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Correspondence

Application of metagenomic next-generation sequencing with brain tissue biopsy for diagnosing intracranial lesions in people with HIV

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Dear Editor,

The central nervous system (CNS) is a target of the human immunodeficiency virus (HIV) [1]. Late presenters with advanced HIV infection may develop opportunistic cerebral infections or lymphoma, most often Epstein–Barr virus (EBV)-related. Abnormal neurological examination or changed mental status are first evaluated by imaging for diagnosis [2], however, specificity remains poor. In the absence of suspected intracranial hypertension, cerebrospinal fluid (CSF) analysis is a diagnostic tool for CNS disorders. However, in the absence of meningitis results are frequently not contributive for a diagnosis.

Metagenomic next-generation sequencing (mNGS) is an unbiased technique, capable of simultaneously detecting multiple pathogens, and has recently demonstrated its effectiveness in the diagnosis of CNS disorders [3]. However, research has focused on CSF analysis by mNGS testing. Such an approach has its limitations as CSF samples have to be stored for a long time or are sometimes improperly preserved [4]. Furthermore, when patients have received anti-microbial therapy before CSF collection, or when the site of the lesion is compartmentalized, negative results may be obtained [3]. Such negative results do not rule out the possibility of CNS lesions.

Based on the above observations, in people with advanced HIV with concurrent neurological symptoms, brain or meningeal tissue biopsy samples have to be assessed for earlier diagnosis and treatment using mNGS.

We enrolled 13 people with HIV (PWH) with intracranial lesions who underwent brain tissue biopsy from January 2021 to March 2024 at the First Affiliated Hospital of Zhejiang University School of Medicine. All brain biopsies were subjected to mNGS examination. Participant clinical records were reviewed retrospectively to obtain clinical information, including demographic information, clinical signs and symptoms, laboratory testing, brain MRI imaging, treatment, and participant outcome.

These 13 participants included 11 males and 2 females, ranging in age from 28 to 68 years. These patients had a median CD4 of 91 cells/μl and a median CD8 of 537 cells/μl upon admission. The median course of HIV infection was 12 months (supplementary Table 1, see online supplementary material). The peripheral blood tests for *Cryptococcus* antigen and T-SPOT were all negative for the 13 PWH. Participants 1, 3, 5, and 8 underwent blood culture due to fever. *Talaromyces marneffei* was cultured in the blood sample of participant 8, while the results for the other patients were negative. Lumbar punctures revealed the acid-fast bacilli *Cryptococcus* antigen, and cytomegalovirus (CMV)-DNA tests and the culture of CSF in the 13 patients were all negative. Detailed results of the peripheral blood and CSF analyses are summarized in supplementary Table 2, see online supplementary material. Brain MRI with contrast showed obvious imaging lesions in all patients (supplementary Figs. 1–13A, see online supplementary material).

mNGS testing of CSF was undertaken in 8 patients (patients 1–4, 9, 10, 12, and 13), and EBV was detected in 7 of them. One read of John Cunningham virus (JCV) DNA was detected in patient 4, and one read of *Mycobacterium kansasii* DNA was detected in patient 12. Three reads of CMV were detected in patient 13.

Among the brain biopsy tissues from the 13 patients, mNGS technology successfully identified five infectious pathogens causing disease in 9 patients, including four cases of JCV, two cases of HIV-1, one case of *Toxoplasma gondii*, one case of *Mycobacterium avium*-intracellular complex (MAC), and one case of *T. marneffei*. These mNGS results were consistent with the pathological diagnoses. Meamwhile, mNGS results from 4 patients showed the presence of EBV, and their pathological diagnoses were lymphoma. Table 1 presents the mNGS results for the CSF and brain biopsy tissue samples and the pathology results.

After confirmatory diagnosis, 12 patients received corresponding treatment. Patient 5 died due to intractable convulsions. A total of 8 PWH showed significant improvement in their condition over 12 months (supplementary Figs. 1–4B, 7B, 10B, 11B, and 13B, see online supplementary material). Four patients were lost to follow-up.

We presented 13 cases of unexplained brain lesions after serum testing for fungal infection and imaging and with CSF

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Table 1. NGS of CSF and brain tissue and pathology results of the 13 PWH with intracranial lesions.

