# Assessment of association between PD-L1 expression and clinicopathological characteristics of Indonesian patients with high grade bladder urothelial carcinoma

**Hanggoro Tri Rinonce, PhD, Theresia Hening Dwi Ambarwati, MD, Muh Syaebani, MD, Maria Fransiska Pudjohartono, MD, Stella Adevita, MD, Paranita Ferronika, PhD, Irianiwati Widodo, PhD**

Department of Anatomical Pathology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/ Dr. Sardjito Hospital, Sleman, Yogyakarta, Indonesia

**ABSTRACT**

**Introduction: Urothelial carcinoma (UC) is the most common type of bladder cancer. One of the treatments that are currently being explored for UC involves the use of immune checkpoint inhibitors, especially those targeting PD–1/PD–L1 interaction. This interaction has been previously suggested to aid in the prediction of outcomes. This study was aimed to investigate PD–L1 expression in high grade UC cases in Indonesia, both at mRNA and protein levels, and assess its associated clinicopathological characteristics.**

**Materials and Methods: The study involved analysis of 51 formalin-fixed paraffin-embedded (FFPE) tissue samples, obtained from patients diagnosed with high grade UC. PD–L1 expression was measured using immunohistochemistry (IHC) and quantitative real-time polymerase chain reaction.**

**Results: PD-L1 IHC staining was found to be positive in five (9.80%) cases. Upregulated expression of PD-L1 mRNA was reported in the patients belonging to older age group ($p$ = 0.049) and mainly involved less invasive cases ($p$ = 0.019), when compared with normoregulated group. Age was positively correlated with PD-L1 expression, as observed in IHC (r = 0.281, $p$ = 0.046). In comparison to this, mitotic index was found to be inversely correlated with PD-L1 mRNA levels (r = −0.369, $p$ = 0.008).**

**Conclusion: IHC staining results showed that PD-L1 expression was lower as compared to previous studies, which might suggest poor response to anti PD-1/PD-L1 agents. The results of the study showed that higher mRNA levels were associated with lower proliferation and less invasive behavior. Thus, the study suggested the potential of PD-L1 mRNA levels to be used as a predictive factor for patient outcomes.**

**KEYWORDS:**
*Bladder Cancer, Immune Checkpoint Inhibitors, Immunohistochemistry, Immunotherapy, Real-Time PCR*

## INTRODUCTION

Urothelial carcinoma (UC) is known to be the most common type of bladder malignancy. It accounts for over 500,000 new cases and over 200,000 deaths per year.[1] Patient outcomes for UC patients at later stages remain dismal, necessitating the development of new treatment strategies. One of the treatment modalities that are currently being explored for treatment of UC involves the use of immune checkpoint inhibitors (ICI), especially the ones that target PD-1/PD-L1 interaction. Currently, there are five ICI agents targeting PD-1 or PD-L1 that have been approved for UC patients.[2]

Despite the advancements in this field, responses of patients toward ICI therapy have been reported to be significantly variable. It has been previously suggested that testing for PD-L1 expression using immunohistochemistry (IHC) staining might help in predicting therapy response and patient outcomes, in general.[3] However, very limited studies have previously explored patient characteristics and outcomes in relation to PD-L1 expression in UC cases. The results in most of these studies were mainly inconclusive. Interestingly, one of the studies reported that PD-L1 mRNA levels could predict prognosis better than PD-L1 expression assessed using IHC.[4]

In Indonesia, studies focused on the associations between PD-L1 status and clinicopathological features are still limited. In fact, the results for these studies are quite conflicting. A study involving colorectal cancer (CRC) patients reported lower levels of mRNA expression for PD-L1 in peripheral blood as compared to healthy controls. Importantly, these mRNA levels correlated with gender but showed no correlation with age, stage, histological type, patient status, and body mass index.[5] Inversely, Al Azhar et al. (2021) reported higher PD-L1 mRNA expression in peripheral blood of nasopharyngeal carcinoma patients as compared to healthy controls. These levels were not associated with any of the clinicopathological features that were analyzed.[6] A study involving analysis of formalin-fixed paraffin-embedded (FFPE) tissue of bladder UC showed an association of PD-L1 expression with depth of invasion.[7]

Although ICI treatment has not been routinely used in case of most of Indonesian patients, data for the expression of immune checkpoint molecules would assist clinicians in considering the potential effectiveness of these treatment strategies in Indonesia. This study was aimed to investigate PD-L1 expression, at mRNA and protein levels, in high grade UC cases in Indonesia, and assessed its association with clinicopathological characteristics.

