Clinical usefulness of metagenomic next-generation sequencing for the diagnosis of central nervous system infection in people living with HIV

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**A B S T R A C T**

**Objectives:** To evaluate the clinical utility of metagenomic next-generation sequencing (mNGS) for the diagnosis of central nervous system (CNS) infection in people living with human immunodeficiency virus (PLWH) in a real-world situation.

**Methods:** Cerebrospinal fluid (CSF) was sent for mNGS for PLWH who tested negative on all conventional tests but were still suspected to have CNS infection. A retrospective analysis was undertaken of the results and the clinical effect of mNGS on this cohort. The final diagnosis was adjudicated by a panel discussion following hospital discharge when the results of all tests and patients’ responses to the empiric therapy were available.

**Results:** Eighty-eight eligible PLWH, including 51 (58%) patients suspected of encephalitis and 34 (46.7%) patients suspected of meningitis, were included in the analysis. Sixty-eight (77.3%) patients were diagnosed with CNS infection, of which 50 were based on the pathogens identified by mNGS. The most common disease missed by mNGS was clinically suspected tuberculous meningitis, followed by clinically suspected non-tuberculous mycobacterial meningitis. The results from mNGS led to modification of treatment in 21 (23.9%) patients, and increased confidence in continuation of original therapy in 30 (34.1%) patients. During hospitalization, two (2.3%) patients died and 66 (75%) patients improved.

**Conclusions:** mNGS of CSF is a useful tool for the diagnosis of CNS infection among PLWH. Further investigations are warranted to improve its sensitivity.

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**Introduction**

The advent of antiretroviral therapy (ART) has significantly improved the life expectancy and quality of life of people living with human immunodeficiency virus (PLWH). However, many PLWH are unaware of their status and are diagnosed at a very late stage. In patients with advanced immunosuppression, central nervous system (CNS) conditions remain significant contributors to morbidity and mortality, particularly in middle- and low-income countries (Ji et al., 2017). Therefore, early identification of pathogens or exclusion of CNS infection is essential to guide treatment and reduce morbidity and mortality.

In PLWH, in addition to CNS infections that occur in immunocompetent individuals, opportunistic infections associated with acquired immunodeficiency syndrome (AIDS), such as toxoplasma, progressive multifocal leukoencephalopathy and tuberculous meningitis, also have high incidence (Bowen et al., 2016; Thakur et al., 2019). CNS infection may be suspected following a physician’s assessment of a patient’s history, clinical presentation, imaging results and subsequent serial laboratory testing. Although several tests, including smear, culture and molecular assays, for one or a few pathogens are available, identification of the precise aetiology of CNS infection is particularly challenging, as diagnostic tests for some rare pathogens are lacking. The requirement for invasive procedures,
such as lumbar puncture or brain biopsy, further impedes diagnosis. More importantly, multiple infections are common among PLWH, which are difficult to diagnose using conventional procedures (Collazos, 2003).

Metagenomic next-generation sequencing (mNGS), which could identify a comprehensive spectrum of pathogens by a single assay, has recently shown its efficacy in the diagnosis of infectious diseases (Wilson et al., 2014, 2019; Goldberg et al., 2015; Gu et al., 2019). In PLWH, a number of case reports have also demonstrated the usefulness of mNGS, particularly in patients presenting with atypical clinical or radiographic patterns (Hu et al., 2018; Xia et al., 2019; Du et al., 2020; Fang et al., 2020). However, due to its high cost, the application of mNGS is very limited in the clinical setting, and its utility in PLWH is still not well established. This study reports the performance of mNGS in a PLWH cohort who tested negative on conventional tests but were still suspected to have CNS infection.

**Methods**

**Study population and procedures**

This was a retrospective cohort study in which PLWH admitted to Shanghai Public Health Clinical Centre (SPHCC) with clinical suspected CNS infection were screened. SPHCC is the only hospital in Shanghai that treats patients with HIV infection, and is also a tertiary referral hospital for difficult-to-treat and complicated cases of HIV infection for eastern China.

