGS group. A. The number of patients who altered the medication regimens according to the mNGS results were listed and the percentages were calculated. We listed the specific types of regimens alterations. B. The specific changes of medication decisions were listed. We calculated the composition ratio of various detailed reasons for drug-use-intensity reduction. *Among the patients with decreased drug use intensity, 1 patient took a lesser dose and fewer types of drugs. The medication was adjusted. Another patient took fewer types of drugs and downgraded medication. All medications were adjusted. Two patients experienced a reduction and adjustment of drug types.

with high homogeneity of the CNS infection, ensuring that the same group of doctors made their medication plan, reflecting the changes in medication usage and prognosis accurately under the influence of mNGS. Our study had limitations inherent to its retrospective design. These included the small sample size of parasite CNS infections and those unclassified in our study. We could not conclude the value of clinical medication guidance of mNGS in these groups. The results of mNGS were interfered with by many factors. A larger sample size, multi-center, and more detailed study is needed to verify the application value of mNGS in CNS infections.

Conclusion

This retrospective cohort study demonstrated that mNGS exhibited superiority over traditional culture and detected CNS infections better. mNGS helped to shorten hospital stays and improved patient outcomes, especially for severe patients and bacterial infection. The application of mNGS reduced the daily drug intensity used on CNS infection patients with better consciousness on admission. For clinical decision-making, mNGS can be applied to assist in rational and precise drug use, reducing the abuse of antibiotics, preventing antibiotic resistance.

Acknowledgements

We thank all participants in the study, and we send thanks to all the health care workers involved in patients' care for their professionalism and compassion. This work was supported by research grants from the Shanghai Science and Technology Committee (20dz2260100, 20Z11901100 and 20dz2210400 to W.H.Z), National Natural Science Foundation of China (82002141 to J.W.A; 82041010, 92169212 and 82161138018 to W.H.Z), Shanghai Municipal Science and Technology Major Project (HS2021SHZX001 to W.H.Z).

Informed consent was obtained from all individual participants included in the study.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jing-Wen Ai, Disease of Infectious Disease, Huashan Hospital, Fudan University, 12 Wulumuqi Zhong Road, Shanghai 200040, China. Tel: +86-17317958269; E-mail: jingwenai1990@126.com

References

[1] The global burden of meningitis. Available from: https://www.meningitis.org/mpt.

[2] Forrester JV, McMenamin PG and Dando SJ. CNS infection and immune privilege. Nat Rev Neurosci 2018; 19: 655-671.

[3] Rhein J, Huppler Hullsiek K, Tugume L, Nuwagira E, Mpoza E, Evans EE, Kiggundu R, Pastick KA, Ssebambulidde K, Akampurira A, Williams DA, Bangdiwala AS, Abassi M, Musubire AK, Nicol MR, Muzoora C, Meya DB and Boulware DR. Adjunctive sertraline for HIV-associated cryptococcal meningitis: a randomised, placebo-controlled, double-blind phase 3 trial. Lancet Infect Dis 2019; 19: 843-851.

[4] Bijlsma MW, Brouwer MC, Kasanmoentalib ES, Kloek AT, Lucas MJ, Tanck MW, van der Ende A and van de Beek D. Community-acquired bacterial meningitis in adults in the Netherlands, 2006-14: a prospective cohort study. Lancet Infect Dis 2016; 16: 339-347.

[5] McGill F, Heyderman RS, Panagiotou S, Tunkel AR and Solomon T. Acute bacterial meningitis in adults. Lancet 2016; 388: 3036-3047.

[6] Schwartz S, Kontoyiannis DP, Harrison T and Ruhnke M. Advances in the diagnosis and treatment of fungal infections of the CNS. Lancet Neurol 2018; 17: 362-372.