*Corresponding Author: Hanggoro Tri Rinonce*
*Email: hanggoro_rinonce@ugm.ac.id*

## MATERIAL AND METHODS

This cross-sectional study included 51 FFPE tissue samples, obtained from patients diagnosed with high grade UC at Dr. Sardjito Hospital, Yogyakarta, Indonesia (longitude of –7.768393056636577 and latitude of 110.37345273846378), during January 2014 to December 2019. Grading for tissue samples was determined on the basis of 2016 WHO Classification of Tumors of the Urinary System and Male Genital Organs.[8] Cases with incomplete clinicopathological data or degraded specimens were excluded from the study.

PD-L1 expression was measured using IHC and quantitative real-time polymerase chain reaction (RT-PCR). IHC staining was performed on 3 µm-thick tissue sections, using PD-L1 antibody clone 22C3 (Dako Agilent Technologies, Santa Clara, CA, USA). UltraTek® HRP Anti-Polyvalent Lab Pack (Scytek Laboratories, Logan, UT, USA) was used for visualization. Normal tonsil tissue was used as positive control. The expression of PD-L1 was determined using combined positive score (CPS), which was calculated using the following formula:

$$\text{CPS} = \frac{\text{number of PD-L1 staining cells (tumor cells, lymphocytes, and macrophages)}}{\text{total number of viable tumor cells}} \times 100$$

Samples with CPS ≥10 was classified as PD-L1 positive, while samples with CPS <10 were classified as PD-L1 negative.

Tumor RNA was extracted from FFPE tissue using GeneAll® Ribospin™ II (GeneAll Biotechnology, Seoul, South Korea). Quantification of PD-L1 mRNA was done using RT-PCR with AccuPower® GreenStar™ RT-qPCR PreMix on Exicycler™ 96 (Bioneer Corp., Daejeon, South Korea). RT-PCR was performed according to the protocol previously described by Vassilakopoulou et al.[9] In particular, this study provided sequences for the primer pairs and thermocycler conditions used in the present study. PD-L1 mRNA levels were calculated and normalized from the quantification cycle (Cq). GAPDH was used as an internal control. Samples were classified as normoregulated if the expression was lower or equal to the average of PD-L1 mRNA levels of the entire subject group, while the samples with levels above the average were classified as upregulated.

Detailed clinical data (age, sex, and lymph node metastasis) were obtained from the medical records. The collected pathological data included information regarding invasion of muscularis propria, mitotic index, and tumor-infiltrating lymphocytes (TILs). Muscle invasion and TILs were assessed from hematoxylin-eosin slides, while mitotic index was measured using Ki-67 IHC stained slides. Presence of muscle invasion was assessed in terms of whether tumor cells had invaded muscularis propria layer or not. If muscle invasion could not be assessed from the sample, the sample was excluded from analysis for muscle invasion. TILs were grouped as intense and non-intense using a 10% cut-off, according to previous studies.[10,11] The samples with 10% or more stromal TILs were classified as intense, while the samples with less than 10% stromal TILs were classified as non-intense. For mitotic index assessed using Ki-67 IHC stained slides, observed under the microscope, 20% cut-off value was used. The number of tumor cells with brown-stained nuclei was calculated for at least 1000 tumor cells.

Chi-square analysis was used to compare clinicopathological characteristics for PD-L1 positive and negative groups for the categorical variables. T-test and Mann–Whitney test were used to compare continuous variables for normally distributed variables and non-normal distribution, respectively. Correlation between continuous variables was analyzed using Pearson correlation. The experiments performed in this study were approved by the Medical and Health Research Ethics Committee of Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia (KE/FK/0733/EC/2019).