In the authors’ real-life clinical practice, patients suspected to have CNS infection underwent lumbar puncture and brain (and/or spine) magnetic resonance imaging (MRI)/computer tomography after informed consent. Patients under treatment for an established diagnosis (e.g. cryptococcal meningitis, tuberculous meningitis) who developed new CNS symptoms were also enrolled. Cerebrospinal fluid (CSF) samples were sent for conventional testing, including bacterial and fungal smear; acid-fast stain; and culture of bacteria, fungal organisms and *Mycobacterium* species. As cryptococcal meningitis is common in PLWH, a cryptococcal antigen test (waived for patients in whom cryptococcal meningitis had already been diagnosed) and Chinese ink staining were also conducted in all patients. In patients with suspected tuberculous meningitis or encephalitis, GeneXpert MTB/RIF test in CSF was requested (Bahr et al., 2018). All patients received empirical treatment before the results were available. Patients who tested negative for all of the above tests (when mycobacterium culture results were not available) were advised to undergo mNGS on CSF.

**mNGS on CSF and interpretation**

After informed consent, 1 mL of CSF that had been stored at −20 °C was sent for mNGS and analysis (BGI Genomics Co. Ltd., Shenzhen, China), as described previously (Miao et al., 2018). Briefly, DNA specimens extracted with the TIANamp Micro DNA Kit (DP316, Tiangen Biotech, Beijing, China) were used for construction of DNA libraries after DNA fragmentation, end repair, adapter ligation and polymerase chain reaction (PCR) amplification. The DNA libraries were sequenced using the BGISEQ-50/MGISEQ-2000 platform after quality qualification (Jeon et al., 2014). Internal references and no-template controls were used as positive and negative controls, respectively. The sequencing data were cleaned by removing the human host sequences mapped to the human reference genome (hg19) using Burrows–Wheeler alignment and low-quality reads. The remaining data were analysed according to the RefSeq databases which include bacteria, fungi, viruses and parasites associated with human diseases (ftp://ftp.ncbi.nlm.nih.gov/). The sequencing data for each sample are categorized into four tables, representing bacteria, fungi, viruses and parasites. Microbes (species level) whose read numbers identified by mNGS were in the top 10 of the complete list were deemed positive results. Virus and *Mycobacterium tuberculosis* were considered positive when at least one read was identified.

Once the mNGS results were returned, a discussion was held between at least three physicians. The effect of the mNGS results on clinical reasoning, patient care management or both have been documented in Table 2.

**Chart review**

Clinical data, including age, gender, signs and symptoms, and laboratory results and images were collected by retrospective, in-depth chart review. Final clinical diagnoses of the patients were adjudicated after hospital discharge when the results of all the laboratory tests (including mycobacterium culture) and patients’ responses to therapy were available. This was done through a group discussion based on clinical manifestations, pathogens identified by mNGS, laboratory testing, CSF characteristics, imaging and treatment response. This study was approved by the Ethics Committee of SPHCC.

**Statistical analysis**

Depending on the data distribution, continuous variables were reported as mean ± standard deviation or median and range, respectively. Student’s t-test was undertaken to evaluate differences between the two groups. Wilcoxon rank sum tests were used.

