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BRIEF COMMUNICATION

CSF TACI and BAFF levels in patients with primary CNS lymphoma as novel diagnostic biomarkers

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Abstract

We used an enzyme-linked immunosorbent assay to measure pretreatment B cell-activating factor belonging to the tumour necrosis factor family (BAFF) and transmembrane activator and CAML-interactor (TACI) levels in CSF and serum collected from patients with primary central nervous system lymphoma (PCNSL) and control groups. The decision tree analysis of CSF TACI and BAFF levels for patients with a PCNSL diagnosis showed 100% sensitivity and 100% specificity when we attempted to differentiate PCNSL from glioblastoma and CNS inflammatory diseases. The combination of CSF TACI and BAFF levels may thus be a novel and useful diagnostic biomarker of PCNSL.

Introduction

BAFF and APRIL play crucial roles in B cell-development, survival, and production of immunoglobulin by binding with receptors.[1] Three BAFF receptors have been identified: BCMA, TACI, and BAFF-R.[2] The two ligands (BAFF and APRIL) and the three receptors (BCMA, TACI, and BAFF-R) are molecules of the BAFF system. High levels of BAFF in the serum occur in several autoimmune diseases.[3,4] Malignant lymphoma cells can evade apoptosis via BAFF, probably in an autocrine manner.[5] PCNSL is a highly aggressive B cell tumor.[6] Therefore, an effective treatment should be started as soon as possible. However, MRI characteristics are not specific for PCNSL, and in most cases diagnosis requires a neuropathological examination.[6] Krumbholz et al. established astrocytes as BAFF producers and suggested that CNS-derived BAFF may promote B cell survival in PCNSL.[1] Thaler et al. reported that CSF and serum TACI and BCMA levels were higher in patients with PCNSL than in control patients.[7]

The present study aimed to evaluate the possibility that CSF and serum levels of BAFF and TACI could be diagnostic biomarkers specific for PCNSL rather than glioblastoma and inflammatory or autoimmune CNS diseases.

Methods

Patients

Patients were recruited at the Departments of Neurology and Neurosurgery, Kumamoto University Graduate School of Medical Sciences, from November 2012 to May 2017. CSF samples were obtained for routine diagnostic

evaluation from nine patients with PCNSL at baseline (i.e. the onset or relapse phase). Serum samples were also obtained from six of these nine patients. All patients with PCNSL were histologically classified as having DLBCL. Control CSF samples were also collected from 73 patients: five with glioblastoma, 11 with multiple sclerosis (relapse phase), nine with aquaporin four IgG-positive neuromyelitis optica (exacerbation), nine with autoimmune encephalitis, five with aseptic meningitis, and 34 with OND (Data S1). Serum samples were also obtained from 57 patients (11 with multiple sclerosis, seven with neuromyelitis optica, six with autoimmune encephalitis, four with aseptic meningitis, and 29 with OND). All serum samples were obtained on the same day and at the same time that CSF samples was obtained. Three of PCNSL patients received prednisolone orally when obtained CSF samples (average: 17.3 mg/day). One multiple sclerosis patient received prednisolone orally when obtained CSF sample (20 mg/day). Five neuromyelitis optica patients received prednisolone orally when obtained CSF samples (average: 13.2 mg/day). Two autoimmune encephalitis patients received prednisolone orally when obtained CSF samples (average: 17.5 mg/day). One aseptic meningitis patient received prednisolone orally when obtained CSF sample (30 mg/day). One patient with other neurological disorder received prednisolone orally when obtained CSF sample (20 mg/day).

Ethics statement

This study was approved by the institutional review board and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment in the study, and the study was approved by the institutional review board of Kumamoto University Hospital (Permit Number: 1391).

CSF and serum sampling

All CSF and serum samples were stored at −20°C until testing and were thawed only once. All CSF samples were obtained by atraumatic lumbar puncture. CSF analysis included cell counts and protein, IgG, and albumin levels. Serum analysis included IgG and albumin levels. Routine analysis of paired CSF and serum specimens comprised cell counts and differentiation; CSF IgG/serum IgG ratio (Q_{IgG}); CSF albumin/serum albumin ratio (Q_{Alb}) to estimate the integrity of the blood-brain barrier; and calculation of intrathecal IgG synthesis by means of quantitative formulae (IgG index). Blood-brain barrier damage was defined as having a Q_{Alb} value higher than normal for the patient's age (i.e. age/15 + 4).[8]

BAFF and TACI detection in CSF and serum samples

A standard ELISA was used to quantify BAFF and TACI (TNFSF13B) in CSF and serum samples—the in vitro SimpleStep ELISA kit (Abcam, Cambridge, UK), according to the manufacturer's protocol (Data S2). We calculated Q_{BAFF} (CSF BAFF/serum BAFF), BAFF index (Q_{BAFF}/Q_{Alb}), Q_{TACI} (CSF TACI/serum TACI), and TACI index (Q_{TACI}/Q_{Alb}).[9] We estimated intrathecal BAFF and TACI synthesis by calculating the BAFF index and TACI index.

