International Immunopharmacology 129 (2024) 111600

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Association of soluble PD-L1 and NLR combination with 1-Year mortality in patients with COVID-19

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ARTICLE INFO

Keywords:
COVID-19
Post-COVID Mortality
sPDL-1
Neutrophil-to-Lymphocyte Ratio
Humoral and cellular immune response

ABSTRACT

Purpose: Understanding the relationship between patient immune characteristics, disease severity, and mortality represents a critical step in the fight against COVID-19. Elevated levels of programmed death ligand-1 (PD-L1) and Neutrophil-lymphocyte ratio (NLR) are linked to increased severity of acute COVID-19 in patients. This study aimed to investigate the association of the combination of sPD-L1 and NLR with 1-year Mortality in patients with COVID-19.

Methods: A prospective study was conducted involving patients with COVID-19 in Karaganda, Kazakhstan. The level of sPD-L1 in the blood serum was evaluated by ELISA. The effect of biomarkers on the development of mortality was analyzed with multivariate regression.

Results: The risk of mortality within one year HR was 2.46 if the plasma sPD-L1 value of more than 277.13 pg/ml, and for NLR more than 2.46 HR was 2.87. The model of combining sPD-L1 and NLR resulted in an improvement in the predictive accuracy of the Hazard Ratio 7.6 (95 % CI: 3.02–19.11).

Conclusion: The combination of two immune-mediated markers (sPD-L1 and NLR), which reflect the systemic inflammatory balance of activation and exhaustion, can complement each other and improve the assessment of the risk of death in patients with COVID-19.

1. Introduction

SARS-CoV-2 has had a significant impact on public health and has forced a re-evaluation of traditional epidemic management approaches. Data from multiple epidemiological studies indicate that individuals previously infected with COVID-19 are at a significantly higher risk of death within the next 12 months [1,2]. Predictors of COVID-19 severity and mortality in the acute period are well-studied; long-term consequences, including mortality, are still under investigation. Understanding the relationship between patient immune characteristics, disease severity, and mortality represents a critical step in the fight against COVID-19. Severe and critical manifestations of COVID-19 are caused by a dysregulated immune response, in which the adaptive immune system driven by T and B lymphocytes plays a fundamental role [3].

Previous studies have established the importance of elevated levels of programmed death ligand-1 (PD-L1) and its receptor PD-1 in the pathogenesis of cancer [4–7] and various infectious diseases, including AIDS and hepatitis [8–10] There is evidence to suggest that elevated levels of PD-L1 might be linked to increased severity of acute COVID-19 in patients [11,12]. The interaction between the checkpoint molecules PD-1 and its ligand PD-L1 helps to reduce inflammation and tissue damage in severe and critical cases of COVID-19 [13,14]. However, due to increased levels of PD-1 expression, T cells can become exhausted and their effector functions may decrease, leading to the progression of the disease. Beserra et al. analyzed the levels of sPD-1 and sPD-L1 in the serum of patients and observed an increase in sPD-L1 in patients with severe and critical symptoms [15]. Additionally, it has been reported that increased levels of sPD-L1 were associated with lower lymphocyte counts and higher CRP levels, and were also linked to longer hospital stays and higher in-hospital mortality rates [16]. However, the mechanisms underlying increased PD-L1 levels in SARS-CoV-2 are not fully understood, and studies on soluble PD-L1 in COVID-19 patients with

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https://doi.org/10.1016/j.intimp.2024.111600
Received 12 December 2023; Received in revised form 16 January 2024; Accepted 24 January 2024
Available online 6 February 2024

varying disease severity are scarce.

It has been established that systemic inflammation is a key factor in COVID-19 development [17–19]. Baseline Neutrophil-lymphocyte ratio (NLR) level has been proposed as a prognostic marker in COVID-19 [20].

Compelling evidence of immune dysfunction associated with COVID-19 and its long-term impact on mortality is essential for effective patient stratification in planning preventive measures and clinical management in the post-covid period. Therefore, this study aimed to investigate the association of the combination of immune-mediated mediators such as sPD-L1 and NLR with 1-year Mortality in patients with COVID-19.

