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Dynamic and direct pathogen load surveillance to monitor disease progression and therapeutic efficacy in central nervous system infection using a novel semi-quantitive sequencing platform

Dear Editor,

Brouwer et al., in this Journal, held the opinion that clinical characteristics failed to differentiate between neurological infections and other diagnoses, and their study emphasized that cerebrospinal fluid (CSF) analysis was the main contributor to the final diagnosis.[1] We are completely in sympathy with the view that rapid and accurate diagnosis of causative agents in central nervous system (CNS) infection remains challenging, let alone the quantitive surveillance of pathogen loads. Currently, the positivity of the CSF culture of CNS infection is only 6%,[1] and CSF analysis and cranial magnetic resonance imaging (MRI) findings are always deceptive. The surveillance of pathogen loads mostly rely on colony culture and specific quantitive polymerase chain reaction (qPCR) of virus, which requires a presumed range of suspicious pathogens. Next-generation sequencing (NGS) has the ability to identify both common and rare pathogens without any prior suspicions needed and can offer a new platform for the quantification of all detected microorganisms. Here we present a cluster of individual cases with similar CNS infection symptoms, where NGS was used for both unknown etiology diagnosis and dynamic semi-quantitive surveillance of pathogen loads in CNS infection, which closely reflected the disease progression.

Case 1, a 1-year-old child was admitted to a local emergency room (ER) for recurrent intermittent fever with no specific symptoms on May 15th. His CSF examinations revealed decreased glucose concentration (1.14 mmol/L) and increased leucocyte count (236×10^6 cells/L) and protein concentration (1428 mg/L), while cranial MRI found abnormal enhanced lesions in the pedunculus cerebri (Fig. 1A). Empirical antibiotic treatment including intravenous vancomycin, meropenem, and linezolid was administered, but the patient's fever was not relieved.

Case 2, a 42-year-old male went to ER because of continuous fever and extreme distended headache on March 16th. Cranial contrasted MRI revealed irregular hyper-intense areas in bilateral symmetrical and basal ganglia and diffuse heterogeneous signal following the convolutions of gyri (Fig. 1B). Intravenous ceftriaxone and mannitol were prescribed but failed to relieve the symptoms.

Case 3, a 41-year-old female went to ER for developing fever (Tmax 38.6 °C) and a persistent distended headache. Her blood routine test was normal but had a markedly increased blood C-reactive protein levels (2.19 mg/L). Cranial MRI revealed heterogeneous leptomeningeal enhancement (Fig. 1C). Oral Saridon was prescribed and symptoms relieved transiently. Over the next 3 days, intermittent low fever with severe diffuse headache recurred, and she was admitted to our hospital.

Jing-Wen Ai, Hao-Cheng Zhang contributed equally to this manuscript.

