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High Serum Level of Soluble Programmed Death Ligand 1 is Associated With a Poor Prognosis in Hodgkin Lymphoma

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Abstract

Blockade of the programmed cell death 1-programmed cell death ligand 1 pathway is a new and promising therapeutic approach in Hodgkin lymphoma (HL). To our knowledge, the impact of soluble programmed cell death ligand 1 (sPD-L1) serum levels on HL patient prognosis has not yet been investigated. In this study, the prognostic value of sPD-L1 was assessed in patients with HL. We measured serum sPD-L1 levels and identified their prognostic value in 108 newly diagnosed HL patients using an enzyme-linked immunosorbent assay (ELISA). We found higher serum sPD-L1 concentrations in HL patients than in healthy controls. The best sPD-L1 cutoff value for predicting disease progression risk was 25.1674 ng/ml. The 4-year progression-free survival (PFS) rates for the high-sPD-L1 and low-sPD-L1 groups were 78.8% and 93.3%, respectively. Multivariate survival analysis showed that advanced stage and higher sPD-L1 levels (>25.1674 ng/ml) were independent prognostic factors for shorter PFS. In addition, higher sPD-L1 levels were positively correlated with advanced stage and negatively correlated with peripheral blood monocyte number. The serum sPD-L1 level is an independent prognostic factor for PFS in HL patients and may allow identification of a subgroup of patients who require more intensive therapy and who may benefit from anti-PD-1 agents.

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Introduction

Hodgkin lymphoma (HL) is a rare cancer that originates from B lymphocytes and accounts for approximately 11% of all lymphoma cases and 0.5% of all cancers [1]. Standard treatment of newly diagnosed HL often involves a combination of multi-agent chemotherapy and radiotherapy, tailored to the stage of disease and the risk of relapse; this

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treatment can cure approximately 80% of patients [2]. Unfortunately, 20% of HL patients still relapse or develop refractory HL, for which effective treatment options are limited [3,4]. Second-line salvage with high-dose chemotherapy (HDC) and autologous stem cell transplantation (auto-SCT) has become the standard care for refractory/relapsed HL, leading to long-lasting responses in approximately 50% of patients [5]. However, disease recurrence or progression after auto-SCT is associated with a very poor prognosis. Thus, alternative therapies, such as antibody-drug conjugates (anti-CD30) [6] and immune checkpoint blockade drugs (anti-PD-1 and anti-PD-L1) [7,8] may be necessary. Furthermore, identification of patients with high risk of relapse is crucial in HL treatment.

Cancer cells have been shown to escape immune surveillance by up-regulating surface molecules that directly induce T-cell suppression [9]. These mechanisms are known as immune checkpoint pathways. Programmed cell death-1 (PD-1), an immune checkpoint expressed on the surface of T-, B- and natural killer (NK) cells, is indicative of this phenotype, and signaling through its ligands, programmed cell death ligand-1 (PD-L1) and programmed death ligand-2 (PD-L2), can attenuate signaling through the T-cell receptor (TCR) and lead to anergy/apoptosis and contribute to immune escape [10,11]. Recent clinical trials have shown that PD-1-blocking antibodies can enhance immunity in solid tumors and several hematologic malignancies, resulting in durable clinical responses [12–16]. Nivolumab and pembrolizumab, PD-1-blocking antibodies, both received breakthrough therapy designation from the FDA for HL patients [17–19].

Previous studies have indicated that PD-L1 overexpression was associated with poor survival in most solid tumors and hematopoietic malignancies [20,21]. However, the value of PD-L1 as a prognostic factor remains controversial [22]. There is an association between PD-L1 protein expression and relative genetic alterations in classical HL (cHL). For example, progression-free survival (PFS) has been shown to be significantly shorter for patients with 9p24.1 amplification, which up-regulates PD-L1 expression [23]. PD-L1 expression can be detected on the surface of tumor and immune cells by immunohistochemistry (IHC) [24] and in blood samples by enzyme-linked immunosorbent assay (ELISA) [25]. Serum sPD-L1 levels are reportedly higher in patients with malignant cancer than in healthy individuals, and high sPD-L1 was found to be a poor prognostic factor for hematopoietic malignancies in recent studies [26]. However, no investigations have assessed the relationship between serum sPD-L1 levels and HL patient prognosis. Therefore, the present study was conducted to address this issue. In addition, we also explored the correlation between serum sPD-L1 levels and the clinicopathological characteristics and immunologic features of HL patients.

