

Elevated serum soluble PD-L1 is associated with adverse outcomes after autologous stem cell transplantation in transplant-eligible multiple myeloma patients

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Abstract

Multiple myeloma (MM) is a plasma-cell malignancy in which immune-evasion mechanisms may contribute to disease progression. Soluble programmed death-ligand 1 (PD-L1) has been associated with adverse outcomes in MM, but its prognostic significance before and after autologous stem cell transplantation (ASCT) remains incompletely defined. To evaluate serum PD-L1 concentrations before and six months after ASCT and to assess their associations with clinical outcomes, including overall survival (OS), disease-free survival (DFS), relapse, and graft failure. This prospective observational study included 40 transplant-eligible patients with MM treated at the Ain Shams University Bone Marrow Center. Serum PD-L1 was measured by ELISA before ASCT and six months after transplantation. Clinical outcomes were assessed during follow-up through 180 days, and associations with survival and adverse outcomes were evaluated using correlation analysis, Kaplan–Meier survival analysis, and receiver operating characteristic (ROC) analysis. Pre- and post-transplant PD-L1 levels were negatively correlated with OS (pre: $r = -0.44$, $p = 0.004$; post: $r = -0.48$, $p = 0.002$). Patients with relapse had higher pre- and post-transplant PD-L1 levels than those without relapse (119.2 vs. 74.6 pg/mL, $p = 0.01$; and 184.4 vs. 63.4 pg/mL, $p = 0.01$, respectively). Post-transplant PD-L1 was higher in patients with graft failure than in those without graft failure (178.7 vs. 87.5 pg/mL, $p = 0.008$). Kaplan–Meier analysis showed poorer OS and DFS among patients with higher PD-L1 according to the study cutoffs. In conclusion, higher serum PD-L1 levels before and after ASCT were associated with adverse clinical outcomes in this cohort. Given the small sample size, limited follow-up, single-center design, and multiple unadjusted comparisons, these findings should be considered exploratory and require validation in larger studies.

Keywords: soluble PD-L1, multiple myeloma, autologous stem cell transplantation, overall survival, disease-free survival, relapse.

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Introduction

Multiple myeloma (MM) is an incurable plasma cell neoplasm distinguished by genetic alterations and a modified bone marrow microenvironment which had crucial role for the survival, proliferation, and evasion of myeloma cells from the immune system.¹

The PD-1/PD-L1 (Programmed Death-Ligand 1) checkpoint pathway is a major way that cancer cells escape from the immune system. PD-L1 is a 33-kDa type 1 transmembrane glycoprotein with 290 amino acids with an extracellular region that contains IgC domains.² Programmed death-ligand 1 (PD-L1) expression is primarily activated by inflammatory cytokines, especially interferon-gamma (IFN- γ), via the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway. This represents an adaptive immune resistance mechanism. Additionally, oncogenic drivers, such as PTEN loss or constitutive activation of the mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF- κ B) pathways, can lead to constitutive, "innate" PD-L1 expression. When PD-1 on T cells binds to PD-L1 on tumor or antigen-presenting cells, it sends an inhibitory signal that makes T cells exhausted with functional impairment tumor microenvironment, thereby facilitating disease progression and immune evasion.³

Research has shown that IFN- γ leads to an increase in PD-L1 in ovarian cancer cells, which is a factor in the progression of the disease. On the other hand, in acute myeloid leukemia mouse models, blocking IFN- γ receptor 1 can decrease PD-L1 expression via the mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and myeloid differentiation primary response 88 (MYD88)/TNF receptor associated factor 6 (TRAF6) pathways.⁴

PD-L1 activates proliferative and survival signaling pathways in cancer cells by attaching to its receptors, making it a pro-tumorigenic factor. This finding provides more evidence that PD-L1 has a role in the growth of tumors. A number of tumor cell types have been demonstrated to be subject to PD-L1's non-immune proliferative actions. In renal cancer

cells, for instance, PD-L1 promoted tumor growth by inducing an epithelial-to-mesenchymal transition (EMT) and stem cell-like characteristics.⁵

An assortment of solid malignancies, including squamous cell carcinoma of the head and neck, melanoma, thyroid, esophagus, lungs, breast, gastrointestinal tract, colorectum, liver, pancreas, kidney, adrenal cortex, bladder, urothelium, ovary, and skin, primarily express PD-L1 on their tumor cells and antigen-presenting cells. There are two pathways in the tumor microenvironment that rely on two binding sites of IRF-1 to induce or constitutively produce PD-L1 on tumor cells and other cells that promote tumor growth. For instance, in melanoma cells with a BRAFV600 mutation, PD-L1 expression can be either constitutive (caused by oncogenic processes) or adaptive (reaction to immunological attacks induced by cytokines).⁶

