Published in final edited form as:

Leuk Lymphoma. 2008 ; 49(0 1): 43–51. doi:10.1080/10428190802311441.

Primary lymphoma of the central nervous system: epidemiology, pathology and current approaches to diagnosis, prognosis and treatment

JAMES RUBENSTEIN¹, ANDRÉS J. M. FERRERI², and STEFANIA PITTALUGA³

¹University of California, San Francisco, CA, USA

²Medical Oncology Unit, San Raffaele H Scientific Institute, Milan, Italy

³National Institutes of Health, Bethesda, MD, USA

Abstract

An overview of the current approaches to the management of patients with primary central nervous system lymphoma (PCNSL) is provided. Although accumulating evidence demonstrates that PCNSL is a curable type of brain tumor, in many cases establishing the diagnosis and overcoming chemotherapeutic resistance remain significant obstacles. The issue of treatment-related neurotoxicity is also a central consideration in treatment planning. The introduction of highly active antiretroviral therapy has had a major impact on this disease in that the incidence of AIDS-related central nervous system lymphoma, once highly prevalent in the 1980s and 1990s, has now virtually disappeared. However, the problem of diagnostic delays secondary to steroid effects, radiation-induced neurotoxicity and methotrexate resistance represent unique and important problems in this disease. The use of anti-CD20 antibody in this disease represents the first application of biologically based targeted therapies for PCNSL; however, the overall impact of this modality in brain lymphoma awaits further evaluation in ongoing studies. The application of proteomic as well as gene expression technologies is yielding insights into PCNSL pathogenesis, in particular specific oncogenic pathways, which may be exploited to develop new therapies.

Keywords

Primary CNS lymphoma; PCNSL; epidemiology; biomarkers

Introduction

Primary central nervous system lymphoma (PCNSL) poses a challenge to physicians and researchers in view of its increasing incidence, unique clinical behaviour and poorly understood pathobiology. The tumor arises within an anatomical site, with structural, biological and immunological characteristics that dictate therapeutic choice and outcome.

Although it has one of the worst prognoses among subtypes of non-Hodgkin lymphoma (NHL), a significant fraction of patients can expect long-term survival and cure.

PCNSL is rare, accounting for only 2–3% of NHL cases. More than 90% of PCNSLs are diffuse large B-cell lymphomas (DLBCL). Immunodeficiency is a known risk factor for PCNSL, which occurred with high frequency in AIDS patients before the era of highly active antiretroviral therapy (HAART). This article, however, will focus on PCNSL in immunocompetent patients, who are usually always Epstein-Barr virus (EBV)-negative.

High-dose methotrexate (MTX) is the cornerstone of treatment for PCNSL due to its excellent activity and CNS penetration; but it is not without toxicity and the agent is rarely curative as monotherapy, thus highlighting the critical need for better treatment. Whereas rituximab is likely to improve outcome, its relative contribution has yet to be determined. Significant advances in treatment for PCNSL likely await the unravelling of its pathobiology. Indeed, molecular and histopathologic subgroups are beginning to be defined in PCNSL. Diagnostic and prognostic protein biomarkers in the cerebrospinal fluid (CSF) are being identified that may facilitate early and non-invasive diagnosis. Such biomarkers may also be useful in the monitoring of disease progression and in the identification of patients whose disease is likely to be refractory to current therapy.

Epidemiology of primary central nervous system lymphoma

The incidence of PCNSL between 1990 and 1994 was approximately 0.43 per 100,000 person-years [1], and the disease accounts for approximately 4% of primary brain tumors [2]. PCNSL is significantly more prevalent among males, and the median age at diagnosis is ~60 years; there are no known racial differences in incidence. Studies from several countries suggest that the incidence is increasing [3,4]. This change is not related to the increased use of organ transplantation or to technological advances in diagnosis [5]. Whereas at one time the spread of AIDS contributed greatly to increasing incidence of PCNSL, studies have also confirmed an increasing incidence in non-AIDS patients, across age groups and sexes [5,6]. Risk factors for PCNSL, other than immunodeficiency, have not been well characterised. In the immunodeficient AIDS/HIV population, the incidence has been falling since the advent of HAART [7]. The known risk factors in patients with HIV are a low CD4 count (<100,000) and a high viral load.

There are important differences in PCNSL between immunocompetent and immunodeficient individuals. In immunodeficient patients, PCNSL is associated with EBV, whereas a viral association with the pathogenesis in immunocompetent patients is not established. The median age at onset is younger in the immunodeficient population – ~30 years – and the disease in this subset of patients generally has a more aggressive course compared with that in immunocompetent patients.

HIV-associated central nervous system (CNS) lymphoma is the second most common mass lesion (after toxoplasmosis) in patients with AIDS. Before the era of HAART, CNS lymphomas affected up to 5% of these patients. A definite decline in the incidence of HIV-associated CNS lymphoma has occurred in the post-HAART era. In one retrospective analysis of HIV-infected patients, the incidence of PCNSL peaked at 5.33 per 1000 person-

years between 1991 and 1994 (pre-HAART) and then declined to 0.32 per 1000 person-years after 1999 (post-HAART) [8]. Moreover, the prognosis of HIV-associated CNS lymphoma has improved with the advent of HAART. In the pre-HAART era, median survival was poor, with death occurring within 3 months of diagnosis. In the post-HAART era, the 2-year survival rate approaches 30% [9].

