Liquid biopsy of cerebrospinal fluid for MYD88 L265P mutation is useful for diagnosis of central nervous system lymphoma

Yuki Yamagishi1,2,3 | Nobuyoshi Sasaki2 | Yoshiko Nakano1 | Yuko Matushita1,3 | Takaki Omura1,4 | Saki Shimizu2 | Kuniaki Saito2 | Keiichi Kobayashi2 | Yoshitaka Narita4 | Akihide Kondo3 | Yoshiaki Shiokawa2 | Motoo Nagane2 | Koichi Ichimura1,3

1Division of Brain Tumor Translational Research, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan
2Department of Neurosurgery, Kyorin University Facility of Medicine, Mitaka-shi, Tokyo, Japan
3Department of Brain Disease Translational Research, Juntendo University Facility of Medicine, Bunkyo-ku, Tokyo, Japan
4Department of Neurosurgery and Neuro-Oncology, National Cancer Center Hospital, Chuo-ku, Tokyo, Japan

Correspondence
Koichi Ichimura, Juntendo University Facility of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo, 113-8421, Japan.
Email: k.ichimura.uk@juntendo.ac.jp

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Abstract
The current standard of diagnosing central nervous system (CNS) lymphoma is stereotactic biopsy, however the procedure has a risk of surgical complication. Liquid biopsy of the CSF is a less invasive, non-surgical method that can be used for diagnosing CNS lymphoma. In this study, we established a clinically applicable protocol for determining mutations in MYD88 in the CSF of patients with CNS lymphoma. CSF was collected prior to the start of chemotherapy from 42 patients with CNS lymphoma and matched tumor specimens. Mutations in MYD88 in 33 tumor samples were identified using pyrosequencing. Using 10 ng each of cellular DNA and cell-free DNA (cfDNA) extracted from the CSF, the MYD88 L265P mutation was detected using digital PCR. The conditions to judge mutation were rigorously determined. The median Target/Total value of cases with MYD88 mutations in the tumors was 5.1% in cellular DNA and 22.0% in cfDNA. The criteria to judge mutation were then determined, with a Target/Total value of 0.25% as the cutoff. When MYD88 mutations were determined based on these criteria, the sensitivity and specificity were 92.2% and 100%, respectively, with cellular DNA; and the sensitivity and specificity were 100% with cfDNA. Therefore, the DNA yield, mutated allele fraction, and accuracy were significantly higher in cfDNA compared with that in cellular DNA. Taken together, this study highlights the importance of detecting the MYD88 L265P mutation in cfDNA of the CSF for diagnosing CNS lymphoma using digital PCR, a highly accurate and clinically applicable method.

KEYWORDS
central nervous system lymphoma, cerebrospinal fluid, digital PCR, liquid biopsy, MYD88

Abbreviations: BCSFB, brain-cerebrospinal fluid barrier; cfDNA, cell-free DNA; CNSL, central nervous system lymphoma; CSF, cerebrospinal fluid; DLBCLs, diffuse large B-cell lymphomas; FFPE, formalin-fixed paraffin-embedded; LMD, leptomeningeal dissemination; MSKCC RPA class, Memorial Sloan Kettering Cancer Center Scoring for PCNSL using recursive partitioning analysis classification; MYD88, myeloid differentiation primary response gene 88; PCNSL, primary central nervous system lymphoma; R-MPV, rituximab-methotrexate-procarbazine-vincristine; SCNSL, secondary central nervous system lymphoma.