2. Materials and Methods

2.1. Study design and participants

A prospective study was conducted at the infectious diseases clinics of Karaganda Regional Clinical Hospital and Karaganda Medical University Hospital between May and August 2021. The main group for this study included patients over 18 years of age who had tested positive for COVID-19. Pregnant and lactating women, and individuals with compromised immune systems, such as those affected by human immunodeficiency virus (HIV) infection, or those currently undergoing treatment for malignancies, were excluded from the study. The study involved 314 COVID-19-positive patients confirmed by RT-PCR analysis of nasopharyngeal swabs for genomic RNA with or without co-existing conditions, undergoing treatment at an infectious diseases hospital.

Clinical data and laboratory results were collected from patients' electronic medical records. All laboratory tests, including complete blood count, plasma C-reactive protein (CRP), ferritin, and D-dimer concentrations, were collected on the same day as plasma sampling. Clinical outcomes (intensive care unit admission, length of hospital stay, mortality) were recorded until hospital discharge. To assess the severity of COVID-19 in patients, WHO criteria were used [21]. Two groups of patients were identified: the first group included those with moderate severity, while the second group consisted of patients with severe and critical disease severity. Electronic medical records were used to collect data on the disease progression, comorbidities, anthropometric indicators, heart rate (HR), oxygen saturation, and percentage of lung tissue damage. Body mass index and Charlson comorbidity index were calculated for all patients [22]. All examinations were carried out on the first day of admission to the hospital. After discharge, patients were monitored via phone and recorded in the medical information system for treatment and death. The endpoint considered all deaths, including cause and date. The observation period for COVID-19 patients was one year from hospital admission, with survivors defined as those alive after this time.

2.2. Laboratory analysis

Blood samples were collected on admission day by venipuncture into 2 vacuum tubes containing 5 ml EDTA. Plasma was collected and aliquoted, and serum aliquots were stored at − 80 ∘C. Enzyme-linked immunosorbent assays (ELISA) were used to quantify plasma sPD-L1 concentrations with a research-only reagent kit (Abcam, #ab277712). Colourimetric determinations were recalculated using a four-parameter logistic curve corresponding to each plate. A complete blood count was performed on a hematology analyzer (Mindray, China).

2.3. Statistical analysis

The data from the study was analyzed statistically using SPSS version 21.0. (IBM Corp., Armonk, NY, USA) и MedCalc version 22 (MedCalc Software Ltd, Ostland, Belgium). Data presented in tables and graphs using GraphPad Prism software version 9.5 (GraphPad Software, Inc., San Diego, California, USA). The normality of the distribution was assessed using the Kolmogorov-Smirnov test. Quantitative measures with non-normal distribution are described by median (Me) and interquartile range. Qualitative characteristics are described with percentages. Comparative analysis of quantitative data between groups utilized the Mann-Whitney score for nonparametric distributions and Fisher's exact tests for categorical variables. The discrimination score was determined by the area under the curve (AUC) of the receiver operating characteristic (ROC). AUCs with 95 % confidence intervals were calculated to estimate the diagnostic value of NLR and sPD-L1. Optimal cut-off values for these biomarkers were determined based on the maximum Youden's J index and further used to estimate survival considering high and low biomarker concentrations using Kaplan-Meier survival analysis. Hazard ratios were based on the log-rank (Mantel-Cox) test. Using multivariate regression, the effect of biomarkers on the development of mortality was analyzed, adjusting for already proven risk variables such as gender, age, comorbidity, and disease severity. Statistical test differences were considered significant if p values were < 0.05.

2.4. Ethical declare

This study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of Karaganda Medical University No. 18, dated 14 April 2021. Written informed consent was obtained from the participants.

3. Results

3.1. Demographic data and clinical and laboratory characteristics of patients

The study population (n = 314) was divided into groups based on disease severity (moderate disease [n = 245] vs. severe disease [n = 69]: 49 severe patients (71 %) and 20 patients (29 %) with critical disease severity) and outcome (survivors [n = 275] vs. non-survivors [n = 39]). Table 1 displays patient characteristics, grouped by disease severity and mortality. The patients who contracted COVID-19 had an average age of 63 years. There was no significant difference in the median age between patients with moderate and severe severity. However, the median age of those who died was 72 years, while the median age of survivors was 61 years (p = 0.0001). Patients with severe disease and those who died had a higher prevalence of comorbidities. Out of the 184 patients (58.5 %) had chronic diseases, including hypertension (57.6 %), diabetes mellitus (26.1 %), cardiovascular diseases (42 %), chronic pulmonary diseases (2.5 %), and chronic kidney disease (4.7 %). The group of patients with severe COVID-19 and the group of deceased patients had higher levels of leukocytes, neutrophils, NLR, and ESR, as well as inflammatory markers such as CRP, ferritin, and D-dimer (Table 1). The median hospitalization time for patients with COVID-19 differed between the moderate and severe disease groups (p = 0.0001). Moreover, patients with severe illness and those who unfortunately passed away had a higher chance of receiving respiratory assistance and being admitted to the intensive care unit (ICU) (p = 0.0001). Comparable findings were observed while comparing the groups of survived and deceased patients (p = 0.0001).