An association has been suggested between PCNSL and infectious agents, with most studies focusing on viruses, but data are a little disjointed and preliminary. EBV has been detected in tumor cells in AIDS-related PCNSL but not in PCNSL in immunocompetent individuals [10]. DNA from human herpesvirus-8 (HHV-8), EBV and *Toxoplasma gondii* was detected in 19, 10 and 13% of intraocular lymphoma cells, respectively [11,12]. However, HHV-8 DNA was not detected in either AIDS-related or immunocompetent PCNSL [13,14]. More recently, polyomaviruses (SV40, BK, JC) were not detected in AIDS-related and immunocompetent PCNSL [15].

Clinical presentation and diagnosis of primary central nervous system lymphoma

In an international study involving 370 immunocompetent patients with PCNSL, diagnosed between 1980 and 1999, the median age at presentation was 61 years (range 14–85 years) [16]. Males accounted for 58% of cases, and two-thirds had poor performance status (European Cooperative Oncology Group 2–3 or 4); 4% had a prior cancer diagnosis. B symptoms were rare, occurring in only 2% of cases. In this study, lactate dehydrogenase (LDH) serum levels were elevated in 35% of patients. Clinical presentation consisted of motor and sensory focal deficits in 50% of patients, personality changes in 30%, headaches in 55%, nausea in 35%, vomiting in 10%, and uveitis in 20%. On neuroimaging, multiple lesions were observed in 34% of patients, with the frontal lobe the most common site. Deep brain lesions (i.e. infiltrating corpus callosum, basal ganglia, periventricular areas, brain stem or cerebellum) were seen in 40% of patients.

In some patients with an intracranial mass, corticosteroid treatment results in shrinkage or disappearance of the mass within a few days or weeks. A presumptive diagnosis of PCNSL is often made in these patients. However, other vascular, inflammatory, and neoplastic disorders can share a similar radiographic appearance as well as steroid-sensitivity. In one retrospective report of the clinical and radiological characteristics of 12 patients with cerebral masses that exhibited regression in response to corticosteroids, only half were found to have PCNSL [17]. The probability of a diagnosis of PCNSL appeared to be lower in those patients who had complete regression of the lesion.

Biopsy is mandatory for an accurate diagnosis and to facilitate definitive therapy in PCNSL. The initial diagnostic work-up involves stereotactic brain biopsy, because a gross total resection does not seem to enhance outcome and is associated with greater morbidity, given the highly infiltrative nature of these tumors. Brain biopsy has a diagnostic sensitivity of greater than 80% [16,18,19]. Total or partial resection should be reserved for patients in whom a condition other than PCNSL is suspected or in those with PCNSL in whom a decompressive approach could be followed by a fast improvement in performance status. In

some cases, diagnosis can be made from CSF cytology and/or flow cytometry, or by vitrectomy in the case of intraocular presentation [20]. In HIV-positive patients, a presumptive diagnosis of PCNSL can sometimes be made without biopsy in patients with increased thallium uptake on single photon emission computed tomography and in patients with positive CSF for EBV DNA [21]. However, this approach should be reserved for patients whose tumors are not easily amenable to biopsy.

Following diagnosis, patients should undergo staging, with an assessment of prognostic factors as well as fitness to tolerate chemotherapy [22]. Conventional lymphoma staging should be accompanied by a complete ophthalmologic evaluation and CSF examination. Renal and hepatic function testing and the exclusion of hepatitis are mandatory. Five potentially important prognostic factors have been identified: age, performance status, serum LDH level, CSF protein concentration, and the involvement of deep regions of the brain [23]. These risk factors can be used to derive the International Extranodal Lymphoma Study Group (IELSG) prognostic score, which categorises patients into three risk groups [24].

Intraocular presentation is seen in 5–20% of cases of PCNSL. Patients with this presentation should undergo ophthalmologic examination, fluorescein angiography, and colour photography. Intraocular presentation precedes brain lesions in 95% of patients; both eyes are involved in 80% of cases [25,26]. Diagnosis can be difficult; patients generally complain of floaters and blurred vision, which are caused by uveitis. Molecular analyses may complement the use of cytopathology.

All patients with PCNSL should undergo a lumbar puncture for CSF cytology and flow cytometry, unless medically contraindicated. CSF analysis should be carried out using 3–10 mL samples collected by lumbar puncture. CSF studies should include cytology examination, cell count, biochemical profile, immunocytochemistry, β_2 -microglobulin levels, immunoglobulin heavy chain (*IgH*) gene rearrangement, and flow cytometry. Positive cytology is detected in approximately 15% of cases, although this may be an underestimate [25]. In patients with negative cytology/flow cytometry, CSF alterations are nonspecific, but may be useful for diagnostic orientation. For example, increased protein concentration is present in more than 60% of patients with PCNSL whereas glucose concentrations are usually unaltered [25]. Tumor markers such as LDH isoenzymes, β -glucuronidase, and β_2 -microglobulin may provide indirect evidence of leptomeningeal lymphoma. Proteomic studies are yielding potential CSF biomarkers which may also have diagnostic utility (see below).