**Table 1**

Clinical and laboratory characteristics of the study population.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) of men</td>
<td>78 (88.6%)</td>
</tr>
<tr>
<td>Age, years (median, IQR)</td>
<td>35 (31–51)</td>
</tr>
<tr>
<td>Onset of symptoms/signs</td>
<td></td>
</tr>
<tr>
<td>Fever, n (%)</td>
<td>73 (13.6)</td>
</tr>
<tr>
<td>Headache</td>
<td>64 (25.0)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>36 (15.9)</td>
</tr>
<tr>
<td>Weakness in the arms/legs</td>
<td>26 (20.4)</td>
</tr>
<tr>
<td>Unsteady gait</td>
<td>14 (8.0)</td>
</tr>
<tr>
<td>Vision changes</td>
<td>8 (6.8)</td>
</tr>
<tr>
<td>Vocal changes</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>Duration of symptoms</td>
<td></td>
</tr>
<tr>
<td>&lt;1 week, n (%)</td>
<td>20 (22.7)</td>
</tr>
<tr>
<td>1–2 weeks, n (%)</td>
<td>15 (17.0)</td>
</tr>
<tr>
<td>2 weeks–3 months, n (%)</td>
<td>36 (40.9)</td>
</tr>
<tr>
<td>&gt;3 months, n (%)</td>
<td>17 (19.3)</td>
</tr>
<tr>
<td>History of CNS infection</td>
<td></td>
</tr>
<tr>
<td>Cryptococcal meningitis, n (%)</td>
<td>11 (12.5)</td>
</tr>
<tr>
<td>Tuberculous meningitis, n (%)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>Duration of ART</td>
<td></td>
</tr>
<tr>
<td>ART naive, n (%)</td>
<td>41 (46.6%)</td>
</tr>
<tr>
<td>&lt;6 months, n (%)</td>
<td>23 (26.1%)</td>
</tr>
<tr>
<td>&gt;6 months, n (%)</td>
<td>24 (27.3%)</td>
</tr>
<tr>
<td>Laboratory test</td>
<td></td>
</tr>
<tr>
<td>HIV RNA &lt;50 copies/mL, n (%)</td>
<td>15 (17.0)</td>
</tr>
<tr>
<td>CD4 T-cell count (cells/mm³) (median, IQR)</td>
<td>63 (24–168)</td>
</tr>
<tr>
<td>Cell count in CSF (10⁶/L) (median, IQR)</td>
<td>4 (1–8)</td>
</tr>
<tr>
<td>Abnormal MRI, n (%)</td>
<td>76 (86.4%)</td>
</tr>
<tr>
<td>Length of hospital stay, days (median, IQR)</td>
<td>19 (9–30)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>Cured</td>
<td>4 (4.6)</td>
</tr>
<tr>
<td>Improved</td>
<td>65 (73.8)</td>
</tr>
<tr>
<td>Unchanged</td>
<td>14 (15.9)</td>
</tr>
<tr>
<td>Deteriorated</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>Died</td>
<td>2 (2.3)</td>
</tr>
</tbody>
</table>

IQR, interquartile range; ART, antiretroviral therapy; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging.

a Data were available from 60 patients.

b Data were available from 84 patients.
for non-normally distributed data. All analyses were performed using STATA v12.0 (StataCorp., College Station, TX, USA) and GraphPad Prism v7.0 (GraphPad, San Diego, CA, USA).

**Results**

**Patient characteristics**

Between 1 October 2017 and 30 September 2019, 88 eligible patients were enrolled in this study, and their CSF samples were sent for mNGS to identify the infectious pathogens or to exclude CNS infection. Detailed clinical characteristics are listed in Table 1.

Among them, 88.6% (78/88) of patients were male, and the median age was 36 (range 31–51) years. Forty-one (46.6%) patients were naïve to ART, and 24 (27.3%) patients had received ART for more than 6 months. Patients were at an advanced stage of immunosuppression with a median CD4 T-cell count of 63 (range 24–168) cells/μL. The cohort primarily included patients with suspected encephalitis [51 patients (58.0%)] and suspected meningitis [34 patients (46.7%)], while only 2.3% (two patients) presented with myelitis. In total, 39.7% (35/88) of patients were admitted to the hospital within 2 weeks of symptom onset. Eleven patients had a history of laboratory-confirmed cryptococcal meningitis, while two patients were clinically diagnosed with tuberculous meningitis upon admission, but secondary CNS infection was still being considered.