3.2. sPD-L1 concentrations in patients with COVID-19

Severe COVID-19 patients have been found to have higher plasma concentrations of sPD-L1 NLR levels compared to patients with moderate disease. Fig. 1A shows that sPD-L1 levels were 279.1 [183.4–401.3] pg/mL for severe patients and 225.9 [150.7–329.5] pg/mL for moderate patients, indicating a statistically significant difference (p = 0.011). Similarly, Fig. 1B shows that the NLR levels were 3.4 [2.4–5.3] pg/ml for severe patients and 2.4 [1.5–3.5] pg/mL for moderate patients (p = 0.0001). Similarly, non-survivors had higher plasma sPD-L1 concentrations (Fig. 1C: 295.6 [177.8–390.4] pg/ml vs 228.6 [154.4–336.2] pg/ml, p = 0.045) and NLR (Fig. 2D: 3.4 [2.4–8.4] pg/ml vs. 2.4

Table 1
Demographic and clinical characteristics of COVID-19 patients.

	All patients (n = 314)	Moderate illness (n = 245)	Severe illness (n = 69)	p-value*	Survivors (n = 275)	Non-survivors (n = 39)	p-value**
Demographic							
Age (year)	63 (51–71)	61 (50–71)	67 (56–72)	0.082	61 (49–70)	72 (63–82)	0.0001
Sex, n (%)							
Male	121 (38.6)	97 (39.6)	24 (34.8)	0.469	170 (61.8)	16 (41)	0.733
Female	193 (61.4)	148 (60.4)	45 (65.2)		105 (38.2)	23 (59)	
BMI (kg/m^2)	29.4 (25.4–34.1)	28.9 (25.4–33.6)	30.8 (25.7–35.5)	0.152	29.4 (25.5–34.2)	28.8 (23.6–33.9)	0.368
Comorbidities or coexisting disorders, n (%)							
Hypertension	181 (57.6)	134 (54.6)	47 (68.1)	0.046	158 (57.4)	23 (58.9)	0.857
Cardiovascular disease	132 (42)	92 (37.5)	40 (57.9)	0.002	112 (40.7)	20 (51.2)	0.211
Diabetes mellitus	82 (26.1)	60 (24.4)	22 (31.8)	0.217	72 (26.1)	10 (25.6)	0.943
Pulmonary disease	8 (2.5)	7 (2.8)	1 (1.4)	0.883	6 (2.18)	2 (5.1)	0.443
Chronic renal failure	15 (4.7)	6 (2.4)	9 (13.01)	0.002	13 (4.7)	2 (5.1)	0.913
Charlson Comorbidity Index, grade							
0–1	201 (64)	166 (68)	35 (51)	0.015	183 (67)	18 (46)	0.002
2–3	85 (27)	61 (25)	24 (35)	0.015	74 (27)	11 (28)	0.002
>4	28 (9)	18 (7)	10 (14)	0.015	18 (6)	10 (26)	0.002
Vital signs at day of sampling, n (%)							
Heart rate (bpm)	80 (76–87)	80 (76–86)	82 (76–90)	0.248	80 (76–86)	80 (76–90)	0.516
Temperature (°C)	36.7 (36.5–37.2)	36.7 (36.5–37.2)	36.7 (36.6–37.3)	0.337	36.7 (36.5–37.2)	36.8 (36.6–37.5)	0.128
Respiratory rate (vpm)	19 (18–21)	19 (18–20)	20 (18–22)	0.004	19 (18–20)	22 (19–22)	0.0001
Oxygen saturation, %	95 (93–98)	96 (94–98)	93 (90–96)	0.0001	95 (94–98)	95 (91–98)	0.116
Infiltrate on chest radiograph, %	25 (12–40)	20 (8–32)	56 (45–66)	0.0001	25 (12–40)	40 (20–52)	0.012
Hospital length of stay (days)	10 (9–12)	10 (8–11)	11 (9–15)	0.0001	10 (9–11)	11 (8–15)	0.383
Invasive mechanical ventilation	20 (6.3)	–	20 (29)	–	8 (2.9)	12 (30.8)	0.0001
Mortality	39 (12.4)	22 (9)	17 (24.6)	0.0001	NA	NA	NA
Laboratory findings							
Leukocytes x 10^9/L	5.2 (3.9–6.5)	5.1 (3.9–6.2)	5.8 (4.2–7.0)	0.027	5.2 (3.9–6.3)	5.9 (4.5–7.2)	0.043
Neutrophils x 10^9/L	3.29 (2.30–4.49)	3.17 (2.20–4.34)	3.95 (2.81–5.47)	0.001	3.24 (2.24–4.36)	4.07 (2.91–5.58)	0.004
NLR	2.6 (1,6–3.8)	2.4 (1,5–3.5)	3.4 (2.4–5.3)	0.0001	2.4 (1.6–3.7)	3.4 (2.4–8.4)	0.0001
ESR, (mm/h)	15 (10–22)	15 (10–23)	15 (10–20)	0.0001	15 (10–22)	15 (8–20)	0.597
CRP (mg/l)	15.1 (6.1–74.7)	12.0 (6.0–37.3)	35.9 (12.0–145.2)	0.0001	12.0 (6.0–46.4)	47.1 (8.2–104.8)	0.031
Ferritin (μg/l)	238 (148–375)	233 (130–356)	254 (186–440)	0.039	234 (148–361)	244 (163–435)	0.335
D-dimer, (ng/ml)	301 (181–441)	286 (165–423)	378 (205–523)	0.004	286 (169–425)	425 (305–549)	0.0001
sPD-L1 (pg/ml)	232.5 (159.1–344.5)	225.9 (150.7–329.5)	279.1 (183.4–401.3)	0.011	228.6 (154.4–336.2)	295.6 (177.8–390.4)	0.045