		NGS									
		CSF					Brain tissue				
Patient number	Pathological results	Sequence type (DNA/RNA)	Bacterial reads (No.)	Viral reads (No.)	Fungal reads (No.)	Parasite reads (No.)	Sequence type (DNA/RNA)	Bacterial reads (No.)	Viral reads (No.)	Fungal reads (No.)	Parasite reads (No.)
1	Fungal spores	DNA	–	EBV (15)	–	–	DNA	–	–	TM (1729)	–
		RNA	–	HIV-1 (91)	–	–	RNA	–	HIV-1 (3)	TM (28)	–
2	Acid-fast bacteria stain (+)	DNA	–	EBV (11)	–	–	DNA	MAC (3260)	EBV (11)	–	–
		RNA	–	HIV-1 (1)	–	–	RNA	–	–	–	–
3	Many gathered foamy tissue cells and massive lymphocytic and plasmacytic infiltrations	DNA	–	EBV (23)	–	–	DNA	–	JCV (174161), EBV (14)	–	–
		RNA	–	EBV (20)	–	–	RNA	–	JCV (16909), EBV (8), HIV-1 (4)	–	–
4	Infiltration of many acute and chronic inflammatory cells	DNA	–	JCV (1), EBV (3)	–	–	DNA	–	JCV (73033), EBV (77)	–	–
		RNA	–	–	–	–	RNA	–	JCV (3064), EBV (4)	–	–
5	Glial cell proliferation, with some atypical cells having a large and vacuolated nucleus. Oligo-2 (+), SV40 (+)	ND					DNA	–	JCV (326477)	–	–
		ND					RNA	–	JCV (39705), HIV-1 (42)	–	–
6	Necrotic tissue with infiltration of acute and chronic inflammatory cells; small red-stained particles were observed in the tissue cells	ND					DNA	–	–	–	TG (9743)
		ND					RNA	–	HIV-1 (2945)	–	TG (67148)
7	Glial cell proliferation, lymphocyte infiltration, and focal vasculitis	ND					DNA	–	–	–	–
		ND					RNA	–	HIV-1 (457)	–	–

Table 1. (*Continued*)

		NGS								
		CSF					Brain tissue			
Patient number	Pathological results	Sequence type (DNA/RNA)	Bacterial reads (No.)	Viral reads (No.)	Fungal reads (No.)	Parasite reads (No.)	Sequence type (DNA/RNA)	Bacterial reads (No.)	Viral reads (No.)	Fungal reads (No.)	Parasite reads (No.)
8	Glial cell proliferation, infiltration of interstitial lymphocytes, and no obvious abnormal nuclear large cells	ND					DNA	–	–	–	–
		ND					RNA	–	HIV-1 (16551)	–	–
9	Glial cell proliferation and inflammatory cell infiltration	DNA	–	EBV (7)	–	–	DNA	–	JCV (12892), EBV (10)	–	–
		RNA	–	–	–	–	RNA	–	JCV (75), HIV-1 (3)	–	–
10	B-NHL	DNA	–	–	–	–	DNA	–	EBV (31)	–	–
		RNA	–	–	–	–	RNA	–	–	–	–
11	EBER (+), B-NHL	ND					DNA	–	EBV (5687)	–	–
		ND					RNA	–	EBV (1247)	–	–
12	EBER (+), B-NHL	DNA	MK (1)	EBV (6)	–	–	DNA	–	EBV (403)	–	–
		RNA	–	EBV (3), HIV-1 (350)	–	–	RNA	–	EBV (822), HIV-1 (4)	–	–
13	EBER (+), B-NHL	DNA	–	EBV (4)	–	–	DNA	–	EBV (5337)	–	–
		RNA	–	HCMV (3)	–	–	RNA	–	EBV (17033)	–	–

–, Negative; CSF, cerebrospinal fluid; HCMV, human cytomegalovirus; TM, *Talaromyces mameffei*; MAC, *Mycobacterium avium*-intracellular complex; TG, *Toxoplasma gondii*; MK, *Mycobacterium kansasii*; EBER, Epstein–Barr virus encoded RNA; B-NHL, B-cell non-Hodgkin's lymphoma; ND, not done.

