

# Biomarkers Reflecting The Destruction Of The Blood-Brain Barrier Are Valuable In Predicting The Risk Of Lymphomas With Central Nervous System Involvement

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**Objective:** We aimed to identify the biomarkers in cerebrospinal fluid (CSF) that facilitate the diagnosis of lymphomas with central nervous system (CNS) involvement.

**Methods:** Four cases of non-Hodgkin's lymphoma (NHL) patients with/without CNS involvement were enrolled respectively, and non-CNS tumor patients (n=3) were selected to be the controls. Lab biomarkers, cytokines, and tight junction proteins (TJs) in CSF and serum were measured.

**Results:** When comparing the CNS to non-CNS group, cytokine including MMP-9 (15.24 vs 0.36 ng/mL), CCL-2 (1922.04 vs 490.68 pg/mL), and sVCAM-1 (61.36 vs 9.00 pg/mL), TJs including OCLN (6.68 vs 2.59 pg/mL), and ZO-1 (710.04 vs 182.98 pg/mL) in CSF were significantly higher in lymphomas patients with CNS involvement than those without CNS involvement. However, serum biomarkers were not significantly elevated. Contrary to the major findings, all conventional biomarkers and MRI results showed no significant change.

**Conclusion:** CSF biomarkers affecting BBB disruption are valuable in mirroring the risk of lymphoma CNS metastasis. Further study with a larger sample size is needed to verify these biomarkers in predicting lymphoma CNS involvement.

**Keywords:** lymphoma, CNS metastasis, cytokines, tight junction protein

## Introduction

CNS involvement is a fatal complication for non-Hodgkin lymphoma (NHL),<sup>1</sup> which incidence may arrive up to 30% for highly-aggressive lymphomas if there is no promptly CNS prophylaxis.<sup>2</sup> The risk of CNS relapse can be reduced with high-dose methotrexate containing prophylactic chemotherapy,<sup>3</sup> however, high-dose methotrexate is a resource-demanding therapy with considerable toxicity, it should be limited to patients with a high risk of CNS recurrence.<sup>4</sup> Radiation therapy, although effective, often associates unacceptable late adverse effects (i.e., secondary neoplasm, endocrinopathy, neurocognitive dysfunction, and neurotoxicity, etc.).<sup>2</sup> Presently, there is no consensus regarding the best type of prophylaxis; different therapeutic modalities have been used: systemic chemotherapy, radiation therapy, or a combination. The accurate risk evaluation of lymphoma CNS metastasis is vital for determining subsequent therapy, and the laboratory biomarkers are desperately needed to define the patients in need of CNS prophylactic treatment.<sup>4</sup>

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Currently, the patient selection for CNS prophylaxis is carried out by clinical risk assessment, including high International Prognostic Index (IPI) score, advanced tumor stage, elevated lactate dehydrogenase (LDH), age > 60 years and involvement of extranodal sites, etc.<sup>4</sup> Unfortunately, high-risk patients selection based on aforementioned standards has poor specificity and sensitivity.<sup>5</sup> It is assumed that CNS relapse in NHL is likely due to occult malignant cells that have presented in CNS but may not be able to detect when the tumor is initially diagnosed.<sup>5</sup> Peripheral tumor cells have metastasized into the CNS and developed neurologic signs for weeks, but only 20% of patients can be found by clinical symptoms.<sup>4</sup> Presently, the evaluating system includes imaging studies such as magnetic resonance imaging (MRI) and computed tomography (CT) scan, cytologic test, and immunophenotypic biomarkers. The subclinical CNS tumor sites may remain undetected by conventional contrast-enhanced MRI scanning behind an intact neurovascular unit or blood-cerebrospinal fluid barrier, due to contrast enhancement is related to blood-brain barrier (BBB) integrity rather than actual tumor size.<sup>6,7</sup> Then, it is difficult to make a definitive diagnosis of lymphoma CNS metastasis.<sup>8</sup> Based on imaging techniques, the differential diagnoses also include glioma, multiple sclerosis, acute disseminated encephalomyelitis.<sup>9,10</sup> Some biological parameters have no significant difference in both primary and secondary CNS lymphomas. Therefore, lymphoma cells in CSF are still the golden standard in diagnosis lymphoma CNS involvement; however, it is generally accepted that CSF cytology has a low sensitivity though it has high specificity, the CSF-flow cytometry and CSF-polymerase chain reaction (PCR) found similarly low sensitivities (11–16%).<sup>11</sup> Nowadays, lacking diagnostic biomarkers with both high sensitivity and specificity was a major obstacle for CNS lymphomas diagnosis and treatment.

