A Systematic Approach to the Diagnosis of Suspected Central Nervous System Lymphoma

Brian J. Scott, MD; Vanja C. Douglas, MD; Tarik Tihan, MD; James L. Rubenstein, MD, PhD; S. Andrew Josephson, MD

Central nervous system (CNS) lymphoma can present a diagnostic challenge. Currently, there is no consensus regarding what presurgical evaluation is warranted or how to proceed when lesions are not surgically accessible. We conducted a review of the literature on CNS lymphoma diagnosis (1966 to October 2011) to determine whether a common diagnostic algorithm can be generated. We extracted data regarding the usefulness of brain and body imaging, serum and cerebrospinal fluid (CSF) studies, ophthalmologic examination, and tissue biopsy in the diagnosis of CNS lymphoma. Contrast enhancement on imaging is highly sensitive at the time of diagnosis: 98.9% in immunocompetent lymphoma and 96.1% in human immunodeficiency virus–related CNS lymphoma. The sensitivity of CSF cytology is low (2%-32%) but increases when combined with flow cytometry. Cerebrospinal fluid lactate dehydrogenase isozyme 5, β2-microglobulin, and immunoglobulin heavy chain rearrangement studies have improved sensitivity over CSF cytology (58%-85%) but have only moderate specificity (85%). New techniques of proteomics and microRNA analysis have more than 95% specificity in the diagnosis of CNS lymphoma. Positive CSF cytology, vitreous biopsy, or brain/leptomeningeal biopsy remain the current standard for diagnosis. A combined stepwise systematic approach outlined here may facilitate an expeditious, comprehensive presurgical evaluation for cases of suspected CNS lymphoma.

Variability in testing to diagnose CNS lymphoma may result in a prolonged time to diagnosis, incomplete pre-surgical evaluation, or treatment with corticosteroids before diagnosis. A thorough understanding of the available imaging, laboratory tests, and biopsy targets can be used to guide the evaluation and avoid unnecessary testing or delays in diagnosis. We reviewed the literature on all these diagnostic tools to propose a uniform approach to this difficult neurologic problem.

RESULTS

IMAGING

Immunocompetent Patients

Most CNS lymphomas demonstrate abnormalities on brain imaging. Uniformly enhancing mass lesions in contact with the subarachnoid space and without necrosis are characteristic. In a pooled analysis of 6 studies of immunocompetent patients with primary CNS lymphoma (PCNSL), a total of 363 initial imaging studies were available. Of these, 358 were performed with contrast, and 354 of 358 patients (98.9%) had contrast-enhancing lesions. The lesions were solitary in 50% to 81% of the cases (Table 2).

Immunosuppressed Patients

The radiographic features and clinical differential diagnosis are different for suspected CNS lymphoma in the immunosuppressed population. Whether occurring in the presence of human immunodeficiency virus (HIV) infection (AIDS-related lymphoma) or after organ transplant (CNS posttransplant lymphoproliferative disorder), imaging is more likely to show multifocal abnormalities and a peripheral rather than homogeneous enhancement pattern. In a combined analysis of 4 studies (126 patients with AIDS-related lymphoma), 122 patients (96.8%) had contrast enhancement on brain imaging. Unlike CNS lymphoma in immunocompetent patients, most cases (56%-71%) had multiple lesions (Table 2). Peripheral enhancement may make it difficult to radiographically distinguish CNS lymphoma in AIDS from cerebral toxoplasmosis or progressive multifocal leuko-
encephalopathy. Specialized imaging may help to discriminate between these entities. For example, increased uptake on thallium 201 single-photon emission computed tomography (CT) has been used to differentiate lymphoma from infectious brain lesions.

**Leptomeningeal Lymphoma**

Definitive diagnosis of leptomeningeal lymphoma is made by a positive cerebrospinal fluid (CSF) cytology; however, it may also be detected on imaging. Radiographic features on brain imaging suggestive of leptomeningeal lymphoma include abnormal enhancement of the leptomeninges, cranial nerves, or periventricular region. Leptomeningeal enhancement may be focal or diffuse, and contrast-enhanced magnetic resonance imaging (MRI) is more sensitive to detect leptomeningeal disease compared with CT (71% vs 36%). In a series of 96 patients with PCNSL, 12 had MRI findings suggestive of leptomeningeal involvement at diagnosis, but only 7 of these patients (58.3%) had a positive CSF cytology.

