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Feasibility of Circulating Tumor DNA Detection in the Cerebrospinal Fluid of Patients With Central Nervous System Involvement in Large B-Cell Lymphoma

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We explored the utility of cerebrospinal fluid (CSF) circulating tumor DNA (ctDNA) sequencing as a noninvasive diagnostic tool for detecting central nervous system (CNS) involvement in patients with diffuse large B-cell lymphoma (DLBCL). Secondary CNS involvement in DLBCL, although rare (~5% of cases), presents diagnostic and prognostic challenges during systemic disease progression or relapse. Effective treatment is impeded by the blood–brain barrier. This was a prospective cohort study (Samsung Lymphoma Cohort Study III) involving 17 patients with confirmed CNS involvement. High-throughput sequencing was conducted using targeted gene panels designed to detect low-frequency variants and copy number alterations pertinent to lymphomas in ctDNA extracted from archived CSF samples. Despite challenges such as low DNA concentrations affecting library construction, the overall variant detection rate was 76%. Detected variants included those in genes commonly implicated in CNS lymphoma, such as *MYD88*. The study highlights the potential of CSF ctDNA sequencing to identify CNS involvement in DLBCL, providing a promising alternative to more invasive diagnostic methods such as brain biopsy, which are not always feasible. Further validation is necessary to establish the clinical utility of this method, which could significantly enhance the management and outcomes of DLBCL patients with suspected CNS involvement.

Key Words: Central nervous system, Cerebrospinal fluid, Circulating tumor DNA, Lymphoma, Relapse

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Secondary central nervous system (CNS) involvement in diffuse large B-cell lymphoma (DLBCL), although relatively rare (occurring in ~5% of cases), is associated with a grim prognosis [1, 2]. It can manifest during the treatment of systemic DLBCL as a sign of disease progression, or as a relapse, with or without systemic involvement. Treatment strategies for secondary CNS involvement typically differ from those used for systemic DLBCL, primarily because of the need to penetrate the blood–brain barrier. High-dose methotrexate chemotherapy regimens are the mainstay of treatment because of their ability to penetrate the CNS [3]. However, most patients do not achieve long-lasting remission, leading to disappointing outcomes [4, 5]. Therefore, early detection and management of CNS involvement are crucial for improving patient outcomes [6]. However, brain biopsy is not

always feasible, and conventional cerebrospinal fluid (CSF) analyses via cytopathology or flow cytometry and diagnostic magnetic resonance imaging (MRI) have shown suboptimal sensitivity and discriminative capacity to enable the diagnosis of CNS involvement without tissue confirmation. The development of methods that overcome these limitations and allow reliable noninvasive identification of CNS involvement would be transformative for the clinical care of patients with suspected secondary CNS involvement by DLBCL.

Liquid biopsy, which involves analyzing circulating tumor DNA (ctDNA) in the blood or body fluid, is revolutionizing cancer diagnosis and surveillance. ctDNA originating from tumor tissue or lysed circulating tumor cells offers a noninvasive method for monitoring cancer progression and treatment responses [7]. Advances in next-generation sequencing (NGS) have enabled the analysis of variants in ctDNA, allowing tumor genotyping using blood samples [8]. High-throughput ctDNA sequencing can provide comprehensive genetic information regarding the tumor, serving as a surrogate for sequencing the entire tumor genome. This approach holds promise for the identification of therapeutic targets and early detection of relapse or residual disease. We have shown that ctDNA can be detected in plasma from patients with various subtypes of B-, T- or NK-cell lymphomas, correlating with tumor volume and patient outcomes [9-12]. However, only a minority of patients with primary CNS B-cell lymphomas have detectable ctDNA in their plasma, possibly because of the blood–brain barrier [13]. Consequently, ctDNA from CSF has emerged as a promising biomarker for CNS involvement of B-cell lymphomas. In this study, we optimized a customized targeted sequencing approach to achieve ultrasensitive ctDNA profiling and investigated its potential for identifying CNS involvement without the need for biopsy.

