Serum levels of soluble programmed death-ligand 1 (sPD-L1) in patients with primary central nervous system diffuse large B-cell lymphoma

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Abstract

Background The interaction of programmed death-1 (PD-1) and programmed death-1 ligand (PD-L1) produces immunosuppressive activity, protecting tumor cells from anti-tumor immunity and possibly releasing soluble PD-L1 (sPD-L1) from PD-L1 expressing tumor cells. Therefore, we measured serum levels of sPD-L1 in patients with primary central nervous system lymphoma (PCNSL) and explored its clinical implications. Methods Sixty-eight patients with newly diagnosed PCNSL had diffuse large B-cell lymphoma and were treated with high-dose methotrexate-containing chemotherapy. The measurement of sPD-L1 and cytokines was performed using serum samples archived at diagnosis, and the tissue expression of PD-L1 was also analyzed from archived paraffin-embedded tissue blocks. Disease relapse, progression-free survival (PFS), and overall survival (OS) were analyzed according to the extent of sPD-L1 in serum and PD-L1 in tissue. Results The median level of serum sPD-L1 (0.429 ng/mL) was higher than in healthy control patients (0.364 ng/mL). The occurrence of relapse was more frequent in the high sPD-L1 (78%) than the low sPD-L1 group (50%), though the groups did not have different clinical or pathological characteristics at diagnosis. As a result, the OS and PFS for the high sPD-L1 group were significantly lower than those in the low group. PD-L1-positive tumor cells were found in 35 patients (67%), and the extent of PD-L1-postive tumor cells was positively associated with serum sPD-L1 levels ($r = 0.299$, $P = 0.031$). Among the 34 cytokines analyzed, only the serum level of IL-7 correlated with the serum level of sPD-L1 ($r = 0.521$, $P < 0.001$). Conclusions Serum levels of sPD-L1 could reflect the expression of PD-L1 in PCNSL tumor cells and predict patient survival outcomes. Therefore, sPD-L1 in serum could be a feasible biomarker for determining a risk-adapted treatment strategy for PCNSL patients.

Background

Primary central nervous system lymphoma (PCNSL) is a rare but aggressive non-Hodgkin lymphoma (NHL) that is confined to the brain, spinal cord, leptomeninges, and eyes [1]. Most cases of PCNSL have diffuse large B-cell lymphoma (DLBCL) histopathology, but PCNSL accounts for less than 3% of all primary tumors of the CNS and 1 to 2% of all NHLs [2-4]. As the treatment of PCNSL has evolved during the past few decades, the outcomes of patients with PCNSL have improved. Currently, high-dose methotrexate (HD-MTX) is the backbone of the multi-agent chemotherapies used in patients newly diagnosed with PCNSL because of its adequate penetration of blood-brain barrier (BBB) [5, 6]. HD-MTX-containing chemotherapy with or without whole brain radiotherapy (WBRT) has produced response rates of 70 to 90% [7-11]. However, almost 50% of patients relapse within the first two years after diagnosis, and one-third become refractory to conventional chemotherapy [12-14]. Salvage treatment options for relapsed/refractory PCNSL include high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) or WBRT [15-17]. However, responses to those treatments are usually not durable, and ASCT cannot be used for frail elderly patients. Given the high probability of treatment failure and limited treatment options for frail elderly patients, two prognostic scoring systems for PCNSL, the International Extranodal Lymphoma Study Group score and Memorial Sloan-Kettering Cancer Center

score consider both age and performance status [18, 19]. However, those systems might not reflect the biological characteristics of PCNSL.

A genetic evaluation of PCNSL demonstrated frequent copy-number alterations in *9p24.1/PD-L1/PD-L2* that increase the expression of programmed cell death protein 1 (PD-1) ligands PD-L1 and PD-L2 in PCNSL [20]. PD-1 is an inhibitory receptor expressed on activated T cells, and its interaction with PD-L1 plays an important role in suppressing T-cell mediated immune response [21], which could decrease T-cell receptor activation and reduce T-cell proliferation, allowing tumor cells to escape from anti-tumor immunity. Given the role of immune escape in the development of neoplasm, the expression of PD-L1/PD-L2 might play a role in the biology of PCNSL [22]. Indeed, blocking PD-1 with nivolumab, an immune checkpoint inhibitor, has shown single-agent activity in relapsed and refractory PCNSL patients, implying its potential as a salvage treatment for relapsed or refractory PCNSL [23]. However, PD-L1 could also be expressed in tumor-infiltrating non-malignant cells. For example, a recent study revealed the overexpression of PD-L1 in the macrophages/microglia of tissue sections from patients with PCNSL [24]. This might be a troublesome issue for clinical manipulation of PD-L1 expression. Furthermore, tissue biopsies of the brain are not always possible due to the risk of post-biopsy complications such as bleeding or neurologic sequelae. Thus, a marker that could reflect the biological characteristics of tumor cells and be obtained from a liquid biopsy, such as blood sampling, would be more feasible and useful than markers in brain tissue. The soluble programmed death-ligand 1 (sPD-L1) can be secreted from PD-L1 positive cells and has immunosuppressive activity. Thus, sPD-L1 could be used as a biomarker representing the tissue expression of PD-L1. Elevated levels of sPD-L1 were first reported to affect overall survival in DLBCL patients in a previous French multi-center trial [25]. sPD-L1 can easily be measured using an enzyme-linked immunosorbent assay (ELISA), making it practical for clinical use, especially in patients without enough tumor tissue to biopsy [26]. However, the prognostic value of sPD-L1 has never been reported in patients with PCNSL. Therefore, we measured the level of sPD-L1 in patients with PCNSL and analyzed its clinical relevance as a prognostic marker, as well as its correlation with PD-L1 expression in tumor cells.

