Diagnostic Biomarkers in Cerebrospinal Fluid in Primary Central Nervous System Lymphoma: A Protocol for Systematic Review and Meta-analysis

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Abstract

Background: Primary central nervous system lymphoma (PCNSL) is a highly aggressive non-Hodgkin’s lymphoma with an unfavorable prognosis. Currently, the diagnosis of PCNSL relies on brain excisional biopsy, which is an invasive procedure that carries the risk of complications such as intracranial hemorrhage and functional impairment. Finding effective biomarkers will help us to diagnose PCNSL faster and safer.

Methods: A systematic review and meta-analysis will be conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocol (PRISMA-P) 2015 guidelines. We will search databases including PubMed, Cochrane Library, Medline, Web of Science, EMBASE, and CNKI. Studies that demonstrated the diagnostic value of certain biomarkers in cerebrospinal fluid (CSF) in PCNSL will be included. The standardized mean difference (SMD) and their 95% confidence intervals (CI) will be calculated for quantitative values. The outcomes are the mean difference in biomarker levels in CSF between PCNSL patients and controls.

Discussion: In this systematic review and meta-analysis, we will analyze biomarkers in cerebrospinal fluid for the diagnosis of PCNSL. This research can help us to identify biomarkers with diagnostic value for PCNSL, making the diagnosis of PCNSL easier, faster, and safer.

Systematic review and meta-analyses registration: PROSPERO CRD42020218143.

Keywords: Diagnostic biomarkers, cerebrospinal fluid, primary central nervous system lymphoma, systematic review, meta-analysis
BACKGROUND
Primary central nervous system lymphoma (PCNSL) is a rare subtype of non-Hodgkin's lymphoma that tends to occur in older people with a median age of 65 years. The common primary sites are the brain, leptomeninges, spinal cord, and eyes, without infiltration of other parts. PCNSL can occur in immunocompromised individuals, such as HIV-infected hosts and post organ transplant recipients. In recent years, an increasing number of cases in immunocompetent hosts have been reported. PCNSL is particularly the activated B cell-like (ABC) subtype, characterized by constitutive activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, which is frequently accompanied by myeloid differentiation factor 88 (MYD88) and CD79B mutations. PCNSL is a highly aggressive malignant lymphoma with an unfavorable prognosis. It responds poorly to conventional chemotherapy, and the overall 5-year survival rate is only approximately 33%. Combination chemotherapy with high-dose methotrexate, whole-brain radiotherapy, and autologous hematopoietic stem cell transplantation can benefit PCNSL patients. In recent years, novel targeted therapeutics have emerged as potential treatments for PCNSL. For instance, the Bruton Tyrosine Kinase (BTK) inhibitor ibrutinib significantly improves the prognosis of PCNSL patients, especially for those with MYD88 and CD79B double mutations. The immunomodulator lenalidomide also enhances the efficacy of chemotherapy in PCNSL.

The clinical manifestations of PCNSL patients are nausea, headache, vomiting, limb weakness, etc. Imaging examinations such as computed tomography (CT) and magnetic resonance imaging (MRI) can find intracranial space-occupying lesions. Several studies have reported that PCNSL has certain changes in imaging features, such as lesions that are mostly located in the supratentorial area, MRI hypointense on T1-weighted images, isohypointense on T2-weighted images, homogeneous enhancement and restricted diffusion, little associated vasogenic edema, and no central necrosis, which can be distinguished from other tumor types such as glioma. However, brain biopsy is still the gold standard for diagnosis. Nevertheless, brain biopsy is an invasive procedure that carries the risk of complications such as intracranial hemorrhage and functional impairment. The procedure may also be challenging because of the difficulty in reaching deep tumor sites. Exploring a high-efficiency and less invasive diagnostic strategy is an urgent need for the diagnosis of PCNSL.

Recently, several studies have shown that certain diagnostic biomarkers could be detected in cerebrospinal fluid (CSF) and peripheral blood in PCNSL patients, providing ideas for the diagnosis of PCNSL without brain excisional biopsy. These biomarkers include tumor cells, tumor DNAs, and proteins. CSF biomarkers have better sensitivity and auxiliary diagnostic value for PCNSL than blood biomarkers. Because lumbar puncture is routine and less traumatic in patients with intracranial lesions, CSF biomarkers are potentially the most suitable biomarkers for PCNSL diagnosis.

AIM AND OBJECTIVES
The aim of this systematic review and meta-analysis is to evaluate the diagnostic performance of frequently reported biomarkers in CSF in PCNSL patients, with an objective to identify promising biomarkers that will help us to diagnose PCNSL faster and safer.

METHODS
This “systematic review and meta-analysis” was registered on the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42020218143) on the 29th of November 2020. This systematic review and meta-analysis will be conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocol (PRISMA-P) 2015 guidelines [Additional file 1].

Eligibility criteria
Type of studies
This review will include all types of studies with human subjects that evaluate the diagnostic value of biomarkers in CSF in PCNSL. Case reports, letters, animal studies, and laboratory studies will be excluded.