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1 INTRODUCTION

CNSL is defined as a rare non-Hodgkin lymphoma that occurs in the brain, spinal cord, and eye, and accounts for approximately 3%-4% of primary malignant brain tumors.\(^1\)-\(^3\) The majority of CNSL is DLBCLs; however, approximately 10% belongs to very rare pathological entities.\(^4\),\(^5\) CNSL typically develops in the elderly and is one of the most common primary malignant brain tumors, along with glioblastoma and metastatic brain tumors, among patients aged >60 y.\(^1\),\(^2\),\(^6\) It is difficult to reliably distinguish CNSLs from other tumors preoperatively using imaging alone. Therefore, confirmation of histopathological diagnosis by stereotactic biopsy or craniotomy is the gold standard.\(^2\)-\(^4\) However, stereotactic brain biopsy is a relatively high-risk surgical procedure, with reported complications of approximately 10% and mortality of 1.0%.\(^7\),\(^8\) To avoid this risk, liquid biopsy using cerebrospinal fluid (CSF) has attracted attention as a minimally invasive method to diagnose CNSL.\(^9\)-\(^17\) However, the obstacle to successful liquid biopsy, such as of CSF, is the low DNA yield and quality. Therefore, it is necessary to select a diagnostic method with high sensitivity. Digital PCR is a highly sensitive test that can easily detect a single-point mutation and is suitable to use for mutation detection of samples obtained using liquid biopsy that have a lower tumor content.\(^18\) Digital PCR directly measures the number of DNA molecules rather than the amplified signal. Therefore, it is highly accurate and less prone to artefacts than other methods such as real-time PCR, making it the method of choice to detect a small fraction of a target DNA in liquid biopsy samples.

The myeloid differentiation primary response gene 88 (MYD88) L265P mutation is an excellent target for this purpose because the mutation is very common and highly specific to PCNSL.\(^3\),\(^19\)-\(^23\) However, CSF liquid biopsy has several pitfalls and limitations, and the experimental conditions and criteria for judgment of mutation need to be established for clinical application.\(^24\) Here, we report the successful detection of the MYD88 L265P mutation in CSF by digital PCR in the largest number of CNSL samples. We also discuss the experimental conditions that are essential for establishing clinically applicable tests.

2 MATERIALS AND METHODS

2.1 Sample collection

Samples from 50 patients histopathologically diagnosed with CNS lymphoma were collected at Kyorin University Hospital and National Cancer Center Hospital between November 2009 and December 2019. Eight patients were excluded due to the absence of matched surgical tumor specimens or CSF pair or were diagnosed with non-B-cell lymphoma (Figure 1). The study was approved by the Institutional Review Board (IRB) of Kyorin University (No. 61) and National Cancer Center (No. 2013-042). Informed consent was obtained from all patients. The CSF was collected by lumbar puncture before chemotherapy was initiated. No patients showed marked increase in the intracranial pressure due to brain tumors. The CSF was frozen within 4 h and stored at −80°C until use.

2.2 Extraction of DNA

For DNA extraction, the entire amount of CSF was thawed and centrifuged at 1000 g at 4°C for 10 min. DNA extraction was performed using pellets and supernatant. DNA was extracted from the pellet using a DNeasy Blood and Tissue Kit (Qiagen), eluted in 100 μL of AE buffer, and stored at −20°C. DNA was extracted from the supernatant using the QIAamp Circulating Nucleic Acid Kit (Qiagen), eluted in 60 μL of AVE buffer, and stored at −20°C. For extraction of tumor DNA, a fresh-frozen surgical tumor specimen or a FFPE tumor sample was used. DNeasy Blood & Tissue Kit (Qiagen) was used for extracting DNA from frozen tumor specimens, and the GeneRead DNA FFPE Kit (Qiagen) was used for FFPE samples. The extracted DNA was eluted with 100 μL of AVE buffer and stored at −20°C until use. All DNA was quantified using a Qubit 2.0 Fluorometer (High sensitivity; Thermo Fisher Scientific).
2.3 | Assessment of DNA fragment length

For the assessment of DNA fragments extracted from each fraction, a Bioanalyzer High Sensitivity DNA Analysis Kit (Agilent) and a 2100 Bioanalyzer Instrument (Agilent) were used. The measurement was performed in accordance with the manufacturer’s protocol using 1 ng of DNA per sample.