In order to improve the diagnostic efficacy, there is emerging interest in finding new biomarkers, such as circulating tumor cells (CTC), proteins, as well as micro-RNA and DNA.<sup>7,12,13</sup> CNS disease typically developed within a few months before the initial clinical presentation, raising the question: whether occult CNS localization was already present at the time of diagnosis.<sup>14</sup> The BBB and the blood-cerebrospinal fluid barrier (BCSFB) are the effective barriers to inhibit large molecules and cells into the CNS. The previous study presumed that the malignant cells originate from extracranial sites but end up coming into the CNS due to highly selective CNS tropism, where

adhesion-, migration- and ECM-related molecules play a pivotal role in CNS involvement.<sup>15,16</sup> Successful binding of lymphoma cells to the blood vessel walls of the BBB is the essential process for lymphoma invasion of the CNS. Vascular cell adhesion molecule (VCAM) results in the formation of cell connections through interactions with integrins, which promotes the cell adhesion and diapedesis, resulting in tumor growth and angiogenesis.<sup>17</sup> It has been suggested that soluble forms of VCAMs may play an important role in cancer metastasis, and the increased expression of soluble CAMs may be a predictor of malignant disease.<sup>18</sup> The degradation of the extracellular matrix (ECM) of blood or lymph vessels is critical to tumor metastasis. The matrix metalloproteinases (MMPs) hydrolyze ECM components in the tissues surrounding the tumor, which facilitates the invasion of tumor cells through the basement membrane to distant organs and results in metastasis.<sup>19,20</sup> MMP-9, secreted by tumor cells, is the main member of MMP family member that plays a critical role in the degradation of type IV collagen (a major constituent of the ECM). MMP-9 can digest TJs of the BBB, enabling tumor cells to infiltrate the brain; thus, MMP-9 activities are directly correlated with their BBB migration capability.<sup>21</sup> Monocyte chemoattractant protein-1 (MCP-1/CCL2), a chemokine secreted by fibroblasts, endothelial/epithelial cells, monocytes, and some tumor cells, plays roles in the recruitment of monocytes and macrophages into inflammatory sites and regulating their activities. CCL2 recruits blood-borne cells to the sites of brain injury by offering a specific driving stimulus.<sup>22</sup> Increased CCL2 is correlated with CNS metastasis and may be one of the mechanisms involved in CNS leukemia.<sup>23</sup>

BBB failure is a critical event in brain tumor metastasis.<sup>24</sup> The quantity of proteins and cells in CSF is dependent on CSF/blood-brain barrier integrity, biomarkers in CSF may have more diagnostic value compared with those in blood.<sup>25–28</sup> Moreover, biomarkers in CSF can reflect not only the status of malignant cells but also the tumor microenvironment in CNS. Our previous study demonstrated that CSF biomarkers, including chemokines, cytokines, and TJs had specificity and high positive predictive value in reflecting BBB damage and the potential of leukemia CNS metastasis.<sup>27,28</sup> Compare with leukemia, and lymphoma has its unique pathophysiologic features. Presently, no clinical study shows whether the aforementioned biomarkers have equal efficacies to lymphomas CNS, and there is sparse information elucidating the role

of these biomarkers in lymphoma CNS metastasis. In this study, we analyzed the diagnostic efficacy of the biomarkers, which will ensure optimal treatment while avoiding unnecessary therapies.<sup>29</sup>