Pathology of primary central nervous system lymphoma in immunocompetent patients

The great majority of PCNSLs are high grade [27], with DLBCL the most common histological subtype. Low-grade PCNSL is rare and is associated with atypical or mild symptoms, which can often result in delayed diagnosis. Low-grade disease is typically located in the spine. In most cases, at least two of the following are present at magnetic resonance imaging: hyperintensity of T2-weighted images, moderate or absent gadolinium contrast enhancement, and heterogeneous contrast enhancement [27]. The low-grade

category with the highest incidence is immunocytoma, followed by marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type [28]. T-cell lymphomas arising in the CNS are rarer, but presentation and prognosis appear to be similar to those reported for B-cell PCNSL [29].

Diffuse large B-cell lymphoma

The median age at presentation for diffuse large B-cell PCNSL is approximately 60 years and, in immunocompetent patients, the male to female ratio is 1.2–1.7:1 [30]. Approximately 75% of PCNSL tumors are supratentorial masses, and 20–40% of patients present with multiple lesions; intraocular involvement occurs in 10–20% of patients. Diffuse large B-cell PCNSL is uniformly positive for CD20 and negative for CD138. In immunocompetent patients, the tumor is usually negative for EBV. Aberrant somatic hypermutation of multiple proto-oncogenes such as *Pim-1*, *RhoH/TTF* and *c-MYC* have been reported in PCNSL; the mean mutation frequency of these genes may be between 2- and 5-fold higher in PCNSL compared with extra-cerebral DLBCL [31].

Characterisation of the immunophenotype of PCNSL reveals that these tumors exhibit overlapping features of a germinal center and activated B-cell differentiation: immunohistochemical analysis has demonstrated that brain lymphomas typically express MUM-1, a marker of activated B-cells, and BCL-6, a marker of germinal centers [32,33]. This finding was also suggested by microarray-based gene expression analysis of PCNSL [34], and could provide an explanation for how a particular subtype of large B-cell lymphoma, with some characteristic features of germinal centre lymphoma (including the expression of BCL-6), is, in general, associated with an inferior outcome relative to systemic lymphomas of the same histology and stage [35].

Rare lymphoma subtypes

Intravascular large B-cell lymphoma is a rare subtype of extranodal DLBCL. It is usually widely disseminated at presentation, and can present in the CNS. Presentation in the CNS resembles ‘lacunar stroke syndrome’, with blood vessels blocked by tumor cells. In contrast to most other PCNSL types, B symptoms are common, as are hepatosplenomegaly and pancytopenia. The disease has an aggressive clinical course, and the prognosis is very poor.

Another rare subtype is marginal zone B-cell lymphoma of the dura. The median age at presentation is around 49 years, and there is a female predominance of 4:1 [36]. The prognosis for this subtype is highly favourable and clinical presentation may mimic that of meningioma. From the database of the National Cancer Institute’s Laboratory of Pathology, 17 patients were identified with marginal zone B-cell lymphoma of the dura. Of these, 12 showed a dura-based mass on radiological examination, three showed meningeal thickening, and one had a lytic lesion [author’s personal communication]. The presenting symptoms were localised neurological deficits (58%), seizures (33%) and syncope (25%). Three patients had another site of involvement outside the brain. As expected, clinical characteristics of these patients were very similar those reported by Tu et al. [36].

T-cell lymphomas account for about 2% of PCNSLs [29]. Median age at presentation is approximately 60 years, and over half of the tumors are supratentorial. The prognosis is similar to that for B-cell CNS lymphoma patients treated with high-dose systemic MTX [29]. Extremely rarely, human T-lymphotropic virus-1-associated adult T-cell lymphoma can be present in the CNS without systemic involvement [29].

Histiocytic sarcoma is an extremely rare presentation of PCNSL. Only five cases have been reported in the literature, with age at diagnosis ranging from 20 months to 69 years. The disease is extremely aggressive. Differential diagnoses include Langerhans cell histiocytosis and Rosai–Dorfman disease. Histiocytic sarcoma can easily be overlooked and misdiagnosed as an inflammatory process [37,38].

Anecdotal cases of primary CNS plasmacytoma and Hodgkin lymphoma have been reported. Radiotherapy alone has provided acceptable disease-free survival in both entities.

Treatment of primary central nervous system lymphoma

The prognosis for PCNSL is generally poor relative to localised large-cell lymphomas outside the CNS, but it is hoped that this will change with advances in the understanding of the biology of PCNSL and the optimisation of chemotherapy regimens. Treatment for PCNSL differs from that for non-CNS NHL; in particular, there is no role for anthracycline-based chemotherapy, probably owing to a combination of failure of these drugs to pass through the blood–brain barrier, and biological differences between PCNSL and other NHL subtypes.

Current knowledge of therapeutic strategies for PCNSL result from a small number of published prospective trials, meta-analyses and retrospective multicentre series. Notably, the paucity of prospective, randomised trials and the presence of selection bias or methodological pitfalls in some published trials mean that questions regarding the best therapeutic approach remain unanswered [2]. Further well-designed, randomised trials will help to determine the optimal treatment strategies for PCNSL.