Statistical analysis

This study is an observational cross-sectional one. To ascertain a normal distribution of variables, the Shapiro–Wilk test was performed. Values of CSF TACI, serum TACI, and CSF BAFF could not be determined for some patients because of the lower ELISA detection limits. In such cases, the values that were half the values of the lower limits for each variable were substituted.[10] Pearson's partial correlation coefficients were used to investigate correlations between two variables by removing the third variable's influence. In multivariate analysis, the objective variable was CSF TACI or CSF BAFF, and explanatory variables were age, sex, and group. In this model, the distributions of the objective variable were adapted from the generalized gamma distribution with log link. Incorporation of variables into models was determined by discussion with neurologists and reference to the Akaike information criterion. To differentiate between PCNSL and glioblastoma or multiple sclerosis via two variables, CSF TACI and CSF BAFF, we used a decision tree analysis and Gini index to evaluate the split points, as described previously.[11] Analyses were performed by using R version 3.3.2 (The R Foundation for Statistical Computing, Vienna, Austria), with the level of statistical significance set at $P < 0.05$.

Results

CSF and serum levels of TACI and BAFF in patients with PCNSL

Table 1 summarizes the clinical features and CSF indices of each group. Patients with inflammatory neurological diseases (multiple sclerosis, neuromyelitis optica, autoimmune encephalitis, and aseptic meningitis) had higher IgG index values compared with patients with PCNSL. Patients with PCNSL had significantly higher CSF TACI values (mean: 135.1 pg/mL) compared with patients in other groups ($P < 0.001$; Fig. 1A and Data S3). The

serum TACI values in patients with PCNSL (mean: 14.5 pg/mL) were higher than those in other groups (Data S4). CSF levels of BAFF in patients with PCNSL (mean: 1.0 ng/mL) and aseptic meningitis (mean: 1.4 ng/mL) were significantly higher than those in other groups ($P < 0.001$; Data S3 and S4). Serum BAFF levels were not elevated in PCNSL patients and other groups (Data S4). With regard to Pearson's partial correlation coefficients, CSF BAFF levels strongly correlated with CSF cell counts (coefficient: 0.50) but not with CSF protein (coefficient: 0.29). We detected no correlation of CSF TACI with CSF cell counts and CSF protein (Data S5).

Additionally, we found no associations among the clinical characteristics, MR findings including leptomeningeal involvement and CSF TACI or BAFF of the patients with PCNSL. CSF cytology samples were obtained from six of nine PCNSL patients, and CSF cytology revealed no malignant cell in all six patients.

Q_{TACI}, Q_{BAFF}, TACI index, and BAFF index in patients with PCNSL

Q_{TACI} in patients with PCNSL was significantly higher than that in other groups (Data S4). Q_{BAFF} in patients with PCNSL and aseptic meningitis were higher than that in other groups. BAFF and TACI indices in patients with PCNSL were significantly higher than those in other groups; the BAFF index was also increased in patients with aseptic meningitis (Data S4).

Decision tree

To differentiate PCNSL and the other disorders – glioblastoma or multiple sclerosis or neuromyelitis optica or autoimmune encephalitis – a decision tree analysis with CSF levels of TACI and BAFF was performed (Fig. 2). CSF TACI levels of > 12.65 pg/mL in the first step and CSF BAFF levels of > 0.12 ng/mL in the second step were said to lead to a diagnosis of PCNSL. The terminal nodes categorized the study samples into three diagnostic groups according to the probability of PCNSL diagnosis. The combination of CSF BAFF and TACI levels for a diagnosis of PCNSL showed 100% sensitivity and 100% specificity.

Discussion

This study aimed to evaluate the possibility that CSF and serum levels of BAFF and TACI could be diagnostic biomarkers of PCNSL. The study had two major findings: (1) CSF levels of TACI and BAFF increased in patients with PCNSL and (2) serum levels of TACI increased in patients with PCNSL.

