Clin. Lab. 2023;69:XXX-XXX

SHORT COMMUNICATION

Soluble Programmed Cell Death Ligand-1 (sPD-L1) Levels in Various Cancer Types and Normal Populations

Seung Hyun Lee [1], Hye Jee Park [1], Joo Young Moon [1], Yun Tae Kim [1], Kyoung Ryul Lee [1], Min Jung Kwak [2], Young Jin Kim [3]

[1] *Department of R&BD, Seoul Clinical Laboratories, Yongin, Korea*
[2] *Deparment of Data Information and Statistics, Pyeongtaek University, Korea*
[3] *Department of Biobank, SCL, Yongin, Korea*

SUMMARY

Background: **To date, PD-L1 expression in tumor tissue, assessed by immunohistochemical stain, is clinically applicable as a predictive companion biomarker for PD-1/PD-L1 inhibitor which has been highlighted over the past several years. Before blood-based sPD-L1 enters clinical use, it is critical to establish the reference range. This study was designed to investigate soluble sPD-L1 levels in various cancer patients and normal population.**
Methods: **For the detection of sPD-L1, 4 cancer groups (hepatocellular carcinoma, lung cancer, bladder cancer, gastric cancer) and healthy volunteers' samples were analyzed using an ELISA kit. Using a receiver operating characteristic curve, optimal sPD-L1 cutoff levels were determined.**
Results: **The mean serum sPD-L1 level of the normal population was 59.97 pg/mL (range; 23.780 - 115.2 pg/mL). In various cancer types, serum sPD-L1 levels ranged from 38.696 pg/mL to 228.77 pg/mL, and cutoff values under AUC ranged from 60.307 pg/mL to 64.371 pg/mL.**
Conclusions: **sPD-L1 can be used as a screening biomarker in various cancer patients referring to optimal cutoff levels suggested.**
(Clin. Lab. 2023;69:xx-xx. DOI: 10.7754/Clin.Lab.2022.220701)

Correspondence:
Young Jin Kim, MD, PhD
Medical Director
Department of Biobank, Seoul Clinical Laboratories
13 Heungdeok 1 Ro
Giheung-gu, Yongin City
Gyeonggi-do, 16954
Korea
Phone: +82 2-330-2011
Fax: +82 31-660-7143
Email: yjkim@scllab.co.kr

Short Communication accepted August 1, 2022

KEYWORDS

sPD-L1, immunohistochemical stain (IHC), cutoff value, immune checkpoint inhibitor (ICI)

INTRODUCTION

Recently, immune-checkpoint inhibitors (ICIs) have emerged as effective alternative therapeutics for various cancers such as gastric cancer, hepatocellular carcinoma, bladder cancer, and lung cancer. Companion-diagnostic biomarkers play an important role in enhancing the therapeutic effect of ICI, such as nivolumab and pembrolizumab [1]. Currently, the FDA-approved immunohistochemical stain (IHC)-based PD-L1 commercial companion-diagnostic assays are used on pre- and post-treatment tissues for cancers such as lung and bladder cancer [2]. However, the performance of the IHC assays has been disappointing, as most positive cutoffs

would exclude a considerable number of responders in the range of 10 - 20% [3]. Moreover, intratumor heterogeneity is a recognized challenge that can confound the determination of PD-L1 expression in small biopsy samples. In the meantime, several studies have suggested that the sPD-L1 in the blood can be used as a biomarker with prognostic value in cancer patients [4]. However, studies regarding the prognostic value of the sPD-L1 in cancer patients have not yielded consistent results because most ELISA Kits used in such studies are for research use only (RUO) and the normal ranges suggested by each ELISA Kit manufacturer vary. Several studies suggested that sPD-L1 is a potential predictive biomarker; however, such studies were carried out by regarding pre-treatment sPD-L1 level which showed variations among individuals at baseline. Since the ELISA kits used in studies suggested different normal ranges, it was difficult to interpret the result of such studies. Currently, it may make more sense to consider the reference range with clinically diagnostic values. Therefore, we aimed to investigate the soluble sPD-L1 levels in various cancer patients and normal population and to determine the optimal sPD-L1 cutoff level for discrimination between normal population and cancer patient groups.

