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Review

Updates on the potential role of immune checkpoints sPD-1 and sPD-L1 in the stratification of many diseases

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ABSTRACT

To determine the latest information on the soluble programmed death-1 (sPD-1) and soluble programmed death-ligand 1 (sPD-L1) immune checkpoints, we assessed the benefits and role of these two soluble proteins in various diseases. Eighty clinical studies in humans were discovered in the initial search among the 201 articles selected from the Pubmed electronic database. We grouped 80 clinical studies based on their disease pathophysiology, and selected 5 groups namely cancer, acute infectious/inflammatory, chronic infectious/inflammatory, autoimmune, and other diseases. Plasma or serum concentration is a common parameter used to assess the level of sPD-1/sPD-L1. In this review we discuss the comparison of sPD-1/sPD-L1 level in patients compared to healthy subjects, the correlation between the level of soluble form and membrane-bound PD-L1, as well as their association with the severity of diseases, treatment response, and other inflammatory markers. The sPD-1 and sPD-L1 significantly increased in cancer patients, and in patients with acute and chronic infection/inflammation. The increase also correlated with its bound shape in membrane and can assess the pathogenesis of disease and treatment response, except for the role of sPD-1 in the cancer treatment response which requires further studies. In autoimmune patients, diverse sPD-1 and sPD-L1 were reported compared with healthy subjects. A relatively small number of samples might be the cause. However, the same relationship was shown by all clinical studies in all diseases, reporting that increase in sPD-1/sPD-L1 correlated with markers of inflammatory indicators, like c-reactive protein (CRP) and various inflammatory cytokines. Since an increase in inflammatory markers occurred in other diseases such as metabolic syndrome disease, more clinical studies are required to confirm the correlation with sPD-1/sPD-L1.

KEYWORDS: soluble programmed death-1, soluble programmed death-ligand 1, immune checkpoint.

INTRODUCTION

Immune checkpoints are particles that can decrease and increase the immune system signals. At the moment, the immune checkpoint is considered as a critical factor in the treatment of infections and autoimmune diseases. Even in cancer disease, the immune checkpoint is used as a target in performing therapy [1]. Basically, the working system of an immune checkpoint is by inhibiting or stimulating signals in immune cells and regulating the function of such immune cells; therefore immune checkpoint plays a crucial role in the regulation of immune homeostasis [2]. Moreover, an immune checkpoint can also transfer signals even though it is at different immune cells, change its activities, and regulate cytokine secretion as a response [3]. The

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immune system can be divided into two groups: a group of particles that works by stimulating, for example, TCR or MHC, and a group of molecules that plays role in inhibition, for example, PD-L1 or PD-1 [4].

PD-L1 is a glycoprotein immune modulator expressed in an antigen-presenting cell (APC), including macrophage [5]. The PD-L1's binding against its PD-1 receptor that exists in the surface of the T-cell contributes to the regulation of immune response. This binding suppresses the production of cytokine, proliferation, and differentiation of T cells. The binding of PD-L1 and PD-1 functions as a negative regulator in normal immune response and antitumor immunity mediated by T cells [6]. Currently, the immune checkpoints PD-L1 and PD-1 are targetted in the treatment of cancer. PD-L1 is being used as a marker in monitoring the progress of cancer [7].

The soluble receptors and ligand of PD-1 and PD-L1 have been documented in several studies. Soluble PD-1 (sPD-1) and soluble PD-L1 (sPD-L1) have been found in the serum of patients suffering from a tumor [8]. This soluble form is easier to be examined compared with its membrane shape and provide real-time information during therapy without invasive method [9]. These two soluble forms are produced by the expression of mRNA or through proteolytic cleavage in membranes (such as soluble tumor necrosis factor) and are found free in plasma [10].

The immune checkpoint sPD-1 is reported as a monomeric protein produced from the expression of mRNA [10]. The increase of sPD-1 predicted may prevent the interaction of PD-1 with its ligand, thus, there is a binding competition between sPD-1 and PD-1 with PD-L1 [11]. Based on this, sPD-1 might be more effective and stronger than PD-1 (mPD-1) antibody, because sPD-1 can suppress the interaction of PD-L1 with PD-1, and PD-L1 with CD80 that inhibit T cells response [12]. Besides, sPD-L1 is produced by the tumor cell, matured dendrite cell, and macrophage cell. The mechanism of sPD-L1 molecular production (splicing) is not entirely clear, but the production of PD-L1 in humans is coded by CD274 genes consisting of seven exons in the 9^{th} chromosome [13]. Le and colleagues reported that the increase of MMPs correlates with the production of sPD-L1 from PD-L1, which indicates that sPD-L1 is produced from the activation of proteolytic cleavage from PD-L1 protein, bound in the cell membrane. SPD-L1 circulates to the entire body through the blood and lymph, and it deploys an inhibitory effect by interacting with PD-1 [10].

Many clinical studies have documented the contribution of sPD-1 and sPD-L1 in the monitoring of cancer treatment since PD-1 and PD-L1 have been used as markers in monitoring the progress of cancer. All of the studies reviewed here support the hypothesis that these two soluble proteins could be potential cancer biomarkers and future therapeutic targets. In addition, the application of these two soluble proteins has been stated in other clinical disease studies such as sepsis, rheumatoid arthritis, and hepatitis disease, with a variety of results. This article will give an update of the most recent researches concerning the contribution of sPD-1 and sPD-L1. We focus on reviewing the potential role of this soluble protein in the pathogenesis of many diseases.

METHODOLOGY

Figure 1 illustrates the searching process adopted in our article. Clinical studies of sPD-1 and sPD-L1 were searched in the PubMed electronic database in February 2020 with medical subject headings (MeSH). The keywords were "Immune Checkpoint" [MeSH term] or "Immune Checkpoint" [All Fields] and ("sPD-1" [Subheading] or "sPD-1" [All Fields] or "sPD-L1" [Subheading] or "sPD-L1" [All Fields] or "sPD-1" and "sPD-L1" [All Fields] or "soluble PD-1" [All Fields] or "soluble PD-L1" [All Fields] or "Circulating PD-1" [All Fields] or "Circulating PD-L1" [All Fields]. The keywords used yielded 201 articles, 80 of which were immune checkpoint sPD-1 and sPD-L1 clinical studies in humans.

In this article, we simplified the various goals in each clinical study and focused on sPD-1 and sPD-L1. We also studied several clinical studies even though the examination of sPD-1 and sPD-L1 was not stated as the primary goal in those studies. Using this approach, we reviewed the roles of these two soluble proteins based on data provided in these clinical studies. We classified the clinical studies into 5 primary groups based on the proximity of pathogenesis underlying the diseases, namely cancer, acute conditions of

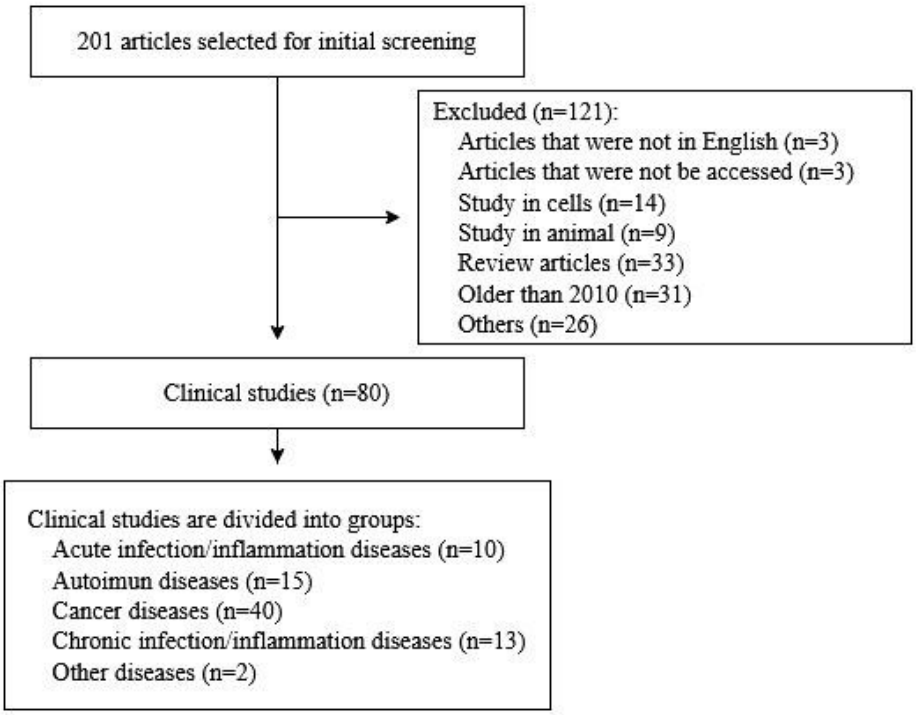

Figure 1. Flowchart of the literature search.