Serum and supernatants commonly contain the soluble form of PD-L1 (PD-L1), and the content of this form is highly correlated with the expression level of PD-L1. High levels of PD-L1 in bone marrow plasma were linked to worse overall survival rates and worse responses after autologous stem cell transplantation (ASCT) in MM patients.⁶ PD-L1 levels predicted treatment response and progression-free survival (PFS) in patients newly diagnosed with MM (NDMM) as well. Plasma cells of MM patients express PD-L1, and patients with minimal residual illness who continue to have the disease have high levels of both PD-L1 and PD-1. This suggests that PD-L1 expression in MM might be useful for prognosis.⁷

The aim of the study was to assess a correlation between PD-L1 serum levels before and after autologous stem cell transplantation and clinical outcomes and disease prognoses in patients with multiple myeloma.

Patients and Methods

This prospective observational study included 40 consecutive transplant-eligible patients with multiple myeloma who underwent ASCT at the Ain Shams University Bone Marrow Center during a six-month recruitment period. Patients were followed for up to 180 days after transplantation. Eligible participants were adults

aged ≥ 18 years with transplant-eligible MM. Patients with relapsed/refractory myeloma or other hematological malignancies were excluded. Multiple myeloma was defined according to International Myeloma Working Group diagnostic criteria.¹⁸ Disease stage was assessed using the International Staging System (ISS), which classifies MM into stages I–III according to serum $\beta 2$ -microglobulin and albumin concentrations.¹⁹ All patients underwent history taking, clinical examination, complete blood count, lactate dehydrogenase, renal and hepatic function testing, viral markers, calcium, creatinine, $\beta 2$ -microglobulin, Bence Jones protein, serum protein electrophoresis, immunofixation, skeletal survey, bone marrow biopsy, flow cytometry, cytogenetic analysis, MRI, and PET imaging. Disease response was assessed before transplantation and at day 100 after transplantation according to International Myeloma Working Group response criteria.²⁰

All patients received six cycles of induction therapy with a proteasome inhibitor- and/or immunomodulatory agent-based regimen before stem-cell mobilization. Induction regimens were bortezomib, lenalidomide, and dexamethasone (VRd; $n=22$, 55%) or bortezomib, cyclophosphamide, and dexamethasone (VCd; $n=18$, 45%). Stem-cell mobilization was performed using granulocyte colony-stimulating factor (G-CSF) alone or with cyclophosphamide. Conditioning consisted of melphalan 200 mg/m² ($n=34$, 85%) or melphalan 140 mg/m² ($n=6$, 15%) in patients with renal impairment. The median CD34+ cell dose infused was 3.2×10^6 /kg (range, 2.1 – 5.8×10^6 /kg). Lenalidomide maintenance (25 mg) was administered to 28 patients (70%) beginning on day 100 after ASCT.

Overall survival (OS) was defined as the time from the date of autologous stem cell transplantation (ASCT) to death from any cause. Patients who were alive at the last follow-up were censored at the date of their last documented follow-up.¹⁸

Disease-free survival (DFS) was defined as the time from the date of ASCT to the occurrence of disease relapse or progression, or

death from any cause, whichever occurred first. Patients who remained alive without documented relapse or progression were censored at the date of their last documented follow-up.

International Staging System (ISS)

Disease stage was classified according to the International Staging System (ISS) for multiple myeloma, based on serum $\beta 2$ -microglobulin and serum albumin levels at diagnosis. ISS stage I was defined as serum $\beta 2$ -microglobulin < 3.5 mg/L and serum albumin ≥ 3.5 g/dL. ISS stage III was defined as serum $\beta 2$ -microglobulin ≥ 5.5 mg/L. ISS stage II included patients who did not meet the criteria for either stage I or stage III. The ISS classification was used for disease staging, rather than the Revised International Staging System (R-ISS).¹⁹

Graft Failure

Primary graft failure was defined as failure to achieve hematopoietic engraftment following autologous stem cell transplantation (ASCT). The occurrence of primary graft failure was determined based on a review of the patients' medical records and documented post-transplant hematopoietic recovery. Eight patients were identified as having primary graft failure during the post-transplant follow-up period.²⁰

Assessment of PD-L1 by ELISA

Serum PD-L1 levels were measured using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit (Human PD-L1 ELISA Kit, catalog number ab214565, Abcam, Cambridge, UK) according to the manufacturer's instructions. The assay detection range was 15.6–1000 pg/mL, with a lower limit of detection of 7.8 pg/mL. Intra-assay and inter-assay coefficients of variation were $< 8\%$ and $< 10\%$, respectively. Blood samples were collected in serum separator tubes, centrifuged at $3000 \times g$ for 10 minutes at 4°C , aliquoted, and stored at -80°C until analysis. Samples underwent a maximum of one freeze–thaw cycle before analysis.