**Corticosteroids and Imaging**

Corticosteroids may reduce or eliminate abnormal contrast enhancement, which may complicate the interpretation of enhanced MRI. Marked reduction in enhancement or clinical improvement following corticosteroid therapy has been suggested to be highly suspicious for CNS lymphoma. However, corticosteroid responsiveness must be interpreted cautiously, since it can also be seen in conditions such as demyelinating disease or, more rarely, in metastatic carcinoma or glioma.

**LABORATORY EVALUATION**

**Serologic Studies**

There are no serologic studies that are diagnostic for CNS lymphoma. Early determination of HIV status in cases of suspected lymphoma as well as CD4 count nadir is important, since the presence of HIV infection increases the likelihood of CNS lymphoma and infection as causes for progressive neurologic symptoms.

**Serum Lactate Dehydrogenase**

Lactate dehydrogenase (LDH) is a marker of rapid cell turnover. Its elevation in systemic lymphoma is an independent predictor of poor prognosis in non-Hodgkin lymphoma, follicular lymphoma, and mantle cell lymphoma. Elevated serum LDH with no established lymphoma diagnosis, however, is highly nonspecific and may be seen in many other medical conditions (liver failure, tissue ischemia, hemolysis, or infection), limiting its usefulness in the diagnosis of CNS lymphoma.

**CSF STUDIES**

**Routine Indices (Cell Count, Protein, Glucose)**

At least one of the routine CSF indices is abnormal in more than 80% of CNS lymphomas at the time of diagnosis. Series that have looked at CSF in a variety of lymphomas with CNS involvement showed that cell counts are normal in 33% to 60% of patients and CSF protein is normal in 33% to 55% of patients. One study found of 42 patients with non-Hodgkin lymphoma found that elevated cell count (>10 cells/μL) was correlated with positive cytology results. However, other investigations have found that CSF cell count or protein abnormalities do not reliably predict CSF involvement.

Central nervous system lymphoma is among the diseases that can result in a low CSF glucose level. Among 120 CSF samples from 38 patients, the CSF glucose level was less than 50 mg/dL in 54% and less than 15 mg/dL in 19% of the patients; malignant cells were seen on examination in 24% of the patients. Another series found a median CSF glucose level of 73 mg/dL and a low glucose level in 10% of 68 immunocompetent individuals with PCNSL undergoing lumbar puncture. Among 63 patients with leukemia and lymphoma (15 of whom had proven CNS involvement), low glucose level had a sensitivity of 27%.

**Immunoglobulin Index/Oligoclonal Bands**

Immunoglobulin index and oligoclonal bands (OCBs) are measures of intrathecal immunoglobulin synthesis and are useful tests in the diagnosis of inflammatory neurologic disorders. Testing for elevated CSF immunoglobulin index and OCBs is a routine part of the evaluation for multiple sclerosis. When added to the determination of cell count and protein, OCB testing contributes to the sensitivity of CSF analysis in diagnosing paraneoplastic syndromes.

Few studies have looked at immunoglobulin index and OCBs in patients with suspected CNS lymphoma. In PCNSL, the IgG index was elevated (>0.7) in 1 of 5 cases (20%) and the IgM index was elevated (>0.08) in 1 of 4 cases (25%). In systemic non-Hodgkin lymphoma with CNS involvement, 2 of 7 cases (29%) had an elevated IgG index and IgM index was elevated in 3 of 7 cases (43%). Between 2 and 10 OCBs were found in 8 of 14 patients (57%) with non-Hodgkin lymphoma, and in 4 of 6 patients (67%) with chronic lymphocytic leukemia. The production of OCBs was isolated to the CSF in 2 of 12 patients (17%). Testing for immunoglobulin index and OCBs in CSF therefore adds little to the diagnostic evaluation of CNS lymphoma.

