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The Role of Cerebrospinal Fluid Cytokines in the Diagnosis of Lymphoma

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Abstract

Objective: Our objective was to determine if quantification of cytokines from the cerebrospinal fluid (CSF) can be an adjunct in the diagnosis of lymphoma. In this work, we evaluated the role of interleukin (IL)-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), and IL-10/IL-6 to detect central nervous system (CNS) and systemic lymphoma.

Methods: Retrospective case series of 22 consecutive patients undergoing neurologic evaluation at Mayo Clinic, Rochester, MN, between 1996 and 2011 Part of the work-up included a diagnostic lumbar puncture. CSF cytokine levels were measured using an electrochemiluminescent enzyme-linked immunosorbent assay (ELISA).

Results: 10/22 patients had a diagnosis of lymphoma; 3 of these 10 patients with lymphoma had CNS involvement. The control group comprised 12 patients with final diagnoses, ranging from functional behavioral spells to prostate cancer. Interleukin-8 levels were significantly higher in patients with lymphoma than in the control group (two sample t-test; p=0.009). No significant difference was detected for any of the cytokines between CNS+ lymphoma and the control group. In a subgroup analysis of patients with lymphoma, none of the cytokines demonstrated a significant difference between CNS+ and CNS- disease.

Conclusion: This is the first study to suggest an association between elevated CSF levels of IL-8 and lymphoma. Conclusions are limited by small sample sizes. However, this data supports additional research to elucidate the role of IL-8 as a possible diagnostic marker of lymphoma.

Keywords: Interleukin-8; CNS lymphoma; Lymphoma; CSF; Cytokines.

Introduction

Central nervous system (CNS) lymphoma can arise either primarily or secondary to local or hematogenous spread. Primary central nervous system lymphoma (PCNSL), which accounts for 3%-5% of primary brain tumors, is a subset of non-Hodgkin lymphoma [1]. The five-year survival rate is poor [2], and most patients expire from disease progression or CNS recurrence. Chemotherapy and whole brain radiation are the mainstays of treatment. Although refinements to this protocol have improved median survival [3,4], overall prognosis remains quite poor. For instance, in one retrospective interventional study of patients with PCNSL, those who received combination chemotherapy and/or radiation plus intravitreal methotrexate (MTX) for coexisting ocular disease (if present) had a median survival only slightly greater than 30 months [5]. Therefore, early diagnosis and intervention remains the best bet for prolonging progression-free survival time. Unfortunately, diagnosis of CNS lymphoma is difficult,

and brain biopsy with histopathological findings remains the gold standard. Less invasive methods of diagnosis are currently under evaluation, including cerebrospinal fluid (CSF) cytology, molecular analysis for gene rearrangements, flow cytometry to establish lymphoid clonality, and measurement of serum and CSF cytokines, especially interleukin (IL)-10, IL-6, and the IL-10/IL-6 ratio.

Previous studies have suggested that elevated serum IL-10 may correlate with poor prognosis in non-Hodgkin lymphoma [6]. Furthermore, in a subset of PCNSL patients with vitreoretinal lymphoma, studies have suggested an association between lymphoma and elevated IL-10 and IL-10/IL-6 in aqueous and vitreous [7-10]. Conversely, IL-6 is preferentially expressed in inflammatory conditions.

Less has been written about IL-8 in lymphoma, although elevated serum IL-8 levels in patients with some forms of hematologic malignancy, including non-Hodgkin lymphoma, have been reported [11]. Elevated serum IL-8 was also found to correlate independently with high-risk features in diffuse large B-cell lymphoma, including poorer response to treatment [12]. Although the pathogenesis of IL-8 is not clearly understood, a proposed mechanism in chronic lymphocytic leukemia is through IL-8-mediated attachment of leukemic cells to stromal cells, potentiating their effect [13].

The goal of this study was to evaluate the role of four measureable CSF cytokines (i.e., IL-6, IL-8, IL-10, and VEGF) and the ratio of IL-10 to IL-6 (IL-10/IL-6) to detect lymphoma and specifically CNS+ lymphoma from a control group. We also compared cytokine levels between CNS+ and CNS- lymphoma to test for any difference between these two groups.