We define graft failure was defined as failure to achieve neutrophil engraftment (absolute

neutrophil count $\geq 0.5 \times 10^9/L$ for three consecutive days) by day 28 after ASCT, or failure to achieve platelet engraftment (platelet count $\geq 20 \times 10^9/L$ without transfusion support for seven consecutive days) by day 60 after ASCT, in the absence of disease progression or death. Overall survival (OS) was defined as the time from ASCT to death from any cause; patients alive at last follow-up were censored at that time. Disease-free survival (DFS) was defined as the time from ASCT to documented disease relapse, progression, or death from any cause, whichever occurred first; patients alive without relapse or progression at last follow-up were censored at that time.

Statistical Analysis

Data were summarized as mean $\pm$ standard deviation (SD) for continuous variables and as number and percentage for categorical variables. Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Correlation coefficients (r) were used to assess relationships between PD-L1 levels and continuous clinical or laboratory variables. Kaplan–Meier methods were used to estimate OS and DFS, and survival curves were compared using the log-rank test. ROC analysis was used to assess the discriminatory performance of pre- and post-transplant PD-L1 for mortality and relapse and to identify study-specific cutoff values. A two-sided p -value < 0.05 was considered statistically significant. Because of the small sample size and multiple comparisons, no formal correction for multiple testing was applied; therefore, the results should be interpreted as exploratory and hypothesis-generating.

Results

The study included 40 patients with MM. The mean age was 58.1 ± 5.8 years (range, 45–74 years), and 23 (57.5%) were male. ECOG performance status was 0 in 3 patients (7.5%), 1 in 29 patients (72.5%), 2 in 7 patients (17.5%), and 3 in 1 patient (2.5%). According to the ISS, 16 patients (40%) were stage I, 13 (32.5%) stage II, and 11 (27.5%) stage III. Pre-transplant disease response was CR in 8 patients (20%),

VGPR in 14 (35%), PR in 12 (30%), and SD in 6 (15%).

During the post-transplant follow-up, all patients experienced fever; bacterial infections occurred in 47.5%, sepsis in 35%, and bleeding in 7.5%. Mean OS was 142.75 ± 65.41 days (range, 21–180), and mean DFS was 120.25 ± 52.27 days (range, 21–150). Graft failure occurred in 8 patients (20%), relapse in 14 (35%), and death in 10 (25%).

The mean overall survival (OS) was 142.75 ± 65.41 days (range: 21–180), while the mean disease-free survival (DFS) was 120.25 ± 52.27 days (range: 21–150). Graft failure occurred in 20% of patients, 35% experienced relapse, and 25% died, with 12.5% of deaths related to transplantation and 12.5% due to unrelated causes as shown in Table 1.

Table 1. Clinical and laboratory characteristics of the studied patients.

Characteristic	Value
Age, years	58.1 ± 5.8 Range: 45–74
$\beta 2$ -microglobulin, mg/L	5.03 ± 1.57 Range: 1.7–8.9
Sex	Male: 23 (57.5%) Female: 17 (42.5%)
Calcium, mg/dL	11.85 ± 1.62 Range: 8.9–14.5
ECOG performance status	0: 3 (7.5%) 1: 29 (72.5%) 2: 7 (17.5%) 3: 1 (2.5%)
Creatinine, mg/dL	2.21 ± 0.64 Range: 0.9–3.2
ISS	I: 16 (40%) II: 13 (32.5%) III: 11 (27.5%)

SD: Standard deviation, ISS: International Staging System, OS: Overall survival, DFS: Disease free survival.

The mean of pre-transplant PD-L1 was 90.25 ± 53.6 pg/ml, with a range of 20 to 220. The mean of post-transplant PD L1 was 106 ± 89.9 pg/ml. (range 20–350). There was no statistically significant difference found between PD-L1 levels before and after transplantation ($p > 0.05$) (Table 2).

Table 2. Comparison of serum PD-L1 levels before and after transplantation.

Variable	Pre-transplant (n=40)	Post-transplant (n=40)	Mean difference (Pre – Post)	Percentage change (Pre – Post)	p-value
PD-L1 (pg/mL)	90.25 ± 53.6 Range: 20–220	106.0 ± 89.9 Range: 20–350	–15.7 ± 73.0 Range: –230 to 160	–34.3 ± 107 Range: –450 to 88.8	NS

$p > 0.05$ is not significant (NS).

There was positive correlation between pre-transplant PD-L1 and performance score ($r=0.40$, $p<0.05$), this highlighting higher PDL-1 level pre-transplant associated with functional impairment and increased disease burden but no significant correlation with age or pre-

transplant laboratory data. Pre-transplant PD-L1 showed a mild positive correlation with LDH ($p<0.05$). PD-L1 showed a mild negative correlation with OS and DFS, both pre-transplant ($r = -0.44$) and post-transplant ($r = -0.48$). ($p<0.0004$, 0.002) respectively (Table 3).