infection/inflammation, chronic conditions of infection/inflammation, autoimmune diseases, and other diseases.

a. Cancer diseases

Table 1 lists 40 articles about sPD-1 and sPD-L1 in the clinical studies of cancer. The studies were conducted in European, American, Australian, and Asian countries. The vast majority of studies on the role of sPD-1 and sPD-L1 were conducted in cancer patients since PD-L1 measurement has already been integrated into the daily clinical procedure and has currently become a marker in monitoring cancer treatment [14]. Several drugs for cancer treatment have received FDA approval, and hence its monitoring requires fast, easy, and safe markers for patients.

Overall, these clinical studies highlight the comparison of sPD-1/sPD-L1 level in patients compared to healthy subjects, the comparison of its level compared to membrane tumor markers, the correlation of its level with inflammatory markers and severity of diseases. All clinical studies in cancer are presented in Table 1, which reported an increase in sPD-L1 levels compared to healthy subjects. However, sPD-1 levels in cancer patients showed mixed results.

The difference in the sPD-L1 level in cancer patients compared with healthy subjects was explained in the following studies. The clinical study of Koukourakis *et al*. in 32 patients with epithelial ovarian cancer (EOC) and 8 healthy subjects reported that sPD-L1 level was significantly higher in the plasma of patients compared to healthy women [15]. Another clinical study in epithelial ovarian cancer patients stated that the increase in sPD-L1 level was significantly associated with the presence of tumors and sPD-L1 helped in predicting the prognosis of EOC patients [16]. Another clinical study in gastric cancer (GC) patients reported that the level of sPD-L1 was significantly higher in patients than in control subjects. The increase in sPD-L1 level is comparable with the increase in PD-L1 expression in GC tissue. Hence, the pre-surgical sPD-L1 level could be used as a predictive marker for recurrence and prognosis in GC patients [17]. Positive sPD-L1 status in gastric cancer was associated with older age, male sex, and intestinal histology, compared to negative sPD-L1 status [18].

Table 1. Clinical studies in cancer diseases.

Type of disease	N	Results*		Key findings	Ref.
		sPD-1	sPD-L1		
EOC	N = 40 (patients = 32; HC=8)	-	patients = 83 pg/mL (29-205) HC = 63 pg/mL (47-98)	A significantly higher level of sPD-L1 in the plasma of EOC patients compared to HC	15
	N = 112 (patients = 83; HC = 29)	-	patients = 6.0 pg/mL (0–32.9) HC = 2.5 pg/mL (0-13.7)	• Increasing sPD-L1 correlates with the presence of tumor • sPD-L1 contributes to predicting the prognosis of EOC patients	16
GC	N = 180 (patients)	-	male = 0.26 ± 0.21 ng/mL female = 0.24 ± 0.17 ng/mL	• A significantly higher level of sPD-L1 in GC patients compared to HC • The increasing level of sPD-L1 correlates with increasing of PD-L1 in GC tissue	17
	N = 592 (patients)	-	NA	PD-L1 expression in tissue samples correlated with the expression of sPD-L1 in serum. The level of sPD-L1 combined with high microsatellite instability (MSI-H) could be an indicator of prognosis for GC.	18
HCC	N = 52 (patients)	-	sPD-L1 = 62.3 pg/mL (33.7–119.6) PD-L1(+) = 75.3 pg/mL (51.6–119.6) PD-L1(-) = 59.8 pg/mL (33.7–100.6)	PD-L1 expression in tissue samples correlated with the expression of sPD-L1 in serum	19
	N = 130 (patients = 81; HC = 49)	-	Patients = 5.129 ng/mL (0.140-12.391) HC = 0.836 ng/mL (0.105-2.168)	Level of sPD-L1 significantly increased in patients with HBV-related HCC. The increase in sPD-L1 is correlated with PD-L1 expression in tumors	20
	N = 181 (patients = 153; HC = 28)	-	patients = 234 ± 91 pg/mL HC = 93 ± 31 pg/mL	A significantly higher level of sPD-L1 in HCC patients compared to HC	21
	N = 120 (patients)	82.7 μg/mL (7.6–2886.8)	5.2 μg/mL (0.1–130.0)	• Level of sPD-L1 positively correlated with sPD-1; • sPD-L1 and sPD-1 levels positively correlated with HBV viral load and CRP	22

Table 1 continued..

	N = 53 (HCC patients)	NA	NA	sPD-1 and sPD-L1 increased at the 2nd week after treatment	25
PC	N = 60 (patients)	-	Baseline = 1.7 ng/mL (0.4–5.7 ng/mL) Cut-off = 4.6 ng/mL	High sPD-L1 level indicates a worse OS	23
	N = 41 (patients)	high CRP = 490 pg/mL normal CRP = 230 pg/mL	high CRP = 60 pg/mL normal CRP = 18 pg/mL	High levels of sPD-1 and sPD-L1 were present in patients with high CRP	24
Lymph	N = 123 (patients = 108; HC = 15)	-	patients = 20.5039 ng/mL HC = 0.722 ng/mL	• A significantly higher level of sPD-L1 in the lymphoma patients compared to HC • sPD-L1 levels (> 25,1674 ng / ml) are independent prognostic factors for shorter progression-free survival (PFS)	26
	N = 80 (patients = 68; HC = 12)	-	Patients = 0.429 ng/mL (0.324–0.757) HC = 0.364 ng/mL (0.329–0.390)	• sPD-L1 is significantly higher in patients compared to HC • The optimal cut off for predicting OS is 0.432 ng / mL	27
	N = 30 (patients)	-	Diagnosis: patients = 43.2 ± 10.9 pg/mL HC = 20.5 ± 3.5 pg/mL End of treatment: patients = 22.3 ±10.2 pg/mL HC = 20.5 ± 3.5 pg/mL	• Level sPD-L1 at diagnosis is significantly higher than HC • Level of sPD-L1 at diagnosis significantly decreased after treatment	28
	N = 40 (patients = 17; HC = 23)	-	Patients = 850 ± 738 pg/mL HC = 324 ± 296 pg/mL	A higher level of sPD-L1 in patients is correlated with positive PD-L1 expression in CD56 cells lymphoma	29
	N = 57 (patients = 37, HC = 20)	-	Patients = 1.696 ng/mL HC = 0.729 ng/mL	The level of sPD-L1 in patients is higher than HC, and positively correlated with the level of IFN-γ	30
	N = 155 (patients = 80, HC=75)	-	Patients = 76.6 ± 17.11 pg/ml HC = 23.43 ± 0.4956	Level of sPD-L1 is significantly higher in patients than HC, and positively correlated with PD-L1 in tumor tissue	31
	N = 87 (patients)	NA	NA	sPD-1 and sPD-L1 play a role in assessing the efficacy of novolumab in NSCLC patients	32

Table 1 continued..