Materials and Methods

Patients

In total, 108 consecutive patients diagnosed with HL and treated in Sun Yat-Sen University Cancer Center between May 2005 and April 2015 were enrolled in our study. The criteria included a primary diagnosis of HL, serum at diagnosis was available, and complete follow-up information. This study was approved by the Sun Yat-Sen University Cancer Center Research Ethics Board and informed consent for use of patient samples and publication was obtained from all patients.

Treatments and Response Evaluation

Patients were clinically staged according to the Ann Arbor staging system and treated with risk-adapted treatment strategies. First-line treatment involved ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) or COPP (cyclophosphamide, vincristine, procarbazine and prednisone) chemotherapy, and some advanced stage patients underwent BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) chemotherapy in standard doses. The treatment courses, which comprised four to eight cycles, were based on the chemotherapy response. Radiotherapy was conducted depending on patients' age, risk group, residual tumor and response to chemotherapy. Treatment response was evaluated after every two cycles based on the World Health Organization (WHO) evaluation criteria. Routine follow-up imaging analyses were performed every 3 months for the first 2 years, every 6 months for the next 3 years, and annually (or whenever clinically indicated) thereafter.

Soluble PD-L1 Measurement

Patient serum was collected at diagnosis before treatment from all 108 patients and from 15 healthy individuals matched for sex and age with enrolled patients and stored as 500 ml aliquots at –80°C. sPD-L1 was measured using an enzyme-linked immunosorbent assay (PDCD1LG1 ELISA kit, USCN Life Science, catalogue: SEA788Hu) according to the manufacturer's instructions. The minimum detectable concentration of sPD-L1 was 0.057 ng/ml. Each sample was analyzed in duplicate. The intra-assay and inter-assay coefficients of variation were below 20%. Briefly, samples and standards were added to a microplate precoated with a PD-L1-specific monoclonal antibody. After enzyme reagent and any unbound antibody were removed by washing, a substrate solution was added to the wells, Stop Solution was used to terminate color development, and the absorbance value was read at 450 nm using a spectrophotometer (Tecan, Mannedorf, Switzerland). The sPD-L1 concentrations were calculated using a standard curve, which was constructed using the standards provided in the kit.

Statistical Analysis

Receiver operating characteristic (ROC) curve analysis was performed to determine the best cutoff value for the sPD-L1 concentration [27]. In this ROC curve, the point with the maximum sensitivity and specificity was selected as the cutoff value. Correlations between sPD-L1 concentration and various clinicopathological parameters were assessed using a Mann-Whitney *U* test or Wilcoxon-matched test, and a chi-squared test or Fisher's exact test was used for categorical values. Overall survival (OS) was defined as the time between the first day of diagnosis and the date of death from any cause; the follow-up of surviving patients was censored at their latest follow-up date. PFS was defined as the time between the first day of diagnosis and the date of disease relapse or progression; the follow-up of surviving patients was censored at their latest follow-up date. OS or PFS was analyzed using Kaplan-Meier curves, which were compared using log-rank tests. Multivariate prognostic analyses of OS or PFS were performed using the Cox proportional hazards regression model. All statistical analyses were performed using SPSS version 21.0. The results were considered statistically significant when $P < .05$.

Results

Patient Characteristics and Baseline Serum sPD-L1 Levels

Patient Characteristics. In total, 108 HL patients were enrolled in our study. The median age at diagnosis was 34.6 years of age (range, 4~76 years), and the study included more male patients (68 cases) than

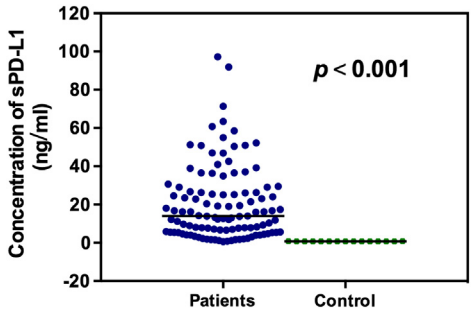

Figure 1. Serum sPD-L1 levels in patients with HL and in healthy controls.

female patients (40 cases). Most patients were diagnosed with cHL, and 9 were diagnosed with nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). According to the Ann Arbor stage, patients were divided into Stage I (7, 6.5%), Stage II (57, 52.8%), Stage III (24, 22.2%) and Stage IV (20, 18.5%). There were 44 patients with B symptoms and 11 with bulky disease (mediastinal mass ratio ≥10 cm or ≥0.33×). All patients received first-line chemotherapy or chemo-radiotherapy treatment.