MATERIALS AND METHODS

For the detection of sPD-L1, a commercially available ELISA kit was used (human PD-L1 ELISA kit; ab-277712, Abcam, USA) according to the manufacturer's specifications manual. The detection range of the ELISA kit used is 7.81 - 500 pg/mL and intra-assay and inter-assay CVs are 2.7% and 4.3%, respectively. Samples from healthy volunteers (n = 87; M = 31, F = 56) between 20 and 71 years of age (median: 41 years) for periodic medical checkup gave informed consent were analyzed and had values ≤ upper limit of normal for all measured laboratory parameters. Cancer patients' sera for each group (hepatocellular carcinoma n = 40, bladder cancer n = 28, gastric cancer n = 40, lung cancer n = 30) were obtained from remnant samples before any treatment including ICI. Each sample was analyzed in duplicate. This study was approved by the Clinical Studies Ethics Committee of Seoul Clinical Laboratories in 2021 (IRB File No. IRB-21-026-04).

Non-parametric Wilcoxon rank sum test and two-sample *t*-test were used. p-value < 0.05 was considered statistically significant. All analyses, including drawing of receiver operating characteristic (ROC) curves and estimation of the area under the ROC curve (AUC), were performed using R programming language.

RESULTS

The mean PD-L1 concentration was determined to be 59.976 pg/mL with a range of 23.780 - 115.27 pg/mL in healthy volunteers. On the other hand, the mean sPD-L1 concentration of the 4 cancer patients groups were determined to be 80.615 pg/mL with a range of 38.696 - 204.32 pg/mL in hepatocellular carcinoma, 93.810 pg/mL (range; 48.12 pg/mL - 228.77 pg/mL) in bladder cancer, 80.447 pg/mL (range; 41.00 pg/mL - 206.82 pg/mL) in gastric cancer, 98.664 pg/mL (range; 53.185 pg/mL - 220.37 pg/mL) in lung cancer. The mean sPD-L1 levels in the 4 cancer groups ranged from 80.447 to 98.664 pg/mL, significantly higher than normal population, which was 59.976 pg/mL ($p < 0.05$) (Figure 1). We used a receiver operating characteristic (ROC) curve to determine the optimal sPD-L1 cutoff level for discrimination between normal population and cancer patient groups. The cutoff values by ROC curve in the 4 cancer groups ranged from 60.307 pg/mL to 64.371 pg/mL as shown in Figure 2.

DISCUSSION

We explored the articles about normal ranges of PD-L1 or pre-treatment levels of sPD-L1 in cancer patients before we started this study. Sample sizes for reference range establishment in other studies were not large enough according to Clinical and Laboratory Standards Institute (CLSI) guideline [5]. Hence, this study aimed to evaluate the usefulness of a soluble sPD-L1 assay by comparing the blood concentrations of cancer patients to normal population with enough numbers; however, we did not fully meet the guideline. Oh et al. mentioned that the mean sPD-L1 value in the patients with cancer was not significantly different from the mean level in healthy volunteers (13.5 pg/uL versus 10.6 pg/uL) [6]. The pre-treatment levels of sPD-L1 in cancer patients reported in other studies were slightly different from our result which range from 101.78 pg/mL to 0.5 ng/mL [7-9]. For this reason, there may be variations among individuals, and this could be interpreted as a result of using ELISA kits that suggest different normal ranges, detection ranges, and units of measurements in each study. In our previous study using another manufacturer's ELISA kit (Invitrogen, #MAN0016580 Human PD-L1 ELISA Kit, Vienna, Austria), different results were shown, median serum sPD-L1 levels 1.882 pg/mL (range; 0.613 - 15.301 pg/mL) in the same normal population samples which were tested in this study. As other study groups described [10], higher PD-L1 expression levels in high CRP, LDH, WBC samples also showed similar findings as in our study (data not shown, unpublished observations). Meanwhile, among the results of the sPD-L1 cutoff values in 4 cancer groups, a cutoff value of 62.616 pg/mL under AUC 0.839 (sensitivity 83%, specificity 62%) in the lung cancer group and a cutoff value of 64.371 pg/mL (sensitivity 82%, specificity 64%) under