NSCLC	N = 43 (patients)	-	initial diagnosis = 39.81 pg/ml (29.75 – 59.21) drug initiation = 49.86 pg/ml (36.11 – 65.91) tumor evaluation = 51.57 pg/ml (31.91 – 72.06)	sPD-L1 higher in the first tumor evaluation and increasing and is associated with PFS and worse OS	33
	N = 39 (patients)	-	2.24 ng/ml (0.98 - 4.32) cutoff point for sPD-L1 = 3.357 ng/mL	• 59% of low plasma sPD-L1 levels and 25% of high sPD-L1 levels achieve complete or partial responses • 22% of patients with low plasma sPD-L1 levels and 75% of patients with high sPD-L1 levels develop progressive disease	34
	N = 75 (patients)	-	Mean value of absorbance of conventional ELISA= 0.051 ± 0.027 Binding capacity ELISA = 0.292 ± 0.461	The average absorbance with new ELISA is significantly higher than the conventional ELISA. The new ELISA can evaluate the capacity of sPDL1 to bind with PD-1 receptors	39
	N = 112 (patients = 85; HC=27)	-	NA	No significant difference of sPDL1 profile between NSCLC patients and HC, but sPD-L1 correlates with the tumor size	40
	N = 20 (patients)	-	pre-treatment: Progressive disease = (346 ± 85 pg / ml) Partial response = (272 ± 70 pg / ml)	No significant difference of sPD-L1 in patients with progressive disease and patients with partial response	41
	N = 38 (patients)	Pre-treatment = 59 pg / mL (20 – 808) Post-treatment = 87 pg/mL (10-496)	-	Serum sPD-1 is found to be higher during treatment with erlotinib than pre-treatment	42
LGC	N = 26 (patients = 21; HC=5)	-	pre-treatment = 469.7 ± 298.8 pg/ml post-treatment = NA HC = 323.0 ± 108.4 pg/ml	Significant correlations between reduction in sPD-L1 and tumor regression were observed after four treatment cycles	35
LC	N = 136 (patients)	-	NA	Preoperative infusion chemotherapy combined with hyperthermia can reduce sPD-L1 and increase the immune response	36

Table 1 continued..

	N = 145 (patients = 115, HC = 30)	-	Adenocarcinoma (EGFR wild type) = 161.4 ng/L (104.9–272.7) Adenocarcinoma (EGFR mutation +) = 134.4 ng/L (86.0–322.5) Squamouscellcarcinoma = 196.1 ng/L (98.0 –317.2) PD-L1+ NSCLC = 830.3 ng/L (413.0–1185.0) SCLC= 147.3 ng/L (84.4–371.7) HC= 31.0–182.5 ng/L	Level of sPD-L1 significantly higher in the patients compared to HC	37
	N = 96 (patients)	-	patients = 6.95 ± 2.90 ng/ml HC = NA	• Level of sPD-L1 is higher compared to HC • associated with a poor prognosis	38
BC	N = 1016 (patients = 516; HC = 500)	patients = 128.24 ± 11.13 pg/mL HC = 88.63 ± 23.44 pg/mL	-	The levels of positive expression of sPD-1 and PD-1 in patients with cancer pain are higher than HC	43
	N = 125 (patients = 66; HC = 59)	before NAC = 549.3±58.76 pg/mL after NAC = 494.2 ± 79.64 pg/mL HC = 379.2 ± 17.30 pg/mL	before NAC = 227.7 ± 23.99 pg/mL after NAC = 190.8±26.07 pg/mL HC = 195.0 ± 8.49 pg/mL	sPD-1 and sPD-L1 decreased in patients with total remission or partial compared to patients with bad NAC responses	44
MM	N = 39 (patients)	Mean Value= week 0= 54,566 pg/mL week 4= 52,787 pg/mL	-	A very low level of baseline sPD-1 or decreased sPD-1 after dendritic cell vaccine (DCV) treatment is a marker for survival prediction	45
	N = 77 (patients = 37, HC = 40)	pre-IMRT = 2.4 ± 7.8 pg/mL post-IMRT = 30.0 ± 20.6 pg/mL HC = 69.7 ± 38.8 pg/mL	pre-IMRT = 4.7 ± 3.2 pg/mL post-IMRT = 11.1 ± 3.3 pg/mL HC = 13.7 ± 5.6 pg/mL	Level of sPD-1 significantly increased in NPC patients after IMRT	46

Table 1 continued..

NPC	N = 35 (patients)	-	Stage III-IVa (50.76 ± 28.15 pg/ml) Stage I-II (19.87 ± 11.38 pg/ml) Stage N2–3 (52.03 ± 28.98 pg/ml) Stage N0-1 (32.88 ± 23.75 pg/ml)	Level of sPD-L1 is positively correlated with clinical stage	47
PAC	N = 32 (patients)	8.93 ng/mL (0-25) Cut-off = 8.6 ng/mL (AUC = 0.85; p value < 0.001)	0.44 ng/mL (0-1.23) Cut-off = 0.36 ng/mL (AUC = 0.89, p value < 0.001)	Patients with high levels of sPD-L1 and sPD-1 have shorter survival	48
EC	N = 71 (patients = 47; HC=24)	Patients= 0.180 ng/mL (0.11–0.920) HC= 0.155 ng/mL (0.11–1.565)	Patients = 20 pg/mL (15–165) HC = 20 pg/mL (15–35)	No significant difference of sPD-L1 in patients compared to HC, but sPD-1 significantly higher in patients than HC but not related with treatment response rates	49
MSO	N = 62 (patients = 40; HC = 22)	-	Patients = 0.07 ng/mL (0.01-0.15) HC = 0.05 ng/mL (0.03-0.06)	Level of sPD-L1 significantly different in mesothelioma and HC patients	50
RC	N = 117 (patients)	NA	NA	sPD-L1 increased after CRT, suggesting that anti-PD-L1 therapy might be a potential treatment strategy in combination with CRT	51
RCC	N = 154 (patients = 117; HC=37)	-	primary renal cancer = 27.8 pg/mL renal cancer during progression = 35.2 pg/mL benign kidney tumor = 19.3 pg/mL HC = 13.0 pg/mL	sPD-L1 increased in patients with primary kidney cancer and tumor development compared to HC	52
TC	N = 101 (patients)	-	Serum: Patients = 0.48 ng/mL (0.05–4.91) HC = 0.37 ng/mL (0.30–0.79) Plasma: Patients = 0.21 ng/mL (0.01–1.71) HC = 0.19 ng/mL (0.08–0.82)	Level of sPD-L1 significantly higher than HC and correlates with disease free-survival (DSF) patients	53

Table 1 continued..

OSA	Non OSA = 132 Mild OSA = 109 Moderate OSA = 70 Severe = 49	-	Non OSA = 64.4 pg/mL Mild OSA = 60.2 pg/mL Moderate OSA = 86.1 pg/mL Severe OSA = 88.5 pg/mL	Level of sPD-L1 is higher in severe OSA compared to patients with mild OSA or non-OSA	54
GLM	N = 142 (glioma = 73; meningioma = 20; HC = 49)	-	Glioma = 0.5594 ng/mL (0–1.4235) Meningioma = 0.0688 ng/mL (0.0454–1.4117) HC = 0.1107 ng/mL (0–0.5908)	Level of sPD-L1 is significantly higher in glioma patients than meningioma and HC. sPD-L1 showed a significant value in diagnosis and stratification of gliomas than inflammatory markers	55

*The sPD-1/sPD-L1 data listed above are in accordance with the results in each clinical studies, in mean or median value. All data were interpreted based on comparison with HC or other indicators available in each of the clinical studies.

Abbreviations: EOC, epithelial ovarian cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; Lymph, lymphoma; PC, pancreatic cancer; NSCLC, non-small cell lung cancer; LGC, lung and gastric cancer; LC, lung cancer; BC, breast cancer; MM, metastatic melanoma; NPC, nasopharyngeal carcinoma; PAC, pancreatic adenocarcinoma; EC, esophageal cancer; MSO, mesothelioma; RC, rectal cancer; RCC, renal carcinoma; TC, thyroid cancer; OSA, obstructive sleep apnea; GLM, glioma; HC, healthy control group; OS, overall survival; PFS, progression free survival; IMRT, intensity-modulated radiation therapy; NA, data not available.