Data are presented as median with interquartile ranges or n (%). Patient data were compared using the chi-square test, or Fisher's exact test for categorical variables and one-way analysis of variance. Mann–Whitney, nonparametric *t*-test was used for continuous variables. $p < 0.05$ was considered statistically significant. *Moderate versus severe illness. **Survivors versus non-survivors. Abbreviations: Body mass index (BMI), Blood pressure (BP), Intensive care unit (ICU), C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR).

[1.6–3.7] pg/ml, p = 0.0001) compared with survivors. Additionally, the relationship between markers and disease severity was separately assessed in subgroups of survivors and deceased to provide evidence that plasma concentrations of sPD-L1 and NLR are associated with mortality, independent of disease severity (Supplementary Fig. A1). In the deceased group, there were no statistically significant differences in the concentrations of sPD-L1 and NLR in patients with moderate and severe COVID-19.

3.3. Discriminatory impact of sPD-L1 and NLR integration on mortality

The diagnostic value of sPD-L1 and NLR in patients with COVID-19 was assessed using ROC curves. To assess discrimination between survivors and deceased based on concentrations, the results are presented as ROC curves (Fig. 2). Table 2 presents the characteristics of the ROC analyses. The calculated AUC for sPD-L1 was 0.609 (95 % CI: 0.552–0.663, p = 0.033), indicating moderate discrimination between survivors and deceased. The discriminatory power of NLR was slightly higher and also statistically significant (AUC = 0.684, 95 % CI: 0.642–0.723; p = 0.0001). Threshold values for these markers were based on the maximum Youden's J index obtained from the ROC curves. Survival of patients was stratified based on these markers. Hazard ratios were calculated using the log-rank test (Mantel-Cox). The study found that patients with plasma sPD-L1 levels greater than 277.13 pg/mL had a higher risk of mortality within a year (HR = 2.46, 95 % CI: 1.27–4.74, shown in Fig. 3A). Similarly, patients with a high NLR greater than 2.46 (shown in Fig. 3B) had a hazard ratio for mortality of 2.87 (95 % CI: 1.49–5.53; p = 0.0015). When creating the model, combining the cutoff values of sPD-L1 and NLR, the Hazard Ratio was 7.6 (95 % CI: 3.02–19.11, Fig. 3C), thereby proving a synergistic relationship between sPD-L1 and NLR in deceased.