2.4 | Digital PCR

The digital PCR primer/probe mix of MYD88 L265P was obtained from Bio-Rad (Refseq NC_000003.11, NG_023225.1, NT_0251718.1, and NG_016954.1). For the detection and quantification of nucleic acids, the QuantStudio 3D PCR system (Thermo Fisher Scientific) was used. We mixed 7.5 μL of 2x Quant Studio 3D digital PCR Master Mix (Thermo Fisher Scientific), 0.75 μL of 20x target and wild probe (MYD88) (Bio-Rad), and 10 ng of DNA to prepare a total volume of 15 μL. A 14.5 μL aliquot of the mixture was transferred to the Sample Loading Blade, and applied to the QuantStudio 3D Digital PCR 20K Chip using the Chip Loader. Amplification was performed using the Dual Flat Block Gene Amp PCR System 9700 (Thermo Fisher Scientific). In the analysis, the specific signal of the target and wild probe × signal was detected as a VIC signal. In this system, the fraction of the MYD88 L265P mutation dye signal over the total dye signal from the target gene (termed “Target/Total” within the QuantStudio™ 3D AnalysisSuite™ Software, Thermo Fisher Scientific) in the sample was calculated using the following formula:

\[
\text{Target/Total} = \frac{(\text{FAM}) + (\text{FAM} + \text{VIC})}{(\text{FAM}) + (\text{FAM} + \text{VIC}) + (\text{VIC})} \times 100\% \]

The thresholds used were those automatically determined by the system and were readjusted manually only when the thresholds clearly crossed the cluster.

2.5 | Pyrosequencing

Pyrosequencing was performed to identify mutations at L265 in MYD88 at a high sensitivity using AmpliTaq Gold 360 (Applied Biosystems) and PyroMark Q24 Advanced (Qiagen) as described previously (Figure S1).19 The primers used for PCR and pyrosequencing are listed in Table S1.

2.6 | Statistical analysis

Statistical analysis was performed using JMP software version 10.0 (Statistic Discovery™, SAS). All tests were two-sided, and a P-value < .05 was considered statistically significant. Analysis of variance between 2 or more groups was performed using the Wilcoxon test. Logistic regression analysis was used to statistically examine the relationship between Target/Total value and MYD88 status.

3 | RESULTS

3.1 | Patient characteristics

The median age of the patients was 69 y (range: 39-90 y), and 27 were men and 15 were women. There were 39 primary CNSL cases (PCNSLs) and 3 secondary CNSL cases (SCNSLs). There were 37 newly diagnosed cases and 5 recurrent cases. In the Memorial Sloan Kettering Cancer Center Scoring for PCNSL using recursive partitioning analysis classification (MSKCC RPA class),25 5 cases were Class I, 14 Class 2, and 21 Class 3. All tumors were histologically diagnosed as DLBCL. The CSF was collected by lumbar puncture in all patients before initiating chemotherapy. The median CSF collection volume was 3.0 mL. The CSF was collected before surgery in 24 patients and postoperatively in 18 patients. There was no significant difference in the DNA yield or Target/Total value between the 2 CSF collection methods (data not shown). The CSF cytology was determined as Class I 5 cases, Class II 27 cases, Class III 5 cases, Class IV 3 cases, and only 1 case had a definitive diagnosis by Class V. Based on the pyrosequencing analysis of the brain tumor tissues, the MYD88 L265P mutation was detected in 33 cases (78.6%) (Tables 1 and S2).