Exploration of sPD-L1 roles in Hepatocellular Carcinoma (HCC) was frequently carried out and reported for various purposes. The clinical study of Itoh *et al*. with the purpose to examine the correlation between PD-L1 and sPD-L1 on HCC patients reported that the expression of PD-L1 cancer cells in tissue samples correlated with the formation of sPD-L1 in serum. These results indicated that PD-L1 expressed on the surface of tumor cells might become a source of sPD-L1 serum, and the testing of sPD-L1 serum might have a role in the assessment of HCC disease [19]. The clinical study in patients with HBV-related HCC reported that sPD-L1 serum concentration increased and was positively correlated with PD-L1 expression in tumors. Lower pre-treatment serum sPD-L1 level was a better predictor of disease-free and overall survival [20]. Another clinical study with the purpose to clarify the role of PD-L1 serum in HCC patients and their sources reported that an inflammatory condition may induce inconsistent PD-L1 signaling in the liver of patients with fibrosis. This inflammatory condition also elevated sPD-L1 level; hence this soluble protein was not only produced by tumor tissue in HCC patients, but also by the inflammatory process [21]. The clinical study of Chang and co-workers reported that the sPD-L1 level in HCC patients was associated with HBV viral load and was correlated with inflammatory markers like c-reactive protein (CRP). HCC patients with CRP serum level (> 3 mg/L) had significantly higher sPD-L1 levels than patients with low CRP levels. High IL-10, IL-17, and TNF-α serum levels also correlated with high sPD-L1 and sPD-1 serum levels in HCC patients [22].

The sPD-L1 level was also reported to increase in pancreatic cancer patients, and it was stated that there was an association between increased systemic inflammation and bad sPD-L1 results in pancreatic cancer [23]. The sPD-L1 was expressed as a systemic inflammatory marker in pancreatic cancer and a high sPD-L1 level was present in patients with high CRP [24]. Another clinical study reported the exploration of sPD-L1 expression which is linked with inflammatory markers. Guo *et al*. who researched sPD-L1 expression in Hodgkin Lymphoma patients

reported that higher sPD-L1 level was positively correlated with advanced stages and negatively correlated with the amount of peripheral blood monocyte, and became an independent predictive element for shorter progression-free survival (PFS) [26]. The clinical study of Cho *et al*. in 68 patients with primary central nervous system lymphoma (PCNSL) reported that sPD-L1 serum level might reflect PD-L1 expression in PCNSL tumor cells and sPD-L1 in serum could become a viable biomarker to determine risk-based adjusted treatment strategies for PCNSL patients [27]. The same was reported in other lymphoma clinical studies namely diffuse large B cell lymphoma (DLBCL) [28] and Nasal natural killer/T-cell lymphoma (NNKTL) [29]. Moreover, the group of high sPD-L1 showed a higher IL-7 level than the group of low sPD-L1 [27]. The sPD-L1 level in Peripheral T Cell Lymphoma (PTCL) patients was also reported to be significantly higher than in healthy subjects [30], and sPD-L1 level was positively correlated with IFN-γ level [31].

The sPD-L1 role in monitoring cancer treatment responses was reported in several clinical studies. The clinical study of Tiako *et al*. in 87 Non-Small Cell Lung Cancer (NSCLC) patients reported that sPD-L1 had a role in reviewing the effectiveness of nivolumab used in NSCLC treatment [32]. High sPD-L1 concentration in the first tumor evaluation was associated with worse PFS and overall survivor (OS), which indicated the success or failure of nivolumab treatment [33]. Another clinical study in 39 NSCLC patients treated with nivolumab showed that 59% of patients with low plasma sPD-L1 level and 25% with a high sPD-L1 level achieved complete or partial responses. As many as 22% of patients with low plasma sPD-L1 level and 75% of patients with high sPD-L1 level developed progressive diseases. Therefore, sPD-L1 plasma level might represent a new biomarker for the prediction of nivolumab therapy efficacy against NSCLC [34]. Moreover, a significant correlation was reported between the reduction of sPD-L1 level and tumor regression observed after four treatment cycles in patients with NSCLC and gastric cancer. The sPD-L1 level might reflect the total active cancer cells in individuals [35]. Clinical studies in lung cancer patients reported that pre-surgical infusion chemotherapy combined with hyperthermia could reduce sPD-L1 level, improve immune response, inhibit tumor growth, and extend patient survival. Hyperthermia has been known to be an effective method in cancer treatment [36].

Unlike sPD-L1, the clinical studies of sPD-1 in cancer are still limited and not as many as sPD-L1. Table 1 reports 12 clinical studies of sPD-1 conducted simultaneously with sPD-L1. Similar to sPD-L1, we simplified sPD-1 roles into 5 groups according to the results of clinical studies, namely assessment of its expression against healthy subjects, correlation with tumor and sPD-L1 developments, correlation with inflammatory markers, assessment of treatment response, and assessment of severity to predict patient survival.

All clinical studies in Table 1 reported the increase of sPD-1 in patients with cancer compared with healthy subjects, correlated with an increase in inflammatory markers. A clinical study reported that sPD-1 expression in breast cancer was higher than in healthy subjects; the median values of PD-1 in breast cancer and sPD-1 were 128.24±11.13 pg/mL and 88.63±23.44 pg/mL, respectively. An increase in sPD-1 value correlated with PD-1 expression on the membrane [43]. An increase in sPD-1 value was positively correlated with an increase in sPD-L1. High sPD-1 level correlated with IL-10, IL-17, and TNF-α expressions, as well as the IFN level of serum [22]. The clinical study of Kruger *et al*. in 41 advanced pancreatic cancer patients reported that high sPD-1 levels occurred in patients with high CRP levels [24].

The sPD-1 expression was reported to vary in several clinical studies in assessing drug response and its effect on patient survival. The clinical study of Li *et al*. with the purpose of analyzing changes of sPD-1 and sPD-L1 at the onset and end of Neoadjuvant Chemotherapy (NAC) and to assess NAC response in triple-negative breast cancer (TNBC) patients reported that patients with partial or total suspension responses after NAC significantly experienced decreased sPD-L1 level compared to the patients with bad NAC responses. The clinical study also reported that sPD-1 serum level was significantly increased compared to healthy control, indicating strong immune suppression in patients [44]. Another clinical study in 39 metastatic melanoma patients reported that a

decrease in sPD-1 level after vaccination was associated with better survival among patients treated with dendritic cell vaccine (DCV) but not in patients treated with tumor cell vaccine (TCV) [45].

Different results were reported in the clinical study of 38 patients with advanced NSCLC, positive for epidermal growth factor receptor (EGFR) mutation and they received erlotinib treatment. This study aimed to compare the sPD-1 level at the time of initial treatment with erlotinib and at the time when clinical resistance to erlotinib was shown. The clinical study reported that sPD-1 level at pre-treatment and during the progression were 59 pg/mL (20-808) and 87 pg/mL (10–496), respectively. Only three patients showed decreased level of sPD-1 during erlotinib treatment. These results suggest that sPD-1 serum concentration was higher in the treatment with erlotinib than before treatment [41]. Another clinical study in 77 nasopharyngeal carcinoma patients with intensity-modulated radiotherapy (IMRT) reported an increase in sPD-1 after therapy compared to initial conditions and healthy subjects. Patients with high sPD-1 (> 10.19 pg/ml) out of 18 patients had better survival compared to other patients with low sPD-1. Therefore, it was predicted that sPD-1 can increase antitumor immunity [46]. However, a clinical study in 32 pancreatic adenocarcinoma patients reported that patients with higher sPD-1 than cut-off median of 8.6 ng/mL had an overall survival median of 3.4 months compared to 20.0 months for patients with low sPD-1 level [48].