Baseline Serum sPD-L1 Levels. The mean sPD-L1 concentration for HL patients was 20.5039 ng/mL, which was much higher than that of healthy controls (0.722 ng/mL, $P < .001$; Figure 1).

Correlation of Serum sPD-L1 Levels with Clinical Characteristics and Inflammatory Markers

The Best Cutoff Value. An optimal cutoff value of 25.1674 ng/mL was defined by ROC curves for newly diagnosed HL patients, with an area above the curve (AUC) value of 0.643[95% confidence interval (CI) 0.473–0.814, $P = .107$] (Figure 2). According to this cutoff value, 33 patients (30.6%) were placed into the high-sPD-L1 group (>25.1674 ng/ml), and the remaining 75 patients (69.4%) were placed into the low-sPD-L1 group (≤25.1674 ng/ml). The sensitivity and specificity were 58.3% and 72.9%, respectively.

Correlation Between Serum sPD-L1 Levels and Clinical Characteristics. There was no significant correlation between sPD-L1 level and gender, pathological type, clinical stage, bulky disease, age, a less than partial response (PR) after 2 cycles of chemotherapy or relapse rate($P > .05$). However, higher sPD-L1 levels were positively correlated with advanced stage ($P = .036$) (Table 1).

The association between sPD-L1 levels and inflammatory markers was explored. As shown in Table 2, patients with a low peripheral blood monocyte number ($P = .029$) had higher serum sPD-L1 levels. There were no correlations between serum sPD-L1 levels and other inflammatory markers ($P > .05$).

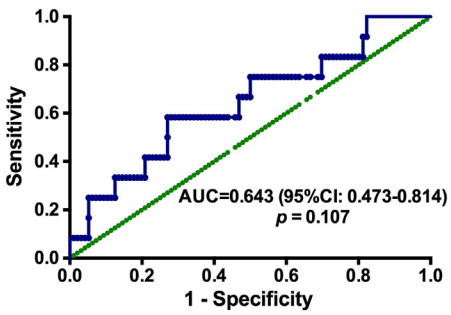

Figure 2. ROC curve analysis for the optimal cutoff point of serum sPD-L1 concentration.

Table 1. Correlation Between Serum sPD-L1 Levels and HL Patients' Clinical Characteristics

Characteristics	Serum sPD-L1 Level		*P*
	High (>25.16 ng/ml) (n=33)	Low (≤25.16 ng/ml) (n=75)	
Age			.096
≤35	15	47	
>35	18	28	
Gender			.597
Male	22	46	
Female	11	29	
Histological type			.345
cHL	29	70	
NLPHL	4	5	
Ann Arbor Stage			.036
Limited	20	44	
Advanced	13	31	
B symptoms			.258
Yes	17	27	
No	16	48	
Bulky			.258
Yes	5	6	
No	28	69	
TR after 2 cycles of chemotherapy			.091
CR or PR	14	45	
Less than PR	19	30	
Relapse or not			.079
Yes	6	12	
No	27	63	

Abbreviations: cHL, classical Hodgkin lymphoma; NLPHL, nodular lymphocyte-predominant Hodgkin lymphoma; TR after 2 cycles of chemotherapy, treatment response after 2 cycles of chemotherapy; CR, complete response; PR, partial response; sPD-L1, soluble programmed death ligand-1.

The Survival Rate of All HL Patients

The Survival Rate of All Newly Diagnosed HL Patients. The median follow-up time was 47 months (1-178 months). The 4-year PFS rate was 80%, with 12 patients relapsing with HL after treatment, and the estimated 4-year OS rate was 95%, with 4 patients dying of cancer. In addition, of all the patients, three developed a second cancer after HL treatment, including NK/T-cell lymphoma (1), infantile fibrosarcoma (1), and gastric carcinoma (1). With suitable and timely treatment, all 3 patients have survived thus far.

