Dear Editor,

Central nervous system lymphomas (CNSL) are difficult to diagnose. Although flow cytometry (FCM) and cytology using tumor cells in cerebrospinal fluid (CSF) are conventionally performed, the sensitivity is still problematic. Recently, cell-free circulating tumor DNA (ctDNA) has been detected in the CSF of patients with malignancies [1–3]. Here, we report a CNSL showing spinal cord masses in which ctDNA was detectable in CSF with amplicon-based droplet digital PCR (ddPCR) with high sensitivity prior to FCM and cytological diagnosis.

A 62-year-old man presented with a 1-month history of motor/sensory disturbance of the extremities. He had a history of a left orchitis and underwent high orchiectomy 1 year ago. MRI showed enhanced masses in the spinal cord at the C5-7 and Th2-3 level (Fig. 1). A fluoro-deoxyglucose (FDG)-PET scan showed no additional lesion. His CSF total cell count was 64 \times 10^9/\mu L, total protein level was 168 mg/dL, and sIL-2R was 251 U/mL. Cytological diagnosis and FCM did not detect lymphoma cells (Fig. 1, CSF-1 and -2). Sequential CSF analysis revealed CD19+/CD20+/Ig-lambda+ clonal B-cell expansion 1 month later (CSF-3), and a diagnosis of CNSL was made. Systemic and intrathecal chemotherapy and radiotherapy diminished the mass. The B-cell clone in CSF also became undetectable (CSF-4). However, 12 months later, FDG-PET revealed a systemic relapse.

After obtaining an informed consent, we performed MYD88L265P and CD79BY196 mutational analysis with ddPCR using cell-free DNA (cfDNA) from CSF (Table S1). Gel electrophoresis of DNA from CSF supernatant showed a similar ladder pattern as plasma-cfDNA (Figure S1) [4, 5]. Because of the lower concentration of CSF-cfDNA compared to plasma-cfDNA [6], amplicon-based ddPCR was established (Supplementary methods and Figure S2). MYD88L265P and CD79BY196N mutations were detected in both DNA from CSF supernatant and the pellet obtained at diagnosis (CSF-3). Then we performed ddPCR using CSF-cfDNA obtained 24 and 17 days before diagnosis (CSF-1 and -2) and after chemotherapy without obvious clonal B-cell population in FCM (CSF-4). The MYD88L265P and CD79BY196N mutations were detected in mostly all the CSF-cfDNA samples analyzed.

We also analyzed DNA from the formalin-fixed paraffin-embedded specimen of the testis obtained 1 year before diagnosis. DNA was extracted from both B-cell rich and sparse lesion. Those mutations were detected only in DNA from the B-cell rich lesion with VAF of 10% and 10.7%, respectively. This phenomenon may suggest that the lymphocytes in his testicular lesion were in pre-lymphoma state.

Our results indicate that tumor DNA in CSF was detected even at the time of negative results with cytology and FCM, and almost 1 month earlier than diagnosis. Genetic analysis with CSF-cfDNA to detect MYD88/CD79B mutations may be a more sensitive strategy to detect CNSL than cytology and FCM, even in the period when a pathological diagnosis has not been made. We analyzed the most frequently reported mutations of MYD88L265P and CD79BY196 [3, 7, 8]. However, about 15% of CNSL do not have these mutations.

Akihiro Tomita
atomita@fujita-hu.ac.jp

1 Department of Hematology, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi 470-1192, Japan
2 Department of Neurology, Fujita Health University School of Medicine, Toyoake, Japan
3 Department of Analytical Neurobiology, Faculty of Pharmacy, Meijo University, Nagoya, Japan
This necessitates careful interpretation of negative test results. Further careful prospective studies are warranted to determine whether the presence of these mutations in CSF-cfDNA is sufficient evidence to diagnose CNSL.

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Author contribution C.I., K.M., A.O., H.Y., W.H., and A.T. took care of the patient. C.I. and K.M. harvested patient’s CSF and blood samples. S.I., C.I., K.M., K.A., A.S., E.I., and R.Y. prepared the DNA samples and performed molecular analyses. A.T. and C.I. designed the study. C.I. and A.T. wrote the paper. M.O., T.M., H.W., and A.T. supervised this work.

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Declarations

**Ethics approval**  The study protocol was approved by the institutional review boards at Fujita Health University (approval number, HG17-032, HG18-017 and HG20-055).

**Conflict of interest** A.T.: research funding: Chugai Pharmaceutical, Astellas Pharma, Eisai, Osuka Pharmaceutical, Ono Pharmaceutical, Kyowa Kirin, Shionogi, Sumitomo Dainippon Pharma, Taisho Pharmaceutical, Takeda Pharmaceutical, Teijin, Nippon Shinyaku, Nihon Pharmaceutical, Pfizer Japan, Mochida Pharmaceutical, Yakult Honsha, and Perseus Proteomics. Lecture fee: Chugai Pharmaceutical, Kyowa Kirin, Eisai, Takeda Pharmaceutical, Astellas Pharma, Nippon Shinyaku, Jansen Pharmaceutical, Zenyaku Kogyo, Abbvie GK, Bristol-Myers Squibb, and SymBio Pharmaceutical.

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