The methods used in the detection of sPD-1 and sPD-L1 in cancer clinical studies (Table 1) varied, with serum or blood plasma as the types of samples. Conventional enzyme-linked immunosorbent assay (ELISA) remains the most used method besides the modification method of ELISA, namely multiplexed fluorescent bead-based immunoassays [25], antibody array assay [22], and binding capacity of ELISA [39]. The basic principle of bead-based immunoassays is the addition of a bead that can distinguish various types of proteins to be detected since over 2 proteins can be detected at once [25]. The use of arrays allows the detection of multiple cytokines in one processing [22]. Another method which is also the development of ELISA is the binding capacity method. This new ELISA can assess sPD-L1 glycosylation, while conventional ELISA can only calculate the quantity of sPD-L1. The combination of these two methods may be able to assess the percentage of sPD-L1 glycosylation to total sPD-L1 [39].

b. Acute infectious/inflammatory diseases

Table 2 presents the clinical studies of sPD-1/sPD-L1 in acute infectious/inflammatory diseases. The studies were conducted in European, American, and Asian countries. PubMed search results contained 4 clinical study groups in this category, namely acute pancreatitis (AP), acute respiratory distress syndrome (ARDS), sepsis, and hantavirus infection. We combined clinical studies on acute infections in this sub-section since infection is one inflammation source. A total of 1677 patients were involved in sPD-1 and or sPD-L1 examinations, and the method used in all clinical studies was ELISA. Of the 10 articles, 7 articles discussed sPD-1 and or sPD-L1 in sepsis and other articles about AP, ARDS, and hantavirus infection, 1 article each.

Clinical studies in acute diseases regarding sPD-1 and sPD-L1 were not as complex as in cancer. There were common goals for all clinical studies that led to 4 points, namely the comparison of these two soluble proteins in patients and healthy subjects, comparison with bound proteins in the membrane, correlation with inflammatory markers, and estimation of deterioration and severity of patients.

The sPD-L1 level showed an increase in all clinical studies (Table 2). In AP conditions, sPD-L1 level significantly showed an increase compared to control subjects. The increased level of sPD-L1 could be the initial parameter for the prediction of infectious complications in patients with AP [56]. Clinical studies in Hantavirus patients with the purpose to investigate how hantavirus replication modulates sPD-L1 reported that patients infected with Puumala virus (PUUV) or Dobrava-Belgrade virus (DOBV) had high sPD-L1 level compared to healthy subjects. SPD-L1 level was directly proportional to PD-L1 expression that occurred *in vitro* in dendritic cells. PD-L1 gene expression was controlled by inflammatory signals due to the virus infection [57]. Another clinical study of 108 sepsis patients reported increased sPD-L1 levels

Table 2. Clinical studies in acute infection/inflammation diseases.

Type of disease	N	Results*		Key findings	Ref.
		sPD-1	sPD-L1		
AP	N = 77 (patients = 56; HC = 21)	-	Patients = 63.87 pg/mL, HC = 48.15 pg/mL	A significantly higher level of sPD-L1 in AP patients compared to HC ($p<0.001$)	56
HV	NA	-	NA	Level of sPD-L1 significantly higher in patients infected with PUUV or DOBV compared to HC	57
Sepsis	N = 108 (patients = 88; HC = 20)	NA	NA	Level of sPD-L1 significantly higher in patients than HC	58
	N = 100 (patients = 85; HC = 15)	-	NA	Transcription of HIF1α significantly increased in the SaO2 group (≤ 92%), and associated with high PD-L1 expression in circulating monocytes and increase of sPD-L1 in serum.	59
	N = 483	-	NA	Continual improvement in inflammation and immunosuppression biomarkers occured in two-thirds survival sepsis patients, corelated with worse long-term outcomes	60
	N = 157 (patients = 112; HC = 45)	NA	NA	•Levels of sPD-1 and sPD-L1 are positively correlated with the severity of sepsis • The peripheral blood levels of sPD-1 and sPD-L1 are higher in unsaved survivors	61
	N = 160 (sepsis = 101; non-infectious critical illness = 28; HC = 31)	HC = 2.9 ng/mL (0.9-9.1) Sepsis = 1.3 ng/mL (0.6-5.8) ICU = 2.4 ng/mL (0.7-5)	-	Level sPD-1 significantly lower in the sepsis group compared to HC, but no significant difference compared to the ICU or between ICU and HC	62
	N = 655 (patients = 595; HC = 60)	HC = 0.76 ng/mL (0.31-1.29) SIRS = 0.80 ng/mL (0.43–1.30) Sepsis = 1.01 ng/mL (0.55–1.58) Severe Sepsis = 1.72 ng/mL (1.07–2.94) Septic Shock = 2.70 ng/mL (1.31–4.83)	-	• Level of sPD-1 is higher in sepsis than SIRS group and HC • Level of sPD-1 in sepsis, severe sepsis, and septic shock groups showed significant differences in pairwise comparisons ($P <0.001$)	63

Table 2 continued..

	N = 120 (patients = 91; HC = 29)	Sepsis = 2.09 ng/mL HC = 0.98 ng/mL	Sepsis = 1.33 ng/mL HC = 0.45 ng/mL	Level of sPD-L1 and sPD-1 significantly increased in sepsis patients than HC. Level of sPD-L1 significantly higher in non-survivors than survivors, but no difference in sPD-1 level	64
ARDS	Blood: N = 20 (patients = 10; HC = 10) BAL: N = 18 (patients =13; HC = 5)	Blood patients = 11,429 pg/mL BAL patients = 6,311 pg/mL Blood HC = 8,061 pg/mL BAL HC = 90.7 pg/mL	-	Levels of sPD-1 are increased in both the serum and BAL fluid in patients with ARDS	65

*The sPD-1/sPD-L1 data listed above are in accordance with the results in each clinical studies, in mean or median value. All data were interpreted based on comparison with HC or other indicators available in each of the clinical studies.
Abbreviations: AP, acute pancreatitis; ARDS, acute respiratory distress syndrome; HV, hantavirus; HC, healthy control group; BAL, bronchial alveolar lavage; PUUV, puumala virus; DOBV, dobrava-belgrade virus; NA, data not available.

associated with decreased T cell activation and T cell apoptosis in cancer [58]. An increase in sPD-L1 was also correlated with the oxygen saturation level and related to high PD-L1 expression in circulating monocytes [59].

Similar to clinical studies in patients with hantavirus, a clinical study in 483 sepsis patients reported that a continuous increase in hs-CRP and sPD-L1 occurred in two-thirds of patients who survived hospitalization for sepsis and was associated with worse long-term results [60]. An increase in sPD-L1 was also correlated with the severity of sepsis patients with a low survival [61].

Level of sPD-1 showed an increase in 4 out 5 clinical studies. In sepsis patients, 3 out of 4 clinical studies reported an increase in sPD-1 level. An exception was found in the study of Lange *et al*. that reported that sPD-1 level decreased in sepsis subjects compared to normal control subjects [62]. Meanwhile, 3 other types of research showed an increase in sPD-1. The increase in sPD-1 level may indicate immune dysfunction in sepsis patients. It is also possible to use these soluble proteins as an immunological biomarker for early assessment of severity and deterioration of sepsis [61] and to make sPD-1 a better marker than sPD-L1 [63]. The difference in sPD-1 level observed in the study by Lange *et al*. compared to others was the sPD-1 level in the healthy controls was relatively higher (median of 2.9, interquartile of 0.9-9.1 ng/mL). Meanwhile the sPD-1 level in sepsis patients was lower than that in healthy controls. Another possibility was a difference in the time of sample acquisition. For this reason, further clinical studies need to be carried out to know whether sPD-1 has a different level based on time change in sepsis [64]. In ARDS patients, sPD-1 level showed an increase. This result indicated that sPD-1 could be used to diagnose ARDS [65].

c. Chronic infectious/inflammatory diseases

Thirteen clinical studies on chronic infectious and inflammatory conditions that were categorized into 8 disease groups are presented in Table 3. These studies were carried out in Asia (mostly), Europe, and Latin America. Clinical studies of the Hepatitis B virus (HBV) are the most frequent in this group. Other reported clinical studies include one clinical study each for hepatitis C virus (HCV), HIV, cystic echinococcosis (CE), dermatomyositis, oral lichen planus (OLP), psoriatic, and allergic rhinitis (AR). The method used for measuring sPD-1/sPD-L1

Table 3. Clinical studies in chronic infection/inflammation diseases.