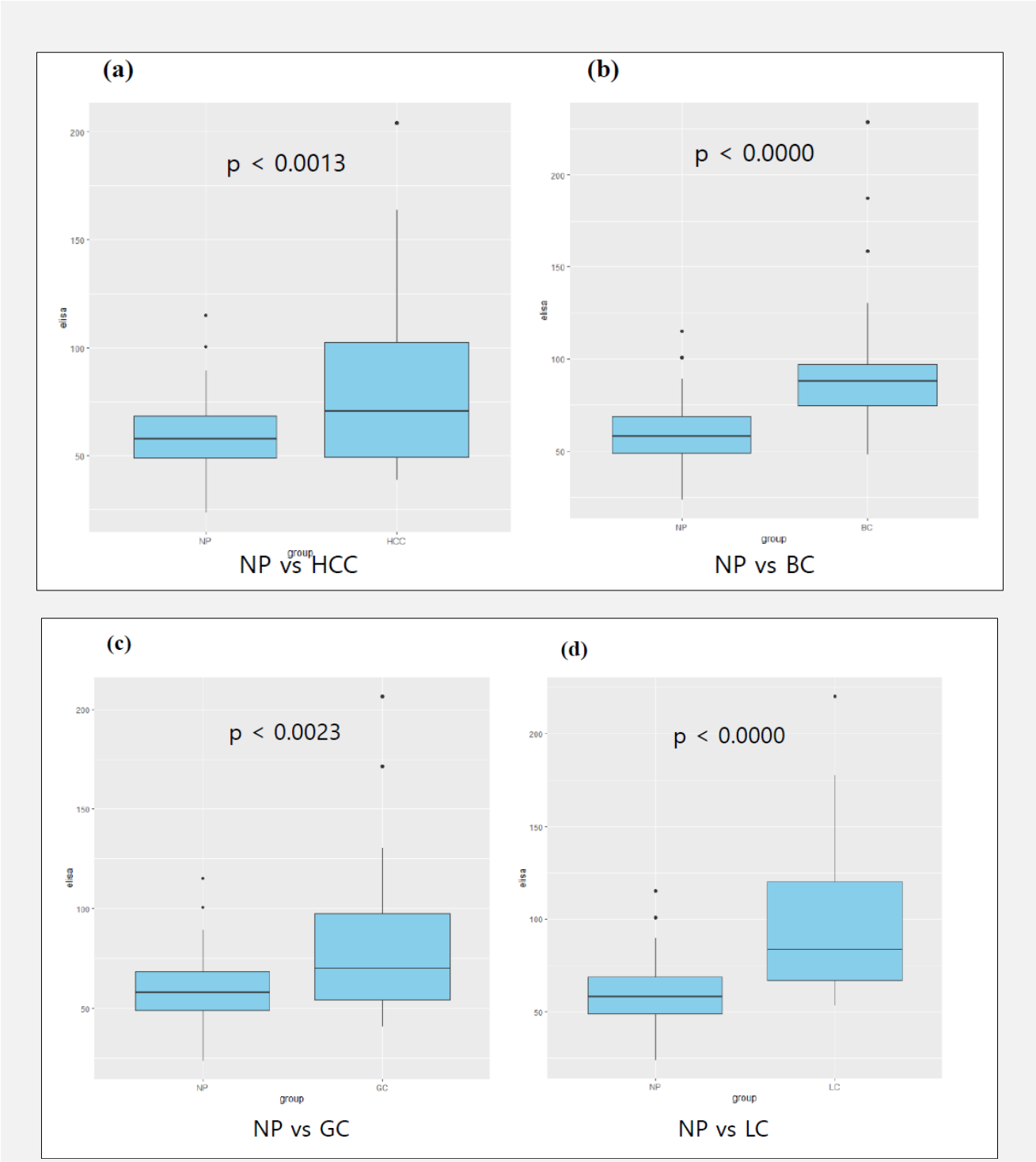

Figure 1. The mean sPD-L1 levels in normal population vs. 4 cancer groups.

The mean sPD-L1 levels in the 4 cancer groups ranged from 80.447 to 98.664 pg/mL, significantly higher than normal population, which was 59.976 pg/mL ($p < 0.05$). (a) NP vs. HCC, (b) NP vs. BC, (c) NP vs. GC, (d) NP vs. LC. Abbreviations: NP - normal population, HCC - hepatocellular carcinoma, BC - bladder cancer, GC - gastric cancer, LC - lung cancer.
*** - p-value for Wilcoxon rank sum test.**