**CSF Cytology**

Cerebrospinal fluid cytology can provide definitive diagnostic information in CNS lymphoma (Figure 1), and, with the aid of immunohistochemical studies, it has been possible to identify atypical lymphoid cells as monoclonal or neoplastic. Sharp nuclear notches, irregular cytoplasm, and increased cell size (>2.5 times the upper limit of normal) are suggestive of lymphoma. There is some overlap in the morphologic features of neoplastic and inflammatory lymphocytes, and this can make the interpretation of cytology difficult.

The sensitivity of CSF cytology varies widely (2%-32%), potentially reflecting differences in the populations studied and in the volume, handling, and interpre-
Flow cytometry can be performed on blood, bone marrow, vitreous, or CSF specimens. Cerebrospinal fluid flow cytometry is a useful adjunct to CSF cytology and has been shown to increase the ability to detect CNS involvement in high-risk individuals.11,37 Cytology examines morphologic features, and flow cytometry has the potential to provide information about the immunophenotype of the lymphocytes in a sample. When samples for cytology and flow cytometry were obtained and interpreted independently, up to 80% of lymphoma cases with CSF involvement were detected on the first CSF sample.11 If CSF flow cytometry is suspicious for lymphoma, a matching abnormal immunophenotype in a blood or bone marrow sample supports a concurrent systemic lymphoma diagnosis. Rarely, a false-positive CSF result may occur if a CSF sample is contaminated by peripheral blood lymphocytes in an individual with active systemic lymphoma.

Flow Cytometry

Flow cytometry provides information about cell surface protein expression,37 and immunoglobulin heavy chain (IgH) rearrangement testing analyzes the clonality of the antibodies being produced. Using PCR analysis of CSF or vitreous fluid, IgH rearrangement studies amplify the CDR-3 region of the IgH. In cases of a neoplastic proliferation of lymphocytes, a unique variable/diversity/joining arrangement is produced, resulting in a single sharp band on agarose gel. In contrast, nonneoplastic proliferation of lymphocytes as seen in inflammatory processes produces a wide band reflecting multiple different heavy chain sequences. The technique was initially reported by Yamada et al55 when evaluating lymph nodes and was later applied to CSF analysis.56 In a retrospective comparison with conventional cytology, IgH rearrangement analysis detected monoclonality in 2 of 4 specimens (50%) with positive cytology and an additional 10 of 20 cases (50%) that

MOLecular DIAGnostic TESTING—CURRENT TECHNOLOGIES

β2-Microglobulin

β2-Microglobulin is an HLA antigen–associated cell surface protein for which elevation reflects increased cell turnover. Although serum levels are not able to distinguish systemic vs CNS involvement, CSF β2-microglobulin has been correlated with the presence of CNS disease in leukemia and lymphoma in small numbers of patients,49 as well as response to therapy.57 Levels of CSF β2-microglobulin were elevated in 15 of 22 patients (68%) with leukemia or lymphoma with CSF involvement.43 Seven of 28 patients (25%) without CNS involvement also had an elevated CSF β2-microglobulin level, potentially because the sensitivity of CSF β2-microglobulin exceeded the study criterion standard (CSF cytology and clinical examination).43 The CSF β2-microglobulin level may be elevated in other neurologic disorders, including bacterial meningitis, reducing its specificity.

LDH Isozyme 5

In the CNS, aerobic isozymes of LDH dominate (LD1 and LD2), reflecting the dependence of the brain on aerobic metabolism. However, disease states, such as malignant brain tumors, result in an increased fraction of anaerobic LDH concentrations (LD3 and LD4) in CSF.48 Studies on CSF from 93 patients with hematologic cancer found that CSF LD1 proportions greater than or equal to 2.8% of total LDH were 93% sensitive for CNS involvement, with a negative predictive value of 98%.49 Unfortunately, the specificity of CSF LD1 isozyme elevation is limited, since elevations may also be seen in conditions such as bacterial meningitis or glioma.48