Type of Disease	N	Results*		Key findings	Ref.
		sPD-1	sPD-L1		
CE	N 71 (patients = 51; HC = 20)	Pre-treatment = 218.6 ± 73.4 pg/mL Post-treatment = 209.9 ± 67.6 pg/mL HC = 181.8 ± 48.2 pg/mL	Pre-treatment = 387.5 ± 196.5 pg/mL Post-treatment = 254.3 ± 75.0 pg/mL HC = 185.0 ± 51.0 pg/mL	Level of sPD-L1 significantly higher in patients with CE compared to HC, but no difference before and after treatment	66
DM	N = 158 (patients = 128; HC = 30)	-	sDM = 12.3 ng/mL (8.4-16.2) CRDM = 18.5 ng/mL (13.8-22.4) sCRDM = 8.5 ng/mL (6.8-11.8) HC = 1.3 ng/mL (0.4-2.2)	Level of sPD-L1 increased in DM patients, and might be a diagnostic indicator for malignancy, especially in patients with anti-TIF1γ antibody	67
OLP	N = 54 (patients = 36; HC = 18)	patients = 26.10 ng/L (8.81- 40.00) HC = 17.65 ng/L (0.00-26.10)	patients = 29.53 ng/L (21.47-36.76) HC = 22.79 ng/L (1.19-28.29)	Level of sPD-1 and sPD-L1 significantly higher in OLP patients than HC, but no correlation with OLP clinical characteristics	68
HIV	N = 49	-	NA	Level of sPD-L1 in HIV (+) group significantly higher than LV, ART and HIV (-) groups. sPD-L1 correlated with activated CD8 Tc and fibrinogen	69
AR	N = 80 (patients)	-	NA	• Level of sPD-L1 correlated with IFN-γ, IL-4 dan IL-7 in allergic rhinitis patients. • A negative correlation between sPD-L1 with IL-4 and IL-17	70
HBV	N = 65 (patients = 45; HC = 20)	NA	NA	Level of sPD-L1 significantly higher in HBV/HIV coinfection compared to HC	71
	N = 278 (patients = 218; HC = 60)	patients = 4.409 pg/mL (3.435-5.306) HC = 0.3665 pg/mL (0.2425-0.5010)	-	• Level of sPD1 correlated with chronic HBV infection Level of sPD-1 is higher in CHB patients compared to HC	72
	N = 427 (patients = 220; HC = 207)	Baseline = 32.01 ± 6.05 ng/L 12 weeks = 33.86 ± 7.12 ng/L 24 weeks = 39.69 ± 7.60 ng/L 48 weeks = 47.58 ± 8.44 ng/L HC = 51.18 ± 10.58 ng/L	-	Level of sPD-1 in HBeAg-positive CHB patients before treatment significantly lower than HC. After entecavir treatment, sPD-1 gradually increases	73

Table 3 continued..

	N = 371 (patients=285 ; HC=86)	pre-treatment = 3.80 ng/mL (0.08-48.38) post treatment = 0.74 ng/mL (0.09-16.56) HC = 0.33 ng/mL (0.04-5.15)	-	Level of sPD-1 significantly increased in patients with chronic HBV infection compared to resolvers of HBV infection or healthy HC	74
	N = 1281 (patients=126 ; HC=1155)	>282 pg/mL	-	Increasing sPD-1 in plasma chronic hepatitis B patients correlates with level of PD-1, viral load and disease development	75
HCV	N = 93 (patients = 63; HC =30)	NA	-	Level of sPD-1 significantly higher in chronic hepatitis C patients compared to HC	76
PsO	N= 86 (patients=57; HC=29)	NA	-	No significant difference of sPD-1 in patients and HC	77

*The sPD-1/sPD-L1 data listed above are in accordance with the results in each clinical studies, in mean or median value. All data were interpreted based on comparison with HC or other indicators available in each of the clinical studies.
Abbreviations: CE, cystic echinococcosis; DM, dermatomyositis; HBV, hepatitis b virus; HCV, hepatitis c virus; HIV, human immunodeficiency virus; OLP, oral lichen planus; PsO, psoriatic; AR, allergic rhinitis; HC, healthy control group; sDM, without malignancies; CRDM, cancer related dermatomyositis; nCRDM, new onset cancers dermatomyositis; sCRDM, stable cancers dermatomyositis; CHB, chronic HBV; LV, low viraemic; ART, antiretroviral therapy; NA, data not available.

was mostly ELISA. As discussed in the sections on cancer and acute diseases, in general, we classified the roles of the two soluble proteins in chronic infection and inflammation into 3 groups, namely its comparison with healthy subjects, correlation with inflammatory markers it generates and degree of infection/inflammation, and assessment of treatment response.

In chronic diseases according to Table 3, increased sPD-1 and sPD-L1 levels were reported in almost all clinical studies. Clinical studies in CE patients investigating the roles of sPD-1 and sPD-L1 by calculating both soluble proteins before and after treatment stated that there was an increase in sPD-L1 and it was significantly higher in CE patients compared to healthy subjects. However, sPD-1 expression was statistically not significantly higher than healthy subjects before and after treatment. This clinical study also explained the relationship between sPD-1 and sPD-L1 with several inflammatory cytokines. Levels of IFN-γ and IL-2 in the serum of CE patients showed no significant difference compared to healthy controls. However, IL-4, IL-6, and IL-10 cytokines in patient serum increased significantly. IL-17 level in the patient's serum was found to increase before treatment but not significant compared to healthy control subjects [66]. Another clinical study on dermatomyositis subjects affirmed that serum sPD-L1 level increased significantly, and very high sPD-L1 level could be a diagnostic indicator for malignancy [67]. An increase in sPD-L1 also occurred in OLP patients. The sPD-L1 and sPD-1 protein levels in patients with OLP were significantly higher compared to healthy subjects, but sPD-1 and sPD-L1 expressions were not related to the clinical characteristics of OLP. This increase in the soluble protein might have a role in the pathogenesis of the disease [68].

A high sPD-L1 level could also be used as a diagnostic and prognostic indicator in dermatomyositis patients [67]. A clinical study in patients with HIV suggested that an increase in sPD-L1 level correlated with sCD14, IFN-γ, and inflammatory cytokines such as IL-21, IL-7 IL-4, and IL-17. Higher level of sPD-L1 was also found in cell culture supernatants stimulated with TNF-α

or LPS (Lipopolysaccharide). In addition, it was also reported that MMP-2 concentration in conventional dendritic cells (CDC) increased in HIV^+ viraemic people. CDC is one of the cells that has higher MMP-2 levels, besides monocytes. These results also suggested that these cells might be able to produce sPD-L1 [69]. A clinical study of allergic rhinitis (AR) patients also showed a relationship between sPD-L1 variables with IFN-IL, IL-4, and IL-7. The results of this study also emphasised the role of sPD-L1 in AR prevention, characterized by an inverse correlation between sPD-L1 and IL-17 and IL-4 [70].

A clinical study in 45 HBV/HIV patients and 20 healthy subjects also reported significantly higher sPD-L1 in HBV/HIV co-infection compared to healthy subjects. This co-infection was associated with TNF-α and several inflammatory cytokines including IL-6, IL-8, IL-10, and IL-12p70 [71]. In chronic viral infections, including HBV and HIV, continuous exposure to high concentrations of viral antigens causes T-cell fatigue [71].