Correlation of Serum sPD-L1 Level and Other Clinical Factors With Survival. Patients with sPD-L1>25.1674 ng/ml had significantly lower 4-year-PFS compared with those with PD-L1≤25.1674 ng/ml (78.8% vs 93.3%, $P = .028$; Figure 3, *A-1*), but there was no difference in OS for different sPD-L1 levels (Figure 3, *A-2*). At the same time, advanced staged and<PR after two cycles of chemotherapy were also related to lower 4-year-PFS rates (Figure 3, *B-1* and *C-1*). Specifically, the patients with lower sPD-L1 levels (≤25.1674 ng/mL), limited stage and complete response (CR) or PR after two cycles of chemotherapy had significantly longer PFS ($P < .05$). On the other hand, only patients with limited stage HL had a longer 4-year-OS ($P = .013$) (Figure 3, *B-2*).

Correlation Between Serum sPD-L1 Levels and Prognostic Factors

As shown in Table 3, patients with lower sPD-L1 levels (≤25.1674 ng/ml), limited stage and good treatment response (CR or PR) after 2cycles of chemotherapy had higher PFS rates ($P < .05$). However, histological type, B symptoms, bulky disease, and radiotherapy or not, did not affect

Table 2. Correlation Between Serum sPD-L1 Levels and Inflammatory Markers in HL Patients

	r	*P*
WBC	0.149	.123
LYM	0.118	.222
NEU	0.160	.102
MON	- 0.210	.029
HBG	0.092	.346
PLT	0.155	.110
Albumin	0.125	.210

Abbreviations: WBCs, peripheral blood white blood cells.

long-term outcomes ($P > .05$). A multivariate survival analysis, including stage, treatment response, and sPD-L1 level, showed that limited stage, lower sPD-L1 levels (≤25.1674 ng/ml) and good treatment response (CR or PR) after 2cycles of chemotherapy were independent prognostic factors for longer PFS, but none were predictive of OS.

Discussion

We investigated serum levels of sPD-L1 in HL patients to identify any correlations with patient characteristics and survival outcomes. We found that serum sPD-L1 concentrations in HL patients were much higher than in healthy individuals. In HL patients, serum sPD-L1 levels were correlated with Ann Arbor stage and the immune-related factor peripheral blood monocyte number. Of note, PFS was significantly shorter for patients with an increased pretreatment serum sPD-L1 level. Furthermore, a Cox regression model, including serum sPD-L1 level, stage and treatment response after 2 cycles of chemotherapy, suggested that higher serum sPD-L1 level (>25.1674 ng/mL), advanced stage and poor early chemotherapy response were noteworthy adverse independent prognostic factors for PFS.

A prognostic role of serum sPD-L1 level had been reported for several tumor types, and the majority of data suggest that sPD-L1 is relevant to poor prognosis [28–32]. Among the studies, more than three directly compared sPD-L1 levels in patients with those in healthy controls and showed that cancer patients had significantly higher sPD-L1 levels [26,33]. In addition, the work of Wang H et al. indicated that post-treatment sPD-L1levels were lower than pretreatment sPD-L1 levels in ENKTCL patients who achieved complete remission after standard treatment [34]. Here, we measured serum sPD-L1 levels in HL patients and in healthy volunteers and confirmed that serum sPD-L1 levels were higher in HL patients than in healthy controls.