## Methods And Patients

The study protocol was approved by the Ethics Committee of Shantou University Medical College. This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from patients or their guardian. Patients with clinically or radiologically suspected were selected. The diagnosis of lymphoma was determined according to the WHO standard, CNS metastasis based on the following criteria: the presence of any number of blasts in the CSF. The inclusion criteria also included lymphoma patients suffering from typical clinical symptoms of meningitis. Accordingly, 4 cases of lymphoma with CNS metastasis, 4 cases of lymphoma without CNS metastasis were enrolled in this study, at the same time, 3 cases of acute myeloid leukemia were included as the controls. Patients underwent a lumbar puncture before intrathecal therapy injection, and the controls underwent a lumbar puncture for diagnostic purposes. The procedures were carefully controlled to avoid blood contamination due to puncture, and samples with  $RBC \geq 100/mm^3$  was excluded. CSF and serum samples were collected, the paired samples were centrifuged (1,700g, 5 min, 4°C) immediately upon isolation, the serum or cell-free CSF supernatants were collected, and the aliquots were stored at -80°C until analysis.

## Cytokines, Chemokines And TJs Proteins Measurement

Cytokines and Chemokines were measured by using ELISA kits from R&D systems. The limits of sensitivity for the CCL2, sVCAM-1, and MMP-9 assays were 0.57 pg/mL, 0.17, and 0.156 ng/mL, respectively. TJs measurements were conducted by ELISA method (Cusabio, America).

All process was done according to the instruction of kits.

## CSF And Serum Conventional Biomarkers Analyses

The conventional biomarkers included cytological analyses: erythrocyte count, WBC count, and lymphoma cells detection. CSF was centrifuged, and the cell pellets were used for cytological examination for the presence of tumor cells in the CSF. CSF biochemical parameters

include albumin (ALB) glucose and chloride. The biochemical parameters in the serum were measured according to the same protocol.

BBB value: the ratio of CSF/serum albumin, was used to evaluate the BBB integrity.<sup>25,30</sup>

## Statistical Analyses

Continuous variables were reported as the medians with interquartile ranges (IQR). The Kruskal–Wallis H, Mann–Whitney U, Wilcoxon rank, and Chi-square tests were used to evaluate differences among each group. SPSS for Windows version 13.0 was used for statistical analyses (SPSS Incorporated, Chicago, IL, USA). Values of  $p < 0.05$  were considered to be statistically significant.

## Results

General characteristics of patients with CNS involvement were summarized in Table 1. Laboratory parameters including complete blood count (CBC), electrolytes, and renal and liver function tests were unremarkable except for a patient had significant elevated LDH (768.0U/L; reference range: 135–225 U/L) and thrombocytopenia of  $24.00 \times 10^9/L$  (reference range:  $100\text{--}300 \times 10^9/L$ ). However, CSF measurements showed significant change. The cytology test showed marked lymphocytic pleocytosis (patients' WBC were: 110, 1500, 115, 3540/ul, respectively;), with many immature lymphoid cells, compatible with lymphoma CNS relapsed (Figure 1). To all patients with/without CNS involvement, the CT and MRI scans of the brain were no obvious changes (Figure 2), three patients had another organ invasion.