PCNSL is a rare variant of extranodal non-Hodgkin lymphoma and is usually of B cell origin. The establishment of PCNSL biomarkers will be useful for early diagnosis and may provide evidence about pathogenesis. TACI and BCMA play major roles in normal and malignant B cells in B cell homeostasis and lymphoid cancers.[12] Other studies reported that CSF levels of TACI and BCMA have potential as useful diagnostic biomarkers.[13,14] In the present study, CSF BAFF and TACI levels in patients with PCNSL were significantly higher than those in other groups. We used a decision tree to confirm the value of CSF BAFF and TACI levels for differentiating PCNSL from glioblastoma and CNS inflammatory diseases. Steroids can work the malignant B cells and reduce inflammation. So, they could affect CSF levels of TACI and BAFF. The average CSF levels of TACI in three PCNSL patients who were receiving steroids (81.7 pg/mL) were lower than those not receiving steroids (161.3 pg/mL). On the other hands, the average CSF levels of BAFF in three PCNSL patients who were receiving steroids (1.5 ng/mL) were higher than those not receiving steroids (0.8 ng/mL). However, this study had no fixed rules of treatments with steroids due to study design. As mentioned above, a prospective, multi-center, clinical interventional study will be necessary to confirm the

Table 1. Clinical features and CSF indices.

	GBM	PCNSL	MS	NMO	AE	AM	OND
n	5	9	11	9	9	5	34
Age, years	52.2 ± 24.1	63.0 ± 9.2	39.5 ± 11.6	53.1 ± 18.9	37.3 ± 14.2	38.6 ± 18.8	67.7 ± 10.2
Sex, female (%)	0 (0.0)	4 (44.4)	8 (72.7)	7 (77.8)	4 (44.4)	2 (40.0)	12 (35.3)
CSF cell count,/μL		17.3 ± 18.9	5.5 ± 7.1	5.1 ± 5.5	7.8 ± 10.2	142.4 ± 158.5	3.5 ± 11.6
CSF protein, ng/dL		166.9 ± 115.8	40.5 ± 17.5	49.9 ± 24.9	47.9 ± 23.9	117.1 ± 100.2	50.9 ± 20.4
Q_{IgG}		0.019 ± 0.017	0.005 ± 0.002	0.005 ± 0.002	0.008 ± 0.006	0.019 ± 0.025	0.004 ± 0.002
Q_{alb}		0.030 ± 0.024	0.006 ± 0.003	0.008 ± 0.004	0.008 ± 0.006	0.021 ± 0.023	0.007 ± 0.003
IgG index		0.606 ± 0.155	0.822 ± 0.470	0.725 ± 0.306	1.071 ± 0.454	0.751 ± 0.182	0.570 ± 0.101