We analyzed archived CSF samples collected between July 2020 and October 2022 from patients diagnosed as having large B-cell lymphoma who participated in a prospective cohort study (Samsung Lymphoma Cohort Study III, Institutional Review Board of Samsung Medical Center, File No. SMC 2017-12-068; ClinicalTrials.gov Identifier: NCT03117036). All patients were pathologically confirmed as having large B-cell lymphoma using immunohistochemistry [14]. As we monitored the patients enrolled, we identified those suspected of CNS involvement either based on clinical neurological symptoms and/or manifestations or radiologically by detecting parenchymal or leptomeningeal abnormalities in the brain or spine MRI scans. From these patients, we collected CSF samples in EDTA tubes via lumbar puncture or using an Ommaya reservoir (Integra LifeSciences, Inc., Princeton, NJ, USA). The samples were aliquoted into cryotubes and stored at −80°C until analysis. We selected a cohort of 17 patients with confirmed CNS involvement for a detailed study. CNS involvement was confirmed via brain biopsy in cases of parenchymal abnormalities and via cytological CSF examination for leptomeningeal cases. Patients with leptomeningeal involve-

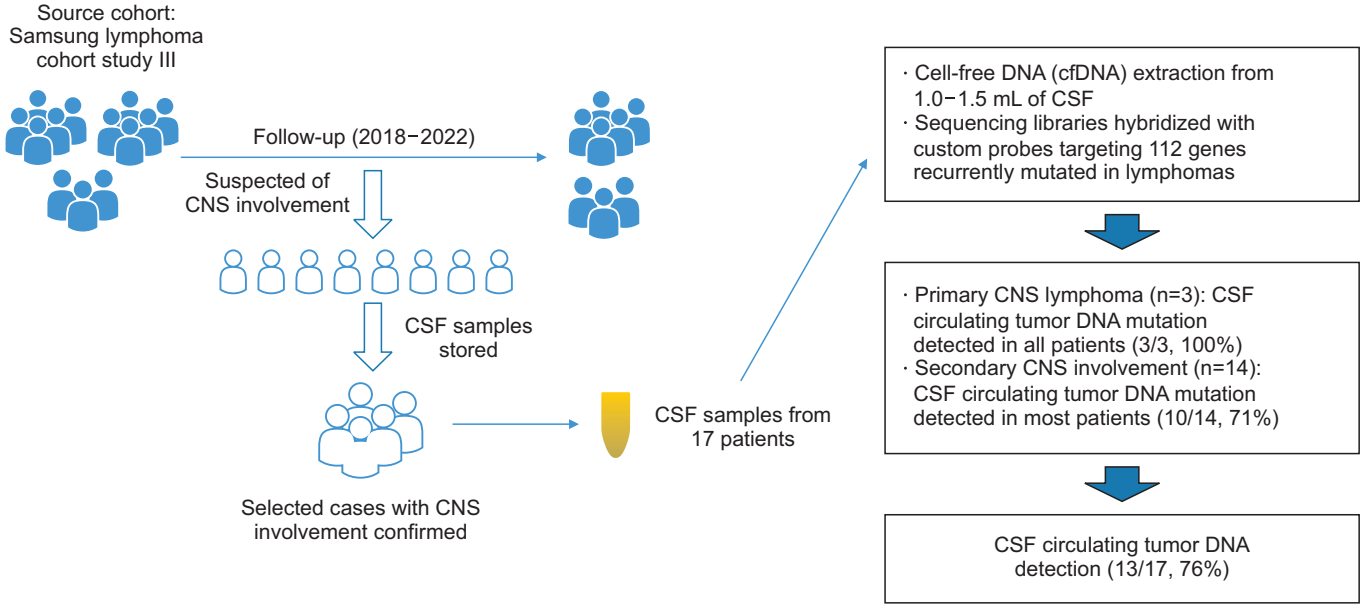

Fig. 1. Study flow.
Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid.

ment who were cytology-negative were identified based on their response to CNS-directed therapy and imaging findings. We monitored all patients' clinical outcomes, including survival status, via follow-up MRI.