![Figure 1](image-url) **Figure 1.** Performance of metagenomic next-generation sequencing (mNGS) in cerebrospinal fluid. (A) Aetiologies of the study population. (B) Difference in CD4 T-cell counts between patients with established diagnoses and patients with unknown diagnoses. (C) Pathogens identified by mNGS which led to diagnosis of central nervous system infection. (D) Diseases missed by mNGS, EBV, Epstein–Barr virus; CMV, cytomegalovirus; HAND, HIV-associated neurocognitive disorder; HHV-8, human herpesvirus 8; HSV, herpes simplex virus; JCV, John Cunningham virus; NTM, non-tuberculous mycobacterium; TB, tuberculosis; VZV, varicella zoster virus.
Aetiological diagnoses were made in 83.0% (73/88) of the study patients (Figure 1A). Patients with established diagnoses had significantly lower median CD4 T-cell counts than patients with unknown diagnoses [50 (range 22–107) vs 207 (range 74–392) cells/μL, P < 0.001, Figure 1B]. Of the individuals diagnosed, 77.3% (68/88) had CNS infection (Figure 1A).

The median turnaround time for mNGS results from CSF submission was 2.5 days. CNS infection was diagnosed based on the pathogens identified by mNGS in 56.8% (50/88) of patients. In these subgroups of patients, the most common pathogens reported were John Cunningham virus (JCV [25]), cytomegalovirus (CMV [8]) and varicella zoster virus (VZV [6]) (Figure 1C). Rare pathogens including Aspergillus spp. (two cases), Listeria monocytogenes, Fusarium oxysporum, Ralstonia pickettii and Acinetobacter parus were also identified as causes of disease by mNGS (Figure 1C). Multiple infections were diagnosed in 15.9% (14/88) of cases (Table 2). CD4 T-cell counts were comparable between patients with one infection and those with multiple infections [median 39 (range 17–62) vs 50 (22–148), P = 0.19].

However, mNGS found no pathogens in 20.5% (18/88) of patients who were also diagnosed with CNS infection based on symptoms, other laboratory tests and response to empirical therapy. The most common disease missed by mNGS was clinically suspected tuberculous meningitis, followed by HIV-associated neurological diseases and non-tuberculous mycobacterial (NTM) meningitis (Figure 1D). Two patients were ultimately diagnosed with neurosyphilis based on clinical symptoms, CSF characteristics and response to penicillin. However, Treponema pallidum was not detected in CSF from these two patients. In a patient who was treated with empirical antitoxoplasma for more than 2 weeks before mNGS, mNGS did not detect any pathogens. mNGS was also unsuccessful in identifying pathogens in another patient who did not respond to empirical ceftriaxone, but recovered rapidly after receiving vancomycin.

EBV DNA was detected in 31.8% (28/88) of patients. These patients had a median CSF cell count of 3 (1–13) *10^6/L but without any evidence of primary CNS lymphoma on MRI. However, EBV was only considered aetiological in two patients, as most of the other patients responded well without anti-EBV treatment. Similarly, CMV DNA was detected in 17.0% (15/88) of patients with a median CSF cell count of 11 (range 2–160) *10^6/L. However, CMV was not deemed to be the cause of disease in seven patients.

Consequences of mNGS and reasoning

The results of mNGS led to the modification of treatment in 23.9% (21/88) of patients (Figure 2). In 12 patients, antiviral treatment (ganciclovir or aciclovir) was added as compared to CMV, EBV or VZV infection was established based on mNGS results. In another three patients, antifungal treatment was used after mNGS identified Aspergillus spp. and Fusarium spp. Anti-Toxoplasma gondii treatment was also initiated in three cases based on mNGS results. Antibacterium therapy was given in two cases after bacterial meningitis was diagnosed based on mNGS results. Empirical treatment was stopped with the help of mNGS which excluded CNS infection in one patient. Surgery was recommended for this patient, in whom multiform glioblastoma was diagnosed after surgery. mNGS increased confidence in continuation of the original therapy in 34.1% (30/88) of patients. It also helped physicians to exclude CNS infection in 6.8% (6/88) of patients. Over the average 19-day (range 9.3–30) hospitalization period, the overall death rate was 2.3% (2/88). One patient died of clinically suspected tuberculous menigitis while another patient died of pneumonia. Three-quarters [73.8% (65/88)] of patients demonstrated improvement, while 15.9% (14/88) were stable and 3.4% (3/88) deteriorated. Interestingly, among the 21 patients who received modified treatment based on mNGS results, 90.5% (19/21) showed improvement after treatment. The cause of deterioration in three patients was disseminated NTM, progressive multifocal leukoencephalopathy and clinically suspected tuberculous meningitis, respectively.