## RESULTS

The characteristic features for the whole group and group comparisons made on the basis of PD-L1 expression are presented in Table I. The study mainly included male patients (76.47%), with an average age of 65.78 years (range: 45–83 years). PD-L1 IHC staining was reported to be positive in five (9.80%) cases. Representative results for PD-L1 immunohistochemistry are shown in Fig. 1.

Fig. 2 shows the distribution of PD-L1 mRNA expression levels of the PD-L1 expression groups based on immunohistochemistry assessment. Patients with upregulated PD-L1 mRNA belonged to older age group ($p$ = 0.049). In fact, these cases were less invasive cases ($p$ = 0.019) as compared to normoregulated group (Table I). No other differences were found to be statistically significant in case of clinicopathological characteristics.

Pearson correlations between continuous parameters are shown in Table II. Age was found to be positively correlated with PD-L1 expression, as assessed using IHC examination (r = 0.281, $p$ = 0.046). In contrast to this, mitotic index was inversely correlated to PD-L1 mRNA levels (r = –0.369, $p$ = 0.008).

## DISCUSSION

This study investigated the associations between PD-L1 expression (both at mRNA and protein levels) and clinicopathological characteristics of high-grade UC cases in Indonesia. The results for IHC staining showed that PD-L1 expression was positive in 9.80% of the cases. Upregulated mRNA levels were found to be associated with less invasive behavior and lower mitotic index. Importantly, higher PD-L1 expression was associated with older age.

For IHC staining, five out of 51 samples tested positive for PD-L1, resulting in a prevalence of 9.80%. This rate is comparatively lower as compared to previous studies, wherein it ranged from 15.1%–46.39%.[12–15] It has been previously reported that differences in populations and histological variants might affect PD-L1 expression.[13] Another factor that might contribute is the variation in the antibody clone used, which is known to cause differences in sensitivity and specificity.[16] Low PD-L1 expression might suggest that ICI treatment in Indonesian patients would exhibit poorer response. However, the predictive role of PD-L1 still remains inconclusive, thus requiring further research.

**Table I:Associations between PD–L1 expression and clinicopathological characteristics**

| | All subjects (n = 51) | PD–L1 expression on IHC | | | PD–L1 mRNA expression | | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | *p* ** | Upregulated | Normoregulated | *p* ** |
| Age, mean ± SD* | 65.78 ± 8.76 | 71.00 ± 9.54 | 65.20 ± 8.59 | 0.163 | 68.24 ± 8.53 | 63.4 ± 8.47 | 0.049 |
| Age category, n (%) | | | | | | | |
| <65 years | 22 (43.13) | 2 (3.92) | 20 (39.22) | 0.632 | 9 (17.65) | 13 (25.49) | 0.400 |
| ≥65 years | 29 (56.87) | 3 (5.88) | 26 (50.98) | | 16 (31.37) | 13 (25.49) | |
| Sex, n (%) | | | | | | | |
| Male | 39 (76.47) | 4 (7.84) | 35 (68.63) | 0.665 | 20 (39.22) | 19 (37.25) | 0.743 |
| Female | 12 (23.53) | 1 (1.96) | 11 (21.57) | | 15 (29.41) | 7 (13.73) | |
| Lymph node metastasis, n (%) | | | | | | | |
| Present | 7 (13.73) | 1 (1.96) | 6 (11.76) | 0.538 | 1 (1.96) | 6 (11.76) | 0.055 |
| Absent | 44 (86.27) | 4 (7.84) | 40 (78.43) | | 24 (47.06) | 20 (39.22) | |
| Muscle invasion, n (%) | | | | | | | |
| Yes | 37 (72.55) | 4 (8.89) | 33 (73.33) | 0.443 | 15 (33.33) | 22 (48.89) | 0.019 |
| No | 8 (15.69) | 0 (0.00) | 8 (17.78) | | 7 (15.56) | 1 (2.22) | |
| Indeterminate | 6 (11.76) | | | | | | |
| Mitotic index, mean ± SD* | 36.64 ± 15.80 | 47.40 ± 20.11 | 35.47 ± 15.07 | 0.110 | 33.04 ± 13.56 | 40.11 ± 17.24 | 0.111 |
| Mitotic index category, n (%) | | | | | | | |
| ≥20% | 42 (82.35) | 4 (7.84) | 38 (74.51) | 0.638 | 19 (37.25) | 23 (45.10) | 0.213 |
| <20% | 9 (17.65) | 1 (1.96) | 8 (15.69) | | 6 (11.76) | 3 (5.88) | |
| TILs, mean ± SD* | 17.21 ± 10.88 | 11.00 ± 8.21 | 17.89 ± 10.98 | 0.237 | 15.32 ± 11.62 | 19.03 ± 10.00 | 0.128 |
| TILs category, n (%) | | | | | | | |
| Intense (≥10%) | 35 (68.63) | 2 (3.92) | 33 (64.71) | 0.171 | 14 (27.45) | 21 (41.18) | 0.075 |
| Non-intense (<10%) | 16 (31.37) | 3 (5.88) | 13 (25.49) | | 11 (21.57) | 5 (9.80) | |
| PD-L1 mRNA, mean ± SD* | 9.78 ± 4.13 | 6.89 ± 2.54 | 10.10 ± 4.16 | 0.099 | | | |
| PD-L1 mRNA category | | | | | | | |
| Upregulated | 25 (49.02) | 1 (1.96) | 24 (47.06) | 0.187 | | | |
| Normoregulated | 26 (50.98) | 4 (7.84) | 22 (43.13) | | | | |