Type of participants
Patients with PCNSL diagnosis according to histopathology will be included, regardless of sex, age, race, ethnicity, involvement site, host immune status, and severity of PCNSL. Patients with secondary central nervous system lymphoma will be excluded.

Type of index test
The results of the index tests that use CSF-based tumor cells, tumor DNA, and proteins in the detection of PCNSL will be considered for analysis.

Types of intervention
Several cytokines and gene mutations in CSF are reported to be associated with the diagnosis of PCNSL. The concentration or properties of the following biomarkers in the CSF of PCNSL patients and controls will be evaluated in this study.
Biomarkers: MiR-21, miR-19, miR-30c, miR-92a, transmembrane activator, and calcium modulator and cyclophilin ligand interactor (TACI), B cell maturation antigen (BCMA), soluble IL-2 receptor (sIL-2R), interleukin-6 (IL-6), interleukin-10 (IL-10), β2-microglobulin (β2-MG), the C-X-C motif chemokine ligand 13 (CXCL13), T cell immunoglobulin and mucin domain 1 (Tim-1), neopterin (Npt), a proliferation inducing ligand (APRIL), B cell activating factor (BAFF), CD79B, myeloid differentiation factor 88 (MYD88), circulating U2 small nuclear RNA fragments (RNU2-1f), soluble CD27, osteopontin (OPN), antithrombin III (AT III).

Types of outcome measures
Diagnostic value of the biomarkers in CSF in the diagnosis of PCNSL will be measured by calculating the mean difference in biomarker levels between PCNSL patients and controls.

Data sources and search strategy
A literature search will be performed in multiple electronic databases, including PubMed, Cochrane Library, Medline, Web of Science, EMBASE, and CNKI, from their inception to October 30, 2020. There are no language restrictions. The search strategy of Medline is shown in Additional file 2. Other databases will be used by a similar strategy.

Data collection and analysis
Study selection
Two reviewers (FF and LQ) will evaluate the titles and abstracts by searching information sources independently. The full text of potential articles will be assessed by both reviewers if the eligibility of an article cannot be decided by only screening the title and abstract. Any disagreements regarding the eligibility of studies will be adjudicated by a discussion with a third reviewer (YS).

Data extraction
Two reviewers (HY and FF) will extract the data from each included study independently by using a standardized form. The items extracted from the studies will be the first author, study title, publication year, regions, study design, sample size and controls, detection methods, biomarkers and levels, diagnostic value, value type, sensitivity, and specificity. If there will be any missing data, then we will contact the authors for additional information. Any disagreements will be resolved through discussion, and the standardized forms will be checked by NZ.

Risk of bias in individual studies
The quality of the included studies and risk of bias will be assessed independently by two reviewers (HY and FF) using Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria. The quality assessment evaluates the risk of bias and concerns regarding the applicability of all included studies. The results of the quality assessment and studies with a high, low, or unclear risk of bias will be displayed in a table.

Data synthesis
Statistical analysis
The mean and standard deviation of biomarker levels from individual studies will be collected to calculate the standardized mean difference (SMD) and their 95% confidence interval (CI). Heterogeneity will be evaluated by Cochran’s Q test and inconsistency index value (I²). If there is no heterogeneity (I²<40%, p>0.05), we will use a fixed-effects model for the meta-analysis; otherwise, the random-effects model will be chosen. All statistical analyses will be conducted using STATA version 15.0.

Subgroup analysis
To further understand the heterogeneity, we will perform subgroup analyses as follows:
1. The type of biomarkers (tumor cells, tumor DNA, and proteins).
2. Detection methods (Droplet Digital PCR, Next Generation Sequencing, ELISA).

Sensitivity analysis
To determine the stability of the study, we will perform a sensitivity analysis. Studies with a high risk of bias on overall effects will be excluded.

Assessment of reporting bias
Funnel plots, as well as associated regression tests, will be used to test publication bias.

Confidence in cumulative evidence
The Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) will be used to assess the strength of evidence.

DISCUSSION
We will evaluate the role of all the reported biomarkers in CSF in patients with PCNSL in this systematic review and meta-analysis. Additionally, we intend to clarify the optimal concentrations of certain biomarkers for diagnosis. By subgroup analyses, we will compare the different types of biomarkers and the different detection methods for the diagnosis of PCNSL. We hope the findings of this study will help us recognize the significance of liquid biopsy of CSF in
PCNSL patients, making the diagnosis of PCNSL easier, faster, and safer and improving the quality of life.

LIST OF ABBREVIATIONS
PCNSL: Primary central nervous system lymphoma
CSF: Cerebrospinal fluid
ABC: Activated B cell-like
NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B cells
BTK: Bruton tyrosine kinase
CT: computed tomography
MRI: Magnetic resonance imaging
PRISMA-P: Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols
QUADAS-2: Quality Assessment of Diagnostic Accuracy Studies
DNA: Deoxyribonucleic acid
PCR: Polymerase chain reaction
ELISA: Enzyme-linked immunosorbent assay

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
This study is a systematic review and meta-analysis, and the results are based on previously published evidence, and hence is exempted from the need for approval from the ethics committee.