## Cytokines, Chemokines And TJs Proteins Measurement

Subgroup analysis shows that CSF cytokines, including MMP-9, CCL-2, sVCAM-1, TJs including OCLN, and ZO-1 were significantly higher in lymphoma with CNS involvement than those without CNS involvement ( $P < 0.05$ ). CSF MMP-9, CCL-2, sVCAM-1, TJs including OCLN, and ZO-1 in lymphoma patients were higher than in patients with leukemia CNS involvement. Serum cytokines and TJs were measured at the same time. The results revealed that serum cytokines in the CNS metastatic group had no significant difference compared with the non-CNS metastatic group; however, serum TJs in the CNS group was higher than that in the non-CNS group. TJs remain the same trend except for the CLDN5. BBB value in the CNS

**Table 1** General Characteristic Of Patients With Lymphoma CNS Metastasis Survival Time Is Calculated From 2012.11.08 To 2015.03.19

	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	3.00	19.00	34.00	14.00
Gender	Female	Male	Female	Male
WBC <sub>Blood</sub> (10E+9/L)	4.21	2.26	7.16	6.79
RBC <sub>Blood</sub> (10E+12/L)	3.73	2.15	3.87	2.44
Hb <sub>Blood</sub> (g/L)	197.00	66.00	119.00	78.00
PLT <sub>Blood</sub> (10E+9/L)	318.00	24.00	133.00	183.00
LDH <sub>Serum</sub> (U/L)	86.00	768.00	216.00	257.00
AST <sub>Serum</sub> (U/L)	65.00	10.00	23.00	21.00
ALT <sub>Serum</sub> (U/L)	60.00	14.00	21.00	40.00
GGT <sub>Serum</sub> (U/L)	23.00	30.00	27.00	105.00
ALP <sub>Serum</sub> (U/L)	160.00	54.00	68.00	97.00
CHE <sub>Serum</sub> (U/L)	9.42	3.56	5.10	5.74
TP <sub>Serum</sub> (g/L)	62.20	51.00	59.80	56.40
ALB <sub>Serum</sub> (g/L)	45.30	30.60	40.40	37.80
GLB <sub>Serum</sub> (g/L)	16.90	20.40	19.40	18.60
Glu <sub>CSF</sub> (mmol/L)	4.03	3.11	0.72	3.49
Cl <sub>CSF</sub> (mmol/L)	122.00	126.00	113.00	123.00
ALB <sub>CSF</sub> (g/L)	1.13	0.70	0.96	0.25
WBC <sub>CSF</sub> (10E+6/L)	110.00	1500.00	115.00	3540.00
BBB value	24.94	22.88	23.76	26.98
Survival time (days)	676	33	70	135
Blasts in CSF	Not found	Not found	Found	Found
Organ invasion	Not detected	Detected	Detected	Detected

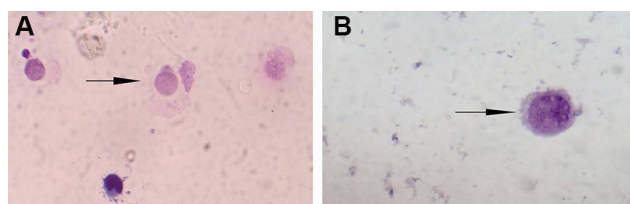
**Abbreviations:** CSF, cerebrospinal fluid; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; PLT, platelet; LDH, L-lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glut amyl transpeptidase; ALP, alkaline phosphatase; CHE, cholinesterase; TP, total protein; ALB, albumin; GLB, globulin; Glu, glucose; Cl, chloride; BBB value,  $\text{ALB}_{\text{CSF}} \times 1000 / \text{ALB}_{\text{Serum}}$ .

group was significantly higher than in a non-CNS group, indicating BBB integrity damaged in the CNS group (Tables 2 and 3).

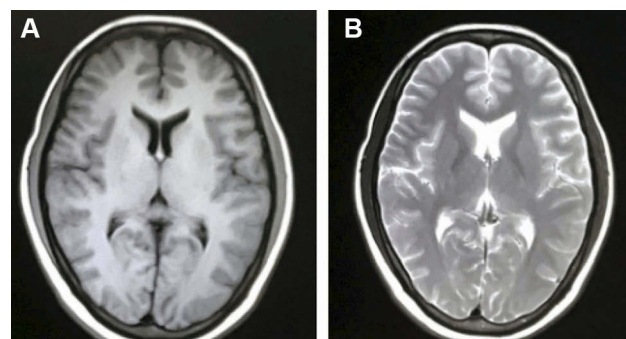
## Discussion

Nowadays, clinical trials are exploring the optimum time of CNS prophylactic treatment should be performed in order to decrease the incidence of lymphoma CNS involvement, meanwhile, minimize CNS toxicity/secondary tumor due to chemotherapy and radiotherapy. Although extensive efforts have been made, the progress is limited. The management of the lymphoma CNS involvement still