There are several established treatment paradigms for PCNSL. High-dose MTX is the most effective drug [2], and high-dose MTX-based regimens can be used either alone or in combination with other drugs or with whole-brain irradiation. The response rate to intravenous high-dose MTX is 52–88% as a single agent and 70–94% when combined with other agents [2]. When followed by whole-brain irradiation, these two regimens result in overall survival rates of 58–72% and 43–73%, respectively [2]. The response rate to whole-brain irradiation is approximately 90%, but unacceptably high rates of neurotoxicity have been reported following this treatment (83% at 1 year in patients aged >60 years) [39]. Because of this high rate of toxicity, there has been increased interest in high-dose chemotherapy regimens that do not require additional whole-brain irradiation. In one important study of high-dose chemotherapy followed by autologous hematopoietic stem cell rescue in 22 patients with relapsed or refractory PCNSL, a regimen based on thiotepa, busulfan and cyclophosphamide produced a 3-year survival rate of 60%, with a 3-year event-free survival of 53% [40].

The largest published multicentre study of treatment of PCNSL is the Radiation Therapy Oncology Group 93–10 study, which involved 102 patients and used the Memorial Sloan-Kettering regimen of combined modality therapy [41]. Patients received five cycles of high-dose MTX (2.5 g/m^2), vincristine, procarbazine and intraventricular methotrexate (12 mg). Whole-brain radiotherapy to a total dose of 45 Gy was then administered. All patients received high-dose cytarabine (two doses of 3 g/m^2) after radiotherapy. Among patients with measurable disease, the complete remission (CR) rate to pre-irradiation chemotherapy was 58%, and 36% of patients had a partial remission, giving an overall response rate of 94%. Median progression-free survival was 24.0 months, and overall survival was 36.9 months. Median survival was 50.4 months in patients younger than 60 years, but only 21.8 months in those aged 60 years or older ($P < 0.001$), supporting the contention that age is an important prognostic factor. Severe delayed neurologic toxicity was evident in 12 patients, of whom eight died. Because of the accumulating evidence that severe delayed neurologic toxicity was associated with whole-brain irradiation in CNS lymphoma, a recent study initiated at Memorial Sloan-Kettering explored the use of reduced-dose whole-brain irradiation after methotrexate-based induction [42].

Conventional-dose combination therapy was associated with a plateau in the survival curve in a phase II study of the MATILDE regimen (MTX, idarubicin, cytarabine, thiopeta) followed by response-tailored radiotherapy. This proved to be an active strategy for patients with PCNSL, particularly for those with low- to intermediate-risk disease. Response rates were 76% after MATILDE and 83% after chemotherapy with or without radiation therapy. A plateau on the overall survival curve was obtained after a median follow-up of 49 months (5-year overall survival $41 \pm 7\%$) [43].

At the University of California, San Francisco, USA, a high-dose chemotherapy approach for newly diagnosed patients has been developed that consists of high-dose MTX (8 g/m^2) with temozolomide ($150 \text{ mg/m}^2/\text{day}$) plus rituximab (375 mg/m^2) (MTR). If patients attain a CR after eight cycles of MTR, they receive eight doses of high-dose cytarabine (2 g/m^2) and infusional etoposide over 96 h as consolidation, without brain irradiation. Data have been collected on a series of 24 immunocompetent patients treated using this regimen since 2001 [44]. The median age of the patients was 60 years (range 40–81 years) and median Karnovsky performance score was 60 (range 40–90). Progression-free survival at 24 months was 50%. Thus far, the MTR combination and cytarabine/etoposide consolidation has been well tolerated, with no treatment-related mortality and no reported evidence of neurotoxicity [44].

Use of rituximab in the treatment of relapsed disease

Several salvage therapy options are available for patients with failed PCNSL, including rituximab, which has shown potential activity in a number of treatment regimens. Rituximab does not appear to penetrate the intact blood–brain barrier in therapeutically meaningful concentrations [45]. This observation has led to interest in the direct intrathecal injection of this and other targeted therapies to treat brain tumors. A phase I dose-escalation study of intraventricular rituximab was carried out in 10 HIV-negative patients with refractory CNS lymphoma [46]. The goals were to define the maximum tolerated dose and the

pharmacokinetics of intraventricular monoclonal antibody administration in humans, to assess safety, and to provide insights into the molecular basis of rituximab action and resistance. The doses used were 10, 25 and 50 mg, given once in week 1 and twice weekly (days 1 and 4) thereafter. Treatment was given for over 5 weeks in total, to a maximum of nine injections. Five patients received all nine injections.

No significant toxicity was detected in the three patients treated at the 10 mg dose or the five patients treated at the 25 mg dose; responses were seen at both doses. However, dose-limiting toxicity (grade 3 hypertension) was seen in the two patients treated at 50 mg; hence, 25 mg was determined as the maximum tolerated dose. Response was assessed in nine patients, of whom four had a complete cytological response and two had a partial cytological response. Two patients had an improvement in intraocular NHL and one had resolution of parenchymal NHL. A subset of the responding patients who achieved a CR to intrathecal rituximab did so after failing to achieve a complete response to intravenous rituximab. Gene expression studies in meningeal lymphoma cells showed that high RNA levels of Pim-2 and FoxP1 were associated with primary rituximab-refractory disease: FoxP1 is an established adverse prognostic factor in large cell lymphoma [47,48].