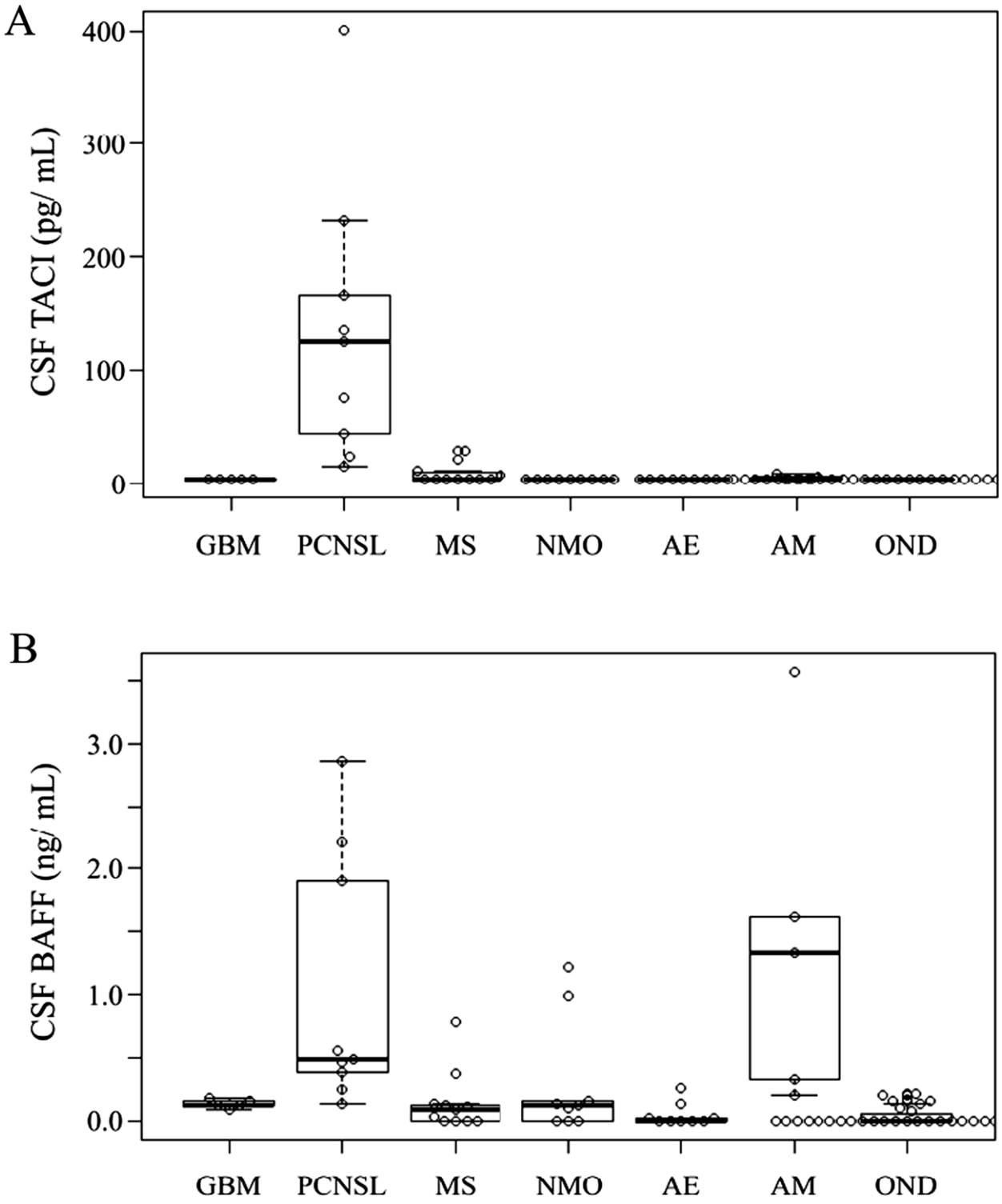

Figure 1. CSF levels of BAFF and TACI as determined by ELISA. (A) CSF levels of TACI in patients with PCNSL (mean: 135.1 pg/mL) were significantly higher than those in other groups ($P < 0.01$). (B) CSF levels of BAFF in patients with PCNSL (mean: 1.0 ng/mL) and aseptic meningitis (mean: 1.4 ng/mL) were higher than those in other groups.