FINANCIAL SUPPORT AND SPONSORSHIP
This work is supported by the Science and Technology Project of Sichuan to Hai Yi (No. 2018JY0583), Scientific Research Project of Sichuan Health Commission to Hai Yi (No. 18PJ358), and Youth Innovation Project of Sichuan Medical Research to Hai Yi (No. Q17004). The sponsors are not involved in design, data collection, data analysis and interpretation, or writing the manuscript.

CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES

DISCLAIMER
All claims made in this article are exclusively those of the writers, and do not necessarily reflect the views of their connected organizations, the publisher, editors, or reviewers. The publication does not guarantee or promote any product that may be evaluated in this article or any claim made by its producer.
## Additional file 1. PRISMA-P 2015 Checklist.

<table>
<thead>
<tr>
<th>Section and topic</th>
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<th>Checklist item</th>
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<td>Identification 1a</td>
<td>Identify the report as a protocol of a systematic review</td>
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<td>Update 1b</td>
<td>If the protocol is for an update of a previous systematic review, identify as such</td>
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<td>Registration 2</td>
<td>If registered, provide the name of the registry (such as PROSPERO) and registration number</td>
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<td>Authors:</td>
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<td>Contact 3a</td>
<td>Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author</td>
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<td>Contributions 3b</td>
<td>Describe contributions of protocol authors and identify the guarantor of the review</td>
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<td>Amendments 4</td>
<td>If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments</td>
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<td>Support:</td>
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<td>Indicate sources of financial or other support for the review</td>
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<td>Role of sponsor/funder 5c</td>
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<td><strong>INTRODUCTION</strong></td>
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<td>Rationale 6</td>
<td>Describe the rationale for the review in the context of what is already known</td>
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<td>Objectives 7</td>
<td>Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)</td>
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<td><strong>METHODS</strong></td>
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<td>Eligibility criteria 8</td>
<td>Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review</td>
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<td>Information sources</td>
<td>9 Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage</td>
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<td>Search strategy</td>
<td>10 Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated</td>
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<td>Study records:</td>
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<td>Data management</td>
<td>11a Describe the mechanism(s) that will be used to manage records and data throughout the review</td>
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<td>Selection process</td>
<td>11b State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)</td>
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<td>Data collection process</td>
<td>11c Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators</td>
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<td>Data items</td>
<td>12 List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications</td>
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<td>Outcomes and prioritization</td>
<td>13 List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale</td>
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<td>Risk of bias in individual studies</td>
<td>14 Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis</td>
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<td>Data synthesis</td>
<td>15a Describe criteria under which study data will be quantitatively synthesised</td>
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<td>15b If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I², Kendall’s τ)</td>
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<td>15c Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)</td>
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<td>15d If quantitative synthesis is not appropriate, describe the type of summary planned</td>
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<td>Meta-bias(es)</td>
<td>16 Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)</td>
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<td>Confidence in cumulative evidence</td>
<td>17 Describe how the strength of the body of evidence will be assessed (such as GRADE)</td>
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Additional file 2. Search strategy applied in MEDLINE database

**MiR-21, miR-19, miR-30c, miR-92a**

Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND (MiR-21 OR miR-19 OR miR-30c OR miR-92a)

**TACI**

Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("transmembrane activator and calcium modulator and cyclophilin ligand interactor" OR TACI)

**BCMA**

Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("B cell maturation antigen" OR BCMA)

**sIL-2R**

Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("soluble IL-2 receptor" OR sIL-2R)

**IL-6, IL-10**

Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("interleukin-6" OR IL-6 OR "interleukin-10" OR IL-10)

**β2-MG**

Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("β2-microglobulin" OR β2-MG)

**CXCL13**

Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("the C-X-C motif chemokine ligand 13" OR CXCL13)

**Tim-1**

Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("T cell immunoglobulin and mucin domain 1" OR Tim-1)
Npt
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("neopterin" OR Npt)

APRIL
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("a proliferation inducing ligand" OR APRIL)

BAFF
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("B cell activating factor" OR BAFF)

CD79B
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("cluster of differentiation 79B" OR CD79B)

MYD88
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("myeloid differentiation factor 88" OR MYD88)

RNU2-If
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("circulating U2 small nuclear RNA fragments" OR RNU2-1f)

Soluble CD27
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("soluble cluster of differentiation 27" OR "soluble CD27")

OPN
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("osteopontin" OR OPN)

AT III
Primary central nervous system lymphoma OR PCNSL AND ("biological"[All Fields] AND "markers"[All Fields]) OR "biomarker"[All Fields]) OR CSF[All Fields] OR cerebrospinal[All Fields]) AND ("Antithrombin III" OR AT III)