It has been widely assumed that intrathecal therapy for brain tumors is unable to elicit a response in the brain parenchyma or in intraocular compartments because of poor access of macromolecules within the CSF to the brain. It was also thought that the absence of complement in CSF would result in rituximab inefficacy. However, these assumptions are now being challenged. Proteomic analyses (see below) have revealed that complement proteins are, in fact, present in the CSF in patients with CNS lymphomas, an observation confirmed using enzyme-linked immunosorbent assay (ELISA) and other techniques [49,50]. Indeed, complement may be produced by the lymphoma cells themselves [50]. In addition, intraparenchymal and intraocular responses have been seen following intraventricular administration of rituximab and MTX, and a rapid lymphocytotoxic effect has been observed with intraventricular rituximab in the treatment of leptomeningeal NHL. The latter may be due to apoptotic mechanisms, complement activation, and/or antibody-dependent cellular cytotoxicity. A safety and efficacy study is currently underway using intrathecal therapy with rituximab plus MTX; two patients have so far been treated without serious treatment-associated adverse events, and both achieved complete responses without significant toxicity.

Ultimately, all of the patients in the phase I study described previously developed resistance to therapy. The molecular and cellular mechanisms responsible for the development of acquired resistance are currently under investigation. Pharmacokinetic studies have shown that intraventricular rituximab has a half-life in the CSF of less than 6 h [46], significantly shorter than when rituximab is given systemically. Whereas some patients who received twice-weekly maintenance rituximab monotherapy for several weeks after achieving a CR showed evidence of a durable response without serious adverse events, ultimately it appears that most patients will develop resistance [46]. Whether the basis for this resistance is associated with pharmacokinetic parameters or other immunologic effects is currently under investigation.

Diagnostic and prognostic biomarkers in primary central nervous system lymphoma

Gene expression profiling

Fundamental insights into the biology of PCNSL are needed to facilitate the development of more successful treatment strategies, with reduced neurotoxicity. In particular, it is important to identify the molecular pathways that are associated with drug resistance. In 2002, a multicentre study was initiated to evaluate gene expression profiles in PCNSL using biopsy specimens. Results of this study, which compared PCNSL to nodal large B-cell lymphoma, provided evidence for unique molecular features of the disease: approximately 460 clones exhibited significant differential expression between PCNSL and nodal large B-cell lymphoma; approximately 100 of these genes were expressed at higher relative levels in PCNSL; the remainder were expressed at higher levels in systemic disease. CNS lymphomas in this series expressed higher levels of the transcriptional regulator X-box binding protein-1 (XBP-1), Pim-1 and c-myc. It was also noted that tumor cells and tumor endothelia in PCNSL express interleukin (IL)-4. High intratumoral expression of IL-4 RNA transcripts, as well as increased expression of activated Stat-6, were each associated with adverse prognosis [34].

In an independent subgroup analysis, gene expression was also used to search for individual protein biomarkers that might be prognostically significant. The objective was to identify gene expression markers associated with primary refractory disease, as defined by a survival time of less than 6 months. In this preliminary analysis, several such associations were found. For example, antithrombin-III (ATIII) was found to be ectopically expressed in these tumors and associated with short survival. Another potential marker was death-associated protein-1 (DAP-1), a downstream mediator of interferon-gamma, which was associated with favourable prognosis. The subset of patients whose lymphoma cells expressed DAP-1 retrospectively exhibited both improved progression-free and overall survival in patients treated with the UCSF regimen (high-dose MTX, temozolomide, rituximab) ($P = 0.035$). Although additional studies are needed, ATIII and DAP-1 may be novel prognostic markers in PCNSL, to be evaluated in future studies [44].

CSF biomarkers for diagnosis and monitoring

In patients whose brain lesions are not amenable to biopsy, the dilemma for physicians is whether to treat without biopsy or, as occurs in many cases, to watch the patient deteriorate while awaiting biopsy feasibility. There is a need to identify protein biomarkers in the CSF for PCNSL and other brain tumors. Such biomarkers would facilitate diagnosis and the detection of minimal residual disease, and would have the potential to act as prognostic indicators, both at diagnosis and at relapse, and to provide insight into the molecular pathogenesis of the disease.

A study was carried out to identify CSF markers using specimens from patients with DLBCL in the CNS; control CSF was obtained from patients with benign brain tumors and other non-malignant CNS conditions [49]. To evaluate the reproducibility of this approach, the analysis was then repeated 1 year later with samples from different patients. A total of 30

CSF specimens were subjected to proteomic profiling to identify candidate CSF biomarkers. Approximately one-third of the lymphoma patients in these initial studies had positive CSF cytology. CSF samples were analysed using two-dimensional liquid chromatography followed by mass spectrometry, and approximately 80 differentially expressed CSF proteins were identified. There was a high degree of concordance between the two sets of results (Spearman correlation 0.71), indicating the highly reproducible nature of this technology when applied to differential protein identification and quantification in the CSF.

The majority of proteins that were upregulated in CNS lymphoma were serine proteases or protease inhibitors, complement mediators and inhibitors, extracellular matrix proteins and serum glycoproteins and lipoproteins. Most of the down-regulated proteins were associated with normal brain function, mostly neuropeptides or proteins involved in the regulation of neuronal signalling. Because one of these markers, ATIII, had been independently identified by gene expression studies described previously, its protein expression was defined in detail. CSF ATIII concentration, as determined by ELISA, was found to be markedly more accurate than CSF cytology in identifying the presence of cancer: in a validation set of 100 cases, a CSF ATIII concentration of >1.2 ng/mL was determined to be associated with 75% sensitivity and 98% specificity for brain cancer (Figure 1). However, this marker was not specific for PCNSL, it was also expressed at high relative concentration in the CSF of patients with other types of brain tumors (such as glioblastoma) and in some cases of breast and lung cancer. There was some evidence that higher ATIII concentrations were associated with worse prognosis at relapse, and also correlated with tumor progression and response, although this retrospective subgroup analysis was carried out in only 14 patients.