Furthermore, the change of sPD-1 level in chronic infection conditions like those caused by viruses has been reported. Three clinical studies on HBV subjects and one clinical study in HCV revealed that there was an increase in sPD-1 level (Table 3). The increase in soluble protein correlated with chronic infection, viral replication, and HBV indicating markers [72]. An increase in sPD-1 also demonstrated its involvement in the pathogenesis of HBV disease and allowed the use of sPD-1 as a biomarker in understanding immune activities and HCC development [74]. The clinical study of Cheng *et al*. revealed that the increase in sPD-1 level observed in the plasma of chronic hepatitis B patients had clinical importance. The increase in sPD-1 level (>282 pg/mL) predicted higher viral load for 4 or more years. High levels of viral load and sPD-1 were associated with HCC development [75]. The quantitative RT-PCR (real-time polymerase chain reaction) result in HCV patients indicated that the mRNA level of PD-1 in peripheral blood mononuclear cell (PBMC) increased in chronic HCV patients compared to normal controls. It was taken into consideration that sPD-1 could be removed from the membranes of these cells, and that the up-regulation of these molecules might be one of the reasons for the high PD-1 level in HCV patients [76].

d. Autoimmune diseases

Table 4 consists of 14 clinical studies on autoimmune and its association with sPD-1 and sPD-L1 examinations. PubMed search results contained 7 clinical study groups in this category, namely 5 rheumatoid arthritis (RA) clinical studies, 2 systemic sclerosis (SSc) clinical studies, and one clinical study each about Sjogren syndrome (SS), antineutrophil cytoplasmic antibody (ANCA), autoimmune hepatitis (AIH), immune thrombocytopenia (ITP), and myasthenia gravis (MG). The assessment of sPD-1 level was discussed in 13 clinical studies and that of sPD-L1 in 7 clinical studies, either discussed together with sPD-1 or independently. We concluded that the objectives of sPD-1 and sPD-L1 clinical studies on autoimmune were categorized into 4 groups, namely comparing its level with healthy subjects, roles in monitoring treatment response, assessment of disease pathogenesis, and correlation with inflammatory markers. In addition to the ELISA method, two clinical studies reported the use of other methods, namely antigen and antibody system, and western blot analysis.

Almost all clinical studies (Table 4) reported an increase in sPD-1 level. In RA patients, sPD-1 levels increased in all of its clinical studies, compared to healthy controls. The clinical study of Wang and coworkers reported that an increase in sPD-1 coincided with the increase of PD-1 percentage in T-cells, as a feedback regulatory mechanism for T-cell fatigue in RA [78]. Increased sPD-1 could also play a role in the pathogenesis of RA diseaseed [80] and be involved in the regulation of Treg effector functions [81]. It was reported that sPD-1 increased significantly only in positive anti-citrullinated protein antibodies (ACPA) but not in negative ACPA in early RA [80]. The RA pathogenesis was also connected with the pro-inflammatory cytokine overproduction which prevent immunological homeostasis maintenance. A clinical study in 82 RA patients reported that sPD-1 was significantly reduced in the treated group compared to the untreated and random

Table 4. Clinical studies in autoimmune diseases.

Type of disease	N	Results*		Key findings	Ref.
		sPD-1	sPD-L1		
RA	N = 44 (patients = 24; HC = 20)	NA	NA	• Level of sPD-1 significantly increased, but no difference of sPD-L1 in patients and HC • Level of sPD-1 positively correlated with PD-1 in cells and CRP in RA patients	78
	N = 414 (patients = 246; HC = 168)	NA	NA	A negative relationship between smoking and sPD-L1 in RA patients	79
	N = 218	NA	-	Level of sPD-1 increased in ACPA (+), but no difference in ACPA (-) early RA	80
	N = 172 (patients = 82; HC = 90)	Within Tregs: Untreated = 1.54 ± 0.16 siRNA-sPD-1 1.02 ± 0.11 Scramble groups 1.51 ± 0.18	-	Level of sPD-1 significantly decreased in siRNA group after treatment compared to pre-treatment and randomized groups. But no differences in sPD-1 between untreated and randomized groups.	81
	N = 84 (patients, blood = 34, sinovial fluid = 30; HC = 20)	Early RA= 0.421 ng/mL (0.04-2.56 ng/mL) Chronic RA = (0.239 ng/mL (0.184-0.584 ng/mL) HC = 0.04 ng/mL (0.04-0,04 ng/mL)	-	• Level of sPD-1 in early and chronic RA increased compared with HC • Level of sPD-1 associated with DAS28 and HAQ score in early stage and inversely related to TSS at 3-5 years • Level of sPD-1 correlated with IgM-RF, anti-CCP antibodies, and IL-21	82
SS	N = 20 (patients = 10; HC = 10)	Patients = 0.618 ± 0.04 pg/mL HC = 0.194 ± 0.018 pg/mL	-	• Level of sPD-1 significantly higher in SS patients than HC • Level of sPD-1 significantly decreased after TGP treatment for 3 months • Level of IL-10 is lower in SS patients than HC, and increased after TGP treatment for 3 months, inversely proportional to IL-17A	83
SSc	N = 141 (patients = 97; HC = 44)	NA	-	Level of sPD-1 and sPD-L2 significantly increased in SSc patients than HC, sPD-1 correlated with sPD-L2	84
	N = 87 (patients = 61; HC = 26)	Patients = 203.7 ± 191.9 pg/mL HC = 174.5 52.9 pg/mL	Patients = 201.56 ± 155.8 pg/mL HC = 73.6 ± 66.2 pg/mL	• Level of sPD-1 and sPD-L1 increased in SSc patients than HC • Level of sPD-1 significantly higher in dcSSc groups than lcSSc group and HC • Level of sPD-1 significantly decreased in icSSc group than HC	85

Table 4 continued..

ANCA	N = 59 (patients)	Severe AAV = 380.7 pg/mL Non Severe AAV = 180.3 pg/mL AAV - ANCA+ve = 235.6 ± 338.8 pg/mL AAV - ANCA-ve = 281.5 ± 381.5 pg/mL	-	• Level of sPD-1 significantly higher in severe AAV patients than non severe patients • Severe AAV is more often identified in patients with serum sPD-1 ≥70.1 pg/mL, and no correlation between ANCA positivity and severe AAV	86
AIH	N = 114 (patients = 67; HC = 47)	AIH active disease = 0.24 ng/mL (0.16-0.28) Incomplete standard treatment = 0.17 ng/mL (0.11-0.22) Standard treatment = 0.11 ng/mL (0.08-0.16) HC = 0.12 ng/mL (0.05-0.16)	-	Level of sPD-1 in AIH patients with active disease and incomplete standard treatment patients significantly increased compared to respondents with standard therapy and HC	87
IPF	N = 23 (patients)	-	Patients = 314.3 ng/L (117.7–483.1 ng/L) HC = 91.0 ng/L (52.4–119.7 ng/L)	Level of sPD-L1 in IPF patients significantly higher compared to HC	88
MG	N = 97 (patients = 97; HC = 25)	NA	NA	• Level of sPD-1 in USMG group significantly higher than RSMG and HC groups • No significant difference between the RSMG and HC group • No differences of sPD-L1 between USMG, RSMG, and HC groups	89
ITP	N = 88 (patients = 67; HC = 21)	ndITP = 5.15 ng/mL (2.07-7.06) cITP = 5.33 ng/mL (2.25-32) HC = 8.91 ng/mL (4.83-52.07)	ndITP = 4.06 ng/mL (1.22-8.71) cITP = 5.47 ng/mL (2.53-15.4) HC = 4.56 ng/mL (2.33-10.23)	• Level of sPD-1 and sPD-L1 in ndITP and cITP patients were not statistically different • Level of sPD-1 in ndITP and cITP patients significantly decreased compared to HC • Level of sPD-L1 was not significantly different with HC	90
	N = 70 (patients n = 40; HC = 30)	NA	NA	• Level of sPD-1 in ITP patients is significantly different from HC • No significant difference of sPD-L1 between patients and HC • Level of sPD-1 is negatively correlated with platelet counts in ITP patients	91

Table 4 continued..

	N = 55 (patients n = 35; HC n = 20)	NA	NA	• Level of sPD-1 in ITP patients before treatment significantly higher than HC • After treatment, level of sPD-1 significantly decreased compared to pre-treatment. But not different than HC	92

*The sPD-1/sPD-L1 data listed above are in accordance with the results in each clinical studies, in mean or median value. All data were interpreted based on comparison with HC or other indicators available in each of the clinical studies.