Methods

Patients

The study population was patients who were diagnosed with PCNSL between January 2009 and February 2017 and registered for our prospective cohort studies after providing written informed consent (NCT00822731 and NCT01877109). Our prospective cohort studies collected serum samples and the pre-treatment characteristics of patients at diagnosis. Treatment and outcome-related data, including treatment regimens, tumor response, date of progression, and date of death, were regularly updated. These cohort studies were approved by the Institutional Review Board of Samsung Medical Center, and all investigations were conducted according to the principles expressed in the Declaration of Helsinki and its contemporary amendments. Because patients with all subtypes of lymphoma were enrolled, the evaluations for work-up and treatments were performed according to our clinical practice for each

subtype. For patients with PCNSL, the initial evaluation was done according to the International Primary CNS Lymphoma Collaborative Group recommendations [27]. Cerebrospinal fluid (CSF) analyses and ophthalmic examinations were also performed in most patients to test for leptomeningeal and ocular invasion. As the primary treatment for newly diagnosed PCNSL, HD-MTX-containing chemotherapy with or without WBRT was used. Response was assessed according to the response criteria for PCNSL recommended by the International Primary CNS Lymphoma Collaborative Group [27]: complete response (CR) was defined as no contrast enhancement in brain magnetic resolution imaging (MRI) and negative findings in ocular and CSF examinations; partial response (PR) was defined as at least a 50% decrease in the enhancing tumor lesion; progressive disease (PD) was defined as at least a 25% increase in the lesion or any new lesion in the CNS or systemic sites; and stable disease (SD) was defined as less than a PR but not PD. Response evaluation was performed after the completion of primary treatment chemotherapy, and surveillance brain MRI was done to monitor the occurrence of disease relapse.

Study design

We retrospectively analyzed 68 patients who had archived serum samples available for measurement of sPD-L1 among patients enrolled in the aforementioned cohort studies, after excluding patients with secondary CNS involvement in systemic DLBCL. Using serum samples and ELISA, we first measured the sPD-L1 levels and correlated them with the clinical and pathological characteristics of the patients at diagnosis. Then, response to first-line therapy and the survival outcomes of patients were compared according to the level of sPD-L1. Second, we analyzed the expression of PD-L1 in tumor cells and non-tumor cells in 52 patients whose paraffin-embedded tissue blocks were available for immunohistochemistry analyses. Third, we measured serum cytokines using multiplex ELISA to explore additional biomarkers that might predict the outcomes of PCNSL patients and influence the level of sPD-L1 or the tissue expression of PD-L1. To confirm the DLBCL histology of our patients with PCNSL, two pathologists (I.C and Y.K) reviewed patients' histopathology slides using the 2017 World Health Organization classification [3]. Relapsed disease was defined as disease recurrence in patients who had no evidence of disease after cessation of therapy, and PD was defined as SD or PD during the primary treatment. Multiple diseases were defined as more than one lesion found in a radiologic evaluation, and deep regions of the brain were defined as the basal ganglia, brainstem, periventricular regions, and cerebellum. We updated the survival status in March 2019 for the survival analysis (IRB No. 2019-05-054).

Measurement of serum sPD-L1

Serum samples were collected at diagnosis and stored at -80°C until analysis. Serum aliquots had not been previously thawed before use in our multiplex chemokine assay. The level of sPD-L1 was measured using ELISA kits (PDCD1LG1 ELISA kit, USCN Life Science, Wuhan, China) according to the manufacturers' instructions. Briefly, the microplate provided in the kit was pre-coated with an antibody specific to PDCD1LG1. Standards or samples were then added to the microplate wells with a biotin-conjugated antibody specific to PDCD1LG1. Next, avidin conjugated to horseradish peroxidase was

added to each microplate well and incubated. After the enzyme-substrate reaction, the color change was measured spectrophotometrically at a wavelength of 450nm. The sPD-L1 values in the blood serum specimens of healthy controls were determined by the same method. The measurement of each sample was done in duplicate.