Table 3. Correlations of pre-transplant and post-transplant serum PD-L1 with demographic, laboratory, and clinical variables.

Variable	Pre-PD-L1 (r)	Pre-PD-L1 (p)	Post-PD-L1 (r)	Post-PD-L1 (p)
Age	0.09	NS	0.16	NS
Eastern Cooperative Oncology Group (ECOG) performance status	0.40	0.01	0.23	NS
Hemoglobin (Hb) (mg/dL)	–0.12	NS	–0.28	NS
Creatinine (mg/dL)	0.06	NS	0.28	NS
Calcium (Ca) (mg/dL)	0.04	NS	–0.30	NS
Serum M-protein (g/dL)	0.10	NS	–0.14	NS
Bone marrow plasma cells (BMPCs) (%)	–0.08	NS	–0.004	NS
β 2-microglobulin (mg/L)	0.13	NS	0.15	NS
Lactate dehydrogenase (LDH) (IU/L)	0.30	0.05	–0.18	NS
C-reactive protein (CRP)	0.11	NS	0.13	NS
Overall survival (OS)	–0.44	0.004	–0.48	0.002
Disease-free survival (DFS)	–0.44	0.004	–0.48	0.002

$p > 0.05$ is not significant (NS).

Association between pre- and post-transplant PD-L1 levels and transplantation outcomes.

The mean post-transplant PD-L1 levels were significantly higher in patients complicated by graft failure a (178.7 ± 93.8 pg/ml (range 70-350) Vs 87.5 ± 80.5 pg/ml (range 20-310), $p= 0.008$), while pre-transplant PD-L1 showed no

significant difference ($p>0.05$). Intragroup analysis revealed a significant increase in PD-L1 in graft failure cases ($p< 0.0003$). Relapsed cases had higher pre and post –transplant PD-L1 levels than non-relapsed cases with statistically significance increase in relapsed cases ($p<0.01$). (the mean pre-transplant PD-L1 119.2 ± 60.6

pg/ml Vs 74.6 ± 42.9 pg/ml, $p = 0.01$ and the mean post-transplant PD-L1 184.4 ± 103.3 Vs 63.4 ± 42 , $p = 0.001$). Overall mortality associated with increased both pre and post-transplant PD-L1 and also transplant related mortality showed statistically significance with elevated both pre and post-transplant PD-L1. The mean pre-transplant overall mortality PD-L1 was

$(141 \pm 66.7$ pg/ml Vs 73.3 ± 36 pg/ml, $p < 0.001$) and the mean post-transplant PD-L1 $(208 \pm 114.8$ Vs 71.6 ± 44.6 pg/ml, $p < 0.001$). Regarding transplant related mortality, the mean pre-transplant PD-L1 $(170.2 \pm 73.4$ pg/ml Vs 78.8 ± 45.4 pg/ml, $p < 0.001$) and the mean post-transplant PD-L1 $(234 \pm 135.7$ Vs 87.4 ± 66 pg/ml, $p < 0.001$) (Table 4).

Table 4. Relation between pre- and post-transplant PD-L1 levels and clinical outcomes.

Outcome group	Pre-PD-L1, mean $\pm$ SD (range)	Post-PD-L1, mean $\pm$ SD (range)	Mean difference (Pre – Post)	<i>p</i> -value
Graft failure - Yes	96.25 ± 56.2 (20–200)	178.7 ± 93.8 (70–350)	-82.5 ± 63.6 (–230 to –30)	0.008
Relapse - No	74.6 ± 42.9 (20–180)	63.4 ± 42.0 (20–220)	10.7 ± 49.3 (–60 to 160)	NS
Relapse - Yes	119.2 ± 60.6 (20–220)	184.4 ± 103.3 (20–350)	-65.0 ± 85.4 (–230 to 160)	0.01
Overall mortality - Yes	141 ± 66.7 (20–220)	208 ± 114.8 (20–350)	-67 ± 101 (–230 to 160)	0.05
Transplant-related mortality - No	78.8 ± 45.4 (20–200)	87.4 ± 66 (20–280)	-8.8 ± 57 (–130 to 160)	NS
Transplant-related mortality - Yes	170 ± 37.4 (120–220)	234 ± 135.7 (20–350)	-64 ± 144 (–230 to 160)	NS

$p > 0.05$ is not significant (NS).

After follow up for 180 days, patients with high pre-transplant PD-L1 (≥ 80 pg/ml) had an inferior OS in comparison to lower pre-transplant PD-L1 (< 80 pg/ml) (OS=63% vs 89%) with p value 0.05 and patients with high post-transplant PD-L1 (≥ 75 pg/ml) had an inferior OS in comparison to lower pre-transplant PD-L1 (< 75 pg/ml) (OS=58% vs 90.5%) with p value 0.01 shown (figure 1)(Table 5).