3.4. Multivariate regression analysis to predict mortality in patients with COVID-19

A multivariate regression model was developed to forecast the possibility of death in patients admitted to the hospital with COVID-19. For this model, patients were classified with high or low levels of sPD-L1 and NLR according to cutoff values (Table 2). The model that included high levels of sPD-L1 and NLR demonstrated a high predictive variable (OR 2.359, p = 0.044), even after adjusting the regression model by gender (OR 1.334, p = 0.480), age over 60 years (OR 4.183, p = 0.006), Charlson Comorbidity Index (OR 2.489, p = 0.147) and severity of illness (OR 1.990, p = 0.108) (Table 3).

4. Discussion

Our study findings provide evidence to support the hypothesis that sPD-L1 levels are increased in patients with severe COVID-19 and those who have died. These results indicate that sPD-L1 may contribute to the progression of COVID-19 and could be a potential target for further immunotherapy.

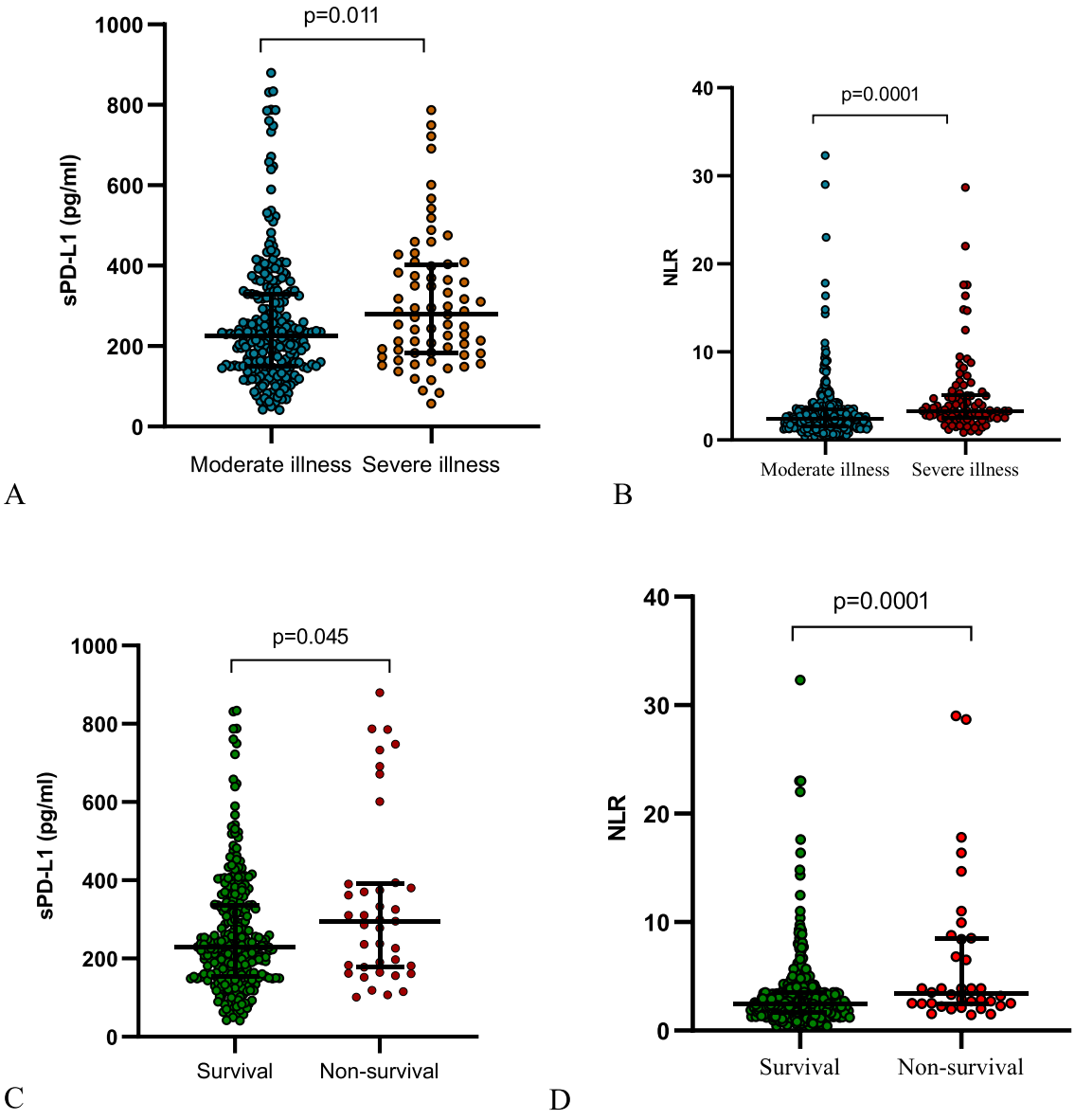

Fig. 1. The plasma concentration of sPD-L1 (A) and NLR (B) moderate and severely ill patients, survivor, and non-survivor patients (C and D). Data are presented as median with interquartile ranges, p-values were calculated with Mann–Whitney U tests.