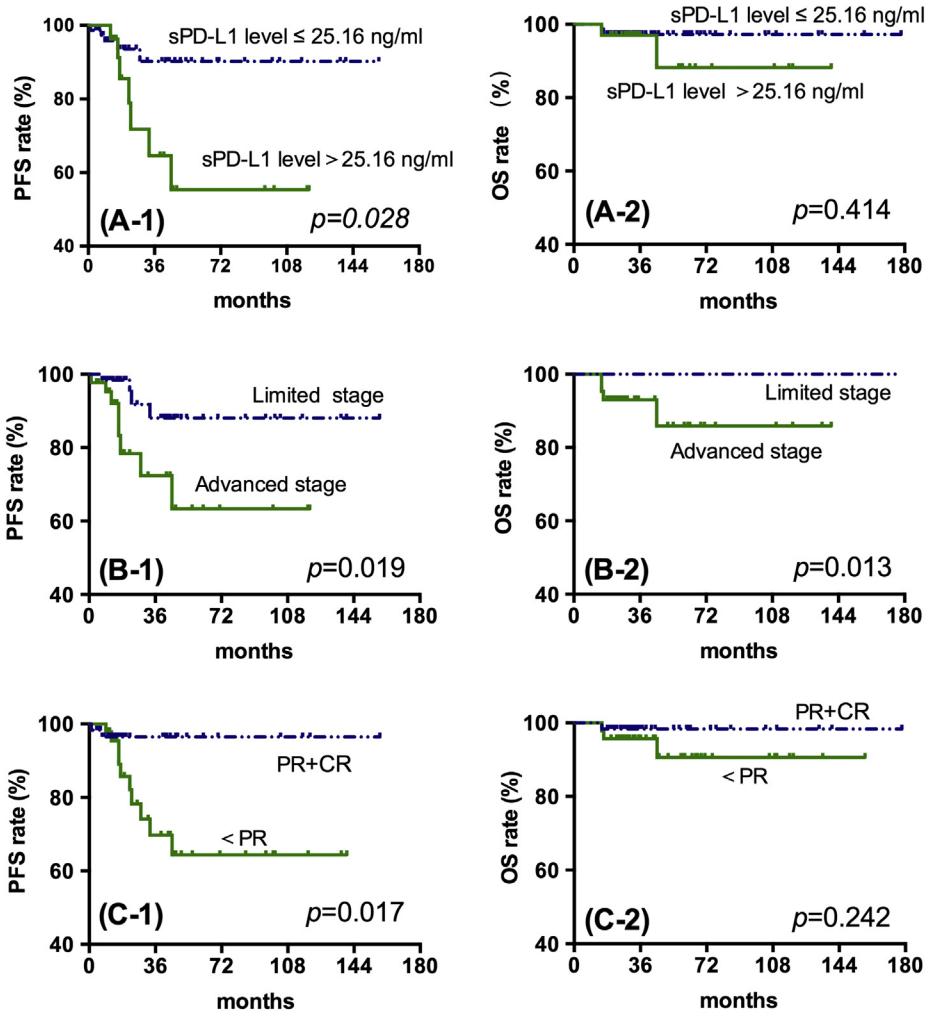

Figure 3. Kaplan-Meier survival analysis for all patients with Hodgkin Lymphoma. A-1and A-2: PFS and OS of HL patients in low- or high-sPD-L1 groups. B-1 and B-2: PFS and OS of HL patients with limited and advanced stages of disease. C-1 and C2: PFS and OS of HL patients with different treatment responses.

Table 3. Univariate and Multivariate Analyses for PFS

Parameters	PFS			OS		
	Univariate Analysis	Multivariate Analysis		Univariate Analysis	Multivariate Analysis	
	P	HR (95%CI)	*P*	*P*	HR (95%CI)	*P*
Histological type	.991			.691	3.136 (3.058-3.255)	
Stage	.013	1.284 (0.237-3.048)	.005	.001	4.701 (4.163-5.449)	.182
B symptoms	.171			.105	1.304 (3.640-4.859)	
Bulky	.859			.192	0.895 (2.992-3.237)	
Radiotherapy or not	.661			.337	0.595 (3.643-3.993)	
TR after 2 cycles of chemotherapy	.014	1.366(0.189-12.47)	.033	.137	1.265 (0.954-4.722)	.174
sPD-L1 level (>25.17ng/mL)	.012	1.021(0.140-2.668)	.046	.795	0.315 (3.636-5.784)	.182

Abbreviations: PFS, progression-free survival; TR after 2 cycles of chemotherapy, treatment response after 2 cycles of chemotherapy; sPD-L1, soluble programmed death ligand.

In addition, we found that a higher sPD-L1 level was correlated with shorter PFS rate (78.8% vs. 93.3%, $P = .028$), but not correlated with OS rate, suggesting that sPD-L1 plays a key role in HL progression and chemotherapy resistance. The mechanisms by which elevated sPD-L1 levels contribute to poor prognosis in HL are not clear, but there are several possible explanations [35]. For example, activation of PD-1 impairs T-cell expansion and function by promoting IL-10 production [23]. PD-1 inhibits T-cell responses by promoting the induction and maintenance of Treg cells via down-regulation of phospho-Akt, mTOR, S6, and ERK2 and the concomitant up-regulation of PTEN, which induces drug resistance in HL cells [36,37]. Further studies are needed to identify how PD1/PD-L1 signaling impacts HL prognosis.