Immunohistochemistry for tissue PD-L1 expression

Immunohistochemistry was performed on paraffin tissue sections (4-μm thick), and the PD-L1 antibody (Spring Bioscience, CA, USA; clone SP142, M4421, rabbit anti-human PD-L1/CD274, monoclonal antibody, 1:25 dilution) was used to assess the expression of PD-L1. The antibody was incubated for 120 min at 37°C using the Ventana BenchMark XT platform after antigen retrieval for 92 min with CC1 buffer. Signal visualization was done using the OptiView DAB immunohistochemistry detection kit (Ventana, Tucson, Azusa) and OptiView Amplification kit (Ventana, Tucson, Azusa). Tonsil squamous epithelium was used as a PD-L1 immunohistochemistry positive control [28]. The slides were semi-quantitatively analyzed by two pathologists (I. C and Y. K). The extent of PD-L1 expression in tumor cells was defined as the proportion of tumor cells showing PD-L1 expression with any intensity in the tumor area [29]. Macrophages and lymphocytes infiltrating the tumor area were considered non-tumor immune cells, and the proportion of PD-L1 expression in them was assessed in the same manner as in the tumor cells. Tumor cells were discriminated from tumor infiltrating lymphocytes using morphology because the tumor cells had unequivocal morphologic characteristics that allowed discernment. The assessment of PD-L1 expression in tumor infiltrating macrophages was done by measuring PD-L1 expression in CD68-positively stained macrophages. PD-L1-positive tumor cells were defined as those positively stained for PD-L1 with a distinct membranous, cytoplasmic, or punctate/granular pattern of any intensity based on previously published descriptions [29, 30]. The following additional antibodies were used to assess CD68 expression and identify the cell of origin: CD68 (Leica Biosystem, Newcastle, NCL-L-CD68, mouse monoclonal, 1:50 dilution), CD10 (Novocastra, Newcastle, NCL-L-CD10-270, mouse monoclonal, 1:100 dilution), BCL6 (Novocastra, Newcastle, NCL-L-Bcl-6-564, mouse monoclonal, 1:80 dilution), and MUM1 (Dako, CA, M7259, mouse monoclonal, 1:100 dilution).

Multiplex cytokine assay

We measured eotaxin-1, GROα, interferon (IFN)-α, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17α, IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, interferon γ-induced protein (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, regulated on activation T cell expressed and secreted (RANTES), stromal cell-derived factor 1α (SDF1α), tumor necrosis factor (TNF)-α, and TNF-β levels in duplicate with a ProcartaPlex™ multiplex immunoassay kit (Invitrogen, Camarillo, CA, USA) and the Bio-Plex Cytokine Assay System (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions.

Statistical analysis

In the survival analysis, overall survival (OS) was designated as the time from the date of diagnosis to the date of death or last follow-up and progression-free survival (PFS) was designated as the time from the date of diagnosis to the date of progression or relapse, death, or last follow-up. The optimal cutoff value for the survival analysis was determined using the receiver-operating characteristic (ROC) curve method. Kaplan-Meier survival graphs and log-rank tests were used for the univariate survival analyses, and Cox proportional regression models were used for the multivariate analyses. A bi-variate analysis of sPD-L1 and tissue expression of PD-L1 was done to analyze their correlation. The chi-square test or Fisher's exact test were used to analyze their associations with clinical and pathological characteristics. In all comparisons, P-values less than 0.05 were considered statistically significant, and all P-values correspond to two-tailed significance tests. Statistical analyses were carried out using SPSS software version 21.0 (IBM, Armonk, New York, USA).

Results

Characteristics and treatment outcomes of patients

The median age of the 68 patients was 55 years (range: 20–77 years), and 40% of the patients were older than 60 years at diagnosis (Table 1). Female patients were predominant, and most patients had good performance status (Eastern Cooperative Oncology Group grade 0–1). The majority of patients had multiple lesions in the CNS, including 8 cases with combined leptomeningeal and parenchymal involvement, and twenty-seven patients (35%) with a tumor in the deep region of the brain (Table 1). Histologically, the activated B-cell (ABC) type (74%) was dominant over the germinal center type (19%, Table 1). All patients received HD-MTX-containing chemotherapy as follows: MTX 3.5g/m^2 on day 1, vincristine 1.4g/m^2 on day 1, and procarbazine 100mg/m^2 on day 1-7 every two weeks (total 5 cycles). Among the 68 patients, 90% completed the planned cycle of HD-MTX chemotherapy (5 cycles in 10 weeks); 7 patients failed to complete the planned treatment due to lack of response or intolerance to treatment (Table 2). Intrathecal chemotherapy and WBRT were performed as adjuvant treatments at the physicians' discretion. Although 97% of patients responded to the primary treatment, disease relapse occurred in 57% of patients, and ASCT was done in 12 patients after salvage therapy. At the time of analysis, 39 patients were alive, and 29 patients had died (Table 2).

Serum level of soluble PD-L1

The median level of patients' serum sPD-L1 (n = 68) was 0.429 ng/mL (range: 0.324–0.757 ng/mL), which was significantly higher than in the healthy control group (0.364 ng/mL; range: 0.329–0.390 ng/mL, $P < 0.01$, Figure 1A). The distribution of serum sPD-L1 among the 68 patients followed the normal distribution (Figure 1B). The optimal cutoff for predicting OS was 0.432 ng/mL, and the area under the curve of sPD-L1 for OS was 0.739 (Figure 1C). According to this cutoff value, we dichotomized patients into low and high groups (< 0.432 ng/mL versus ≥ 0.432 ng/mL). The OS and PFS of the high group were significantly lower than those of the low group (Figure 1D, E). When we compared the low and high group patients' pre-treatment characteristics at diagnosis, we found no significant differences

between them (Table 1). In other words, unfavorable parameters such as multiple lesions, deep brain involvement, ABC type, and high Ki67 were not associated with the high sPD-L1 group. All patients received the same treatment: HD-MTX combination chemotherapy. Furthermore, their initial response to that therapy did not differ between groups either. However, relapse and progression were more frequent in the high sPD-L1 group (78%, 25/32) than in the low sPD-L1 (50%, 18/36) group (P = 0.023, Table 2). As a result, the number of deaths in the high group (n = 20) was significantly higher than in the low group (n = 9, Table 2).