3.2 | Characteristics of DNA extracted from each fraction

The DNA fragments obtained from the pellet fraction ("cellular DNA") invariably showed a single peak at approximately 10 000 bp, suggesting the genomic origin of the DNA. However, the DNA fragments from the supernatant fraction showed an additional peak at approximately 250 bp, representing cell-free DNA (cfDNA) (data not shown). The median amounts of DNA extracted from the pellet and supernatant were 19.1 and 29.0 ng, respectively. The amount of DNA extracted from the supernatant fraction was significantly higher than that extracted from the pellet fraction. (P = .02; Figure 2A). When the clinical factors related to DNA yield were examined, the samples with increased cell counts showed a significantly higher DNA yield than those with decreased cell counts (Table S3). In contrast, the median Target/Total values in cases with the MYD88 mutation were 5.1% for cellular DNA and 17.9% for cfDNA, suggesting a significantly higher tumor-derived DNA content in cfDNA than that in cellular DNA (P = .04; Figure 2B). As a clinical factor related to the Target/Total value—the fraction of mutated DNA and total DNA recovered—large tumor diameter was significantly associated with higher Target/Total value in cellular DNA compared with that in cfDNA (P = .006). In cfDNA, large tumor diameter (P = .04) and increase in protein in the CSF (P = .03) were significantly associated with a higher Target/Total value compared with those in cellular DNA (Table 2).
First, we performed digital PCR with CNSL tumor DNA in which the MYD88 L265P mutation was detected using pyrosequencing, and confirmed that the MYD88 L265P mutation was detected in all cases. Subsequently, the tumor DNA with confirmed mutation
in **MYD88** was used as a positive control and mixed with wild-type DNA (DNA extracted from non-PCNSL patient’s blood) to prepare serial dilutions of 50%, 10%, 1%, 0.5%, 0.3%, 0.2%, and 0.1%. Digital PCR was then performed for each sample (Figure 3). A strong linear correlation between the dilution concentration and the Target/Total ratio was obtained ($R^2 = .999$), confirming that the digital PCR assay could quantitatively detect the **MYD88** L265P mutation.

We then performed digital PCR for a series of negative controls (**MYD88** wild-type) using 20 non-lymphoma (glioma or metastatic brain tumor) blood DNA samples. The median amplified signal for all experiments was 2847, and the range was 2015-4870 (Table S4). The range of the Target/Total values for the negative control samples was 0.00%-0.21% (average 0.05%, standard deviation 0.04, Figure 4), and up to 5 FAM signals were detected. To establish this

**TABLE 2**  Factors related to the Target/Total value

| Factors related to the Target/Total value | Target/Total value of cellular DNA | Target/Total value of cfDNA |
|-----------------------------------------|-----------------------------------|-----------------------------|
|                                        | n  | Median [IQR] | P-value | n  | Median [IQR] | P-value |
| Hans classification                      |    |              |         |    |              |         |
| GCB subtype                              | 7  | 1.04 [0.33-26.3] | .28    | 9  | 17.0 [2.95-56.7] | .80    |
| Non-GCB subtype                          | 7  | 8.89 [1.57-20.2] |         | 10 | 26.5 [4.10-54.6] |         |
| Location                                 |    |              |         |    |              |         |
| Around the ventricle                     | 13 | 8.70 [0.73-28.5] | .26    | 16 | 17.9 [5.77-63.3] | .43    |
| Within substance                         | 4  | 1.31 [0.51-7.06] |         | 8  | 16.7 [2.87-38.9] |         |
| Diameter (T1Gd, shortest radius of the largest tumor) |    |              |         |    |              |         |
| <30 mm                                   | 7  | 0.92 [0.33-3.98] | .006   | 10 | 5.95 [1.40-31.9] | .04    |
| ≥30 mm                                   | 10 | 18.8 [6.79-33.0] |         | 14 | 25.7 [8.55-69.0] |         |
| Cell count in CSF                        |    |              |         |    |              |         |
| ≤5/mm$^3$                                | 2  | 13.9 [1.57-26.3] | .63    | 5  | 13.70        | .88    |
| ≥6/mm$^3$                                | 14 | 6.90 [0.36-20.2] |         | 18 | 23.4 [4.98-45.9] |         |
| Protein in CSF                           |    |              |         |    |              |         |
| <40 mg/dL                                | 1  | 1.57         | .74    | 3  | 2.46 [1.10-3.17] | .03    |
| ≥40 mg/dL                                | 15 | 8.70 [0.53-26.3] |         | 20 | 25.7 [8.52-61.6] |         |
| Cytomorphological classification of CSF  |    |              |         |    |              |         |
| Class I                                  | 1  | 0.33         | .29    | 3  | 63.9 [0.91-77.4] | .72    |
| Class II                                 | 12 | 6.90 [0.63-24.8] |         | 16 | 23.4 [6.33-40.7] |         |
| Class III                                | 3  | 17.3 [1.57-30.7] |         | 3  | 4.10 [2.46-86.7] |         |
| Class IV                                 | 0  | -           | 0      | -  | 0            |         |
| Class V                                  | 0  | -           | 1      | 8.50 |         |         |
| Surgery                                  |    |              |         |    |              |         |
| Before                                   | 9  | 3.98 [0.70-13.1] | .50    | 13 | 24.8 [5.21-48.8] | .79    |
| After                                    | 8  | 14.5 [0.63-29.6] |         | 11 | 8.56 [3.17-66.2] |         |
| Admission of PSL                         |    |              |         |    |              |         |
| Yes                                      | 7  | 8.70 [0.77-29.7] | .90    | 9  | 5.58 [1.98-49.9] | .26    |
| No                                       | 8  | 7.00 [0.27-20.7] |         | 12 | 27.4 [8.65-61.6] |         |
| RPA class                                |    |              |         |    |              |         |
| Class 1                                  | 1  | 1.04 [0.91-63.9] | .88    | 2  | 24.8 [1.50-63.9] | .27    |
| Class 2                                  | 6  | 2.78 [6.33-40.7] |         | 6  | 6.32 [2.46-26.5] |         |
| Class 3                                  | 10 | 13.0 [2.46-86.7] |         | 10 | 28.2 [7.04-71.8] |         |