Patients and Methods

This study was approved by the Mayo Clinic Institutional Review Board (IRB), Rochester, MN. All data were analyzed anonymously, and patient identifying information was concealed to protect patient identity. The sample size was based on consecutive patients evaluated at the Mayo Clinic, Rochester MN, from 1996 to 2011 because of focal neurologic deficits or to rule out CNS involvement in systemic disease. After written informed consent was obtained, each patient underwent a lumbar puncture with removal of 3 to 5 cc of CSF. Lumbar punctures were performed either at initial evaluation or later during the workup as warranted.

Cytokine levels were measured in undiluted CSF using a Meso Scale system on an electrochemiluminescent ELISA. The same kit lot and provider were used in all determinations, and samples were stored at -70 centigrade at the Mayo Clinic Tissue Bank until the time of processing. Four markers of interest (i.e., IL-6, IL-8, IL-10, and VEGF) were chosen for analysis. In addition, IL-10/IL-6 was calculated for each patient. Information pertaining to patient demographics, medical history, workup, and treatment were extracted from the medical records.

Statistical analysis using a two-sample t-test was performed using the statistical software JMP® 10.0.0 (2012) SAS Institute Inc. Cytokine levels were compared between all patients with lymphoma and CNS+ lymphoma against a control group, as well as between patients with CNS+ and CNS- lymphoma. P-values <0.05 were considered statistically significant.

Results

A total of 22 patients were included in the study. The date of initial evaluation at our clinic ranged from 1996 to 2011. Information pertaining to patient demographics, medical history, diagnoses, and treatment course for patients with lymphoma is reported in **Table 1**. Similar information for control patients is summarized in **Table 2.** To conceal patient identifying information, specific dates of diagnosis or treatment are withheld and only the year is reported.

Out of 22 patients, 10 had lymphoma; 7 out of 10 had a diagnosis of lymphoma without any known CNS involvement at the time of writing this manuscript (Cases 4-10), whereas 3 had CNS involvement, either from primary disease (Case 2) or secondary spread (Cases 1 and 3). Case 8 had concomitant diagnoses of lymphoma and gastric adenocarcinoma diagnosed years apart. Of the remaining 12 patients comprising the control group, 1 was diagnosed with prostate adenocarcinoma (Case 11) and underwent lumbar puncture to rule out CNS involvement. One patient was diagnosed with an unresectable, grade 3 fibrillary astrocytoma (Case 12). The remaining 10 patients were seen for various conditions in which lumbar puncture was warranted as part of the diagnostic workup (Cases 13-22). The final diagnoses in this group ranged from functional behavioral spells to Friedreich ataxia.

The limit of detection for the assay was chosen to be 0.6 pg/mL based on best approximation. No threshold of sensitivity is available for the IL-10/IL-6 ratio given that this was a calculated measurement. IL-6, IL-8, IL-10, and VEGF levels in patients with lymphoma are reported in **Table 3** and in control patients in **Table 4.** The IL-10/IL-6 level was zero in all patients except for Case 2 (6.9×10^{-1}), Case 3 (4.2×10^{-2}), Case 5 (9.2×10^{-2}), Case 8 (6.9×10^{-2}), and Case 13 (2.9×10^{-1}).

A two-sample t-test was used to compare distribution of CSF cytokine levels between all lymphoma patients (n=10) and control patients (n=12), CNS+ lymphoma patients (n=3) and control patients, and CNS+ lymphoma patients and CNS- lymphoma patients (n=7). **Table 5** reports the mean value +/- standard deviation and 95% confidence internal (CI) of each cytokine for the lymphoma (all lymphoma, CNS+, and CNS-) and control groups as well as results of the statistical analyses.

In a comparison of lymphoma and control patients, IL-8 (p=0.009) levels were significantly higher in CSF of lymphoma patients than in the control group. Conversely, there was no significant difference in IL-6, IL-10, VEGF, and IL-10/IL-6 levels between the two groups. Comparing CNS+ lymphoma with control groups and in a subset analysis of CNS+ and CNS- lymphoma, there was no significant difference detected for any of the cytokines.