Tissue expression of PD-L1

Out of 52 patients who were analyzed for tissue expression of PD-L1, positively stained tumor cells were found in 35 patients (67%, 35/52), whereas 17 patients did not show the presence of PD-L1-positive tumor cells. Among the 35 patients with PD-L1-positive tumor cells, the median percentage was 0.7% and 20 patients (57%) had less than 1% of positivity. The extent of PD-L1 expression in the first and second quartiles (Q1, Q2) of serum sPD-L1 was lower than that in the third and fourth quartiles (Q3, Q4; Figure 2A). In other words, the distribution of PD-L1-postive tumor cells showed a modest association with serum levels of sPD-L1 (r = 0.299, P = 0.031, Figure 2B). The median percentage of positively stained immune cells, including macrophages, was 2.7% (Q1: 1.2%–Q3: 6.3%); however, the percentage of PD-L1-postive immune cells did not correlate with serum sPD-L1 levels (Figure 2C). The OS and PFS of patients without PD-L1-positive tumor cells did not differ significantly from those of patients with less than 1% or ≥ 1% of PD-L1-positive tumor cells, although the survival curves showed a trend of poor OS and PFS in patients with ≥ 1% of PD-L1-positive tumor cells (Figure 2D, E). When patients were grouped according to the percentage of PD-L1-positive immune cells (0% versus < 3% versus ≥ 3%), the OS and PFS did not differ significantly (Figure 2F, G). In patients without PD-L1-postive tumor cells, the high sPD-L1 group showed a better OS trend than the low sPD-L1 group, although that trend was not significant because of the small number of patients (Figure 3A). Likewise, the high sP-L1 group showed a trend of better OS in patients without PD-L1-postive immune cells (Figure 3B). In other words, the OS of patients with low sPD-L1 and without PD-L1-positive tumor cells did not differ from that of patients with low sPD-L1 with PD-L1-positive tumor cells (Figure 3C).

Serum cytokines and sPD-L1

The serum levels of cytokines from 68 patients were measured to explore their association with survival outcomes and the serum level of sPD-L1. Of the 34 cytokines measured in this study, 15 cytokines (eotaxin-1, GROα, IFN-α, IL-1α, IL-7, IL-10, IL-18, IL-23, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, SDF1α, and TNF-α) could be analyzed; the remaining cytokines were not detectable in the majority of cases. In the ROC analysis for OS with these 15 cytokines, none of them showed more than 0.6 of area under the curve. Thus, an optimal cutoff for OS could not be obtained, and their levels were not associated with OS. However, the serum level of IL-7 did correlate with the serum level of sPD-L1 (r = 0.521, P < 0.001, Figure 3D): the high sPD-L1 group had higher IL-7 levels than the low sPD-L1 group (Figure 3E). The comparison of OS based on the median value of Il-7 showed a trend of worse OS for

patients in the high IL-7 group compared with those in the low IL-7 group, but that trend was not statistically significant (Figure 3F).

Discussion

Since an association between sPD-L1 and prognosis was demonstrated in patients with renal cell carcinoma, the prognostic implications of sPD-L1 have been suggested in several solid cancers and hematologic malignancies [31-35]. A meta-analysis evaluating eight studies and 1,102 patients with cancers of the lung, stomach, liver, and biliary tract; lymphoma; and myeloma indicated that a higher level of sPD-L1 was associated with worse OS (HR = 1.60, 95% CI: 1.21–1.99) [36]. However, the clinical relevance of sPD-L1 in patients with PCNSL has never been reported. In this study, we measured sPD-L1 from the archived serum samples of patients with PCNSL, and the median level in those patients (0.429 ng/mL, range: 0.324–0.757 ng/mL) was lower than the previously reported values in other hematologic malignancies such as DLBCL (1.84 ng/mL), extranodal NK/T-cell lymphoma (2.76 ng/mL), and multiple myeloma (4.15 ng/mL) [25, 34, 37]. The low level of sPD-L1 in PCNSL might be associated with the peculiar characteristics of PCNSL: tumors confined to the CNS have relatively small volume, and the BBB might influence the level of sPD-L1 circulating in blood. Nevertheless, the serum level of sPD-L1 was significantly higher in patients with PCNSL than in healthy controls, and it had prognostic value for survival outcomes. The comparison of clinical and pathological characteristics based on serum sPD-L1 levels showed no differences between the high and low sPD-L1 groups in deep region involvement, multiple lesions, cell of origin, or cell proliferation. Furthermore, even though most patients responded to the HD-MTX-containing chemotherapy, the high sPD-L1 group showed more frequent relapse or progression (78%, 25/32), resulting in more deaths (63%, 20/32, Table 2) compared with the low sPD-L1 group. These results are consistent with the relationship between poor prognosis and high expression of PD-L1 in tumor cells [38, 39]. Because overexpression of PD-L1 in tumor cells is related to the downregulation of effector T-cell function and represents a potent mechanism of tumor immune evasion [40], our findings imply a possible role for sPD-L1 in allowing tumor cells to escape from anti-tumor immunity, similar to the T-cell exhaustion through the immune checkpoint mechanism seen in the PD1/PD-L1 axis [41, 42]. Thus, patients with elevated levels of sPD-L1 might be more likely to have surviving residual tumor cells after HD-MTX-containing chemotherapy. Accordingly, the serum level of sPD-L1 could act as a reliable biomarker to predict the probability of relapse and survival outcome of patients with PCNSL.