Abbreviations: cfDNA, cell-free DNA; CNS, central nervous system; CSF, cerebrospinal fluid; GCB, germinal center B-cell-like; IQR, interquartile range, PSL, prednisolone, RPA, recursive partitioning analysis.

Bold values indicates $P < .05$.

* Arm $\leq 2$ two-way analysis of variance, arm $\geq 3$ median test.
test as a diagnostic alternative to biopsy, it is important to avoid false positives. Therefore, we set the cutoff Target/Total value above the highest level of the negative controls. In addition, when the amplified signals are too few, the noise signals may exceed the cutoff Target/Total value and hamper proper judgment. Therefore, we set a minimal total amplified signal to determine the results.

Based on the above considerations, we set the following criteria to determine the MYD88 L265P mutation:

1. The Target/Total value of $\geq 0.25\%$.
2. Minimum of 2000 total amplified signals (FAM + (FAM + VIC) + VIC).

### 3.4 Sensitivity of detecting MYD88 L265P mutation using digital PCR and CSF-DNA

Of the 42 samples with a matched tumor-CSF pair, the amount of DNA sufficient for digital PCR was obtained in 22 samples (52.4%) of cellular DNA and 31 samples (73.8%) of cfDNA from CSF. The results of digital PCR from all 22 samples of cellular DNA met the above criteria, whereas 4 samples of cfDNA failed to meet the criteria; therefore MYD88 mutation status was successfully determined in 28 cfDNA samples (66.7%) (Figure 1). Taken together, the MYD88 status could be determined in 31 samples (73.8%) using DNA from either of the fractions. Samples that failed to meet the above criteria were found only in cfDNA. In these cases, despite a sufficient amount of extracted DNA, the number of amplified signals failed to meet the criteria, and were identified as undetermined.

The MYD88 mutation status in CSF-DNA was evaluated by digital PCR. Using the above criteria, MYD88 mutations were detected in 22 and 28 samples of cellular DNA and cfDNA, respectively (Table S2). In the cellular DNA, the median Target/Total value was 5.1% (interquartile range (IQR): 0.95%-20.9%) in the cases harboring MYD88 L265P mutation and 0.08% (IQR: 0.04%-0.13%) in those with wild-type status (Figure 4). The sensitivity, specificity, and accuracy were 92.2%, 100%, and 94.1%, respectively. In the cfDNA, the median Target/Total value was 17.9% (IQR: 4.4%-51.7%) in the MYD88 L265P-mutated case and 0.09% (IQR: 0.04%-0.15%) in those with wild-type status (Figure 4). Therefore, all samples were accurately judged, with 100% sensitivity and specificity.