Discussion

PCNSL, which accounts for <5% of brain tumors, is characterized by recurrence and poor survival [1,2]. Therefore, early diagnosis

Table 1 Summary of patient demographics, medical comorbidities, and diagnostic/treatment course in patients with lymphoma.

Case #	Sex	Age at time of diagnosis	Date of initial diagnosis	Site(s) of initial disease	Lymphoma Type	Treatment prior to LP	CNS involved?	Cytology	Date of CNS Diagnosis	CNS specific treatment prior to LP	Date of LP
1	F	69	1994	Systemic CLL	B-cell CLL	Chlorambucil and prednisone × 7 cycles (1996-1997), Fludarabine × 4 cycles (1999-2000), Fludarabine × 6 cycles (2003) to PR.	Yes	Negative	2004	Steroids (dose/ duration not available)	2004
2	M	51	2004	Primary CNS lymphoma	DLBCL	IV Solu-Medrol and oral prednisone taper (2004)	Yes	Negative	2004	Same as initial treatment	2004
3	M	60	2004	Mediastinal and mesenteric adenopathy, pulmonary infiltrates, multiple splenic lesions, bone marrow (60-70% involvement), stage IV-B	DLBCL	R-CHOP × 2 cycles (2004) with relapse in CSF	Yes	Negative	2004	3rd cycle of R-CHOP, prednisone and IT MTX × 7 doses (2004)	Late 2004
4	F	83	Early 2004	Right tonsil, stage I-A	DLBCL	None	No	Negative	N/A	N/A	2004
5	M	57	2004	Right ethmoid sinus with dehiscence of lamina papyracea, infiltration of SO and deviation of MR, stage I-AE	DLBCL	Surgical resection (2004) with residual disease R-CHOP + IT MTX x 2 cycles (2004)	No	Negative	N/A	N/A	2004
6	F	80	2004	Left orbit	Extranodal marginal zone B –cell lymphoma of mucosa associated lymphoid tissue (MALT)	None	6	F	80	2004	Left orbit
7	M	62	2004	Abdominal and pelvic adenopathy, stage II-A	DLBCL	Prednisone	7	M	62	2004	Abdominal and pelvic adenopathy, stage II-A
8	M	51	2004	Liver, ascending colon across the ileocecal valve, BM (<5%)	High grade B-cell lymphoma, Burkett-like	None	8	M	51	2004	Liver, ascending colon across the ileocecal valve, BM (<5%)
9	M	61	2004	Cervical, mediastinal, axillary, and abdominal adenopathy, stage IV-B	DLBCL	R-CHOP x 1 cycle (2004) with mixed response	9	M	61	2004	Cervical, mediastinal, axillary, and abdominal adenopathy, stage IV-B
10	M	52	2003	Left testicle with LN involvement of left renal hilum	DLBCL	Surgical resection 2003 R-CHOP + prophylactic IT MTX with Cytarabine and preservative free hydrocortisone × 1 cycle (2004), R-CHOP + IT MTX × 3 cycles (2004)	10	M	52	2003	Left testicle with LN involvement of left renal hilum

BM: Bone Marrow; CLL: Chronic Lymphocytic Leukemia; CNS: Central Nervous System; CR: Complete Response; CSF: Cerebrospinal Fluid; DLBCL: Diffuse Large B-Cell Lymphoma; IT: Intrathecal; LP: Lumbar Puncture; LN: Lymph Node; MALT: Mucosa Associated Lymphoid; MR: Medial Rectus; MTX: Methotrexate; PR: Partial Response; R-CHOP: Rituximab-Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisone; SO: Superior Oblique

Table 2 Summary of patient demographics, medical comorbidities, and diagnostic/treatment course in control patients.