We extracted cell-free DNA (cfDNA) from 1.0–1.5 mL of CSF using a MagMAX Cell-Free Total Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA yield and size distribution were assessed using a TapeStation 4150 instrument (Agilent Technologies, Santa Clara, CA, USA) and Qubit 4.0 fluorometer (Thermo Fisher Scientific). We utilized 0.04–40 ng of DNA for library construction. The DNA was ligated using a Twist MF Library prep Kit (Twist Bioscience, San Francisco, USA) with Illumina adapters and indexed with unique dual indices for duplex sequencing (Illumina, San Diego, CA, USA). The sequencing libraries were hybridized with custom probes targeting 112 genes known to be mutated in lymphomas (Supplemental Data Table S1). Pooled libraries were paired-end sequenced (2×150 bp) on the NovaSeq 6000 System (Illumina, San Diego, CA, USA). Single-nucleotide variants (SNVs) and insertions/deletions were called using PiSeq (Dxome, Sungnam, Korea) to differentiate low-frequency variants from amplification artifacts and sequencing errors. The analytical sensitivity for SNVs was assumed to be 0.24% [15]. Copy number alterations were identified using ExomeDepth and a custom tool. Variants were visually confirmed using Integrative Genome Viewer (Broad Institute, Cambridge, MA, USA). The variant allele frequency (%) was calculated as the number of sequencing reads of a specific DNA variant divided by the overall coverage at that locus.

We analyzed 17 patients with CNS involvement who had CSF samples available for targeted sequencing (Fig. 1). Fourteen patients had systemic DLBCL with secondary CNS involvement, such as isolated CNS relapse or disease progression including the CNS, whereas three had primary CNS lymphoma (Table 1). However, their CSF cytology showed negative results although

Table 1. Patient clinical characteristics at CSF sampling time and CSF ctDNA detection

Case No.	Age (yrs)	Sex	Diagnosis	Clinical history of CNS involvement	Radiologic CNS involvement pattern	CSF cytology	CSF ctDNA	Survival
1	72	M	DLBCL, ABC type	RCHOP 6 cycles for stage I → Isolated CNS relapse	Leptomeninges	Negative	Not detected-	Dead
2	54	M	DLBCL, ABC type	RCHOP 6 cycles for stage 4 → Isolated CNS relapse	Parenchyma+Leptomeninges	Positive	Detected	Dead
3	41	M	DLBCL, ABC type	RCHOP 6 cycles for stage 1 → Isolated CNS relapse	Leptomeninges	Positive	Detected	Alive
4	34	M	Primary CNS DLBCL	Primary CNS involvement	Parenchyma	Negative	Detected	Alive
5	57	F	DLBCL, ABC type	RCHOP 6 cycles for stage 1 → Isolated CNS relapse	Parenchyma	Positive	Detected	Dead
6	39	F	DLBCL, GC type	RCHOP 6 cycles for stage 4 → Systemic relapse → ICED followed by ASCT → Systemic relapse with CNS involvement	Leptomeninges	Negative	Library construction failed	Dead
7	73	M	DLBCL, GC type	RCHOP 6 cycles for stage 3 → Isolated CNS relapse	Leptomeninges	Positive	Detected	Dead
8	61	M	DLBCL, ABC type	RCHOP 6 cycles for stage 2 → Isolated CNS relapse	Parenchyma	Positive	Not detected	Alive
9	40	M	DLBCL, GC type	RCHOP 3 cycles for stage 4 → Progression with CNS involvement	Leptomeninges	Positive	Detected	Dead
10	72	M	DLBCL, ABC type	RCHOP 6 cycles for stage 4 → Isolated CNS relapse	Leptomeninges	Negative	Detected	Dead
11	52	M	DLBCL, GC type	RCHOP 6 cycles for stage 4 → Systemic relapse with CNS involvement	Parenchyma+Leptomeninges	Negative	Not detected	Dead
12	72	M	Primary CNS DLBCL	Primary CNS involvement	Parenchyma	Negative	Detected	Alive
13	53	M	DLBCL, ABC type	RCHOP 2 cycles for stage 4 → Progression with CNS involvement	Parenchyma	Negative	Detected	Dead
14	73	F	DLBCL, GC type	RCHOP 3 cycles for stage 4 → Isolated CNS relapse	Parenchyma+Leptomeninges	Negative	Detected	Dead
15	71	M	Primary CNS DLBCL	Primary CNS involvement	Parenchyma+Leptomeninges	Negative	Detected	Dead
16	41	F	DLBCL, ABC type	RCHOP 6 cycles for stage 4 → Systemic relapse with CNS involvement	Parenchyma+Leptomeninges	Positive	Detected	Dead
17	68	F	DLBCL, ABC type	RCHOP 6 cycles for stage 4 → Isolated CNS relapse	Parenchyma	Negative	Detected	Dead

Abbreviations: CSF, cerebrospinal fluid; ctDNA; circulating tumor DNA; DLBCL, diffuse large B-cell lymphoma; ABC, activated B-cell like; GC, germinal center; CNS, central nervous system; RCHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone.