### 3.5 Case report

Finally, we followed the above protocol to prospectively detect MYD88 mutations in the CSF-DNA from 1 patient. A 63-year-old woman visited the neurology department with a chief complaint of right hemiplegia that had worsened for several months. MRI showed a contrast-enhancing lesion on the ventral side of the left pontomedullary region (Figure 5A, left panel). Primary malignant brain tumor or neurodegenerative disease was suspected; however, the standard laboratory or cytology tests of CSF showed no significant findings. Biopsy was not performed considering the tumor location and the patient’s age. We therefore performed digital PCR to determine the MYD88 status using cfDNA extracted from the CSF. The results showed a cluster of clear mutation signals (amplified signal 3545; Target/Total value, 5.1%; Figure 5B). Based on the results of imaging, laboratory tests, and digital PCR, PCNSL was suspected and we administered rituximab-methotrexate-procarbazine-vincristine (R-MPV) induction therapy. After 3 cycles of chemotherapy, the contrast-enhancing lesions completely disappeared (Figure 5A, right panel) and clinical symptoms were alleviated.

### 4 DISCUSSION

In this study, we demonstrated that CSF liquid biopsy using digital PCR to detect MYD88 mutation is clinically applicable for the diagnosis of CNSL. We rigorously determined the digital PCR conditions to detect MYD88 mutation in the CSF, and successfully applied it to support the diagnosis and treatment of a PCNSL patient.

The most important point of this study is that the concordance of MYD88 mutations in paired tissue-CSF samples was 100%, which had not been previously achieved. As liquid biopsy for CNSL aims to provide non-invasive diagnostics and therapeutic interventions based on the results, it is of utmost importance to be accurate. To achieve this, we focused on optimizing the condition to detect MYD88 mutations in the CSF of patients with CNSL. The cutoff was rigorously determined to avoid a false-positive judgment. This point has not been sufficiently pursued in the previous studies. Therefore, we proved in this study that a highly accurate liquid biopsy to detect MYD88 mutations in the CSF can be established through rigorous optimization of the experimental conditions, therefore making the test readily applicable to clinical practice.

Previous reports with liquid biopsy using CSF-DNA in CNS lymphomas have shown high sensitivity for detecting mutations,
however few studies have discussed the cutoff for accurately determining mutations. Hattori et al based on a dilution study, suggested that the lower limit of detection of digital PCR was approximately 0.1%.10 Hiemcke-Jiwa et al9 reported that, based on the validation using 98 non-DNA samples as a negative control (ultrapure water), the fraction abundance range was 0.3%-3.5% and that a maximum of 5 mutation signals was detected. In our experiments, the range of the Target/Total value in the negative control was up to 0.21%. The most important requirement for the liquid biopsy of CNS lymphoma is to avoid false detection of mutations, which would lead to erroneous diagnosis and inappropriate intervention of a non-lymphoma case such as lymphoma with the MYD88 mutation. Conversely, a negative result, either by insufficient quantity/quality of DNA, mutation fraction below the cutoff, or absence of the MYD88 mutation in the tumor, would justify surgical biopsy and ensure correct diagnosis. Based on the above principle, we set a conservative cutoff Target/Total value of ≥0.25%.

In the negative control, up to 5 mutation signals ([FAM] + ([FAM + VIC]) were detected, consistent with the pattern described by Hiemcke-Jiwa et al9 The digital PCR data may show some background noise, which could significantly affect the Target/Total ratio, especially when the total amount of amplified signal ([FAM] + ([FAM + VIC] + (VIC)) is low, when the amount of DNA is small, or the DNA used in the experiment is highly fragmented. To reliably prevent false judgment due to the noise signal, we considered the total number of amplified signals in our criteria. Allowing a maximum of 5 false-positive signals, the total number of amplified signals that produced a Target/Total value below the 0.25% cutoff was ≥2000. A 10 ng tumor-derived genomic DNA harboring MYD88-wild-type status showed an amplified signal in the range 2015-4870, indicating that a minimum 10 ng of DNA is required to meet this criterion. Therefore, we set the requirements for determining mutation positivity to ≥2000 for the amplified signal and ≥0.25% for the Target/Total value. When the MYD88 status was examined using the CSF-DNA of CNS lymphoma sample using the above criteria, analyses of both cellular DNA and cfDNA showed extremely high accuracy and, most importantly, detected no false positives.