Abbreviations: RA, rheumatoid arthiritis; SS, sjogren syndrome; SSc, systemic sclerosis; ANCA, antineutrophil cytoplasmic antibody; AIH, autoimun hepatitis; IPF, idiopathic pulmonary fibrosis; MG, myasthenia gravis; ITP, immune thrombocytopenia; HC, healthy control group; ACPA, anti-citrullinated protein antibodies; dcSSc, difuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; AAV, associated vasculitis; USMG, untreated stage myasthenia gravis; RSMG, remission stage myasthenia gravis; ndITP, newly diagnosed immune thrombocytopenia; cITP chronic ITP immune thrombocytopenia, NA, data not available.

groups, and sPD-1 might be involved in the regulation of Treg effector functions at the inflammation site [81]. Another clinical study reported that sPD-1 concentration correlated with IgM-RF, anti-CCP antibodies, and IL-21 [82].

In other autoimmune patients like SS and SSc, the increase in sPD-1 was directly proportional to IL-17 pro-inflammatory cytokine and inversely proportional to IL-10 anti-inflammatory cytokine. It suggested that low IL-10 in both diseases could not effectively inhibit the pro-inflammation of IL-17 cytokines [83, 84]. After treatment with total glucosides of paeony (TGP) for 3 months, sPD-1 level decreased significantly and was inversely proportional to IL-17 level [83].

Patients with severe ANCA-associated vasculitis (AAV) reported higher average sPD-1 serum than those who were not and were more frequently identified in patients with sPD-1 serum of ≥70.1 pg/mL [86]. In clinical studies on AIH and MG, sPD-1 concentration was higher before incomplete treatment or medication than in the group after full treatment or medication and healthy control [87, 89]. Clinical studies in ITP patients showed mixed sPD-1 results. Two clinical studies showed an increase in sPD-1 in ITP patients while the clinical study of Atesoglu *et al*. reported a decrease in sPD-1 at ITP patients compared to healthy subjects even though the difference was not significant [90]. Two other clinical studies reported an increase in sPD-1 in ITP patients, and after treatment, sPD-1 concentration in ITP patients was significantly reduced [91, 92]. It was also reported that IFN-γ and IL-17 serum concentrations in ITP patients were higher compared to control subjects [91]. The different results observed among ITP patients could be due to a relatively small number of samples. It was also suggested that ethnicity played a small role in sPD-L1 level.

The sPD-L1 concentration showed various changes in clinical studies in autoimmune disease groups. The clinical study of sPD-L1 in RA patients was only carried out in 2 out of 5 clinical studies. The research of Wang *et al*. showed that the increase in sPD-L1 was not significantly different from healthy subjects. They stated that the small number of samples was possibly the cause [78]. Clinical studies in ITP and MG patients indicated insignificant different results compared to healthy subject controls [89-91]. MG patients showed that sPD-L1 concentration was only 1/5 of sPD-1 concentration. They considered that excessive sPD-1 could disrupt the binding between PD-1 in Tfh cell membrane and PD-L1 in B-cell membrane, and hence Tfh cell would not accept a relatively strong negative regulatory signal [89].

Changes in sPD-L1 concentration was reported in the following 2 clinical studies. The research of Wasen *et al*. stated that sPD-L1 level decreased in RA patients who smoked. Smoking was reported to limit sPD-L1 response in RA smokers by altering the balance in FcγR stimulation expression and its

inhibition [79]. In SSc patients, there was an increase in sPD-L1 level, but sPD-L1 serum level was not associated with clinical aspect and laboratory data. It seems that the sPD-L1 roles in this autoimmune condition are yet to be clearly understood, but it was reported that an increase in sPD-1 level could reflect dermal sclerosis activities in SSc patients [85].

e. Other diseases

Table 5 describes two other clinical studies that are included under acute or chronic infections and inflammation, namely the studies in kidney transplanted patients and preeclampsia. The clinical study of Malendreras *et al*. in 84 kidney transplanted patients explained that sPD-1 and sPD-L1 levels observed at the time before and after transplantation in some of their patients showed almost similar results. They have become a marker in patients with bad transplantation results, and hence these two soluble proteins were useful as biomarkers of immune status indicators that helped to predict the success of kidney transplants [93]. Other clinical studies in preeclampsia women suggested that sPD-1 level was significantly higher in preeclampsia than in normal pregnancy controls, but not at sPD-L1 level. This clinical study explained that the ratio of sPD-1/sPD-L1 changed in preeclampsia compared to controls, and an increase in sPD-1 level indicated that the immune system was more active in preeclampsia [94]. Further research should be undertaken to investigate the other roles of sPD-1 and sPD-L1 in kidney transplanted patients and preeclampsia.

CONCLUSION AND FUTURE PERSPECTIVES

This article summarized 80 clinical studies of sPD-1 and sPD-L1 in humans conducted from 2010 to 2020. The clinical studies were categorized into 5 disease groups according to the similarity of pathophysiology, namely cancer disease, acute infectious/inflammatory disease, chronic infectious/ inflammatory disease, autoimmune disease, and other diseases that did not belonged to the other groups. This article discussed the comparison of sPD-1/sPD-L1 level in patients compared to healthy subjects, the correlation between the level of soluble form and membrane-bound PD-L1, as well as their association with the severity of diseases, treatment response, and other inflammatory markers. ELISA is the most frequently used method, however, other methods such as multiplexed fluorescent bead-based immunoassays, antibody array assays, ELISA binding capacity, and western blot analysis are also acceptable.

The sPD-1 and sPD-L1 were reported to significantly increase compared to healthy subjects in all clinical

Table 5. Clinical studies in other diseases.

Type of disease	N	Results*		Key findings	Ref.
		sPD-1	sPD-L1		
KTP	N = 84 (patients = 59; HC = 25)	NA	NA	Patients with high soluble molecules showed a progressive and gradual decline in kidney function	93
PE	N = 172 (preeclampsia = 86; normotensive = 86)	preeclampsia = 69 ± 131 pg/mL normotensive = 43 ± 52 pg/mL	preeklampsia = 6262 ± 1860 pg/mL normotensive = 1134 ± 349 pg/ mL	Level of sPD-1 significantly higher in preeclampsia compared to normal pregnant, but level of sPD-L1 not so	94

*The sPD-1/sPD-L1 data listed above are in accordance with the results in each clinical studies, in mean or median value. All data were interpreted based on comparison with HC or other indicators available in each of the clinical studies.

Abbreviations: KTP, kidney transplanted patients; PE, Pre-eclampsia; HC, healthy control group; NA, data not available.

studies of cancer and acute and chronic infections/ inflammation. In autoimmune patients, it was reported that sPD-1 increased in almost all clinical studies. The relatively small number of samples made some clinical studies unable to show improved results. This also resulted in sPD-L1 levels being reported with a variety of results, and hence the role could not described clearly. The increase of sPD-L1 in cancer and cancer-related infectious/inflammatory diseases was equivalent to an increase in membrane-bound PD-L1. The increase in sPD-L1 has become a marker of deterioration and severity of cancer diseases; meanwhile the decrease in sPD-L1 indicated successful treatment of cancer. The increase of sPD-1 in autoimmune patients correlated with PD-1 levels in T-cells, and marked success in the treatment. In cancer disease, more clinical studies have to be conducted to assess the role of sPD-1 in determining treatment responses. The summary of our results also found that sPD-L1 and sPD-1 in all disease groups correlated with the expression of various inflammatory marker cytokines. The increase in these two soluble proteins is correlated with the inflammatory marker CRP, and various pro-inflammatory cytokines such as TNF-α, IL-17, IL-7, IL-12, and IL-21, as well as anti-inflammatory cytokines such as IL-10 and IL-4. Meanwhile, the increased level of sPD-1/sPD-L1 also correlated with IL-6 that acts as a pro-inflammatory and anti-inflammatory cytokine. The correlation with various inflammatory markers is an interesting thing. Other chronic diseases besides the clinical studies discussed here also reported correlation with a variety of inflammatory markers such as CRP as well as pro and anti-inflammatory cytokines. Metabolic syndrome diseases such as obesity, diabetes etc. were reported to correlate with inflammatory markers and could even develop as a trigger for cancer, but there is no clinical study which assesses the description of these two soluble proteins in the condition of metabolic syndrome. In future, further explorations on the levels of sPD-1 and sPD-L1 in patients suffering from these diseases need to be carried out to provide deeper insights that can be useful for clinical applications.

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Not applicable.

CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

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