It is possible that the entire CSF proteome has prognostic significance. Analysis of the overall CSF proteomic pattern revealed two broad groups, A and B [author's unpublished data]. This grouping could distinguish patients with CNS lymphoma from patients without CNS malignancy in 29 of 30 cases. There also appeared to be a relationship with survival: patients with the group A pattern had significantly shorter survival than those with the group B pattern ($P < 0.02$) (Figure 2). The studies described above are the first demonstration that proteomic analysis can yield biomarkers that have greater sensitivity than CSF cytology. Furthermore, a recent gene expression comparison between PCNSL and non-CNS DLBCL has identified a unique genetic 'signature' for PCNSL that may have significant biological relevance for lymphoma research and the development of novel therapeutic strategies [51]. Further work is underway to build on these findings.

References

1. Surawicz TS, McCarthy BJ, Kupelian V, Jukich PJ, Bruner JM, Davis FG. Descriptive epidemiology of primary brain and CNS tumors: results from the Central Brain Tumor Registry of the United States, 1990–1994. *Neuro Oncol.* 1999; 1:14–25. [PubMed: 11554386]
2. Ferreri AJ, Abrey LE, Blay JY, Borisch B, Hochman J, Neuwelt EA, et al. Summary statement on primary central nervous system lymphomas from the Eighth International Conference on Malignant Lymphoma, Lugano, Switzerland, June 12 to 15, 2002. *J Clin Oncol.* 2003; 21:2407–2414. [PubMed: 12805341]
3. Makino K, Nakamura H, Kino T, Takeshima H, Kuratsu J. Rising incidence of primary central nervous system lymphoma in Kumamoto, Japan. *Surg Neurol.* 2006; 66:503–506. [PubMed: 17084198]

4. Haldorsen I, Krossnes B, Aarseth J, Scheie D, Johannesen T, Mella O, Espeland A. Increasing incidence and continued dismal outcome in primary central nervous system lymphoma in Norway 1989–2003, time trends in a 15-year national survey. *Cancer*. 2007; 110:1803–1814. [PubMed: 17721992]
5. Olson JE, Janney CA, Rao RD, Cerhan JR, Kurtin PJ, Schiff D, et al. The continuing increase in the incidence of primary central nervous system non-Hodgkin lymphoma: a surveillance, epidemiology, and end results analysis. *Cancer*. 2002; 95:1504–1510. [PubMed: 12237919]
6. Schabet M. Epidemiology of primary CNS lymphoma. *J Neurooncol*. 1999; 43:199–201. [PubMed: 10563423]
7. Abrey, L.; Ferreri, AJM. Primary and secondary central nervous system lymphomas in immunocompetent and human immunodeficiency virus–positive patients. In: Young, NS.; Gerson, SL.; High, KA., editors. *Clinical Hematology*. 1st. Elsevier; Amsterdam, Netherlands: 2006. p. 564–578.
8. Wolf T, Brodt HR, Fichtlscherer S. Changing incidence and prognostic factors of survival in AIDS-related non-Hodgkin's lymphoma in the era of highly active antiretroviral therapy (HAART). *Leuk Lymphoma*. 2005; 46:207–215. [PubMed: 15621803]
9. Biggar RJ, Engels EA, Ly S. Survival after cancer diagnosis in persons with AIDS. *J Acquir Immune Defic Syndr*. 2005; 39:293–299. [PubMed: 15980688]
10. Bashir R, Luka J, Cheloha K, Chamberlain M, Hochberg F. Expression of Epstein-Barr virus proteins in primary CNS lymphoma in AIDS patients. *Neurology*. 1993; 43:2358–2362. [PubMed: 8232956]
11. Shen DF, Herbort CP, Tuailon N, Buggage RR, Egwuagu CE, Chan CC. Detection of toxoplasma gondii DNA in primary intraocular B-cell lymphoma. *Mod Pathol*. 2001; 14:995–999. [PubMed: 11598169]
12. Chan CC. Molecular pathology of primary intraocular lymphoma. *Trans Am Ophthalmol Soc*. 2003; 101:275–292. [PubMed: 14971583]
13. Montesinos-Rongen M, Hans VH, Eis-Hübingen AM, Prinz M, Schaller C, Van Roost D, et al. Human herpes virus-8 is not associated with primary central nervous system lymphoma in HIV-negative patients. *Acta Neuropathol*. 2001; 102:489–495. [PubMed: 11699563]
14. Corboy JR, Garl PJ, Kleinschmidt-DeMasters BK. Human herpesvirus 8 DNA in CNS lymphomas from patients with and without AIDS. *Neurology*. 1998; 50:335–340. [PubMed: 9484350]
15. Martini F, Dolcetti R, Ferreri AJ, Ponzoni M, Fumagalli L, Reni M, et al. No association between polyomaviruses and primary central nervous system lymphomas of HIV-seronegative and HIV-positive patients. *Cancer Epidemiol Biomarkers Prev*. 2004; 13:1819–1820. [PubMed: 15533914]
16. Ferreri AJ, Reni M, Pasini F, Calderoni A, Tirelli U, Pivnik A, et al. A multicenter study of treatment of primary CNS lymphoma. *Neurology*. 2002; 58:1513–1520. [PubMed: 12034789]
17. Bromberg JEC, Siemers MD, Taphoorn MJB. Is a “vanishing tumor” always a lymphoma? *Neurology*. 2002; 59:762–764. [PubMed: 12221174]
18. Soo TM, Bernstein M, Provias J, Tasker R, Lozano A, Guha A. Failed stereotactic biopsy in a series of 518 cases. *Stereotact Funct Neurosurg*. 1995; 64:183–196. [PubMed: 8817805]
19. Miller DC, Hochberg FH, Harris NL, Gruber ML, Louis NL, Cohen H. Pathology with clinical correlations of primary central nervous system non-Hodgkin's lymphoma. The Massachusetts General Hospital experience 1958–1989. *Cancer*. 1994; 74:1383–1397.
20. Grimm SA, Pulido JS, Jahnke K, Schiff D, Hall AJ, Shenkier TN, et al. Primary intraocular lymphoma: an International Primary Central Nervous System Lymphoma Collaborative Group Report. *Ann Oncol*. 2007; 18:1851–1855. [PubMed: 17804469]
21. Antinori A, De Rossi G, Ammassari A, Cingolani A, Murri R, Di Giuda D, et al. Value of combined approach with thallium-201 single-photon emission computed tomography and Epstein-Barr virus DNA polymerase chain reaction in CSF for the diagnosis of AIDS-related primary CNS lymphoma. *J Clin Oncol*. 1999; 17:554–560. [PubMed: 10080599]
22. Abrey LE, Batchelor TT, Ferreri AJ, Gospodarowicz M, Pulczynski EJ, Zucca E, et al. Report of an international workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma. *J Clin Oncol*. 2005; 23:5034–5043. [PubMed: 15955902]