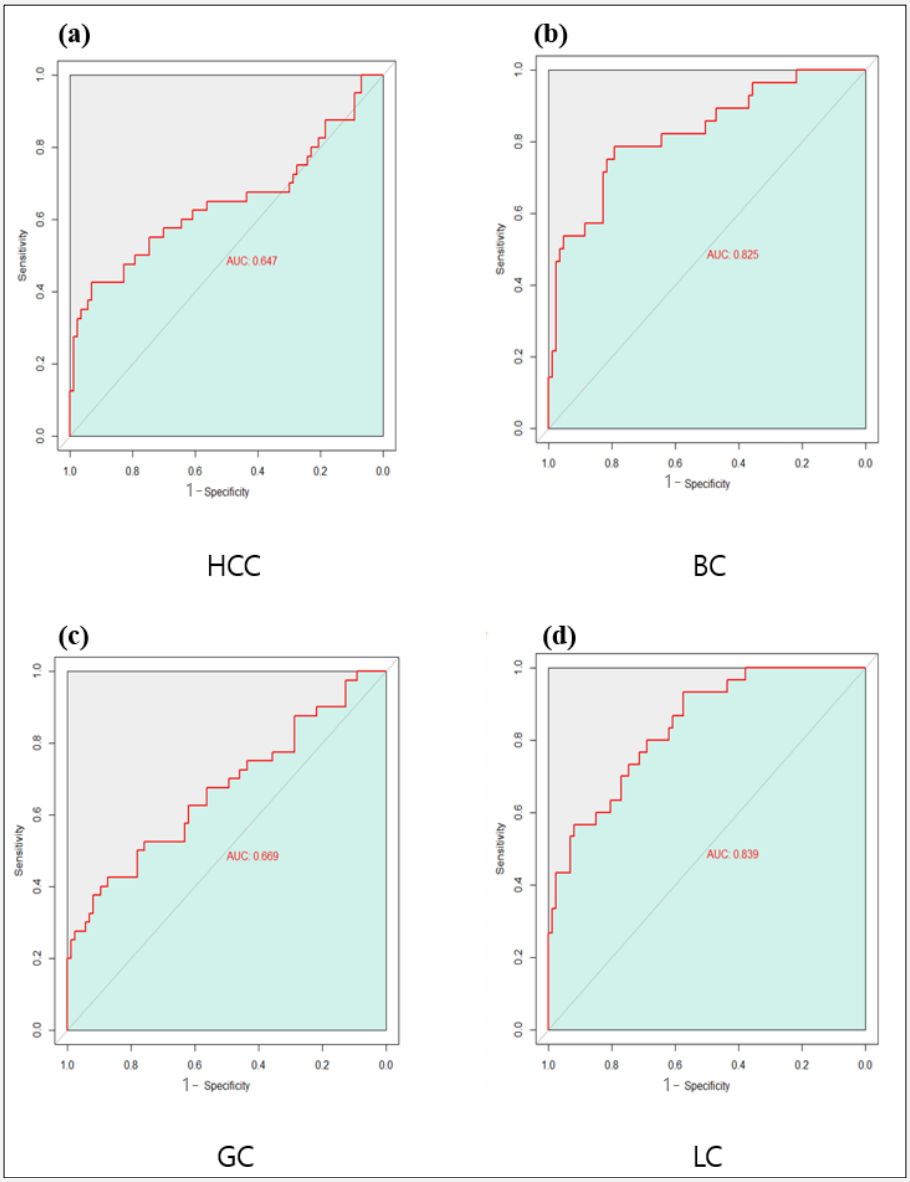

Figure 2. Receiver operating characteristic curve of sPD-L1 for optimal cutoff values.

(AUC, cutoff values, sensitivity, specificity)
(a) HCC: AUC 0.647, cutoff value: 60.307 pg/mL (sensitivity 65%, specificity 56%)
(b) BC: AUC 0.825, cutoff value: 64.371 pg/mL (sensitivity 82%, specificity 64%)
(c) GC: AUC 0.669, cutoff value: 60.372 pg/mL (sensitivity 68%, specificity 56%)
(d) LC: AUC 0.839, cutoff value: 62.616 pg/mL (sensitivity 83%, specificity 62%).

AUC 0.825 in the bladder cancer group showed to be interesting results. These results support that sPD-L1 can be used as a screening tool with optimal cutoff value. In several studies including hepatocellular carcinoma, lung cancer, and gastric cancer patients, the cutoff values as prognostic factor ranged from 0.0183 to 7.32 ng/mL [11,12]. Several studies exploring the prognostic role of pre-treatment peripheral PD-L1 expression in hepatocellular carcinoma noted variable cutoff values by ROC or median in a healthy cohort, from 0.8 ng/mL to 2.825 ng/mL [13]. In contrast to other studies, El-Gebaly et al. showed that the diagnosed cutoff values of

serum PD-L1 level are ≤ 2.522 ng/mL for healthy ones (specificity of 100% and sensitivity of 100% and ≥ 7.42 ng/mL for hepatocellular carcinoma (specificity of 100% and sensitivity of 88%). Therein lies the author's comment that the healthy control sample size (n = 10) included in study was not large enough [14].

There is a clinical unmet need for liquid biomarkers that are broadly relevant to immunotherapy. Attempts to establish new companion diagnostic biomarkers using liquid biopsies are underway. Taken together, our finding supports that sPD-L1 can be used as a screening tool in various cancers referring to the pre-treatment level suggested by other studies, and the cutoff values in various cancer types suggested by our study. Furthermore, if a consensus can be established with regard to the reference range, a soluble sPD-L1 ELISA assay may be clinically useful for therapeutic strategic decisions and predictive prognostic biomarkers for various cancer patients in the near future.