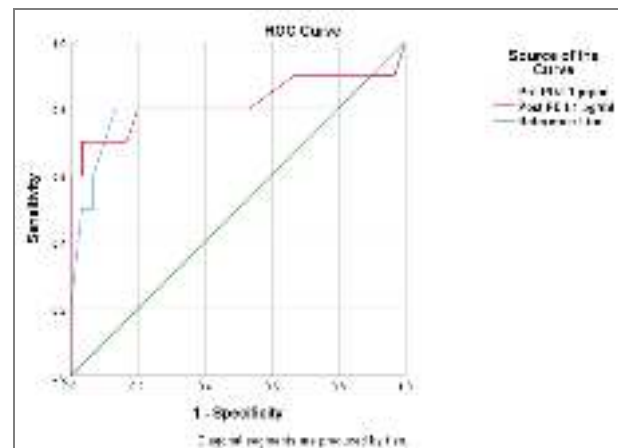


Figure 1. ROC curves for pre- and post-transplant PD-L1 for prediction of mortality.

Table 5. Kaplan–Meier analysis of overall survival (OS).

Variable	Total number	Number of deaths	30-day survival (%)	90-day survival (%)	180-day survival (%)	<i>p</i> -value
All cases	40	10	87.5	75	75	—
Gender- Male	23	5	91.3	78.3	78.3	NS
Gender-Female	17	5	82.4	70.6	70.6	
Performance score-0	3	0	—	—	—	<0.001
Performance score-1	29	5	93.5	84	84	
Performance score-2	7	4	40	20	20	
Performance score-3	1	1	100	0	0	
ISS-I	16	0	—	—	—	<0.001
ISS-II	13	2	100	84.6	84.6	
ISS-III	11	8	—	27.3	27.3	
Pre-PD-L1-Low (<80)	18	2	100	94.4	89	0.05
Pre-PD-L1-High (≥80)	22	8	81.8	63.6	63.6	
Post-PD-L1-Low (<75)	21	2	100	95.2	90.5	0.01
Post-PD-L1-High (≥75)	19	8	79	58	58	
Relapse-No	26	2	100	92.3	92.3	<0.001
Relapse-Yes	14	8	64.3	43	43	
Graft failure-No	32	7	94	78	78	NS
Graft failure-Yes	8	3	62.5	62.5	62.5	

p > 0.05 is not significant (NS).

Also, patients with high pre-transplant PD-L1 (≥80 pg/ml) had an inferior DFS in comparison to lower pre - transplant PD-L1 (<80 pg/ml) (OS=48.5%vs 82.6%) after 180 days with *p* value 0.02 and patients with high post - transplant PD-L1 (≥75pg/ml) had an inferior DFS in comparison to lower pre-transplant PD -L1 (<75 pg/ml) (OS=34.2% vs 90.2%) with *p* value 0.001 shown (figure 2) (Table 6).

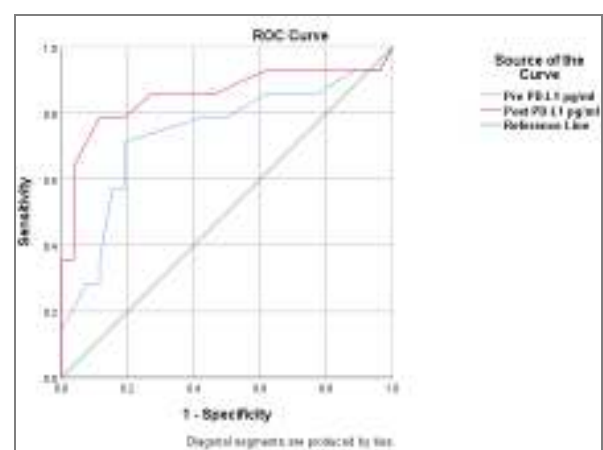


Figure 2. ROC curves for pre- and post-transplant PD-L1 for prediction of relapse.

Table 6. Kaplan–Meier analysis of disease-free survival (DFS).

Variable	Total number	Number of relapses	30-day DFS (%)	90-day DFS (%)	180-day DFS (%)	p-value
Gender-Male	23	6	91.3	82.6	73.4	NS
Gender-Female	17	8	82.4	76	50.6	
Performance score-0	3	1	100	100	66.7	0.005
Performance score-1	29	9	93.5	87	70	
Performance score-2	7	4	40	20	20	
Performance score-3	1	0	—	—	—	
ISS-I	16	3	100	100	81.3	<0.001
ISS-II	13	2	100	100	81.8	
ISS-III	11	9	54.5	27.3	18	
Pre-PD-L1-Low (<80)	18	3	94.4	94.4	82.6	0.02
Pre-PD-L1-High (≥80)	22	11	81.8	68	48.5	
Post-PD-L1-Low (<75)	21	2	95.2	95.2	90.2	<0.001
Post-PD-L1-High (≥75)	19	12	79	62.7	34.2	
Graft failure — No	32	8	94	84.3	74.1	0.005*
Graft failure — Yes	8	6	62.5	62.5	25	

$p > 0.05$ is not significant (NS).