Epstein-Barr Virus

Epstein-Barr virus (EBV) activity has been associated with the development of CNS lymphoma in both HIV and post-transplant lymphoproliferative disorder. Epstein-Barr virus polymerase chain reaction (PCR) on CSF has been proposed as a useful diagnostic tool to identify cases of active EBV infection and to distinguish lymphoma from infectious encephalitides. Studies50-52 that used autopsy confirmation as a criterion standard found the sensitivity of EBV PCR to range from 80% to 100%, with a specificity of 93% to 100%. In posttransplant lymphoproliferative disorder, 93% of biopsy-confirmed cases also had positive CSF EBV PCR results.53 In routine clinical use, however, the positive predictive value of CSF EBV PCR may be considerably lower when attempting to discriminate lymphoma from CNS toxoplasmosis.54

Immunoglobulin Heavy Chain Rearrangement

Flow cytometry provides information about cell surface protein expression,37 and immunoglobulin heavy chain (IgH) rearrangement testing analyzes the clonality of the antibodies being produced. Using PCR analysis of CSF or vitreous fluid, IgH rearrangement studies amplify the CDR-3 region of the IgH. In cases of a neoplastic proliferation of lymphocytes, a unique variable/diversity/joining arrangement is produced, resulting in a single sharp band on agarose gel. In contrast, nonneoplastic proliferation of lymphocytes as seen in inflammatory processes produces a wide band reflecting multiple different heavy chain sequences.

The technique was initially reported by Yamada et al55 when evaluating lymph nodes and was later applied to CSF analysis.56 In a retrospective comparison with conventional cytology, IgH rearrangement analysis detected monoclonality in 2 of 4 specimens (50%) with positive cytology and an additional 10 of 20 cases (50%) that
were either suspicious or negative by conventional cytology. Other investigations reported that IgH rearrangement studies have a sensitivity of 58% and a specificity of 85% in the detection of monoclonal antibody production. Pretreatment with corticosteroids reduces the sensitivity of IgH rearrangement studies.

MOLECULAR DIAGNOSTIC TESTING—FUTURE DIRECTIONS

Proteomics

Proteomic analysis of CSF has revealed numerous proteins that are differentially expressed in CNS lymphoma. Among these, antithrombin III, a serine protease inhibitor that is associated with neovascularization in CNS lymphoma, has been prospectively validated. Elevated levels of antithrombin III were found in the CSF of patients with CNS lymphoma compared with the CSF of patients with other cancers and those with inflammatory neurologic conditions (Figure 2). Elevated antithrombin III levels correlated with a shorter overall survival and less response to chemotherapy. Antithrombin III levels higher than 1.2 μg/mL made possible the detection of CNS lymphoma with 75% sensitivity and 98.7% specificity.

CSF MicroRNAs

MicroRNAs are short, nontranslated fragments of RNA that bind to 3’ untranslated regions of messenger RNA and repress protein translation in several molecular pathways. MicroRNAs may function as oncogenes or tumor suppressor genes, and abnormal expression of microRNAs has been associated with several different cancers, including lymphoma. Recently, 3 microRNAs (miR-21, miR-19b, and miR92a) were shown to be differentially expressed in the CSF of 23 patients with PCNSL compared with control patients with nonneoplastic inflammatory neurologic disorders. A classification tree was devised on the basis of these 3 microRNAs that had a 95.7% sensitivity to detect CNS lymphoma (only 13% of the patients had CSF involvement of lymphoma by cytology or flow cytometry results) and a 96.7% specificity differentiating it from other nonneoplastic CNS diseases (multiple sclerosis, stroke, and headache).

TISSUE

Vitreous Biopsy

It is possible to diagnose CNS lymphoma without brain biopsy in patients with intraocular involvement. Ocular involvement may occur at any time in the course of PCNSL and has been found in 15% to 25% of cases. When looking for intraocular lymphoma, the initial evaluation should include a dilated ophthalmologic examination with slit-lamp. The presence of cells in the vitreous on slitlamp examination is consistent with intraocular lymphoma, but not specific, and is sometimes mistaken for a nonspecific inflammatory reaction. As with CNS lymphoma, successfully making the diagnosis of intraocular lymphoma may be challenging, and several decision algorithms have been proposed. Ultimately, the diagnosis is most commonly made using vitreal biopsy, although chorioretinal biopsy or fine-needle aspiration of subretinal lesions (when present) may also reveal lymphoma cells, which is diagnostic. Fluorescein angiography helps to distinguish intraocular lymphoma from other inflammatory conditions.