[7] Wilson MR, Sample HA, Zorn KC, Arevalo S, Yu G, Neuhaus J, Federman S, Stryke D, Briggs B, Langelier C, Berger A, Douglas V, Josephson SA, Chow FC, Fulton BD, DeRisi JL, Gelfand JM, Naccache SN, Bender J, Dien Bard J, Murkey J, Carlson M, Vespa PM, Vijayan T, Allyn PR, Campeau S, Humphries RM, Klausner JD, Ganzon CD, Memar F, Ocampo NA, Zimmermann LL, Cohen SH, Polage CR, DeBiasi RL, Haller B, Dallas R, Maron G, Hayden R, Messacar K, Dominguez SR, Miller S and Chiu CY. Clinical metagenomic sequencing for diagnosis of meningitis and encephalitis. N Engl J Med 2019; 380: 2327-2340.

[8] Guo LY, Li YJ, Liu LL, Wu HL, Zhou JL, Zhang Y, Feng WY, Zhu L, Hu B, Hu HL, Chen TM, Guo X, Chen HY, Yang YH and Liu G. Detection of pediatric bacterial meningitis pathogens from cerebrospinal fluid by next-generation sequencing technology. J Infect 2019; 78: 323-337.

[9] Goff DA, Jankowski C and Tenover FC. Using rapid diagnostic tests to optimize antimicrobial selection in antimicrobial stewardship programs. Pharmacotherapy 2012; 32: 677-687.

[10] Tansarli GS and Chapin KC. Diagnostic test accuracy of the BioFire® FilmArray® meningitis/encephalitis panel: a systematic review and meta-analysis. Clin Microbiol Infect 2020; 26: 281-290.
[11] Zhou H, Larkin PMK, Zhao D, Ma Q, Yao Y, Wu X, Wang J, Zhou X, Li Y, Wang G, Feng M, Wu L, Chen J, Zhou C, Hua X, Zhou J, Yang S and Yu Y. Clinical impact of metagenomic next-generation sequencing of bronchoalveolar lavage in the diagnosis and management of pneumonia: a multicenter prospective observational study. J Mol Diagn 2021; 23: 1259-1268.
[12] Guo BF, Ding MX, Xue JR, Yan DN and Sun SZ. Clinical utility of metagenomic next-generation sequencing in suspected central nervous system infectious pediatric patients with empirical treatment: a cohort study. 2021.
[13] Teasdale G, Maas A, Lecky F, Manley G, Stocchetti N and Murray G. The Glasgow Coma Scale at 40 years: standing the test of time. Lancet Neurol 2014; 13: 844-854.
[14] Beardsley J, Wolbers M, Kibengo FM, Ggayi AB, Kamali A, Cuc NT, Binh TQ, Chau NV, Farrar J, Merson L, Phuong L, Thwaites G, Van Kinh N, Thuy PT, Chierakul W, Siriboon S, Thiansukhon E, Onsanit S, Supphamongkholchaikul W, Chan AK, Heyderman R, Mwinjiwa E, van Oosterhout JJ, Imran D, Basri H, Mayxay M, Dance D, Phimmasone P, Rattanavong S, Lalloo DG and Day JN. Adjunctive dexamethasone in HIV-associated cryptococcal meningitis. N Engl J Med 2016; 374: 542-554.
[15] WHO Collaborating Centre for Drug Statistics Methodology. Available from: https://www.whocc.no.
[16] Hasan MR, Sundararaju S, Tang P, Tsui KM, Lopez AP, Janahi M, Tan R and Tilley P. A metagenomics-based diagnostic approach for central nervous system infections in hospital acute care setting. Sci Rep 2020; 10: 11194.
[17] Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, Denning DW, Loyse A and Boulware DR. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis 2017; 17: 873-881.
[18] Zhang Y, Cui P, Zhang HC, Wu HL, Ye MZ, Zhu YM, Ai JW and Zhang WH. Clinical application and evaluation of metagenomic next-generation sequencing in suspected adult central nervous system infection. J Transl Med 2020; 18: 199.
[19] Xing XW, Zhang JT, Ma YB, He MW, Yao GE, Wang W, Qi XK, Chen XY, Wu L, Wang XL, Huang YH, Du J, Wang HF, Wang RF, Yang F and Yu SY. Metagenomic next-generation sequencing for diagnosis of infectious encephalitis and meningitis: a large, prospective case series of 213 patients. Front Cell Infect Microbiol 2020; 10: 88.
[20] Perlejewski K, Bukowska-Ośko I, Rydzanicz M, Pawełczyk A, Caraballo Cortès K, Osuch S, Paciorek M, Dzieciątkowski T, Radkowski M and Laskus T. Next-generation sequencing in the diagnosis of viral encephalitis: sensitivity and clinical limitations. Sci Rep 2020; 10: 16173.
[21] Miao Q, Ma Y, Wang Q, Pan J, Zhang Y, Jin W, Yao Y, Su Y, Huang Y, Wang M, Li B, Li H, Zhou C, Li C, Ye M, Xu X, Li Y and Hu B. Microbiological diagnostic performance of metagenomic next-generation sequencing when applied to clinical practice. Clin Infect Dis 2018; 67: S231-S240.
[22] Wang S, Ai J, Cui P, Zhu Y, Wu H and Zhang W. Diagnostic value and clinical application of next-generation sequencing for infections in immunosuppressed patients with corticosteroid therapy. Ann Transl Med 2020; 8: 227.
[23] Mina Y, Schechner V, Savion M, Yahav D, Bilavsky E, Sorek N, Ben-Zvi H and Adler A. Clinical benefits of FilmArray meningitis-encephalitis PCR assay in partially-treated bacterial meningitis in Israel. BMC Infect Dis 2019; 19: 713.
[24] Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, Bridge SC, Bangdiwala AS, Meya DB, Denkinger CM, Muzoora C and Boulware DR. Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. Lancet Infect Dis 2018; 18: 68-75.