23. Ferreri AJ, Reni M. Primary central nervous system lymphoma. *Crit Rev Oncol Hematol*. 2007; 63:257–268. [PubMed: 17590348]
24. Ferreri AJ, Blay JY, Reni M, Pasini F, Spina M, Ambrosetti A, et al. Prognostic scoring system for primary CNS lymphomas: the International Extranodal Lymphoma Study Group experience. *J Clin Oncol*. 2003; 21:266–272. [PubMed: 12525518]
25. Ferreri AJM, Blay JY, Reni M, Pasini F, Gubkin A, Tirelli U, et al. Relevance of intraocular involvement in the management of primary central nervous system lymphomas. *Ann Oncol*. 2002; 13:531–538. [PubMed: 12056702]
26. Chan CC, Buggage RR, Nussenblatt RB. Intraocular lymphoma. *Curr Opin Ophthalmol*. 2002; 13:411–418. [PubMed: 12441846]
27. Jahnke K, Schilling A, Heidenreich J, Stein H, Brock M, Thiel E, et al. Radiologic morphology of low-grade primary central nervous system lymphoma in immunocompetent patients. *Am J Neuroradiol*. 2005; 26:2466–2454. [PubMed: 16286386]
28. DeAngelis LM, Ferreri AJM. Central nervous system lymphoma. In: Marcus R, Sweetenham JW, Williams ME, editors. *Lymphoma: Pathology, Diagnosis and Treatment*. Cambridge University Press; New York: 2007. p. 200–214.
29. Shenkier T, Blay J-Y, O'Neill B, Poortmans P, Thiel E. Primary CNS lymphoma of T-cell origin: a descriptive analysis from the international primary CNS lymphoma collaborative group. *J Clin Oncol*. 2005; 23:2233–2239. [PubMed: 15800313]
30. Batchelor T, Loeffler JS. Primary CNS lymphoma. *J Clin Oncol*. 2006; 24:1281–1288. [PubMed: 16525183]
31. Montesinos-Rongen M, Van Roost D, Schaller C, Wiestler OD, Deckert M. Primary diffuse large B-cell lymphomas of the central nervous system are targeted by aberrant somatic hypermutation. *Blood*. 2004; 103:1869–1875. [PubMed: 14592832]
32. Lin CH, Kuo KT, Chuang SS, Kuo SH, Chang JH, Chang KC, et al. Comparison of the expression and prognostic significance of differentiation markers between diffuse large B-cell lymphomas of central nervous system origin and peripheral nodal origin. *Clin Cancer Res*. 2006; 12:1152–1156. [PubMed: 16489068]
33. Camilleri-Broët S, Crinière E, Broët P, Delwail V, Mokhtari K, Moreau A, et al. A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. *Blood*. 2006; 107:190–196. [PubMed: 16150948]
34. Rubenstein J, Fridlyand J, Shen A, Aldape K, Ginzinger D, Batchelor T, et al. Gene expression and angiotropism in primary CNS lymphoma. *Blood*. 2006; 107:3716–3723. [PubMed: 16418334]
35. Kadoch C, Treseler P, Rubenstein J. Molecular pathogenesis of primary CNS lymphoma. *Neurosurg Focus*. 2006; 21:E1. [PubMed: 17134111]
36. Tu PH, Giannini C, Judkins AR, Schwab JM, Burack R, O'Neill BP, et al. Clinicopathologic and genetic profile of intracranial marginal zone lymphoma: a primary low-grade CNS lymphoma that mimics meningioma. *J Clin Oncol*. 2005; 23:5718–5727. [PubMed: 16009945]
37. Cheuk W, Walford N, Lou J, Lee AK, Fung CF, Au KH, et al. Primary histiocytic lymphoma of the central nervous system: a neoplasm frequently overshadowed by a prominent inflammatory component. *Am J Surg Pathol*. 2001; 25:1372–1379. [PubMed: 11684953]
38. Sun W, Nordberg ML, Fowler MR. Histiocytic sarcoma involving the central nervous system: clinical, immunohistochemical, and molecular genetic studies of a case with review of the literature. *Am J Surg Pathol*. 2003; 27:258–265. [PubMed: 12548175]
39. Abrey L, Yahalom J, De Angelis L. Treatment for primary CNS lymphoma: the next step. *J Clin Oncol*. 2000; 18:3144–3150. [PubMed: 10963643]
40. Soussain C, Suzan F, Hoang-Xuan K, Cassoux N, Levy V, Azar N, et al. Results of intensive chemotherapy followed by hematopoietic stem-cell rescue in 22 patients with refractory or recurrent primary CNS lymphoma or intraocular lymphoma. *J Clin Oncol*. 2001; 19:742–749. [PubMed: 11157026]
41. DeAngelis LM, Seiferheld W, Schold SC, Fisher B, Schultz CJ. Combination chemotherapy and radiotherapy for primary central nervous system lymphoma: Radiation Therapy Oncology Group Study 93-10. *J Clin Oncol*. 2002; 20:4643–4648. [PubMed: 12488408]