Case #	Sex	Age at time of diagnosis	Diagnosis	Date of initial diagnosis	Site of initial disease	Treatment prior to LP	CNS involvement?	Date of CNS Diagnosis	CNS specific treatment prior to LP	Date of LP
11	M	59	Adenocarcinoma of the prostate	2011	Right prostate	Surgical resection and pelvic lymphadenectomy (2011)	No	N/A	N/A	?
12	F	64	Grade 3 unresectable fibrillary astrocytoma	2004	CNS	?; patient returned home for follow up	Yes	Same as initial diagnosis	?; patient returned home for follow up	2004
13	M	35	Friedreich's ataxia	1996	CNS	None	Yes	Same as initial diagnosis	Same as initial treatment	2004
14	M	56	Chronic progressive myelopathy secondary to spinocerebellar ataxia	1996	CNS	None	Yes	Same as initial diagnosis	Same as initial treatment	?
15	F	45	Chronic progressive myelopathy secondary to multiple sclerosis	1996	CNS	None	Yes	Same as initial diagnosis	Same as initial treatment	?
16	M	54	Small vessel ischemic disease with lacunar infarcts	1996	CNS	Aspirin for stroke prevention	Yes	Same as initial diagnosis	Same as initial treatment	1996
17	M	46	Progressive asymmetric right akinetic-rigid syndrome, Parkinsonian-type	1996	CNS	Amantadine and Cylert sometime between 1994-1996	Yes	Same as initial diagnosis	Same as initial treatment	1996
18	F	44	Diagnosed with latent syphilis while undergoing evaluation as kidney donor for son. CSF syphilis studies were negative.	2004	Systemic	IM Penicillin, 2.4 million units once weekly x 3 for latent syphilis	No	None	None	2004
19	F	41	Facial paresthesias, dysphagia to liquids, dizziness, ataxia, dysarthria starting in 2003. No diagnosis made, and patient wished to stop after limited work up.	2004	?	None	?	None	None	2004
20	F	25	Behavioral spells, no organic disease found	2004	?	Zoloft prior to referral to Mayo ? start date	No	Same as initial diagnosis	Same as initial treatment	2004
21	F	37	Intermittent lower extremity weakness and unresponsive spells starting in 1998. Work up normal except for enhancing T1 lesions in the basal ganglia on MRI in 2004 which was felt to be caused by elevated serum magnesium from TPN	2004	CNS	Reformulation of TPN	Yes	Same as initial diagnosis	Same as initial treatment	2004

Case #	Sex	Age at time of diagnosis	Diagnosis	Date of initial diagnosis	Site of initial disease	Treatment prior to LP	CNS involvement?	Date of CNS Diagnosis	CNS specific treatment prior to LP	Date of LP
22	F		Chronic variant migraine exacerbated by medication overuse with possible functional component	2003	CNS	Amerge, toradol, maxalt, axert, ibuprofen, arthrotec, tylenol, ultram, hydrocodone, topamax, morphine XR, keppra, flexeril, trazodone nortriptyline, midrin, thorazine suppositories for breakthrough pain, remeron, Migra health for prevention. verampil for vasospastic transient monocular blindness	Yes	Same as initial diagnosis	Same as initial treatment	2004

Index: CNS: Central Nervous System; IM: Intramuscular; LP: Lumbar Puncture; MRI: Magnetic Resonance Imaging; N/A: Not Applicable; T: Thoracic; TPN: Total Parenteral Nutrition; XR: Extended Release

Table 3 CSF Cytokine levels in lymphoma patients.

Case #	[IL-6] (pg/mL)	[IL-8] (pg/mL)	[IL-10] (pg/mL)	[VEGF] (pg/mL)
1	8.9	106.2	0.0	3.2
2	2.9	31.9	2.0	0.0
3	21.6	120.9	0.9	14.0
4	4.6	61.5	0.0 (0.4)**	1.2
5	6.5	79.5	0.6	7.1
6	3.2	58.6	0.0 (0.2)**	2.2
7	63.6	63.6	0.0	7.3
8	39.2	62.4	2.7	2.6
9	5.9	74.6	0.0 (0.5)**	2.0
10	4.8	159.9	0.0 (0.5)**	9.3

IL: Interleukin; VEGF: Vascular Endothelial Growth Factor
**The value within parentheses denotes the actual measured level according to the assay. The value not in parenthesis is the value used in the statistical analysis when applying a lower limit of detectability of 0.6 pg/ml for the assay.
Results of IL-10/IL-6 are reported in the text.