PD-L1 has a statistically significant ROC result for overall mortality ($p=0.008$), with a cut-off value of >100 pg/ml, 80% sensitivity, and 87% specificity. Post-transplant PD-L1 also showed a statistically significant ROC result for overall mortality ($p=0.003$), with a cut-off value of >100 pg/ml, 80% sensitivity, and 80% specificity. For transplant-related mortality, pre-transplant PD-L1 had a statistically significant ROC result ($p=0.002$), with a cut-off value of >135 pg/ml, 80% sensitivity, and 89% specificity, while post-transplant PD-L1 showed a statistically

significant ROC result ($p=0.04$), with a cut-off value of >170 pg/ml, 80% sensitivity, and 89% specificity. Regarding relapse, pre-transplant PD-L1 showed a statistically significant ROC result ($p=0.01$), with a cut-off value of >85 pg/ml, 71.5% sensitivity, and 81% specificity, whereas post-transplant PD-L1 showed the strongest ROC discrimination, with a statistically significant result ($p<0.001$), a cut-off value of >100 pg/ml, 79% sensitivity, and 88.5% specificity (Table 7).

Table 7. ROC analysis of pre- and post-transplant PD-L1 for prediction of mortality and relapse.

ROC outcome	95% CI	p-value	Cut-off value	Sensitivity	Specificity
Overall mortality - Pre-PD-L1	0.56–0.99	0.008*	>100	80%	87%
Overall mortality - Post-PD-L1	0.61–0.99	0.003*	>100	80%	80%
Transplant-related mortality- Pre-PD-L1	0.83–0.99	0.002*	>135	80%	89%
Transplant-related mortality -Post-PD-L1	0.44–0.99	0.04*	>170	80%	89%
Relapse - Pre-PD-L1	0.56–0.91	0.01*	>85	71.5%	81%
Relapse - Post-PD-L1	0.90–0.99	<0.001*	>100	79%	88.5%

* $p \leq 0.05$ is significant.

ROC analysis showed that the optimal cutoff for overall mortality was 100 pg/mL for both pre- and post-transplant PD-L1. For pre-transplant PD-L1, the AUC was 0.78 (95% CI, 0.56–0.99), with 80% sensitivity and 87% specificity; for post-transplant PD-L1, the AUC was 0.82 (95% CI, 0.61–0.99), with 80% sensitivity and 80% specificity. Kaplan–Meier analyses used the study-specific cutoffs of 80 pg/mL before transplantation and 75 pg/mL after transplantation, as presented in Tables 5 and 6. These survival-analysis cutoffs should not be interpreted as identical to the ROC-derived mortality cutoffs.

Discussion

Multiple myeloma (MM) is a complex plasma-cell malignancy characterized by interactions between malignant plasma cells and the bone marrow microenvironment. These interactions contribute to tumor-cell survival, proliferation, immune evasion, and disease progression. The PD-1/PD-L1 axis is an important component of this immunosuppressive environment, as PD-L1 expression can inhibit T-cell activity and promote immune escape. Beyond its immune-regulatory role, PD-L1 has also been implicated in tumor-cell survival and other pro-tumorigenic processes. Accordingly, soluble PD-L1 (PD-L1) has attracted attention as a potential biomarker of disease activity and prognosis in MM and other hematological malignancies.^{8,13,14}

The present prospective study investigated serum PD-L1 levels in 40 patients with MM undergoing autologous stem cell transplantation (ASCT), with measurements obtained before transplantation and six months after transplantation. The principal finding was that higher PD-L1 concentrations were associated with unfavorable clinical outcomes, including relapse and reduced overall survival (OS) and disease-free survival (DFS). These associations were observed at both time points, while post-transplant PD-L1 showed particularly strong associations with relapse and survival outcomes. These findings support the potential role of circulating PD-L1 as a marker reflecting an unfavorable disease and immune status in patients undergoing ASCT.

The demographic characteristics of our cohort were generally consistent with the known profile of patients with MM. The mean age was 58.1 ± 5.8 years, and males represented 57.5% of the study population. Similar characteristics have been reported in larger MM cohorts, including Kumar et al., in which 61.1% of patients were male and the median age was approximately 59 years.. Most patients in our cohort had a favorable functional status, with 72.5% having an ECOG performance status of 1. Nevertheless, poorer performance status was significantly associated with inferior OS and DFS, emphasizing the importance of baseline functional status when interpreting post-transplant outcomes.