Because circulating sPD-L1 could be secreted by PD-L1-positive cells, we analyzed the association between serum levels of sPD-L1 and the percentage of PD-L1-positive tumor cells. Previous studies suggested that both tumor cells and immune cells could produce sPD-L1 by demonstrating a correlation between sPD-L1 and tissue expression of PD-L1 [43]. However, our results revealed a lack of correlation between serum levels of sPD-L1 and PD-L1 expression in immune cells. Our results might indicate the relative importance of the contribution of PD-L1-positive tumor cells to the serum level of sPD-L1 compared with PD-L1 expression by immune cells. In line with those findings, the survival analysis based on the extent of PD-L1 expression in tumor cells showed a better association with PD-L1 expression in

tumor cells than in immune cells (Figure 2). These findings might indicate that a high sPD-L1 level could be a surrogate marker for tumor PD-L1 expression in patients with PCNSL. However, our study failed to show a significant association between PD-L1 expression in tumor cells and survival outcomes. Although that lack of association could result from various causes, including the relatively small number of patients, our inability to determine an optimal cutoff for survival outcomes might have influenced our results. To date, no optimal cutoffs for PD-L1 expression in PCNSL have been established, and various cutoffs for PD-L1 have been used in PCNSL patients (Table 3). This might be associated with differences in PD-L1 antibody clones, immunohistochemistry protocols, and the scoring systems used. Thus, the measurement of sPD-L1 in serum could be a feasible and reproducible test for assessing the status of tissue PD-L1 expression in patients with PCNSL.

In this study, we also analyzed the association between cytokine profiles and survival outcomes and serum sPD-L1 levels in patients with PCNSL. Cytokines in the tumor microenvironment contribute to the growth and survival of tumor cells, and we previously demonstrated an association between inflammatory cytokines and the outcomes of lymphoma patients [44-46]. However, the 34 cytokines evaluated in our study failed to show a significant association with the survival outcomes of PCNSL patients. On the other hand, serum levels of sPD-L1 were significantly related to serum IL-7, which is mainly produced by non-lymphoid cells regulating T-cell receptor γ rearrangement [47]. Our results were consistent with a previous study reporting that γ-chain cytokines such as IL-2, IL-7, IL-15, and IL-21 could induce PD1 and PD-L1 expression [48]. Thus, although IL-7 failed to show a significant association with survival outcomes in PCNSL, it might increase the serum levels of sPD-L1 by increasing the tissue expression of PD-L1.

Conclusions

In conclusion, our study demonstrated that serum levels of sPD-L1 could reflect the expression of PD-L1 in PCNSL tumor cells and predict the survival outcomes of patients. Thus, sPD-L1 in serum could be a feasible biomarker for determining risk-adapted treatment strategies for PCNSL patients, including the use of PD-1 inhibitors.

Abbreviations

PD-1: programmed death-1; PD-L1: programmed death-1 ligand; PCNSL: primary central nervous system lymphoma; ROC: receiver-operating characteristic; HD-MTX: high-dose methotrexate; BBB: blood-brain barrier; WBRT: whole brain radiotherapy; IFN: interferon: IP-10: interferon γ-induced protein; MCP-1: monocyte chemoattractant protein 1; MIP-1α: macrophage inflammatory protein-1α; RANTES: regulated on activation T cell expressed and secreted; SDF1α: stromal cell-derived factor 1α; TNF)-α: tumor necrosis factor (TNF)-α

Declarations

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Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Authors' contributions

Conception and design: SJ Kim. Acquisition of data: SE Yoon, YH Ko, WS Kim, SJ Kim. Analysis and interpretation of data: I Cho, H Lee, KJ Ryu, YH Ko. Drafting of manuscript: I Cho, H Lee, SJ Kim. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by an ethical board (IRB No. 2019-05-054). Written informed consent was obtained from all eligible participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Patient characteristics

	All patients (n = 68)		Low sPD-L1 (n = 36)		High sPD-L1 (n = 32)		
	n	(%)	*n*	(%)	*n*	(%)	*P*
Age (years)							
≤ 60	41	(60)	22	(61)	19	(59)	0.884
> 60	27	(40)	14	(39)	13	(41)	
Sex							
Male	27	(40)	13	(36)	14	(44)	0.622
Female	41	(60)	23	(64)	18	(56)	
ECOG PS							
0-1	56	(82)	33	(92)	23	(72)	0.054
≥ 2	12	(18)	3	(8)	9	(28)	
Serum Lactate dehydrogenase							
Normal	54	(79)	29	(81)	25	(78)	> 0.999
Elevated	14	(21)	7	(19)	7	(22)	
Number of lesions							
Single	11	(16)	7	(19)	4	(12)	0.521
Multiple	57	(84)	29	(81)	28	(88)	
Brain deep region involvement							
Absence	44	(65)	23	(64)	21	(66)	> 0.999
Presence	24	(35)	13	(36)	11	(34)	
Cell of origin subtype							
GCB	13	(19)	8	(22)	5	(16)	0.719
ABC	50	(74)	25	(69)	25	(78)	
Not evaluated	5	(7)	3	(8)	2	(6)	
Ki67							
< 90	23	(34)	11	(31)	12	(38)	0.769
≥ 90	42	(62)	23	(64)	19	(59)	
Not evaluated	3	(4)	2	(6)	1	(3)	