Table 4 CSF cytokine levels in CSF of control patients.

Case #	[IL-6] (pg/mL)	[IL-8] (pg/mL)	[IL-10] (pg/mL)	[VEGF] (pg/mL)
11	2.6	31.4	0.0	2.9
12	3.0	45.7	0.0	0.0
13	2.1	50.6	0.6	3.2
14	3.7	30.9	0.0	3.6
15	1.6	33.6	0.0	5.0
16	3.1	37.4	0.0 (0.3)**	5.0
17	4.4	64.9	0.0 (0.2)**	2.8
18	2.6	64.3	0.0 (0.1)**	0.5
19	2.9	53.5	0.0	1.6
20	10.7	33.8	0.0 (0.4)**	7.3
21	1.8	36.4	0.0	6.0
22	2.1	28.9	0.0	3.7

Index: IL: Interleukin; VEGF: Vascular Endothelial Growth Factor
**The value within parentheses denotes the actual measured level according to the assay. The value not in parenthesis is the value used in the statistical analysis when applying a lower limit of detectability of 0.6 pg/ml for the assay.
Results of IL-10/IL-6 are reported in the text.

and intervention is important and gives the best chance for prolonging disease-free survival time. Unfortunately, diagnosis of CNS lymphoma is challenging, and CSF cytology is often inconclusive. Although brain biopsy with histopathologic analysis is the definitive method of diagnosis, this is only possible when there is a discrete lesion that is amenable to biopsy. The biopsy procedure is invasive and subjects patients to procedure-based morbidity.

The role of CSF cytokines in diagnosing systemic or CNS lymphoma has not been clearly defined. However, in the ophthalmic literature, several studies have described an association between vitreoretinal lymphoma and elevated levels of intraocular IL-10 and IL-10/IL-6 [7-10]. For example, our group was able to show that, compared with cytology, aqueous IL-10 and IL-10/IL-6 could be used to differentiate cytology-proven vitreoretinal lymphoma from uveitis with a diagnostic sensitivity and specificity of >80% and 100%, respectively [10].

Given these promising results, we chose to evaluate the role of CSF cytokines to detect CNS or systemic lymphoma. Four cytokines were selected for analysis (i.e., IL-6, IL-8, IL-10, and VEGF). Data regarding IL-10 and IL-6 were mentioned above.

Regarding IL-8, recent work has shown that serum IL-8 appears to be elevated in systemic lymphoma, including mantel cell lymphoma [14] and diffuse B-cell lymphoma [15] and that elevated levels may indicate a risk factor for poor treatment response in diffuse large B-cell lymphoma [12]. The pathogenesis of these lymphomas is proposed to be from pro-inflammatory and pro-angiogenic effects of IL-8 [16] that may even serve as a target in untreated and relapsed mantel cell lymphoma [17]. Finally, a recent meta-analysis revealed that overexpression of VEGF from surgically resected non-Hodgkin lymphoma was associated with poor prognosis [18].

To date, few studies have actually evaluated CSF cytokines either as a diagnostic tool or as a way to monitor disease progression

Table 5 Summary statistics and student's t-test results.