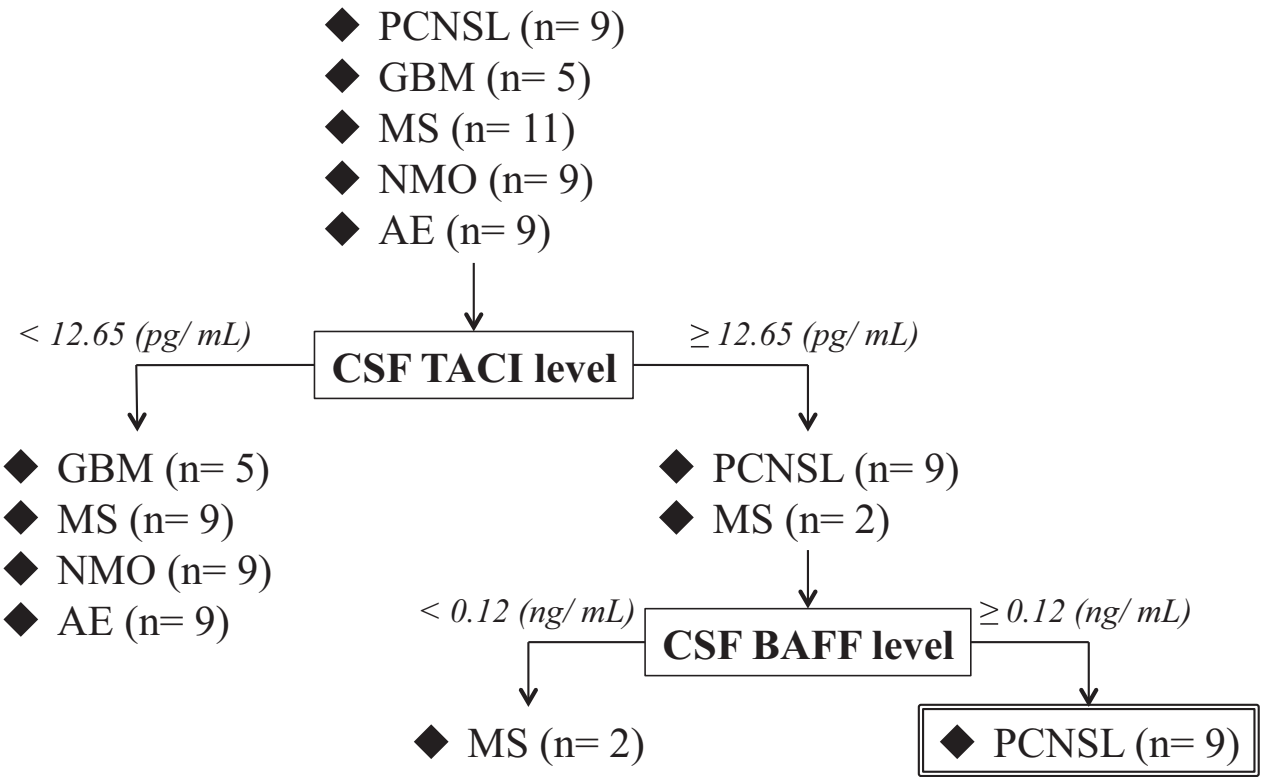

Figure 2. Decision tree. The terminal nodes categorized the study samples into three diagnostic groups according to the probability of PCNSL diagnosis. The combination of CSF BAFF and TACI levels as correlated with diagnosis of PCNSL showed 100% sensitivity and 100% specificity.

relationship among the CSF levels of BAFF or TACI and steroids. Finally we determined, on the basis of the TACI and BAFF indices, that BAFF and TACI were produced intrathecally in patients with PCNSL rather than originating by passive filtration from blood.

The increased CSF BAFF and TACI levels in patients with PCNSL support our hypothesis that malignant lymphoma cells and residual astrocytes, respectively, produce CSF TACI and BAFF in these patients.[1,6,15] In general, BAFF is usually expressed by a few stromal cells, T cells, and most myeloid cell lineages, including monocytes, macrophages, dendritic cells, and stimulated neutrophils,[16] and is a B cell survival factor that is constitutively produced by astrocytes inside the CNS.[9] Although we have not yet defined the cellular source of BAFF and TACI in the CSF, we assume that resident astrocytes and B cells produce BAFF and TACI, respectively.[17] This association may be not only a clue to understanding the pathological mechanism of PCNSL but also an aid to developing treatment applications. Belimumab, a B cell stimulator inhibitor, has significantly reduced systemic lupus erythematosus disease activity.[18] The agents that modulate the BAFF system molecule may thus be useful in PCNSL treatment.

This study had several limitations. First, the study was retrospective and utilized a limited number of CSF and serum samples. Second, institutional bias may exist in this study. A prospective, multi-center, clinical interventional study is necessary to verify the relationships between CSF and serum levels of the BAFF ligand-receptor system, as well as the clinical characteristics including histopathological analysis of PCNSL, because the present study focused on BAFF and its receptor TACI. We will plan to confirm the correlation among these biomarkers, disease activity, and outcome in a forthcoming prospective study.

In conclusion, our analysis of CSF BAFF and TACI levels showed high sensitivity and specificity for the diagnosis of PCNSL. The current study demonstrated that CSF BAFF and TACI levels could be novel, valuable, and noninvasive discriminative biomarkers of PCNSL. Further studies are necessary to clarify the cellular source of BAFF and TACI in the CSF in patients with PCNSL. In addition, a prospective, multi-center, clinical interventional study should be performed to fully elucidate the relationship between the BAFF system molecules in CSF and treatments. Advances in B cell immunology will continue to lead to better understanding of PCNSL pathogenesis and the development of specific B cell-directed therapies.

Author Contributions

Conceived and designed the experiments: HM (Mizutani), SN, and YA. Performed the experiments: HM (Mizutani), SN, KT, NT, and HM (Matsui). Collected the samples and summarized the cases: HM (Mizutani), SN, HN, KT, KM, AM (Mukaino), MW, AM (Mukasa), and YA. Analysed the data: HM (Mizutani), SN, and TI* (* biostatistician). Wrote the paper: HM (Mizutani), SN, TI, AM (Mukasa), and YA.

Conflict of Interest

None of the authors have any conflicts of interest to disclose.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Twenty-five with amyotrophic lateral sclerosis, 4 with Parkinson's disease, 4 with idiopathic normal pressure hydrocephalus, 1 with vitamin B12 deficiency were included in 34 disease controls with OND.

Data S2. To determine BAFF concentrations, serum samples were diluted 1:10 but CSF samples were tested undiluted. CSF and serum samples were both tested undiluted to determine TACI concentrations.

Data S3. Results of multivariate regression model analysis.

Data S4. CSF and serum levels of TACI and BAFF.