Recent studies assessing the prognostic role of PD-L1 expression by IHC in various solid tumors and hematological malignancies have yielded mixed results [24,38–46]. Pin wu et al. performed a meta-analysis of solid tumors and showed that PD-L1 overexpression was associated with poor 3-year OS in all studies analyzed, with the exception of one study on melanoma and lung cancer. Interestingly, few studies of the PD-1 and PD-L1 pathway in HL have been performed [47,48]. HL usually exhibits diffuse and strong PD-L1 positivity in tumor (RS) cells based on IHC [49]. Paydas et al. found that PD-L1 was expressed on >5% of tumor cells in 70% of cHL and 54% of NLPHL, indicating that PD-L1 was not an independent risk factor for prognosis, but co-expression of PD-1 and PD-L1 was an independent risk factor [50]. In contrast, Young WhaKoh et al. reported that 75% of HL cases were positive for PD-L1 and identified significant adverse prognostic effects of PD-1 expression but not PD-1 and PD-L1 co-expression or PD-L1 expression alone [51]. Therefore, the prognostic value of PD-L1 overexpression in HL is still unclear. In addition, there are still some problems with the IHC method used to assess PD-L1 expression, resulting in a lack of clarity, including differences in the cutoff value of PD-L1 positivity (proportion of positive cells: 1, 5, 10%) and determination of positive cells (tumor cells and/or immune cells) [32]. Compared with IHC, there are many benefits to liquid biopsy, which is less invasive than tissue biopsy and may enable evaluation of immune status before and during treatment as opposed to assessing archival tissue samples [52–56]. Above all, we suggest that serum sPD-L1 level measured via ELISA rather than PD-L1 expression assessed with IHC may be a better prognostic factor for HL patients. However, the relationship between PD-L1 expression on IHC and serum sPD-L1 level still needs more research.

Additionally, we found that a higher sPD-L1 level is associated with advanced stage in HL patients, which may imply that sPD-L1 plays an important role or at least is a good cancer activity indicator. Generally, soluble forms of receptors are believed to typically be produced through proteolytic cleavage of membrane-bound proteins such as the sTNF and sB7-H3 or by translation of alternative spliced mRNA, as has been found with sCD86 and sCTLA-4 [57–59]. In addition, some research has found that addition of MMPI reduced the production of sPD-L1 on PD-L1-transfected cells [25]. Guangbo Zhang et al. observed that soluble B7-H3 binds to the B7-H3 receptor (B7-H3R) on activated T cells, which showed that sB7-H3 is a functionally active form [57]. These results indicate that the presence of sB7-H3 as an active form might significantly affect cell–cell interactions and responses to surface-bound B7-H3 [60]. However, the impact of the cleavage of membrane B7-H3 to its soluble form on cell–cell interactions and the role of B7-H3 in the pathological mechanisms of disease require further elucidation.

Our study also found that HL patients with lower peripheral blood monocyte number had a higher serum sPD-L1 level ($P < .1$), which is consistent with previous studies and further suggests that the presence of sB7-H1 may be one mechanism by which tumors compromise immune responses [25,61–63]. Although the International Prognostic Score (IPS) remains a valid predictor of outcome for patients with advanced-stage disease, it is unsuccessful in stratifying patients with limited-stage cHL into subgroups with a poorer prognosis. Therefore, PD-L1 expression is a viable alternative prognostic factor to IPS in limited-stage cHL.

A previous study suggested that HL patients achieving CR after two courses of chemotherapy had a better prognosis, regardless of whether they were newly diagnosed or relapsed/refractory patients [64]. Our study suggested that good treatment response (CR or PR) was a favorable prognostic factor in HL but not an independent prognostic factor. These results illustrated that response-adapted strategies aiming to identify patients in whom therapy may be safely deescalated to minimize long-term toxicity are scientific and practical in clinical management.

Some limitations of our present study include its retrospective design, the short follow-up period for some recent cases, and the small sample size. Furthermore, there is a lack of cytogenetic and molecular abnormality analyses of patients in this study. Further investigations are warranted to clarify and understand the role of sPD-L1 as a prognostic or predictive biomarker of systemic chemotherapy as well as immune checkpoint inhibitors.

Conclusions

In summary, this is the first report measuring serum sPD-L1 protein levels in HL. Our results suggest that serum sPD-1, which can be easily measured in clinical practice, may be an important independent

prognostic factor in cHL and useful for identifying a subgroup of patients with high risk for recurrence or progression. These results suggest a role of sPD-L1 in HL pathogenesis and offer new insights into potential therapeutic strategies.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the Sun Yat-Sen University Cancer Center Research Ethics Board and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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