Brain Biopsy

Brain tissue with infiltration of malignant lymphocytes at biopsy or autopsy is the diagnostic criterion standard for CNS lymphoma. Most of the time, adequate tissue for diagnosis is obtained by stereotactic biopsy, and open biopsy procedures are rarely necessary. The main risk of stereotactic biopsy is intracranial hemorrhage or postoperative neurologic deficit, which occurs in up to 8% of cases. The risk of the procedure is higher if the lesion involves deep brain structures or requires multiple passes to obtain tissue. Resection is not indicated for CNS lymphoma and has been associated with worse outcomes.

SYSTEMIC LYMPHOMA

Most lymphomas presenting with neurologic symptoms are PCNSLs. Most commonly, CNS involvement of systemic lymphoma occurs in advanced disease. However, occult systemic involvement may be present at the time of CNS lymphoma diagnosis.

Body Imaging

Two retrospective studies (144 patients and 1 prospective study (51 patients) using chest, abdomen, and pelvis CT and bone marrow biopsy for staging
found occult systemic lymphoma in 4% to 13% of CNS lymphoma cases at presentation. Compared with contrast-enhanced CT, fludeoxyglucose F 18 positron emission tomography (FDG-PET) has improved sensitivity in the detection of systemic Hodgkin and non-Hodgkin lymphoma (85% vs 98%, respectively). A study of FDG-PET at initial staging for CNS lymphoma found occult systemic lymphoma in 3 of 42 patients (7%) and other abnormalities in 5 of 42 patients (12%). When there is abnormal uptake on PET scanning, CT adds spatial resolution to better target the area of interest for biopsy.

**Testicular Ultrasound**

Testicular lymphoma comprises 1% to 2% of all non-Hodgkin lymphoma, with most cases occurring in men older than 60 years.
involvement occurs in approximately 15% of testicular lymphomas, with CNS involvement at the time of diagnosis occurring in 3% of cases. Body CT and PET scanning are limited in their ability to image the testis; therefore, a bedside testicular examination should be done and testicular ultrasonography should be considered in men with suspected CNS lymphoma. Testicular biopsy is a potential route to a tissue diagnosis in suspected lymphoma with CNS involvement and is especially useful in cases of brain lesions that are not amenable to biopsy. A testicular biopsy should be pursued only in the setting of an abnormal mass on testicular examination that is confirmed by ultrasonography to be soft tissue and potentially consistent with lymphoma.

Serum and Urine Protein Electrophoresis

Serum protein electrophoresis (SPEP) is a useful screening tool in the diagnosis of the monoclonal gammopathies. Urine protein electrophoresis and immunofixation electrophoresis help refine the diagnosis when the SPEP result is abnormal. Although diffuse large B-cell lymphoma is the most common histologic feature in PCNSL, conditions such as multiple myeloma or Waldenström macroglobulinemia occasionally may produce early CNS manifestations associated with an abnormal SPEP finding.

Tissue Biopsy

Abnormally increased metabolic activity on PET may support an alternative diagnosis or suggest systemic lymphoma. Therefore, a biopsy of the PET avid tissue may add diagnostic information.

Table 3. Usefulness and Levels of Evidence for Diagnostic Tests in Suspected CNS Lymphoma