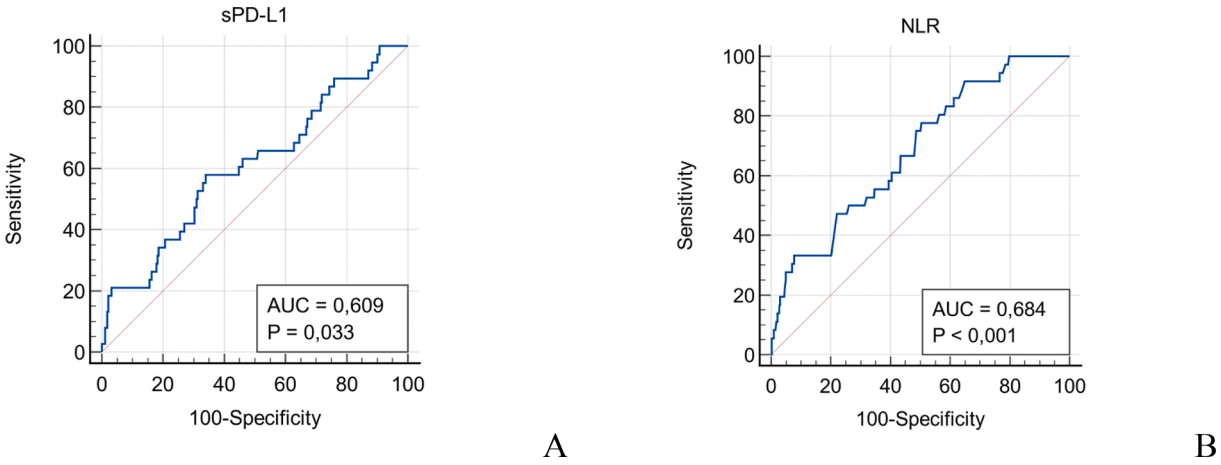

Fig. 2. Receiver operating characteristic (ROC) curves of sPD-L1 (A) and NLR (B) concentrations to predict mortality among patients with COVID-19. The area under the curve (AUC) and the p-values for significant are depicted in the graphic.

Table 2
ROC-test characteristics. Significance of sPD-L1 and NLR at admission in predicting mortality in patients with COVID-19.

	Cut-off	Youden's J	AUC (95 % CI)	Sensitivity, % (95 % CI)	Specificity, % (95 % CI)	p-value
sPD-L1	>277.13	0.2395	0.609 (0.552–0.663)	57.89 (40.8 – 73.7)	66.06 (60.1 – 71.6)	0.033
NLR	>2.46	0.2737	0.684 (0.642–0.723)	77.7 (60.8 – 89.9)	49.59 (45.1 – 54.1)	0.0001