We then performed CSF liquid biopsy in a patient with a brain stem tumor for which biopsy was difficult, and determined the presence of the MYD88 mutation. Based on the results of clinical examination, MRI, and digital PCR, the patient was diagnosed with PCNSL. R-MPV induction therapy was subsequently administered to the patient, and the tumor was radiologically eradicated after 3 cycles of chemotherapy. Based on the treatment response and the radiological and clinical findings, we concluded that the tumor was a PCNSL. Therefore, considering the risk of surgical biopsy, particularly in elderly patients, CSF liquid biopsy may serve as a safe and accurate alternative to diagnose CNSL. Theoretically, up to 60% of the CNSL patients could be diagnosed by CSF liquid biopsy to avoid surgical intervention. The CSF liquid biopsy may therefore change the clinical practice, and allow a less invasive diagnosis of CNS lymphoma in patients with suspected brain tumors at difficult-to-biopsy sites, and in frail and aged patients. We are currently developing a method to detect mutations in CD79B, frequently mutated in CNS
lymphoma cases, as reported by Nakamura et al.Ç,Ç The detection of CD79B mutations by CSF liquid biopsy will increase the possibility of diagnosing CNSL in patients with wild-type MYD88. Approximately 90% of the CNS lymphoma cases harbor mutations in MYD88 and/or CD79B.ÇÇÇÇ By examining mutations in these 2 genes, the vast majority of the patients with CNSL may be diagnosed using liquid biopsy alone. A prospective study to validate the efficacy of CSF liquid biopsy is currently underway.

Several key factors may influence the efficacy of CSF liquid biopsy. In this study, 73.8% of the cases in whom CSF was collected met the criteria and were diagnosed correctly. The remaining cases failed due to insufficient amounts of unfragmented DNA, either because the amount of CSF was less or it was suboptimal, which was a limitation of this retrospective study. To improve the feasibility of CSF liquid biopsy, we recommend sample management in accordance with the consensus protocol of the CSF collection.ÇÇÇ By examining mutations in these 2 genes, the vast majority of the patients with CNSL may be diagnosed using liquid biopsy alone. A prospective study to validate the efficacy of CSF liquid biopsy is currently underway.

In this study, both cellular DNA and cfDNA were used; however, as reported previously,ÇÇÇ cfDNA had a significantly higher DNA extraction and tumor-derived DNA content. Previous studies also indicated that cfDNA is a better material for liquid biopsy as the pellet fraction contains normal monocytes and may show a lower Target/Total value compared with that in cfDNA.ÇÇÇ cfDNA has a very short half-life. Watanabe et al reported that the amount of cfDNA was reduced to approximately one-third after leaving samples at 4°C for 2 h compared with rapid processing.ÇÇ Some of the samples used in this study had been stored at -80°C for several years before the cfDNA was extracted. There was no statistically significant correlation between the storage period of CSF and the DNA yield or the Target/Total value in this study (Figure S3). However, there is a possibility that long-term storage may affect DNA yield and test results. The importance of the proper sample management in accordance with the CSF consensus protocol, such as swift cryopreservation and extraction of cfDNA from CSF, cannot be overemphasized.

In conclusion, we present a clinically applicable condition to determine MYD88 mutations in the CSF of patients with CNSL. The MYD88 mutation is specifically found in the CNSL and not in other types of brain tumors. Therefore, CSF liquid biopsy may serve as a non-surgical diagnostic tool for CNSL. We believe that CSF liquid biopsy has the potential to change the clinical practice of CNSL.

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The authors declare no competing interests.

ORCID
Yuki Yamagishi https://orcid.org/0000-0003-0111-2744
Motoo Nagane https://orcid.org/0000-0002-0018-1652
Koichi Ichimura https://orcid.org/0000-0002-3851-2349

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

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