poses a major challenge in lymphoma therapy. Prophylaxis with intrathecal chemotherapy has resulted in varied outcomes.<sup>31,32</sup> It is currently unknown the efficacy of CNS prophylaxis at CNS relapse, and the debate on the optimal route of CNS prophylaxis (intrathecal versus systemic) is still ongoing.<sup>14</sup> Patient's status may be the most critical factor for therapeutic efficacy, which should be accurately evaluated. In our patients with or without



**Figure 1** Immature cells in CSF that come from different patients (A and B) with lymphoma (black arrows),  $\times 1000$ .



**Figure 2** MRI scans (A and B) of the brain revealed unremarkable changes in lymphoma patients with CNS involvement.

**Table 2** General Characteristics Of Different Groups

	CNSL Group (n=4)	Non-CNSL Group (n=4)	Control (n=3)	AML-Non-CNSL (n=4)
Age (years)	16.50(8.50–26.50)	14.50(8.50–15.50)	19.50(12.00–27.00)	17.00(16.00–29.00)
WBC <sub>Blood</sub> (10E+9/L)	5.50(3.24–6.98)	4.08(2.52–6.10)	8.93(5.33–11.04)	5.80(4.89–6.03)
RBC <sub>Blood</sub> (10E+12/L)	3.09(2.30–3.80)	3.26(2.95–4.19)	3.18(1.84–4.52)	3.39(2.68–3.44)
Hb <sub>Blood</sub> (g/L)	98.50(72.00–158.00)	103.50(86.50–129.50)	106.00(62.50–142.00)	113.00(88.00–115.50)
PLT <sub>Blood</sub> (10E+9/L)	158.00(78.50–250.50)	175.00(145.50–234.50)	16.00(12.00–57.00)	277.00(154.00–280.00)
LDH <sub>Serum</sub> (U/L)	236.50(151.00–512.50)	221.50(179.50–231.50)	292.50(239.50–448.00)	148.00(136.50–156.50)
AST <sub>Serum</sub> (U/L)	22.00(15.50–44.00)	20.00(16.50–74.00)	16.50(14.50–48.00)	19.00(14.00–27.50)
ALT <sub>Serum</sub> (U/L)	30.50(17.50–50.00)	18.50(16.00–302.00)	18.00(13.00–91.50)	13.00(12.50–15.00)
GGT <sub>Serum</sub> (U/L)	28.50(25.00–67.50)	18.00(13.00–42.00)	23.50(19.50–28.50)	28.00(23.00–58.00)
ALP <sub>Serum</sub> (U/L)	82.50(61.00–128.50)	137.00(128.50–178.50)	133.50(93.00–174.50)	120.00(117.50–133.50)
CHESerum (U/L)	5.42(4.33–7.58)	7.46(6.74–8.57)	7.73(7.19–8.44)	5.63(5.24–7.02)
TP <sub>Serum</sub> (g/L)	58.10(53.70–61.00)	64.95(61.95–68.50)	73.60(66.05–76.65)	59.50(59.30–62.35)
ALB <sub>Serum</sub> (g/L)	39.10(34.20–42.85)	41.20(38.50–44.90)	41.35(37.75–48.30)	35.50(35.25–35.90)
GLB <sub>Serum</sub> (g/L)	19.00(17.75–19.90)	23.10(20.75–26.30)	28.60(21.35–35.30)*	24.00(23.40–27.10)**
Glu <sub>CSF</sub> (mmol/L)	3.30(1.92–3.76)	3.12(2.90–3.36)	4.16(2.39–4.70)	3.45(3.37–3.76)
Cl <sub>CSF</sub> (mmol/L)	122.50(117.50–124.50)	125.35(121.85–129.00)	121.10(108.65–129.60)	125.80(125.15–131.90)
ALB <sub>CSF</sub> (g/L)	0.83(0.48–1.05)	0.19(0.12–0.27)	0.37(0.16–3.87)	0.29(0.20–0.42)
WBC <sub>CSF</sub> (10E+6/L)	807.50(112.50–2520.00)	1.50(1.00–2.00)**	39.00(2.50–117.50)**	2.00(1.50–3.50)**
BBB value	24.35(23.32–25.96)	4.57(2.87–6.32)*	8.66(4.25–74.73)	8.17(5.60–11.94)**