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they were suspicious of CNS involvement, such as radiological leptomeningeal involvement in more than half of the cases. The average sequencing coverage depth for CSF samples was 53,733× (range, 12,563–90,023×). The median cfDNA concentration was 45.85 ng/mL (2.15–12,000 ng/mL). Library construction failed for one sample with the lowest cfDNA concentration (2.15 ng/mL, case No. 6), and variants were not detected in three other patients (case Nos. 1, 8, and 11). The overall variant detection rate was 76% (13/17, Fig. 1). In total, 182 variants were detected in 13 patients, with the most common variants occurring in *KMT2D*, *HIST1H1*, *PIM1*, and *MYD88* (Fig. 2A). CSF samples from two patients (case Nos. 9 and 16) were collected serially, with consistent ctDNA variant profiles (Fig. 2B). The inability to detect variants or successfully prepare libraries was significantly associated with the DNA concentration in the CSF (Fig. 2C).

The feasibility of detecting ctDNA in the CSF of patients with large B-cell lymphoma involving the CNS is an area of active research. CNS involvement in large B-cell lymphoma presents diagnostic and therapeutic challenges, and the ability to detect tumor-specific DNA in the CSF may offer valuable insights for diagnosis, monitoring, and treatment decisions. Droplet digital polymerase chain reaction demonstrated a 71% detection rate (10 of 14 cell-free CSF samples) for *MYD88* variants in patients with primary CNS lymphoma [16]. A later study reported a slightly higher detection rate of 76.9% (20 of 26 cases) in a similar cohort [17]. However, these studies were limited to specific variants, such as the *MYD88* variant. Given the variety of variants present in systemic DLBCL, a broader genetic analysis is recommended. For instance, a Spanish study employing a 300-gene panel assessed the feasibility and utility of ctDNA detection in CSF for both primary CNS lymphoma and systemic lymphoma with secondary CNS involvement [18]. Variants were successfully detected in all six patients with CNS involvement [18]. A recent study using CSF samples from 92 patients reported a 100% variant detection rate using cancer personalized profiling