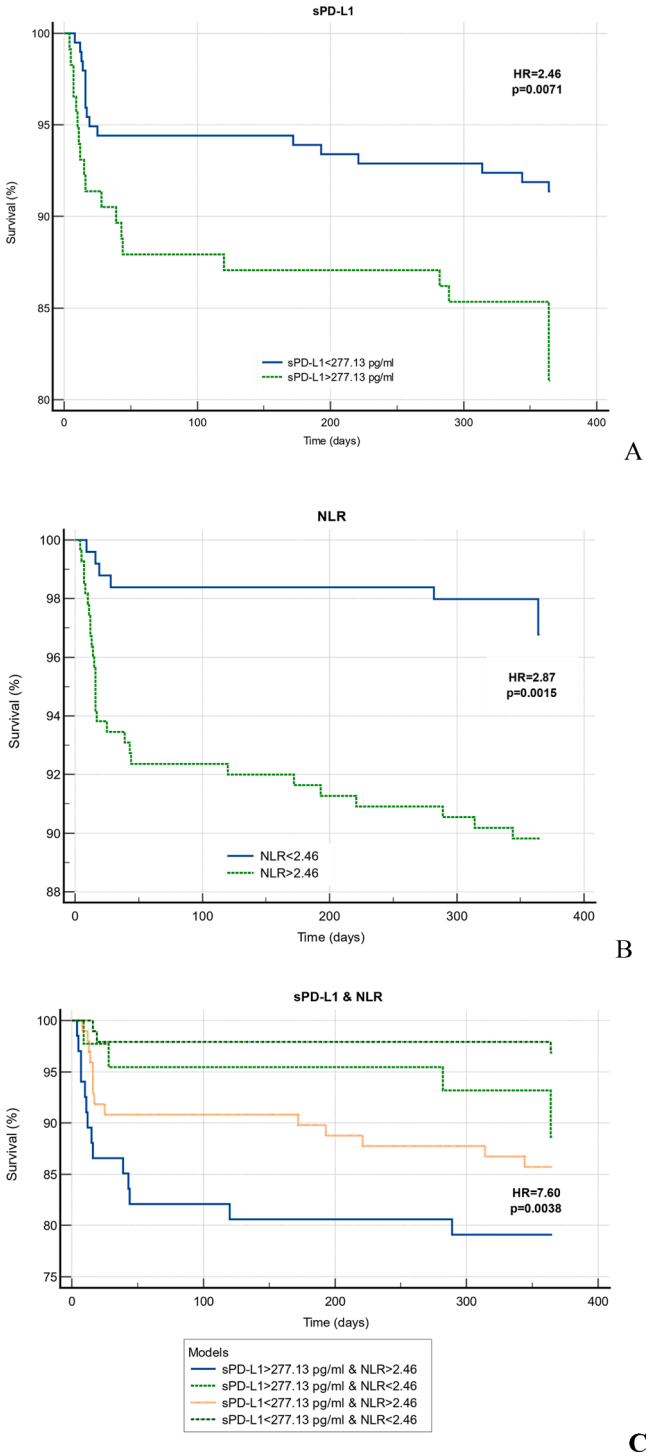

Fig. 3. Survival analysis of patients with COVID-19 for high versus low concentrations of (A) NLR, (B) sPD-L1, (C) integration of sPD-L1 and NLR. Hazard ratios were calculated with the log-rank (Mantel-Cox) test.

Table 3
Multivariate Regression Model for Mortality in patients with COVID-19.

Variable	OR (95 % Confidence Interval)	p-value
Sex (Male)	1.334 (0.600–2.965)	0.480
Age over 60 years	4.183 (1.513–11.599)	0.006
Charlson Comorbidity Index	2.489 (0.725–8.542)	0.147
Severity illness	1.990 (0.861–4.599)	0.108
High sPDL-1 and High NLR	2.359 (1.025–5.429)	0.044

*OR = Odds ratio.

Multiple retrospective studies have shown that uncontrolled immune responses and hyperinflammation are hallmarks of severe COVID-19 [23,24]. The SARS-CoV-2 virus leads to a decrease in the number of lymphocytes, additionally, it can also cause the shrinking of secondary lymphoid organs, such as the spleen and lymph nodes [25,26]. Biomarkers of SARS-CoV-2 infection may include overproduction of cytokines that can cause lung damage, such as interleukin-1β (IL-6β), IL-2, interferon-γ (IFN-γ), tumor necrosis factor α (TNF-α) or transforming growth factor β (TGF-β) [27–30]. CD4 and CD8 T cells in COVID-19 patients express markers of T cell exhaustion, including PD-1 and Tim-3, which contribute to SARS-CoV-2-induced sepsis and death [13]. The main hypothesis for the immunopathogenesis of long-term COVID-associated complications is that it may involve persistent virus or viral antigens and RNA in tissues, leading to chronic inflammation. This can trigger autoimmunity following an acute viral infection, as well as dysbiosis of the microbiome and irreversible tissue damage. All of these factors, to some degree or another, involve the immune system [31].

Previous studies indicate elevated PD-L1 levels in COVID-19 patients with severe symptoms [32]. However, studies on soluble PD-L1 are limited to L. Chavez-Galan et al demonstrated that increased sPD-L1 levels in COVID-19 patients are associated with requiring mechanical ventilation [33]. F. Sabbatino et al showed that higher levels of sPD-L1 were found in deceased COVID-19 patients compared to survivors [16]. Our study suggests that the levels of sPD-L1 continue to be linked with mortality even after one year of hospitalization.