**Note:** \*p ≤ 0.05 and \*\*p ≤ 0.01 when compared with lymphoma group.

**Table 3** Cytokine And Tight Junction Proteins Of Different Groups

	CNSL group(n=4)	Non-CNSL(n=4)	AML-CNSL(n=3)	AML-non-CNSL(n=4)
MMP-9 <sub>CSF</sub> (ng/mL)	15.24(0.49–36.46)	0.36(0.36–0.37)*	0.41(0.39–0.50)	0.37(0.37–0.38)**
MMP-9 <sub>Serum</sub> (ng/mL)	82.44(24.27–184.99)	125.22(68.71–169.40)	79.75(18.60–538.28)	127.00(73.46–793.78)**
CCL2 <sub>CSF</sub> (pg/mL)	1922.04(1039.41–2553.66)	490.68(245.51–658.80)	747.53(286.37–1181.84)**	517.54(459.16–526.88)**
CCL2 <sub>Serum</sub> (pg/mL)	328.40(247.84–698.50)	363.43(198.81–508.20)	495.35(229.16–895.81)	213.99(183.63–282.87)
sVCAM-1 <sub>CSF</sub> (ng/mL)	61.36(9.21–118.24)	9.00(8.09–10.63)	12.01(9.78–21.04)	11.77(9.75–12.15)
sVCAM-1 <sub>Serum</sub> (ng/mL)	1546.95(1148.11–1971.14)	1092.34(859.12–1361.05)	2402.09(1492.87–2898.95)	1410.06(1090.65–1830.87)
CLDN5 <sub>CSF</sub> (pg/mL)	234.92(144.77–635.82)	281.06(238.08–329.27)	200.22(95.65–421.64)	215.42(203.54–257.04)
CLDN5 <sub>Serum</sub> (pg/mL)	115.75(82.18–161.60)	100.93(74.44–153.18)	110.61(91.90–165.48)	93.23(92.03–117.20)
OCLN <sub>CSF</sub> (pg/mL)	6.68(2.80–10.63)	2.59(1.09–3.96)	0.71(0.32–0.93)	0.39(0.20–0.70)
OCLN <sub>Serum</sub> (pg/mL)	7.11(3.26–16.89)	6.94(4.67–9.53)	15.51(11.26–22.11)	7.28(5.83–10.35)
ZO-1 <sub>CSF</sub> (pg/mL)	710.04(590.52–777.18)	182.98(174.11–191.14)*	208.76(143.24–394.13)	122.24(110.16–167.32)**
ZO-1 <sub>Serum</sub> (pg/mL)	1549.60(1121.47–1790.29)	939.75(680.12–1102.58)	1925.50(1718.23–1976.06)	1039.49(900.80–1170.52)

**Note:** \*p ≤ 0.05 and \*\*p ≤ 0.01 when compared with lymphoma group.

CNS involvement, there was no obviously different clinical manifestation between them, indicating patients could be asymptomatic in the early or late phase, and IPI score was not enough for discriminating patients with or without CNS involvement. In addition, there was no extraordinary discovery in MRI, and even patients had significantly higher lymphoma cells in CSF.