Regarding disease stage, 40% of patients had ISS stage I, 32.5% had stage II, and 27.5% had stage III disease. ISS stage was strongly associated with both OS and DFS, with patients with stage III disease showing substantially poorer survival than those with stages I or II. This finding is clinically relevant because disease stage is an established determinant of outcome and may also act as an important confounding factor when evaluating the prognostic value of emerging biomarkers such as PD-L1. Therefore, the association between elevated PD-L1 and poor survival in the present study should be interpreted as an association rather than evidence of an independent prognostic effect, particularly in the absence of multivariable analysis.

The mean pre-transplant serum PD-L1 concentration was 90.25 ± 53.6 pg/mL and increased to 106 ± 89.9 pg/mL after transplantation, although this overall change did not reach statistical significance. Despite the absence of a significant overall change, PD-L1 levels differed according to subsequent clinical outcomes. Patients who subsequently experienced relapse had substantially higher pre-transplant and post-transplant PD-L1 levels than patients who remained relapse-free. Furthermore, post-transplant PD-L1 levels were markedly higher among patients who experienced graft failure. These findings suggest that the absolute level of circulating PD-L1, particularly after transplantation, may provide clinically relevant information that is not

captured by the overall mean change in the cohort.

The biological basis for these observations is plausible. PD-L1-mediated signaling can suppress antitumor T-cell responses and contribute to an immunosuppressive tumor microenvironment. PD-L1 expression has also been associated with mechanisms that may facilitate tumor-cell survival and progression.⁸

In MM, persistent PD-L1 expression may therefore reflect a disease state characterized by immune evasion and an altered tumor microenvironment. Following ASCT, substantial changes occur in the cellular composition and function of the bone marrow microenvironment during immune reconstitution. Persistent elevation of PD-L1 during this period may consequently identify patients in whom immune suppression remains prominent and who may be at increased risk of disease persistence or relapse.

Our findings are consistent with previous observations in MM. Wang et al. reported that serum PD-L1 levels were associated with treatment response and progression-free survival in patients with MM, supporting the potential prognostic value of circulating PD-L1.¹⁴ Similarly, Lee et al. demonstrated that higher PD-L1 expression in bone marrow plasma cells was associated with poorer prognosis and developed a prognostic model incorporating PD-L1 expression.¹² Huang et al. also reported that soluble PD-L1 could predict progression following autologous transplantation in patients with MM.¹⁷ In addition, previous work has linked increased PD-L1 expression with persistent disease and greater bone marrow involvement, supporting a possible relationship between PD-L1 and disease progression.¹⁶ The concordance between these observations and our results strengthens the hypothesis that circulating PD-L1 may serve as a marker of adverse disease biology.

In the present study, pre-transplant PD-L1 showed a significant positive correlation with ECOG performance status. This finding suggests that patients with poorer functional status may have higher circulating PD-L1 concentrations. However, this association should be interpreted cautiously because ECOG performance status

may itself reflect overall disease burden, comorbidity, and general clinical condition. Thus, the observed relationship may represent, at least in part, the effect of underlying disease severity rather than a direct causal relationship. Interestingly, Lee et al. did not identify a significant association between PD-L1 expression and ECOG performance status.¹² The discrepancy may be related to differences in patient populations, disease characteristics, assessment methods, or the relatively small sample size of the present study. We also observed a modest positive correlation between pre-transplant PD-L1 and LDH, whereas no significant relationships were observed with most other clinical or laboratory variables. Taken together, these findings suggest that circulating PD-L1 may be influenced by the broader clinical and biological context rather than by a single disease parameter.

We observed a significant inverse correlation between both pre- and post-transplant PD-L1 levels and OS and DFS. Kaplan–Meier analysis further demonstrated inferior OS and DFS among patients with elevated pre-transplant and post-transplant PD-L1 levels. These findings are in keeping with previous reports showing that increased PD-L1 expression is associated with inferior survival in MM.^{12,17} The consistency of this association across different study populations supports the potential prognostic relevance of the PD-1/PD-L1 pathway in MM. At the same time, the present data cannot establish whether PD-L1 directly contributes to poor outcome or instead reflects underlying disease severity.

An important finding in our study was the particularly strong relationship between post-transplant PD-L1 and relapse. Patients who relapsed had considerably higher post-transplant PD-L1 levels than those who did not relapse, and elevated post-transplant PD-L1 was associated with markedly lower DFS on Kaplan–Meier analysis. This observation is consistent with previous work suggesting that increased PD-L1 expression may be associated with persistent disease and subsequent progression.¹⁶ The post-transplant period may therefore provide an informative window in which elevated PD-L1 reflects residual or

biologically active disease together with an immunosuppressive microenvironment favoring recurrence.