The immune system is damaged during COVID-19 due to a failed antiviral activity of interferons, which results in systemic manifestations. Additionally, there is higher degranulation of neutrophils, and increased levels of cytokines, both in acute and long-term periods [34–37]. Combining different biomarkers shows promise in assessing COVID-19 patient outcomes. During the first wave of the COVID-19 pandemic, lymphopenia and NLR emerged as early prognostic markers [38,39]. The biomarker NLR is a crucial component of the risk score used to predict the likelihood of developing critical illness from COVID-19 [38,40,41]. Its presence is explained by its contribution to the pathogenesis of inflammation and its related complications in a broader sense.

Our study showed that the combination of two immune-mediated markers (sPD-L1 and NLR), which reflect the systemic inflammatory balance of activation and exhaustion, can complement each other and improve the assessment of the risk of death in patients with COVID-19. With a plasma sPD-L1 value of more than 277.13 pg/ml, the risk of mortality within one year HR was 2.46, and for NLR more than 2.46 HR was 2.87. Combining sPD-L1 and NLR while creating a model resulted in a significant improvement in the predictive accuracy of the Hazard Ratio, which was found to be 7.6 (95 % CI: 3.02–19.11).

It can be assumed that patients with more severe disease are at higher risk of death, and therefore there is overlap in groups depending

on disease severity and mortality. It should be noted that sPD-L1 and NLR concentrations (Fig. A1) in the non-survivor group did not differ according to disease severity. It is suggested that elevated plasma concentrations of sPD-L1 and NLR are associated with increased mortality, regardless of disease severity.

The importance of these findings relates to the possibility of using PDL-1 as a therapeutic target. Blockade of the PD-1/PDL-1 pathway is a known therapeutic target in oncology, and α-PDL-1 antibody-based therapies are currently in ongoing clinical trials to evaluate their effect on severe sepsis/septic shock [42]. Our study supports the theory that specific anti-PDL therapy can contribute to both short-term and long-term mortality reduction in COVID-19. Futrhermore, there is another way to utilize this biomarker. The manifestations of COVID-19 vary widely and the severity of the disease is not always predictable. Accordingly, personalizing therapy using PDL-1 inhibitors and corticosteroid therapy for patients with acute infections and post-infectious therapy that induces PD-1/PDL-1 expression can improve patient outcomes.

Our study found that age was not a significant factor in disease severity. However, we did observe a higher mean age in the group of deceased patients, indicating that age may be a contributing factor to poor outcomes ($p = 0.0001$). Comorbidity was higher in both the group of severe patients and the deceased. The differences in CRP, dimer, and ferritin levels correspond with other studies based on disease severity and outcome [43,44] A. Mainous et al. showed that increased levels of CRP, which is one of the indicators of severe COVID-19 in the acute period, are associated with an increased risk of mortality after 12 months of follow-up [45]. There were significant differences in the main inflammatory markers CRP and D-dimer between survivors and deceased, as well as between severe and moderate cases. Additionally, ESR and ferritin levels also differed.

Inevitably, our study has several limitations. Firstly, we did not investigate genetic variations in PD-L1 that may affect the expression and function of this biomolecule [46,47]. Secondly, our study was limited by the lack of analysis of how increased mortality from various causes is associated with sPD-L1 and NLR levels. In this study, cardiovascular failure and acute vascular events were found to be the most common causes of death in the post-COVID period. The limited number of observations in each group was the main limitation to conducting a detailed analysis of sPD-L1 and NLR concentrations based on mortality causes. The study of all-cause mortality allowed for the inclusion of those with confirmed COVID-19 pneumonia who died within one year. Although another limitation such as examining only mortality as a single endpoint, its significant advantage is the relatively extended study duration in the context of COVID-19 research.

5. Conclusions

Soluble PD-L1 and NLR represent distinct aspects of the inflammatory response and are involved in the dysregulated inflammatory state observed in COVID-19 patients. The study results suggest that a combination of these biomarkers may be used to stratify patients with poor outcome.

Ethics statement.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Bioethics Committee of Karaganda Medical University No. 18, dated 14 April 2021. Written informed consent was obtained from all patients/participants who took part in this study.

Funding.

This work was supported by the Ministry of Health of the Republic of Kazakhstan, Program No. BR11065386.

CRediT authorship contribution statement

Lyudmila Akhmaltdinova: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Irina Mekhantseva:** Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Lyudmila Turgunova:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Mikhail Kostinov:** Writing – review & editing, Conceptualization. **Zhibek Zhumadilova:** Methodology, Investigation, Data curation. **Anar Turmukhambetova:** Supervision, Resources, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2024.111600.

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