CSF cytology is still the golden standards for tumor CNS involvement; however, even when cytological examination shows no CNS involvement, approximately 10–35% of ALL patients relapse in the CNS.<sup>33</sup> Under

this circumstance, clinical risk paradigms and conventional parameters are not enough for the identification of patients with CNS metastasis. Combination of diagnostic approaches is needed to timely diagnosis and start an adequate therapeutic regimen.<sup>34</sup> Circulating biomarkers are easily accessible, no need for the expensive instrument, and measured conveniently, which could benefit to determine risk stratification and clinical decisions, and evaluating the outcome of treatment.<sup>35</sup>

The permeability of the BBB is modulated by protein-protein interactions of the TJs proteins.<sup>36</sup> which are the



first barrier that cancer cells must overcome to metastasize.<sup>37</sup> Though the mechanism of lymphoma CNS involvement is unclear, two mechanisms have been postulated: (1): seeding from occult reservoir lesions within the CNS (including eye and CSF), or (2): seeding from the blood and bone marrow.<sup>38,39</sup> To secondary CNS lymphomas, the lymphoma cells need to dissociate from the primary site and enter the peripheral blood where they disseminate and infiltrate the CNS. During this process, cytokines and chemokines may play a critical role. sVCAM-1 is involved in tumor cell arrest and subsequent extravasation across the brain endothelium.<sup>40</sup> Higher sVCAM-1 may accelerate circulating leukemic cells to target and adhere to BMVECs, making the interaction with the endothelial possible, and then enhancing the possibility of tumor cells entering the CNS. MMPs and vascular endothelial growth factor(VEGF) perturbs TJs integrity by decreasing OCLN and ZO-1 expression and causing CLDN5 and ZO-1 protein disruption.<sup>41,42</sup> MMP-9 is critical for the breakdown of the basal membrane of the BBB.<sup>43</sup> In acute leukemia (AL), the degradation of TJs ZO-1, claudin-5, and occludin by MMP-2 and -9 constitutes an important mechanism in the BBB breakdown that contributes to the invasion of the CNS in AL.<sup>21</sup> Previous studies found that MMP-9 secreted by leukemic cells degrade ZO-1, CLDN5, and OCLN, which constitute an important mechanism of the BBB breakdown.<sup>27</sup> MMPs in the CSF may be indicative of the disruption of the BBB.<sup>44</sup>

CXCR4 signaling might participate in the initiation of lymphoma cells homing into the CNS.<sup>4</sup> CXCR4/CXCL12 and CXCR5/CXCL13 axis play an important role in CNS tropism of DLBCL.<sup>45</sup> CCL2 can be produced by neurons, astrocytes, and microglia, as well as from the choroid plexus.<sup>46,47</sup> There may be greater production of CCL2 in the CNS than systemically. In patients with lymphoma CNS metastasis, significantly higher levels of CCL2 was observed in CSF than in serum, high levels of CCL2 in the CSF may represent a major chemoattractant stimulus for the differential recruitment of leukocytes into the subarachnoid space.<sup>48</sup> Compare with serum CCL2, CCL2 in CSF is sufficient for recruiting blood-borne cells to the sites of brain injury by offering a specific driving stimulus, and in response to leukocytes migrates across the BBB.<sup>49</sup>