	Control (Mean ± STD) (n=12)	95% CI	Lymphoma (Mean ± STD) (n=10)	95% CI	CNS+ Lymphoma (Mean ± STD) (n=3)	95% CI	CNS- Lymphoma (Mean ± STD) (n=7)	95% CI	p1 Lymphoma vs. Control	p2 CNS+ Lymphoma vs. Control	p3 CNS+ Lymphoma vs. CNS- Lymphoma
[IL-6] (pg/ml)	3.4 ± 2.4	1.8-4.9	16.1 ± 20.2	1.7-30.6	11.1 ± 9.5	-12.6-34.9	18.3 ± 23.7	-3.7-40.2	0.08	0.29	0.52
[IL-8] (pg/ml)	42.6 ± 12.9	34.4-50.8	81.9 ± 37.2	55.3-108.5	86.3 ± 47.7	-32.2-204.9	80.0 ± 36.0	46.7-113.3	0.009*	0.25	0.85
[IL-10] (pg/ml)	$5.0 \times 10^{-2} \pm 1.7 \times 10^{-1}$	-6.0×10^{-2}-1.6×10^{-1}	$6.2 \times 10^{-1} \pm 9.8 \times 10^{-1}$	-8.0×10^{-2}-1.3	$9.7 \times 10^{-1} \pm 1.0$	-1.5-3.5	$4.7 \times 10^{-1} \pm 1.0$	-4.6×10^{-1}-1.4	0.10	0.25	0.52
[VEGF] (pg/ml)	3.5 ± 2.2	2.1-4.8	4.9 ± 4.4	1.7-8.0	5.7 ± 7.3	-12.5-24.0	4.5 ± 3.3	1.5-7.5	0.37	0.65	0.81
IL-10/IL-6	$2.4 \times 10^{-2} \pm 8.4 \times 10^{-2}$	-2.9×10^{-2}-7.7×10^{-2}	$8.9 \times 10^{-2} \pm 2.1 \times 10^{-1}$	-6.4×10^{-2}-2.4×10^{-1}	$3.7 \times 10^{-1} \pm 3.5 \times 10^{-1}$	-4.9×10^{-1}-1.2	$2.3 \times 10^{-2} \pm 4.0 \times 10^{-2}$	-1.4×10^{-2}-6.0×10^{-2}	0.32	0.23	0.23

Index:CI: Confidence Interval; CNS: Central Nervous System; IL: Interleukin; STD: Standard Deviation; VEGF: Vascular Endothelial Growth Factor, *indicates statistically significant result

or therapeutic response in patients with CNS lymphoma [1,8,19]. One study by Salmaggi et al. (2000) tested CSF and serum IL-10 levels in patients with PCNSL and found that CSF IL-10 was significantly higher in patients with PCNSL compared to a control group of patients with other forms of brain tumor [19]. Interestingly, IL-10 >9 pg/mL tended to correlate with cotemporaneous or impending clinical or radiological worsening of the patients' disease, whereas levels <5 pg/mL correlated with relative disease stability. Only one patient in their study was noted to have cytology-positive disease in the CSF; notably, in this patient, IL-10 was markedly elevated to 65 pg/mL and there was evidence of radiographic spread.

More recently, Sasayama et al. (2012) reported a diagnostic sensitivity and specificity of 71% and 100%, respectively, for distinguishing PCSNL from other brain tumors, using CSF IL-10 [1]. In their study, post-treatment CSF IL-10 levels were lower than pre-treatment levels in all patients. Not unsurprisingly, IL-10 levels were higher in cases of disease recurrence. Together, this suggests that CSF IL-10 may have value as a tool to monitor disease progression and response to therapy.

In our study, IL-10 levels appeared to be elevated in the lymphoma group compared with the control group with a trend toward, but not reaching, significance (p=0.10). One reason for this is that our electrochemiluminescent ELISA is geared to detect disease at the upper range of the assay (i.e., highly elevated levels correlate with disease). However, a significant number of negative samples are still required to establish a lower limit of detection. Therefore, with our current assay, low values may not exclude the presence of disease. In fact, values at the lower range of the assay were found to have considerable overlap between patients with lymphoma and those in the control group. This may partially explain why IL-10 did not demonstrate a significant difference in our study. Based on the standard calibration curve, 0.6 pg/mL was chosen as the best approximation of the lower limit of detectability of the assay.

There were, indeed, patients with lymphoma (Cases 4, 6, 9, and 10) and in the control group (Cases 16, 17, 18, and 20) with cytokine levels >0.0 pg/mL but <0.6 pg/mL (actual values denoted in parenthesis in **Tables 3** and **4**) who were assigned a cytokine value of 0.0 pg/mL in the statistical analysis. Therefore, patients with detectable cytokine levels at the lower range of the assay may be missed. Performing a repeat two sample t-test with a limit of detectability of even 0.5 pg/mL shows a significant difference (p=0.050) with IL-10 levels being higher in lymphoma (mean +/- STD of 7.2×10^{-1} +/- 9.3×10^{-1}; 95% CI 5.5×10^{-2}-1.4) vs. the control group (5.0×10^{-2} +/- 1.7×10^{-1}; 95% CI -6.0×10^{-2}-1.6×10^{-1}). Further development of the assay with recruitment of additional patients is, thus, warranted prior to declaring that there is no difference in IL-10 between the two groups.