The association between PD-L1 and graft failure is another potentially interesting observation. Patients who experienced graft failure had substantially higher post-transplant PD-L1 concentrations than those without graft failure. Previous work has highlighted the complex role of PD-L1 in transplantation and immune regulation, including its effects on alloreactive T-cell responses.¹⁵ However, the number of patients with graft failure in our cohort was small, and this finding should therefore be regarded as exploratory. Larger studies will be necessary to determine whether post-transplant PD-L1 has a reproducible relationship with hematopoietic recovery or graft-related complications.

The post-transplant clinical course in our cohort also illustrates the multifactorial nature of outcomes after ASCT. Fever and infectious complications were common, and graft failure, relapse, and mortality contributed to the observed outcomes. These findings are broadly consistent with the recognized complexity of early post-transplant management, in which immune reconstitution and infection control remain important determinants of clinical outcome.⁹ The survival outcomes in our cohort should, however, be interpreted in light of the relatively short 180-day follow-up and the clinical characteristics of this single-center population.

Our ROC analysis further demonstrated that PD-L1 had potentially useful discriminatory ability for mortality and relapse. Both pre- and post-transplant PD-L1 demonstrated significant associations with mortality, while post-transplant PD-L1 showed particularly strong performance for predicting relapse. These findings suggest that PD-L1 may have potential as a risk-stratification biomarker. Nevertheless, the reported cut-off values were derived from the same small cohort in which their predictive performance was evaluated. Such data-derived thresholds require validation in independent cohorts before they can be considered clinically applicable.

The findings of the present study should also be interpreted in the context of established prognostic factors. Patients with poorer ECOG performance status and higher ISS stage had inferior survival, while relapse was strongly associated with reduced OS and DFS. These observations highlight the multifactorial nature of post-transplant outcomes in MM. Although elevated PD-L1 was associated with adverse outcomes, the current study cannot establish whether PD-L1 represents an independent prognostic factor or instead reflects underlying disease severity and other established prognostic variables. Multivariable analysis would be required to determine the independent contribution of PD-L1; however, the limited sample size and number of outcome events in the present study precluded reliable multivariable modeling.

The biological and clinical implications of these findings are potentially important. Elevated PD-L1 may reflect an immunosuppressive state that enables malignant plasma cells to evade immune surveillance. Previous observations that bone marrow effector-cell populations may be inversely associated with PD-L1 expression further support a possible relationship between PD-L1 and post-transplant immune reconstitution.¹² Nevertheless, the present observational findings should not be interpreted as evidence that PD-L1-targeted therapy should routinely be combined with ASCT. Although the PD-1/PD-L1 pathway represents a biologically attractive therapeutic target, the current study was not designed to evaluate therapeutic interventions. The potential clinical utility of PD-L1-directed strategies in selected MM patients requires dedicated prospective clinical trials.

Overall, the present study provides further evidence that elevated circulating PD-L1 is associated with unfavorable outcomes in patients with MM undergoing ASCT. Both pre-transplant and post-transplant levels were associated with poorer survival, while elevated post-transplant levels showed particularly strong associations with relapse and graft failure. These findings are broadly consistent with previous reports linking PD-L1 expression with disease progression and inferior survival in

MM.^{12,14,16,17} Nevertheless, the small sample size, short follow-up period, single-center design, limited number of outcome events, lack of a non-ASCT control group, and absence of multivariable analysis substantially limit the strength of causal or independent prognostic conclusions.

Therefore, PD-L1 should currently be regarded as a potential prognostic biomarker rather than an established independent predictor of outcome. Larger, multicenter prospective studies with longer follow-up, standardized sampling time points, predefined cut-off values, and appropriate multivariable analyses are required to validate these findings and determine whether serial PD-L1 measurement can improve risk stratification and clinical decision-making in patients with MM undergoing ASCT.

In conclusion, higher serum PD-L1 concentrations before and after ASCT were associated with adverse outcomes, including poorer OS and DFS, relapse, and graft failure, in this cohort of patients with MM. Because of the small sample size, short follow-up, single-center design, and multiple unadjusted comparisons, these findings should be considered exploratory rather than definitive. Larger multicenter studies with longer follow-up and multivariable analysis are required to validate the prognostic significance of PD-L1.

Author Contributions

MMM, MF. and IAME contributed to Idea of research, they are principal investigator who had the research point, they created the study design, contributed to the analysis of the study data. HSM and MGN, contributed to the analysis of data and contributed to writing and revising the manuscript, Furthermore, she is the corresponding author. All authors have read and approved the manuscript , contributed to the laboratory work including performing polymerase chain reaction and contributed to the analysis of data

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Ethical approval

The study's protocol was designed according to the Helsinki Declaration and was approved by Ain-Shams University Research Ethics Committee (ASU-REC) (Approval No. MD 297/2022)..

Informed consent

A signed consent form was obtained from each study participant.

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