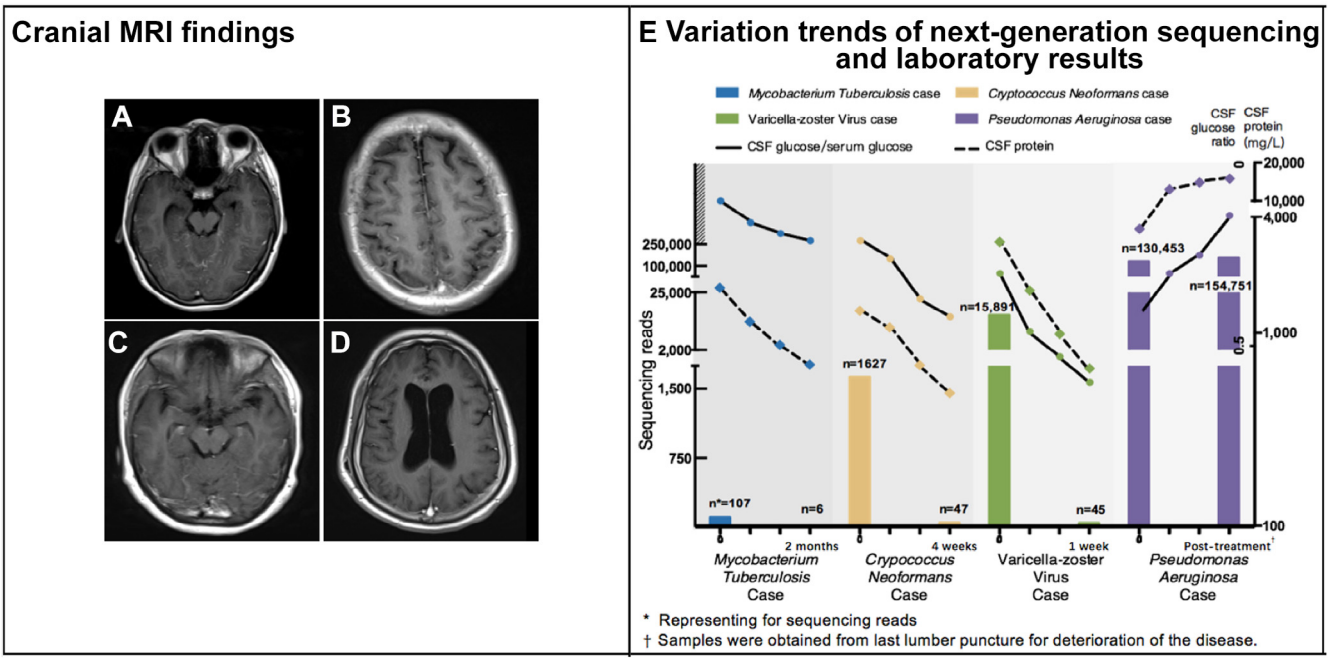

Figure 1 Cranial MRI findings of 4 patients and variation trends in next-generation sequencing and laboratory results. An axial T1-weighted contrast image revealed abnormal nodule-like enhancing lesions beside the pedunculus cerebri (Panel A). An axial T1-weighted contrast image showed heterogeneous leptomeningeal enhancement in bilateral cerebral hemisphere (Panel B). An axial T1-weighted contrast image revealed punctiform ischemic foci within bilateral basal ganglia except for heterogeneous leptomeningeal enhancement (Panel C). An axial T1-weighted contrast image revealed lancual ischemic foci in bilateral frontal lobes with postoperative change in the right part and supratentorial hydrocephaly except for heterogeneous hyperintensities in the pia mater (Panel D). The images above showed similar heterogeneous leptomeningeal enhancement. Panel E shows the parallel variation trends between next-generation sequencing and laboratory results.

Case 4, a 67-year-old male suddenly developed obnubilation and gatism, accompanied with fever of 39 °C. He was immediately admitted to a local hospital, and cranial MRI showed lacunal ischemic foci in bilateral frontal lobes with a postoperative change in the right part and supratentorial hydrocephaly (Fig. 1D). Lumbar puncture revealed decreased glucose concentration (2.4 mmol/L) and increased leucocyte count (330×10^6 cells/L) and protein concentration (3468 mg/L). Ceftriaxone, vancomycin, and sodium valproate prescription showed no efficacy, and the patient became unconscious 3 days later.

All 4 patients were then transferred to our hospital, and NGS of the CSF was performed. NGS detected *Mycobacterium tuberculosis* complex, *Cryptococcus neoformans*, varicella-zoster virus, and *Pseudomonas aeruginosa* in patient 1–4, respectively, all consistent with the simultaneous CSF Xpert, India ink test, PCR confirmation and culture results (Table 1).

After NGS detection, the 4 patients were given the respective specific treatment. At the end of the intensification therapy of patient 1, induction therapy of patient 2, and standard therapy of patient 3, NGS was re-performed. All 3 patients had similar diminution trend of the sequencing reads, which correlated with symptoms and laboratory tests improvements (Fig. 1E). For patient 4, whose condition continuously deteriorated after treatment, a marked increase in *P. aeruginosa* sequencing reads in the CSF was observed (Fig. 1E). These cases implied that NGS might provide a more straightforward means to observe pathogen load changes, which might further assist the surveillance of the disease progression and therapeutic efficacy.

We reported, for the first time, 4 cases in which NGS was used to both identify and monitor dynamic changes in the pathogen load. NGS offers a relatively unprejudiced diagnostic tool for all pathogens included in the database library in a single test, regardless of prior suspicions of candidate pathogens. In CNS infection, multiple case reports have demonstrated the ability of NGS for the detection of viruses from CSF or brain tissue, indicating its ability to identify causative viral agents in CNS infection of unknown etiology.[2–5] To date, most NGS studies in the field of CNS infection are confined to viruses, bacteria, and some uncommon pathogens,[6–10] and our study further demonstrated the overall ability of NGS in the rapid diagnosis of CNS infection caused by fungi, bacteria, viruses, and *M. tuberculosis*.

Currently, dynamic clinical surveillance of CNS infection progression is mostly guided by clinical manifestation, CSF routine laboratory results, and, in some cases, pathogen colony culture. In particular situations, specific immunological test, such as cryptococcal latex agglutination test (CLAT), can indirectly monitor the causative pathogen loads. However, we still lack a comprehensive tool to directly monitor the dynamic trend of the pathogen, which may interfere with the physician's clinical evaluation of disease progression. In our study, we discovered that the primary factor differentiating NGS from traditional laboratory examinations was the degree to which the former can directly and precisely display the dynamic changes in pathogen loads semi-quantitively.