As expected, in our study, IL-6 did not show a significant difference between any of the comparison groups. This was an expected finding given that IL-6 is overexpressed in non-malignant inflammation. We also did not detect any difference in VEGF levels between the lymphoma and control groups. Interestingly, in a meta-analysis, VEGF overexpression was detected in resected tissue but not in serum in association with non-Hodgkin lymphoma [18].

The authors acknowledge that the small sample size in this study with unequal numbers in the lymphoma and control groups made assessing distributional assumptions difficult. The results of our study are preliminary and future validation studies with larger

sample sizes are warranted. Furthermore, of the three patients with CNS+ disease, one received systemic chemotherapy, one received systemic steroids, and one received both systemic and intrathecal chemotherapy before having an LP done at Mayo. How much of an effect this had on blunting cytokine levels is hard to gauge.

Certainly, additional CSF markers also require close evaluation. Among these, elevated CSF β2 microglobulin has been associated with various hematologic malignancies, including acute lymphoblastic leukemia and malignant lymphoma [20-22]. Similarly, studies have suggested a relationship between elevated CSF soluble interleukin-2 receptor (sIL-2R) levels and hematologic malignancies [23]. However, as a preliminary study, one of the primary aims was to assess the effect sizes of the interleukin levels between groups for future validation studies. Interestingly, our study was able to show significantly higher levels of IL-8 in the lymphoma group compared with the control group. To the best of our knowledge, CSF of patients with systemic lymphoma. Of the three patients with CNS+ disease, IL-8 was >100 pg/mL in two patients (Cases 1 and 3). Only one other patient in the lymphoma or control groups demonstrated IL-8 levels >100 pg/mL, and this was in a male with testicular lymphoma with local extension to the renal hilum. This is of unclear significance, although elevated serum IL-8 has been previously reported in inflammatory conditions of the male genital tract [24].

Conclusion

This is a preliminary study, and conclusions are limited by small sample sizes. However, this is the first study, to our knowledge, to suggest an association between elevated CSF levels of IL-8 and lymphoma. Further studies are warranted to elucidate the role of IL-8 as a possible diagnostic marker of lymphoma.