Discussion

 Opportunistic CNS infections, including cryptococcal meningitis, tuberculous meningitis, toxoplasma encephalitis, progressive multifocal leukoencephalopathy and CMV encephalitis, are common among PLWH with advanced immunosuppression (Bowen et al., 2016). However, the tests available to diagnose CNS infection are limited. mNGS is a promising method for PLWH to diagnose or rule out CNS infection. However, its current high cost limits its use to select populations. The current study evaluated the clinical utility of mNGS in diagnosing CNS infection in a population that was most likely to undergo mNGS testing in a real-world situation — patients who were suspected to have CNS infection but who tested negative by all conventional methods (e.g. CSF smear, culture and serology). To the best of the authors’ knowledge, this is the first study to evaluate the clinical utility of mNGS in diagnosing CNS infection in PLWH.

In this study, aetiology was identified in 83.0% of the study population. Importantly, CNS infections were diagnosed based on the pathogens detected by mNGS in 56.8% of patients. Therefore, the results show that mNGS is a useful tool for the diagnosis of CNS infection, even among PWLH (Wilson et al., 2019). Interestingly, patients with established diagnoses had significantly lower CD4 T-cell counts than patients with unknown diagnoses. This may be because patients with advanced immunosuppression are prone to CNS infection. Accordingly, mNGS may be best suited to patients with low CD4 T-cell counts.

Various pathogens were detected in CSF by mNGS, including those not classified as AIDS-related opportunistic pathogens (e.g. Aspergillus spp., Listeria monocytogenes). Infection with these pathogens leads to similar symptoms, non-characteristic imaging and atypical laboratory results such that physicians may never suspect these aetiologies. One example is the identification of R. pickettii as the cause of meningitis in one case. The patient presented with headache, a cell count of 300*10^6 cells/L, and normal levels of glucose, chloride and protein in CSF. His headache was relieved shortly and his CSF normalized 2 weeks after empirical antibacterial treatment. As R. pickettii was identified in CSF from this patient by mNGS, it was attributed as the causative pathogen in this case (Bonatti et al., 2009; Basso et al, 2019; Nasir et al., 2019). A major advantage of mNGS is that it can detect pathogens in an unbiased way, enabling broad identification of known as well as unexpected pathogens, or even the discovery of new organisms (Gu et al., 2019).

Indeed, the mNGS results led to the modification of therapy in more than 23.9% of patients, with consequent favourable outcomes. To that end, PLWH with advanced immunosuppression would be an ideal target population for mNGS.
Multiple pathogens were identified in CSF from more than 15.9% of patients, indicating that multiple infections are not uncommon in PLWH (Tang et al., 2012; Siddiqi et al., 2014; Yang et al., 2017). mNGS is able to identify multiple pathogens in a single test in an unbiased manner, which is of enormous value when used in PLWH because CSF sample volume and availability are often limited. This study did not include patients who tested positive by conventional tests, in whom multiple infections are also possible (Siddiqi et al., 2014; Yang et al., 2017). Therefore, mNGS of CSF may be beneficial to all PLWH suspected to have CNS infection.