*SD = standard deviation.
**$p$ value < 0.05 was considered significant.

**Table II: Correlation between PD-L1 expression and clinicopathological parameters**

| | Mitotic Index | TILs | PD-L1 (IHC) | PD-L1 (mRNA) |
|---|---|---|---|---|
| Age | 0.007<br>(0.959) | -0.060<br>(0.676) | **0.281**<br>**(0.046)** | 0.133<br>(0.351) |
| Mitotic Index | | -0.049<br>(0.732) | 0.233<br>(0.101) | **-0.369**<br>**(0.008)** |
| TILs | | | -0.238<br>(0.093) | -0.027<br>(0.851) |
| PD-L1 (IHC) | | | | -0.196<br>(0.167) |
| PD-L1 (mRNA) | | | | |

Statistically significant values are indicated in bold

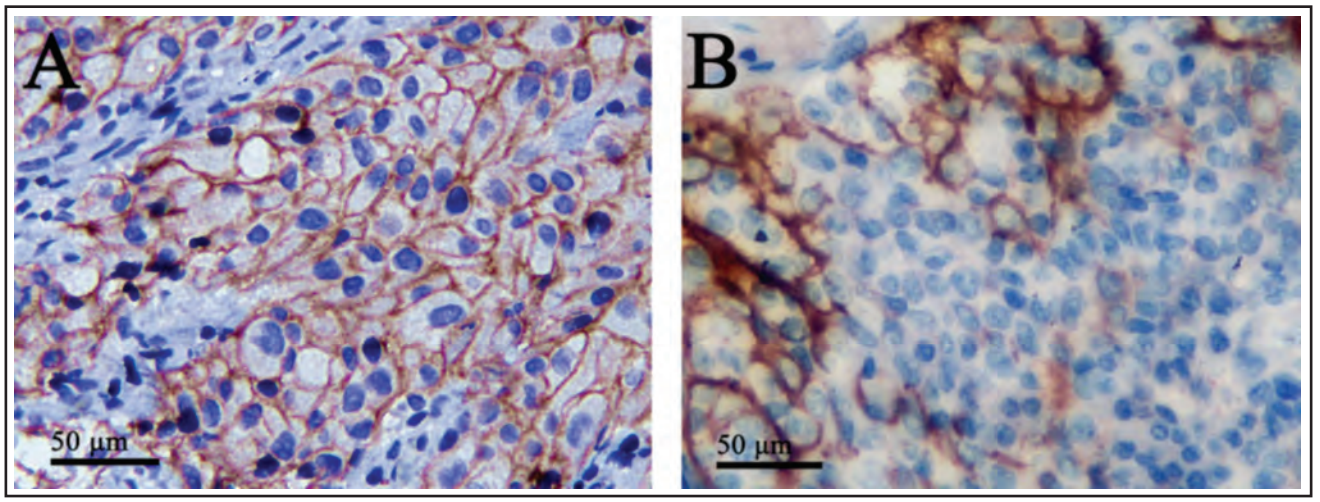


**Fig. 1:** (A) Immunohistochemistry staining image for representative urothelial carcinoma case stained positive for PD-L1, with combined positive score 100 (400× magnification). (B) Normal tonsil tissue as positive control showed positive membranous expression on reticulated epithelium of crypts.

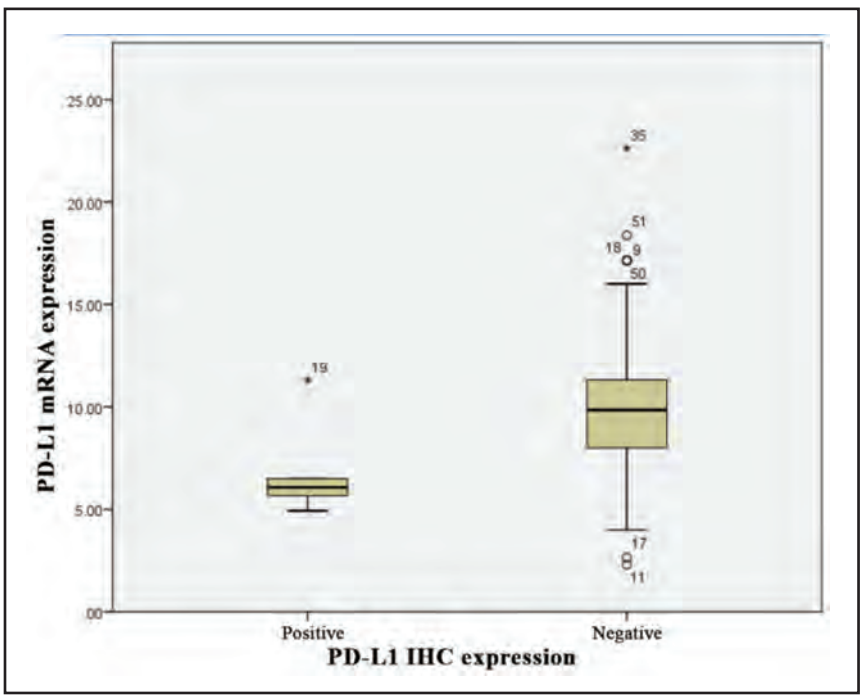


**Fig. 2:** Blox plot showing distribution of PD-L1 mRNA expression levels in positive and negative PD-L1 expression groups based on immunohistochemistry.

Importantly, it was observed that PD-L1 protein expression was not directly proportional to mRNA levels. PD-L1 expression levels observed for IHC and quantitative PCR even appeared to be negatively correlated. However, it was not statistically significant. Studies in UC have been previously shown to be variable in terms of the correlation between expression of PD-L1 mRNA and protein. Le Goux et al. reported a positive correlation between both these molecules.[17] However, Eckstein et al. reported that over 80% of urothelial bladder tumors that showed high expression of PD-L1 mRNA were negative for PD-L1 when IHC staining was performed.[4]

The type of PD-L1 expression (whether constitutive or inducible) might affect the correlation between the expression of PD-L1 mRNA and protein. In steady state conditions, mRNA levels tend to be proportional to protein levels.[18] However, in dynamic cell settings, such as proliferation, correlations between mRNA and protein expression become more variable. In fact, it can even reach negative correlation in certain cases. Variations in the findings of several studies might reflect the involvement of different expression modes in different populations. In this study, lack of positive correlation between PD-L1 mRNA and protein suggested that PD-L1 expression in Indonesian UC patients was inducible or reactive. The mode of expression might affect the prognostic role of PD-L1 in cancers, with reactive PD-L1 expression marking better prognosis and improved response to therapies targeting PD-L1.[19]