Table 2. Treatment and patient outcomes

	All patients (n = 68)		Low sPD-L1 (n = 36)		High sPD-L1 (n = 32)		
	n	(%)	*n*	(%)	*n*	(%)	*P*
HD-MTX chemotherapy							
Completed	61	(90)	32	(89)	29	(91)	> 0.999
Failed to complete	7	(10)	4	(11)	3	(9)	
Response to HD-MTX treatment							
CR/PR	54/12	(97)	30/6	(100)	24/6	(94)	0.486
SD/PD	1/1	(3)	0/0	(0)	1/1	(6)	
Intrathecal chemotherapy							
Combined	26	(38)	10	(28)	16	(50)	0.081
Not combined	42	(62)	26	(72)	16	(50)	
Whole brain radiotherapy							
Done	45	(66)	24	(67)	21	(67)	> 0.999
Not done	23	(34)	12	(33)	11	(34)	
Relapse after primary treatment							
No relapse	29	(43)	18	(50)	11	(34)	0.226
Relapse	39	(57)	18	(50)	21	(66)	
Autologous stem cell transplantation							
Done	12	(18)	7	(19)	5	(16)	0.758
Not done	56	(82)	29	(81)	27	(84)	
Relapse or progression							
None	25	(37)	18	(50)	7	(22)	0.023
Occurred	43	(63)	18	(50)	25	(78)	
Survival status							
Alive	39	(57)	27	(75)	12	(37)	0.003
Dead	29	(43)	9	(25)	20	(63)	

Table 3. Summary of studies evaluating PD-L1 expression in primary CNS lymphoma

	Type of specimen	PD-L1 clone	Cut-off for PD-L1 expression	Staining pattern	Frequency of PD-L1 expression
Berghoff *et al* *[49].*	Whole slide	Clone 5H1 (Abcam)	≥ 5% of tumor cells	Membranous	2/20 (10%)
Four *et al* *[50].*	Tissue microarray	Clone SP142 (Ventana)	≥ 1% of tumor cells	Membranous and cytoplasmic	12/32 (37.5%)
Hayano *et al* *[51].*	NA	Clone E1L3N (Cell Signaling Technology)	NA	Membranous	2/64 (4.1%)
Cho *et al* *[52].*	Tissue slide	Clone ab58810 (Abcam)	≥ 100 cells/HPF of tumor cells	NA	10/76 (13.2%)
Y Sugita *et al* *[53].*	Tissue slide	Clone EPR1161 (Abcam)	no staining (−); 0–30% (1+); 30–60% (2+); > 60% (3+) in both tumor cells and TAM	Membranous	12/17 (70.6%) in EBV-positive cases. 11/22 (50%) in EBV-negative cases.

TAM: tumor associated macrophages; HPF: high power field; NA: not available; EBV: Epstein-Barr virus