References

1. Sasayama T, Nakamizo S, Nishihara M, Kawamura A, Tanaka H, et al. (2012) Cerebrospinal fluid interleukin-10 is a potentially useful biomarker in immunocompetent primary central nervous system lymphoma (PCNSL). Neuro Oncol 14: 368-380.
2. Tuaillon N, Chan CC (2001) Molecular analysis of primary central nervous system and primary intraocular lymphomas. Curr Mol Med 1: 259-272.
3. Abrey LE, Yahalom J, DeAngelis LM (2000) Treatment for primary CNS lymphoma: the next step. J Clin Oncol 18: 3144-3150.
4. DeAngelis LM, Yahalom J, Thaler HT, Kher U (1992) Combined modality therapy for primary CNS lymphoma. J Clin Oncol 10: 635-643.
5. Hong JT, Chae JB, Lee JY, Kim JG, Yoon YH (2011) Ocular involvement in patients with primary CNS lymphoma. J Neurooncol 102: 139-145.
6. Blay JY, Burdin N, Rousset F, Lenoir G, Biron P, et al. (1993) Serum interleukin-10 in non-Hodgkin's lymphoma: a prognostic factor. Blood 82: 2169-2174.
7. Cassoux N, Merle-Beral H, Leblond V, Bodaghi B, Miléa D, et al. (2000) Ocular and central nervous system lymphoma: clinical features and diagnosis. Ocul Immunol Inflamm 8: 243-250.
8. Whitcup SM, Stark-Vancs V, Wittes RE, Solomon D, Podgor MJ, et al. (1997) Association of interleukin 10 in the vitreous and cerebrospinal fluid and primary central nervous system lymphoma. Arch Ophthalmol 115: 1157-1160.
9. Sugita S, Takase H, Sugamoto Y, Arai A, Miura O, et al. (2009) Diagnosis of intraocular lymphoma by polymerase chain reaction analysis and cytokine profiling of the vitreous fluid. Jpn J Ophthalmol 53: 209-214.
10. Raja H, Snyder MR, Johnston PB, O'Neill BP, Caraballo JN, et al. (2013) Effect of intravitreal methotrexate and rituximab on interleukin-10 levels in aqueous humor of treated eyes with vitreoretinal lymphoma. PLoS One 8: e65627.
11. Denizot Y, Fixe P, Liozon E, Praloran V (1996) Serum interleukin-8 (IL-8) and IL-6 concentrations in patients with hematologic malignancies. Blood 87: 4016-4017.
12. Nacinovic-Duletic A, Stifter S, Dvornik S, Skunca Z, Jonjic N (2008) Correlation of serum IL-6, IL-8 and IL-10 levels with clinicopathological features and prognosis in patients with diffuse large B-cell lymphoma. International journal of laboratory hematology 30: 230-239.
13. Yoon JY, Lafarge S, Dawe D, Lakhi S, Kumar R, et al. (2012) Association of interleukin-6 and interleukin-8 with poor prognosis in elderly patients with chronic lymphocytic leukemia. Leuk Lymphoma 53: 1735-1742.
14. Sonbol MB, Maurer MJ, Stenson MJ, Allmer C, LaPlant BR, et al. (2014) Elevated soluble IL-2Rα, IL-8, and MIP-1β levels are associated with inferior outcome and are independent of MIPI score in patients with mantle cell lymphoma. Am J Hematol 89: E223-227.
15. Ansell SM, Maurer MJ, Ziesmer SC, Slager SL, Habermann TM, Link BK, et al. (2012) Elevated pretreatment serum levels of interferon-inducible protein-10 (CXCL10) predict disease relapse and prognosis in diffuse large B-cell lymphoma patients. American journal of hematology 87: 865-869.
16. Waugh DJ, Wilson C (2008) The interleukin-8 pathway in cancer. Clin Cancer Res 14: 6735-6741.
17. Campbell LM, Maxwell PJ, Waugh DJ (2013) Rationale and Means to Target Pro-Inflammatory Interleukin-8 (CXCL8) Signaling in Cancer. Pharmaceuticals (Basel) 6: 929-959.
18. Yang J, Li W, He X, Zhang G, Yue L, et al. (2015) VEGF Overexpression Is a Valuable Prognostic Factor for Non-Hodgkin's Lymphoma Evidence from a Systemic Meta-Analysis. Dis Markers 2015: 786790.
19. Salmaggi A, Eoli M, Corsini E, Gelati M, Frigerio S, et al. (2000) Cerebrospinal fluid interleukin-10 levels in primary central nervous system lymphoma: a possible marker of response to treatment? Ann Neurol 47: 137-138.
20. Hansen PB, Kjeldsen L, Dalhoff K, Olesen B (1992) Cerebrospinal fluid beta-2-microglobulin in adult patients with acute leukemia or lymphoma: a useful marker in early diagnosis and monitoring of CNS-involvement. Acta Neurol Scand 85: 224-227.
21. Jeffery GM, Frampton CM, Legge HM, Hart DN (1990) Cerebrospinal fluid B2-microglobulin levels in meningeal involvement by malignancy. Pathology 22: 20-23.
22. Mavlight GM, Stuckey SE, Cabanillas FF, Keating MJ, Tourtellotte WW, et al. (1980) Diagnosis of leukemia or lymphoma in the central nervous system by beta 2-microglobulin determination. N Engl J Med 303: 718-722.
23. Chang CS, Liu HW, Lin SF, Chen TP (1989) Soluble interleukin-2 receptor levels in cerebrospinal fluid of patients with acute lymphocytic leukemia or with non-Hodgkin's lymphoma. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi 22: 132-137.
24. Lotti F, Maggi M (2013) Interleukin 8 and the male genital tract. J Reprod Immunol 100: 54-65.