Post-transcriptional factors, including miRNA, involved in PD-L1 expression pathway might also affect the correlation between mRNA and protein levels. Several oncogenic miRNAs, such as miR-873 and miR-34a, are known to target the genes that encode PD-L1.[20] Consequently, inhibited translation could result in low protein expression, regardless of mRNA levels. Polymorphism of untranslated regions of PD-L1 mRNA can also affect the mRNA stability, which in turn can affect the measured mRNA levels.

Several post-translational processes, such as N-glycosylation, phosphorylation, and ubiquitination, can also affect PD-L1 protein levels. Glycosylation has been previously shown to protect PD-L1 from degradation, while phosphorylation and ubiquitination marked PD-L1 for degradation.[21] Increased degradation of PD-L1 protein might also account for a poor correlation between PD-L1 mRNA and protein levels.

This discrepancy between PD-L1 mRNA and protein expression might also explain the other associations that were found. Expression of PD-L1 in urothelial cancer cells is known to be associated with more aggressive tumors and higher recurrence.[22,23] Conversely, the results of this study showed that upregulated PD-L1 mRNA is associated with lower muscle invasiveness. Two previous studies also reported that bladder cancer cases with higher PD-L1 mRNA exhibit longer patient survival.[4,24] Differences in the prognostic role of protein and mRNA might arise from their different roles. A failure to express the PD-L1 protein, despite high levels of mRNA, might lead to stronger immune responses toward the tumor, thereby resulting in improved patient outcomes.

Interestingly, both upregulated PD-L1 mRNA and increased PD-L1 protein expression were found to be associated with older age. There is a possibility that PD-L1 transcription increases with age, which resulted in an overall increase in PD-L1 mRNA and protein expression levels. Very few studies have previously reported an association with age. Interestingly, one study conducted in United States of America reported that PD-L1 expression on IHC was linked to younger age.[10]

Although the samples analyzed in this study were limited and retrospective data collection might lead to information bias, the results of this study suggested that PD-L1 mRNA levels have the potential to be used as a prognostic factor for UC cases. Further research with larger sample size is required to investigate the correlation between PD-L1 expression (using both IHC and RT-PCR) and outcomes among subjects who do not receive immunotherapy.

## CONCLUSIONS

The results of this study suggested that PD-L1 expression at mRNA and protein levels exhibited different clinicopathological associations and possibly prognostic implications. The study particularly highlighted the potential of PD-L1 mRNA levels to be used as a predictive factor for patient outcomes. Studies with larger subject group are required to further ascertain the prognostic role of PD-L1 expression in Asian patients.


## ACKNOWLEDGMENT

The authors would like to thank Nur Eka Wiraditya (Department of Anatomical Pathology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia) and Agustina Supriyanti (Department of Integrated Laboratory, Dr. Sardjito Hospital, Yogyakarta, Indonesia) for providing assistance in the laboratory processes during this study.


## CONFLICT OF INTEREST

All authors declare no conflict of interest.

## REFERENCES


1. Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca-Cancer J Clin 2021;71(3):209-49.
2. Kim H,Seo H. Immune checkpoint inhibitors for urothelial carcinoma. Investig Clin Urol 2018; 59(5):285-96.
3. Tang H, Liang Y, Anders R, Taube J, Qiu X, Mulgaonkar A, et al. PD-L1 on host cells is essential for PD-L1 blockade–mediated tumor regression. J Clin Investig 2018; 128(2):580-88.
4. Eckstein M, Wirtz R, Pfannstil C, Wach S, Stoehr R, Breyer J, et al. A multicenter round robin test of PD-L1 expression assessment in urothelial bladder cancer by immunohistochemistry and RT-qPCR with emphasis on prognosis prediction after radical cystectomy. Oncotarget 2018; 9(19):15001-14.
5. Ajoedi A, Azhar M, Nadliroh S, Hartini S, Andalusia R, Witarto A. The mRNA expression profile of PD-1 and PD-L1 in peripheral blood of colorectal cancer patients. Indonesian Journal of Cancer 2019;13(3):80-5.