<table>
<thead>
<tr>
<th>Diagnostic Value</th>
<th>Test Result</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consistent</td>
<td>Mild CSF pleocytosis</td>
<td>Low (44%)</td>
<td>Low</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Elevated CSF protein</td>
<td>Low (70%)</td>
<td>Low</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Low CSF glucose</td>
<td>Low (27%)</td>
<td>Low</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Increased serum LDH</td>
<td>Low</td>
<td>Low</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Increased serum β2-microglobulin</td>
<td>Low</td>
<td>Low</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Clinical/radiographic improvement with corticosteroids</td>
<td>Low</td>
<td>Low</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Contrast enhancement on CT/MRI</td>
<td>High (99%)</td>
<td>Low</td>
<td>3a</td>
</tr>
<tr>
<td>Suspicious</td>
<td>Atypical/suspicious CSF cytology</td>
<td>Unknown</td>
<td>Low</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Atypical/suspicious CSF flow cytometry</td>
<td>Unknown</td>
<td>Low</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Increased CSF LD₅₀</td>
<td>Moderate (93%)</td>
<td>Low</td>
<td>2b</td>
</tr>
<tr>
<td></td>
<td>Increased CSF β₂-microglobulin</td>
<td>Low (68%)</td>
<td>Low (75%)</td>
<td>4b</td>
</tr>
<tr>
<td>Highly suspicious</td>
<td>Positive CSF flow cytometry</td>
<td>Low (73%)</td>
<td>Moderate</td>
<td>3b</td>
</tr>
<tr>
<td></td>
<td>Clonal IgH rearrangement</td>
<td>Low (58%)</td>
<td>Moderate (85%)</td>
<td>3b</td>
</tr>
<tr>
<td></td>
<td>Proteomics: elevated CSF antithrombin III</td>
<td>Low (75%)</td>
<td>High (98%)</td>
<td>2b</td>
</tr>
<tr>
<td></td>
<td>MicroRNA analysis</td>
<td>High (96%)</td>
<td>High (97%)</td>
<td>2b</td>
</tr>
<tr>
<td>Diagnostic</td>
<td>Positive CSF cytology</td>
<td>Low (32%)</td>
<td>High (100%)</td>
<td>1c</td>
</tr>
<tr>
<td></td>
<td>Positive retinal/vitreous biopsy</td>
<td>Moderate</td>
<td>High (100%)</td>
<td>1c</td>
</tr>
<tr>
<td></td>
<td>Positive brain biopsy</td>
<td>Moderate-high</td>
<td>High (100%)</td>
<td>1c</td>
</tr>
</tbody>
</table>

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; IgH, immunoglobulin heavy chain; LD₅₀, lactate dehydrogenase (LDH) component 5; MRI, magnetic resonance imaging.

Bone Marrow Biopsy

The usefulness of bone marrow evaluation in suspected CNS lymphoma is not known. On the basis of 2 reviews, at the time of CNS lymphoma diagnosis (128 and 16 patients), the likelihood of a positive marrow result is low, at 2 of 144 cases (1.4%). With the rarity of bone marrow involvement at the time of CNS lymphoma diagnosis, bone marrow biopsy should mainly be considered in the absence of accessible surgical targets systemically or in the CNS.

In clinical practice, it is difficult to move from a clinical suspicion of CNS lymphoma to a definitive diagnosis when lesions are not amenable to biopsy. Imaging and routine CSF studies are inherently nonspecific, and CSF cytology and flow cytometry have limited sensitivity (Figure 3). Improvements in flow cytometry and the refinement of proteomics and microRNA techniques for the diagnosis of lymphoma hold promise to improve presurgical diagnosis but are not yet widely available.

In the absence of an adequate single diagnostic test, a combination of the available screening and diagnostic tests may be used to establish the diagnosis quickly and at the lowest risk for the patient. Figure 4 outlines a suggested diagnostic algorithm for CNS lymphoma based on the present review. Each test provides some diagnostic value in CNS lymphoma based on available evidence (Table 3). This approach allows for efficient, comprehensive preoperative testing and provides a way to increase the chances of making a diagnosis prior to a brain biopsy.
Ultimately, many cases of suspected CNS lymphoma are diagnosed as other neurologic conditions. This decision tool does not account for tests that may be ordered to exclude other diagnoses, and these should be performed concurrently as appropriate. This diagnostic algorithm requires prospective validation, but it is a step toward a more consistent approach to diagnosis in this complex disease entity.

Accepted for Publication: July 3, 2012.

Published Online: January 14, 2013. doi:10.1001/jamaneurol.2013.606

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Conflict of Interest Disclosures: None reported.

REFERENCES