Figures

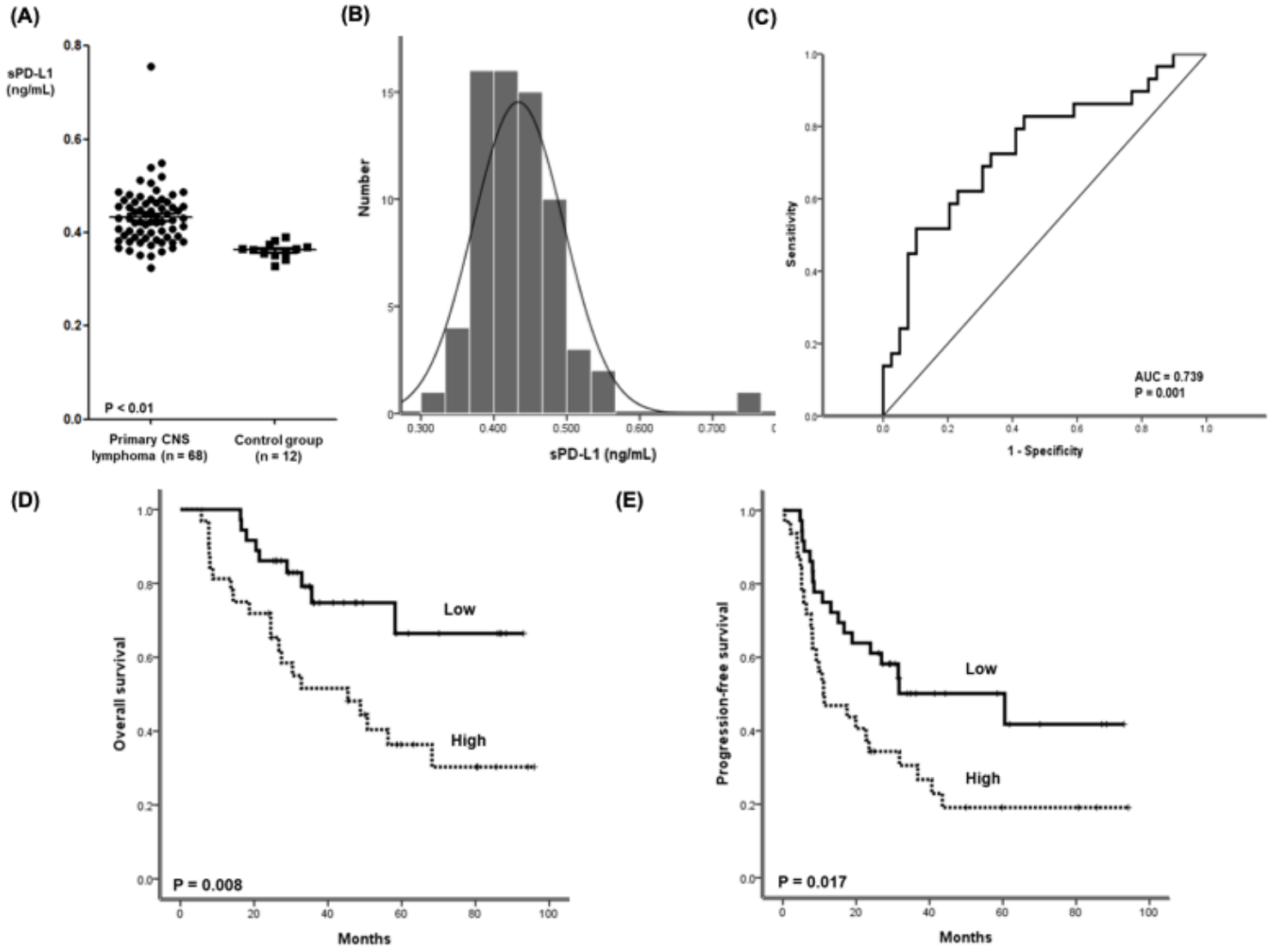

Figure 1

(A) Comparison of serum sPD-L1 between patients with PCNSL (n = 68) and the healthy control group (P < 0.01). (B) The distribution of serum sPD-L1 in 68 patients. (C) The ROC curve of sPD-L1 for overall survival. (D) The overall survival and progression-free survival in the high sPD-L1 group were lower than those in the low sPD-L1 group.

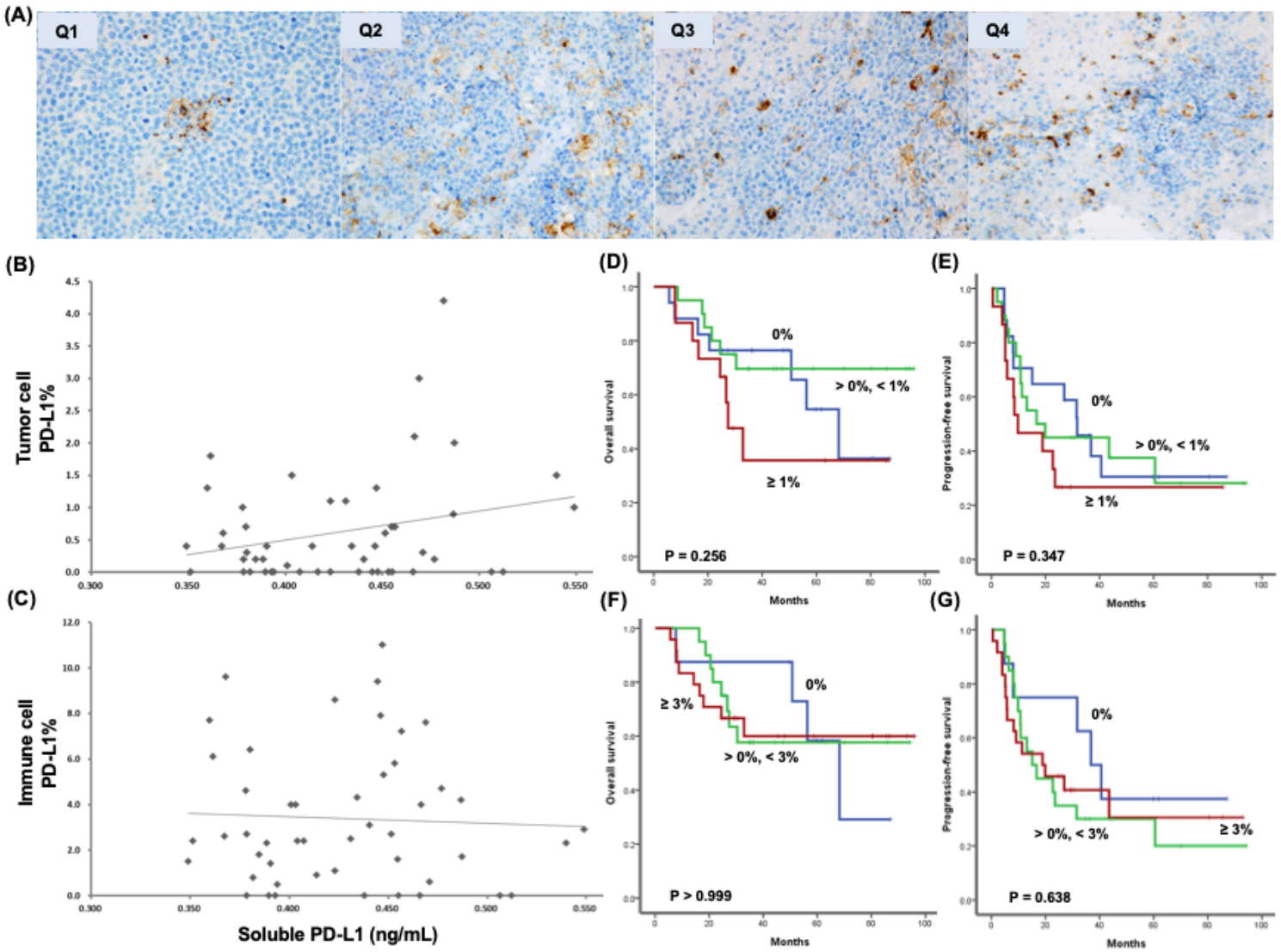

Figure 2

(A) The extent of PD-L1 expression in the first and second quartiles (Q1, Q2) of serum sPD-L1 was lower than that in the third and fourth quartiles (Q3, Q4). (B) The association between PD-L1-postive tumor cells and serum levels of sPD-L1 (r = 0.299, P = 0.031). (C) The percentage of PD-L1-postive immune cells did not correlate with serum levels of sPD-L1. (D, E) Comparison of overall and progression-free survival based on the percentage of PD-L1-positive tumor cells: < 1% versus ≥ 1%. (F, G) Comparison of overall and progression-free survival based on the percentage of PD-L1-positive immune cells: 0% versus < 3% versus ≥ 3%.