However, mNGS did miss 18 cases of CNS infection during this study. With the exception of HIV, which was not covered by the mNGS platform as the authors only sequenced DNA, the most frequently missed pathogens were mycobacterium for clinically suspected tuberculosis and NTM. Indeed, none of the tuberculosis or NTM meningitis cases were diagnosed based on mNGS. Diagnosis of tuberculosis and NTM meningitis are difficult in PLWH (Flor et al., 1996; Vinnard and Macgregor, 2009). The sensitivity of CSF smear microscopy is often low, while culture of tuberculosis has better sensitivity but is time consuming and cannot guide therapy (Bahr and Boulware, 2014). In PLWH, Gene Xpert and Xpert Ultra are useful in the diagnosis of tuberculous meningitis, both with sensitivity of approximately 60% (Bahr et al., 2018; Donovan et al., 2020). In HIV-negative patients diagnosed with TB meningitis (including definitive and clinical diagnosis), mNGS has been reported to have sensitivity ranging from 27.3% to 84.44% when using the final diagnosis as the reference, showing that mNGS should be applied in patients with suspected TB meningitis (Wang et al., 2019; Xing et al., 2020; Yan et al., 2020). In this study, only patients who had negative results on conventional tests were recruited, including CSF smear microscopy for acid-fast bacilli and Gene Xpert (pandering mycobacteria culture). Therefore, the addition of mNGS to traditional tests may not improve sensitivity in the diagnosis of meningitis and meningitis NTM in PLWH. Another two neurosyphilis cases were missed by mNGS in the current study, showing that mNGS may not be suitable in patients for whom the suspected pathogen is typically diagnosed by serology (Ramachandran and Wilson, 2020). Similarly, mNGS has also been shown to be less sensitive than conventional PCR assays in the diagnosis of viral encephalitis when low viral loads are common (Perlewiski et al., 2020). Therefore, negative mNGS results should be interpreted with caution if suspected pathogens are of low abundance or absent in CSF (Wilson et al., 2019).

In addition to the aforementioned shortages, there remain some barriers to the widespread use of mNGS as a diagnostic tool for CNS infection among PLWH. One challenge is how to interpret the mNGS results (Simmer et al., 2018). It is not possible to distinguish whether the pathogens identified by mNGS are growing/replicating actively or if the DNA (e.g. EBV) is the consequence of cell lysis. Therefore, as for all laboratory tests, the results of mNGS have to be interpreted in combination with clinical data, preferably in a multidisciplinary manner (Simmer et al., 2018). In addition, the cost-effectiveness of the broad application of mNGS needs further investigation (Thakur, 2020).

This study had various limitations. Data collection was retrospective. Nevertheless, all procedures were routine clinical practices and all patients were hospitalized with well-documented medical records, which mitigated design flaws. In addition, due to the high cost of mNGS, the relatively low incidence of RNA virus infection in the CNS, and limited approaches to treat RNA virus infection, the untargeted mNGS did not include RNA-Seq, which may have missed some rare RNA virus infections (e.g. human enteroviruses) (Wilson et al., 2019). Therefore, this study may have underestimated the effectiveness of mNGS in the diagnosis of CNS infection, and further studies are warranted. In the current study, mNGS identified various pathogens (e.g. CMV, JCV, VZV) which might be identified by commercial PCR kits. Unfortunately, PCR tests for these pathogens on CSF are not approved by the National Medical Product Administration of China as these kits were developed for urine, plasma or secretions. Therefore, these tests are not performed routinely on CSF for patients in clinical practice, and the authors were not able to evaluate the additional diagnostic value of mNGS on the basis of these tests.

In conclusion, this study found that clinical mNGS of CSF is a useful tool for the diagnosis of CNS infection in PLWH. Further investigations are warranted to improve its sensitivity and specificity.

Conflict of interests

None declared.

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Author contributions

JC and HL conceived and coordinated the study. JC, RZ, LL, TQ, ZW, WS, YT and JS managed the patients and contributed data collection. DL, YL, SX and JY participated in data analysis and critical revisions for intellectual content. All authors critically reviewed, edited and approved the final version of the manuscript.

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