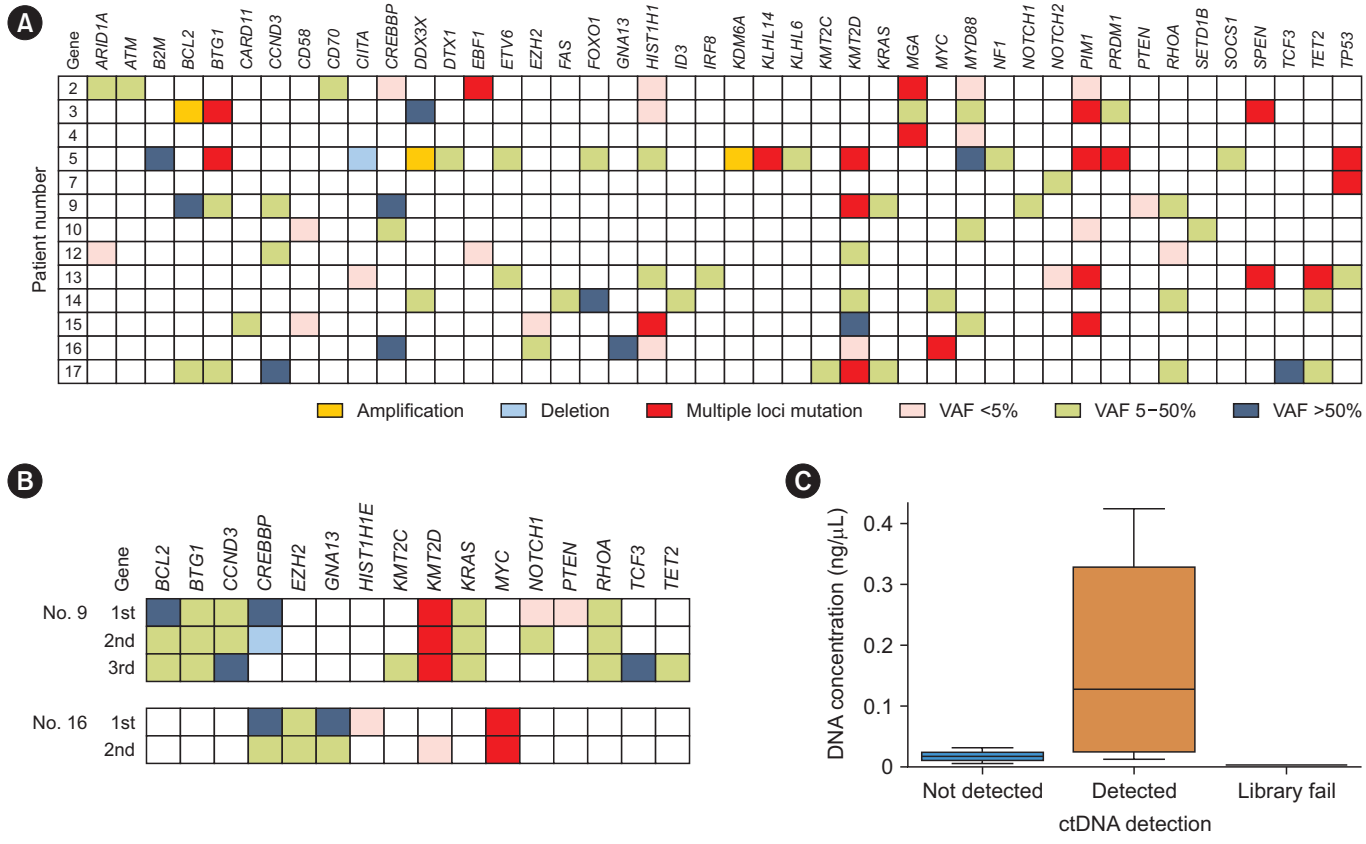

Fig. 2. Mutation profiles and DNA concentration distribution in CSF from patients with CNS involvement. (A) Mutation profiles detected in ctDNA from 14 patients. (B) Consistency of mutation profiles in serial samples of two patients (No. 9 and 16). (C) DNA concentration distribution according to the status of ctDNA detection.
Abbreviations: ctDNA, circulating tumor DNA; CSF, cerebrospinal fluid; CNS, central nervous system.

by deep sequencing (CAPP-Seq) for primary CNS lymphoma or secondary CNS involvement [19]. Despite its effectiveness, CAPP-Seq may not be universally available, making targeted sequencing of a large number of genes a more practical option in some settings. Our variant detection rate aligns with these findings, and our study provides a feasible approach for routine clinical application.

Despite its promising performance in variant detection, CSF ctDNA analysis still has some challenges. One challenge is the low concentration of ctDNA in CSF compared to that in peripheral blood, hampering detection [20]. Additionally, distinguishing tumor-derived DNA from background DNA released from normal cells in the CNS presents a challenge, especially considering the potential for contamination from blood or other sources during CSF collection. Despite these challenges, advancements in NGS techniques have improved the ctDNA detection sensitivity, enabling researchers to detect tumor-specific variants in CSF samples.

Our study demonstrated the feasibility of CSF ctDNA sequencing as a diagnostic tool for detecting CNS involvement in DLBCL despite most patients showing negative CSF cytological results. The development of early detection methods for identifying tumor cells in CSF may significantly impact treatment strategies for patients with systemic DLBCL. This would enable the initiation of targeted therapies for patients who are CSF cytology-negative yet suspected of CNS involvement, potentially altering their disease course. Advancements in CSF-based targeted sequencing may help in predicting the risk of CNS relapse or progression in patients with systemic DLBCL, facilitating the development of preemptive strategies for those at high risk. Given these potential benefits, there is a pressing need for further research to enhance the feasibility and accuracy of CSF targeted sequencing. This may ultimately lead to more precise and effective management of patients with DLBCL with or at risk of CNS involvement.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2024.0257

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AUTHOR CONTRIBUTIONS

Conception and design: Kim SJ; Resources: Yoon SE and Kim WS; Acquisition of data and experiments: Park MR, Park B, Ryu KJ, and Shin S; Analysis and interpretation of data: Kim SJ, Kim JJ, Shin S, and Lee ST; Drafting of manuscript: Kim SJ, Kim JJ, and Shin S. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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