Acknowledgment:

The biospecimens and data used for this study were provided by the Biobank of Kangwon National University Hospital and the Biobank of Keimyung University Dongsan Medical Center, a member of the Korea Biobank Network (KBN-Portal) supported by the Korea Center for Disease Control and Prevention, Republic of Korea.

Declaration of Interest:

The authors declare no conflicts of interest, financial or otherwise.

References:

1. Doroshow DB, Bhalla S, Beasley MB, et al. PD-L1 as a biomarker of response to immune-checkpoint inhibitors. Nat Rev Clin Oncol 2021 Jun;18(6):345-62. (PMID: 33580222)

2. Prince EA, Sanzari JK, Pandya D, Huron D, Edwards R. Analytical Concordance of PD-L1 Assays Utilizing Antibodies From FDA-Approved Diagnostics in Advanced Cancers: A Systematic Literature Review. JCO Precis Oncol 5:953-73. (PMID: 34136742)

3. Grigg C, Rizvi NA. PD-L1 biomarker testing for non-small cell lung cancer: truth or fiction? J Immunother Cancer 2016 Aug 16; 4:48. (PMID: 27532023)

4. Shigemori T, Toiyama Y, Okugawa Y, et al. Soluble PD-L1 Expression in Circulation as a Predictive Marker for Recurrence and Prognosis in Gastric Cancer: Direct Comparison of the Clinical Burden Between Tissue and Serum PD-L1 Expression. Ann Surg Oncol 2019 Mar;26(3):876-83. (PMID: 30565045)

5. Defining, Establishing, and Verifying Reference Interval in the Clinical Laboratory, Approved Guideline- 3rd Edition. CLSI document EP28-A3c. Wayne, PA: Clinical and Laboratory Standards Institute; 2010. https://clsi.org/standards/products/method-evaluation/documents/ep28

6. Oh SY, Kim S, Keam B, Kim TM, Kim DY, Heo DS. Soluble PD-L1 is a predictive and prognostic biomarker in advanced cancer patients who receive immune checkpoint blockade treatment. Sci Rep 2021 Oct 5;11(1):19712. (PMID: 34611279)

7. Mocan T, Ilies M, Nenu I, et al. Serum levels of soluble programmed death-ligand 1 (sPD-L1): A possible biomarker in predicting post-treatment outcomes in patients with early hepatocellular carcinoma. Int Immunopharmacol 2021 May;94:107467. (PMID: 33611059)

8. Ando K, Hamada K, Watanabe M, et al. Plasma Levels of Soluble PD-L1 Correlate with Tumor Regression in Patients with Lung and Gastric Cancer Treated with Immune Checkpoint Inhibitors. Anticancer Res 2019 Sep;39(9):5195-201. (PMID: 31519633)

9. Finkelmeier F, Canli O, Tal A, et al. High levels of the soluble programmed death-ligand (sPD-L1) identify hepatocellular carcinoma patients with a poor prognosis. Eur J Cancer 2016 May; 59:152-9. (PMID: 27039170)

10. Ito M, Oshima Y, Yajima S, et al. Is high serum programmed death ligand 1 level a risk factor for poor survival in patients with gastric cancer? Ann Gastroenterol Surg 2018 Jun 5;2(4):313-8. (PMID: 30003194)

11. Li X, Zheng Y, Yue F. Prognostic Value of Soluble Programmed Cell Death Ligand-1 (sPD-L1) in Various Cancers: A Meta-analysis. Target Oncol 2021 Jan;16(1):13-26. (PMID: 33222017)

12. Wei W, Xu B, Wang Y, Wu C, Jiang J, Wu C. Prognostic significance of circulating soluble programmed death ligand-1 in patients with solid tumors: A meta-analysis. Medicine (Baltimore) 2018 Jan;97(3): e9617. (PMID: 29504990)

13. Sun DW, An L, Huang HY, Sun XD, Lv GY. Establishing peripheral PD-L1 as a prognostic marker in hepatocellular carcinoma patients: how long will it come true? Clin Transl Oncol 2021 Jan;23(1):82-91. (PMID: 32462395)

14. Gebaly FE, Saif SA, Elkadeem M, et al. Study of Serum Soluble Programmed Death Ligand 1 as a Prognostic Factor in Hepatocellular Carcinoma in Egyptian Patients. Curr Cancer Drug Targets 2019;19(11):896-905. (PMID: 31538897)