The process of cancer metastasis consists of linked sequential steps, known as the metastatic cascade that includes detachment, invasion, intravasation, circulation, adhesion, extravasation, and growth in distant organs. The previous study revealed that MMP-9, CCL-2, sVCAM-1

play an important role in the metastatic cancer cascade, affecting leukemia cell CNS transferred.<sup>20</sup> In this study, lymphoma patients with CNS involvement had significantly higher MMP-9, CCL-2, and sVCAM-1 in CSF, all of which are the key factors of metastatic cancer cascade; therefore, they may be closely associated with enhanced CNS tropism. MMP-9 facilitate the invasion of lymphoma cells through BBB by hydrolyzing ECM components in the tissues, digest TJs of the BBB.<sup>19,50</sup> sVCAM-1 over-expression was detected in patients with non-NHL and acute leukemia.<sup>51</sup> In this study, significantly higher MMP-9 in CSF induced higher levels of TJs (OCLN and ZO-1) in patients with CNS lymphomas, demonstrating that higher MMP-9 has a role in promoting CNS metastasis in regulating factors relating to TJs breakdown and tumor invasion. Therefore, increased MMP-9, CCL-2, and sVCAM-1 were correlated with lymphoma CNS metastasis. We further evaluated the diagnostic efficacy of MMP-9, CCL-2, and sVCAM-1 and TJs. Our results showed that patients without CNS involvement had quite lower levels of MMP-9, CCL-2, and sVCAM-1 and TJs in CSF, rather than in serum, indicating that CSF biomarkers had higher specificity than serum biomarkers in predicting lymphoma CNS metastasis. Considering all factors (also include age, biochemical parameters including LDH, CSF albumin, and cytology), CSF MMP-9, CCL-2, sVCAM-1, and TJs were the most significant prognostic factors for predicting lymphomas CNS metastasis, which had more valuable in mirroring the CNS status. If further verified, these biomarkers might become useful in predicting lymphoma CNS involvement.

The main limitation of our study is the small sample size; a longer prospective evaluation of a larger number of patients is necessary to confirm the clinical significance of these CSF biomarkers. In clinical routine, some patients with large, space-occupying brain tumors are not amenable for a CSF analysis due to the risk of hernia ion.<sup>52</sup> then, we could not find suitable controls to compare these cytokines between lymphomas and solid tumors in CNS. Controversies still exist regarding the diagnostic procedure (biopsy or craniotomy), treatment strategies (methotrexate/chemotherapy/target therapy regimen, radiation therapy [RT], immunotherapy, stem cell therapy), and prognostic factors among published results.<sup>53</sup> Although we found that CSF biomarkers are valuable in predicting lymphomas CNS involvement, validation studies with a broad patient spectrum are crucial to identify relevant cutoff values and to assess the respective diagnostic potentials of reliable biomarkers.<sup>52</sup>

## Abbreviations

ALB, Albumin; ALL, Acute lymphoblastic leukemia; ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; AML, Acute myeloid leukemia; AST, Aspartate aminotransferase; BBB, Blood–brain– barrier; BCSFB, Blood–cerebrospinal– fluid barrier; CBC, Complete blood count; CCL-2, Chemokine ligand 2; CHE, Cholinesterase; CNS, Central nervous system; CLDN5, Claudin 5; CT, Computed tomography; CTC, Circulating tumor cell; CSF, Cerebrospinal fluid; CXCR4, C-X-C chemokine receptor type 4; CXCL12, Chemokine (C-X-C motif) ligand 12; CXCR5, C-X-C chemokine receptor type 5; CXCL13, Chemokine (C-X-C motif) ligand 13; DLBCL, Diffuse large B cell lymphoma; Hb, Hemoglobin; IPI, International Prognostic Index; GGT, Gamma-glut amyl transpeptidase; GLB, Globulin; GLU, Glucose; LDH, Lactate dehydrogenase; MMP, Matrix metalloprotein; MRI, Magnetic resonance imaging; NHL, Non- Hodgkin lymphoma; OCLN, Occludin; PCNSL, Primary central nervous system lymphoma; PCR, Polymerase chain reaction; PLT, Platelet; RBC, Red blood cell; RT, Radiation therapy; sVCAM-1, Soluble vascular cell adhesion molecule-1; VEGF, Vascular endothelial growth factor; TJs, Tight junction proteins; TP, Total protein; WBC, White blood cell; ZO-1, Zonula occludens-1.

## Disclosure

The authors report no conflicts of interest in this work.

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