Table S1. Enrollment and exclusion criteria

Enrollment Criteria
Age ≥ 14 years
Patients with a final diagnosis of central nervous system infection
Patients with records of Electronic Medical Records System at Huashan Hospital
Exclusion Criteria
Age < 14 years
Patients with previous etiological diagnosis
Patients who received effective anti-infection treatment before
Patients without records of Electronic Medical Records System at Huashan Hospital
Patients with no records of lumbar puncture during hospitalization

Each of the above items must be met simultaneously.

Table S2. Glasgow coma scale (GCS)

Eye opening (E)
None
To pressure
To speech
Spontaneous
Verbal response (V)
None
Sounds
Words
Confused
Orientated
Best motor response (M)
None
Extension
Abnormal flexion
Normal flexion (withdrawal)
Localizing
Obeying commands

Table S3. The modified rankin scale

Grade	Description
0	No symptoms
1	Minor symptoms not interfering with lifestyle
2	Symptoms that lead to some restriction in lifestyle, but do not interfere with the patients' ability to care for themselves
3	Symptoms that restrict lifestyle and prevent total independent living
4	Symptoms that clearly prevent independent living, and the patient does not need constant care and attention
5	Totally dependent, requiring consistent 24 h help

Grade 0: Good outcome, Grade 1 or 2: Intermediate outcome, Grade 3 to 5: Poor outcome.

Table S4. CDI, DDI, and LOH of non-co-infection patients in the TraE group and the mNGS group

	TraE Group (n=66)	mNGS Group (n=68)	*p* value
CDI	65.83 (11.75-83.43)	57.95 (14.00-353.50)	0.8583
DDI	2.72 (0.84-4.14)	2.25 (1.00-3.05)	0.5874
LOH	20.74 (11.00-26.50)	22.24 (12.00-24.00)	0.8080

TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group.

Table S5. DDI of the mNGS and TraE Group in different infection subgroups without co-infection

	TraE Group	mNGS Group	*p* value
Viral Infection	1.84 (0.22-2.08)	1.36 (0.56-2.32)	0.7390
Bacterial Infection	3.54 (2.00-4.90)	3.20 (2.28-4.60)	0.4777
Fungal Infection	1.74 (1.26-2.31)	1.95 (0.87-2.80)	0.7577

TraE group, traditional examination group. mNGS group, metagenomic next-generation sequencing group.