analysis and culture. Among them, 8 patients underwent mNGS detection of CSF, and we did not obtain a diagnosis. Based on imaging diagnosis [5], patient 1 was thought to be infected with *Toxoplasma* and CSF mNGS was not diagnostic. However, the presence of *T. marneffei* was detected through mNGS of brain tissue and fungal spores were found upon pathological examination. In a multicenter study, cases negative by mNGS due to low titers of pathogens in CSF included those infected with mycobacteria [3]. In patient 2, despite having a history of MAC, no significant pathogens were detected in the mNGS of CSF. However, MAC was detected using mNGS of brain biopsy tissue. Mycobacterial infection appears to be particularly challenging in the mNGS of CSF [6]. In some patients with brain lesions, culture might take weeks or months to determine the pathogen; therefore, most patients in China will receive empirical treatment that might be ineffective or harmful. The condition of patient 5 rapidly deteriorated after empirical anti-mycobacterial treatment. Despite the final accurate diagnosis through mNGS of brain biopsy tissue, his condition worsened despite appropriate therapy. Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease caused by reactivation of latent JCV infection under immunosuppressive conditions [7]. PML might be confused with HIV-related encephalopathy, as they can both manifest as focal subcortical white matter changes, making it difficult to distinguish them on imaging [8]. It is urgent to get laboratory results to assist the diagnosis when clinical suspicion remains. Unfortunately, in the NGS of CSF from patients 3, 4, and 9, no or only one sequence of JCV was found. So, while still strongly suspecting PML, we performed brain tissue biopsies on the patients and accurately and quickly determined the JCV diagnosis after combined NGS testing.

In patient 10, mNGS of brain biopsy tissue did not reveal any pathogenic microbes. Subsequent pathological examination suggested that her brain lesion was large B-cell non-Hodgkin lymphoma, not EBV positive. In patients 11, 12, and 13, mNGS of brain biopsy tissue detected many reads for EBV, and the subsequent pathological results showed diffuse large B-cell lymphoma with EBV-encoded RNA positivity. Considering the patients' clinical symptoms, the brain lesions were diagnosed as EBV+ diffuse large B-cell lymphoma. The large amount of EBV detected in the brain tissue might promote the occurrence and development of lymphoma, rather than EBV encephalitis [9]. The prevalence of chronic and asymptomatic EBV infection is 80%–95% worldwide [10]. The detection of EBV DNA in the brain tissue of PWH should be interpreted cautiously.

Early diagnosis and treatment are crucial when neurological disorders occur in advanced PWH, as they are closely related to their prognosis. However, the spectrum of neurological diseases associated with advanced HIV infection is wide. Diagnosis remains a challenge for clinicians. When neither conventional detection methods nor mNGS of CSF can determine the pathogenic factor, biopsy of brain lesions and mNGS of brain tissue are a promising approach. Compared with brain tissue pathological examination, mNGS yields faster results with a usual turnaround time from sample to results of ~24–48 h, and can contribute to the histopathological diagnostic.

There are some limitations in the study. Firstly, the number of patients meeting the study criteria remains small; secondly, nearly one-third of the patients (5/13) did not have mNGS test results of CSF; and finally, among the remaining 8 patients, mNGS testing of CSF provided almost no positive results for diagnosis. Therefore, it is currently difficult for us to add specific numerical values to demonstrate the effectiveness of both methods.

This is the first report in which mNGS of brain tissue was used to accurately diagnose CNS disorders in severe immunodeficiency PWH, and in most cases, tissue metagenomics may be applied to the target population in which it will be most valuable, especially when there is a high index of suspicion for infection and mNGS of CSF has produced negative results.

Ethics statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of the Medical College of Zhejiang University (No. IIT20230188B) and complies with the principles of the Declaration of Helsinki. All data were analysed anonymously.

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Author contributions

Ye Xiong (Conceptualization, Data curation, Methodology, Project administration, Writing—original draft), Dairong Xiang (Conceptualization, Investigation, Project administration, Visualization), Xiaotang Zhou (Conceptualization, Investigation, Methodology, Project administration, Validation, Visualization), Ying Huang (Data curation, Project administration, Resources), Jean-Pierre Routy (Supervision, Visualization, Writing—review & editing), and Biao Zhu (Conceptualization, Funding acquisition, Supervision, Visualization, Writing—review & editing)

Supplementary data

Supplementary data are available at *PCMEDI* online.

Conflict of interest

None declared.

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