42. Shah GD, Yahalom J, Correa DD, Lai RK, Raizer JJ, Schiff D, et al. Combined immunochemotherapy with reduced whole-brain radiotherapy for newly diagnosed primary CNS lymphoma. *J Clin Oncol.* 2007; 25:4730–4735. [PubMed: 17947720]
43. Ferreri AJ, Dell'Oro S, Foppoli M, Bernardi M, Brandes AA, Tosoni A, et al. MATILDE regimen followed by radiotherapy is an active strategy against primary CNS lymphoma. *Neurology.* 2006; 66:1435–1438. [PubMed: 16682682]
44. Issa M, Hwang J, Karch K, Fridlyand M, Prados T, Batchelor K, et al. Treatment of primary CNS lymphoma with induction high-dose methotrexate, temozolomide, rituximab followed by consolidation cytarabine/etoposide: a pilot study with biomarker analysis. *J Clin Oncol.* 2006; 24 Abstract 7595.
45. Rubenstein JL, Combs J, Rosenberg Levy A, McDermott M, Damon L, Ignoffo R, et al. Rituximab in CNS lymphoma: targeting the leptomeningeal compartment. *Blood.* 2003; 101:466–468. [PubMed: 12393404]
46. Rubenstein JL, Fridlyand J, Abrey L, Shen A, Karch J, Wang E, et al. Phase I study of intraventricular administration of rituximab in patients with recurrent CNS and intraocular lymphoma. *J Clin Oncol.* 2007; 25:1350–1356. [PubMed: 17312328]
47. Barrans SL, Fenton JA, Banham A, Owen RG, Jack AS. Strong expression of FOXP1 identifies a distinct subset of diffuse large B-cell lymphoma (DLBCL) patients with poor outcome. *Blood.* 2004; 104:2933–2935. [PubMed: 15238418]
48. Banham AH, Connors JM, Brown PJ, Cordell JL, Ott G, Sreenivasan G, et al. Expression of the FOXP1 transcription factor is strongly associated with inferior survival in patients with diffuse large B-cell lymphoma. *Clin Cancer Res.* 2005; 11:1065–1072. [PubMed: 15709173]
49. Roy S, Josephson SA, Fridlyand J, Karch J, Kadoch C, Karrim J, et al. Protein biomarker identification in the CSF of patients with CNS lymphoma. *J Clin Oncol.* 2008; 26:96–105. [PubMed: 18056677]
50. Kadoch C, Shuman M, Rubenstein JL. Complement activation and resistance within the cerebrospinal fluid following intraventricular administration of anti-CD20 in the treatment of central nervous system lymphoma. *Blood.* 2007; 110 Abstract 3418.
51. Tun HW, Personett D, Baskerville KA, Menke DM, Jaeckle KA, Kreinest P, et al. Pathway analysis of primary central nervous system lymphoma. *Blood.* 2008; 111:3200–3210. [PubMed: 18184868]

Figure 1.

Sensitivity and specificity for cancer of cerebrospinal fluid (CSF) antithrombin-III (ATIII) >1.2 µg/mL. Roy S et al. Protein biomarker identification in the CSF of patients with CNS lymphoma. *J Clin Oncol.* 2008;26:96–105. Reproduced with permission. © 2008 American Society of Clinical Oncology. All rights reserved.

Figure 2.

Relationship of survival to cerebrospinal fluid (CSF) proteomic pattern. Unsupervised clustering of CSF proteins, which were determined to distinguish CNS lymphoma from patients without CNS malignancy [49], identifies prognostic subgroups. Unsupervised clustering of the expression pattern of CSF proteins distinguishes CSF proteins from CNS lymphoma nearly uniformly from CSF proteins from benign conditions, with the exception of one case. Moreover, clustering of peptides divides the CNS lymphoma patients into two nodes, denoted as Group A and Group B.