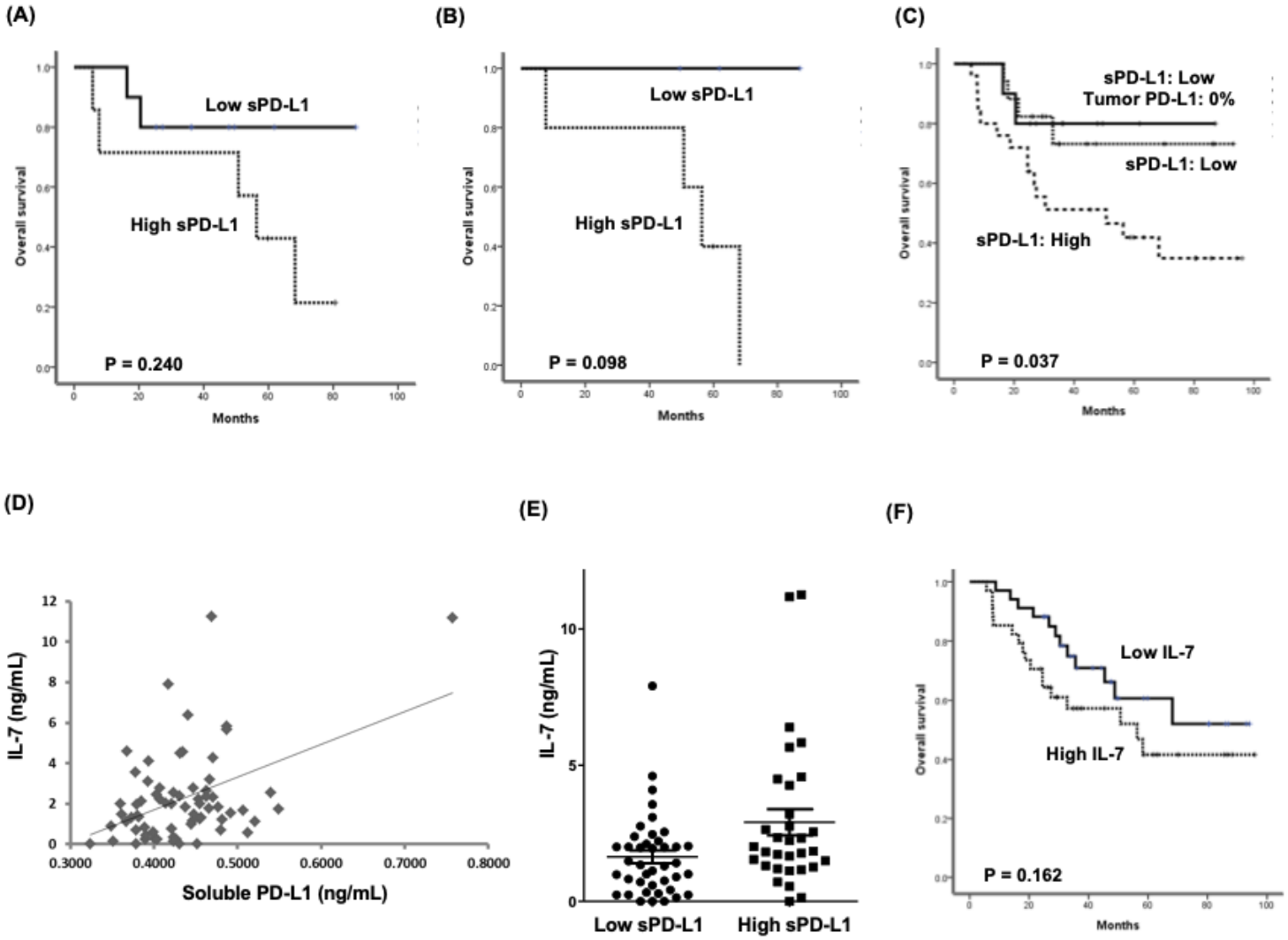

Figure 3

(A) In patients without PD-L1-postive tumor cells, the high sPD-L1 group showed a trend of better OS than the low sPD-L1 group. (B) The high sP-L1 group also showed a trend of better OS in patients without PD-L1-postive immune cells. (C) The overall survival of patients with low sPD-L1 and without PD-L1-positive tumor cells did not differ from that of patients with both low sPD-L1 and tissue PD-L1 expression. (D) The association between serum IL-7 levels and serum sPD-L1 levels ($r = 0.521$, $P < 0.001$). (E) The high sPD-L1 group showed a higher level of IL-7 than the low sPD-L1 group. (F) Comparison of overall survival based on the median value of Il-7 showed a trend of worse OS for patients in the high IL-7 group compared with those in the low IL- group.