6. Al Azhar M, Nadliroh S, Prameswari K, Handoko H, Tobing D, Herawati C. Profile of PD-1 and PD-L1 mRNA expression in peripheral blood of nasopharyngeal carcinoma. Molecular Cellular Biomedical Sci 2020; 4(3):121-7.
7. Widayanti LA, DewiI, Saputra H, Susraini AAAN, Sriwidyani NP, Muliarta IM. Hubungan antara tumor infiltrating lymphocytes (TIL), ekspresi programmed death-ligan 1 (PD-L1) pada sel tumor dan TIL dengan kedalaman invasi pada karsinoma urothelial kandung kemih tipe tidak spesifik di RSUP Sanglah, Bali, Indonesia. di RSUP Sanglah, Bali, Indonesia. Intisari Sains Medis 2020; 11(3):1119-25.
8. Moch H, Cubilla A, Humphrey P, Reuter V, Ulbright T. The 2016 WHO classification of tumours of the urinary system and male genital organs—Part A: Renal, Penile, and Testicular Tumours. EurUrol 2016; 70(1):93-105.
9. Vassilakopoulou M, Avgeris M, Velcheti V, Kotoula V, Rampias T, Chatzopoulos K,et al. Evaluation of PD-L1 expression and associated tumor-infiltrating lymphocytes in laryngeal squamous cell carcinoma. Clin Cancer Res 2015; 22(3):704-13.
10. Rouanne M, Betari R, Radulescu C, Signolle N, Allory Y, Marabelle A, et al. Association of stromal lymphocyte infiltration with tumor invasion depth and high-grade T1 bladder cancer. J Clin Oncol 2018; 36(6_suppl):488.
11. Huang H, Su H, Li P, Chiang P, Huang C, Chen C, et al. Prognostic impact of tumor infiltrating lymphocytes on patients with metastatic urothelial carcinoma receiving platinumbasedchemotherapy. Sci Rep 2018; 8(7485):1-7.
12. Faraj S, Munari E, Guner G, Taube J, Anders R, Hicks J,et al. Assessment of tumoral PD-L1 expression and intratumoral CD8+ T Cells in urothelial carcinoma. Urology 2015; 85(3): 703.e1-e6.
13. Kim B, Lee C, Kim Y,Moon K. PD-L1 expression in muscle-invasive urinary bladder urothelial carcinoma according to basal/squamous-like phenotype. Front Oncol 2020; 10(527385):1-7.
14. Nechifor-Boilă I, Loghin A, Nechifor-Boilă A, Decaussin-Petrucci M, Voidăzan S, Chibelean B,et al. PD-L1 expression in muscle invasive urothelial carcinomas as assessed via immunohistochemistry: correlations with specific clinical and pathological features, with emphasis on prognosis after radical cystectomy. Life 2021;11(5):404.
15. Kim D, Kim JM, Kim JS, Kim S, Kim KH. Differential expression and clinicopathological significance of HER2, indoleamine 2,3-dioxygenase and PD-L1 in urothelial carcinoma of the bladder. J Clin Med 2020; 9(5): 1-15.
16. Reis H, Serrette R, Posada J, Lu V, Chen Y, Gopalan A,et al. PD-L1 expression in urothelial carcinoma with predominant or pure variant histology. Am J Surg Pathol 2019; 43(7):920-7.
17. Le Goux C, Damotte D, Vacher S, Sibony M, Delongchamps NB, Schnitzler A, et al. Correlation between messenger RNA expression and protein expression of immune checkpoint-associated molecules in bladder urothelial carcinoma: a retrospective study. Urol Oncol: Semin Orig 2017; 35(5): 257-63.
18. Liu Y, Beyer A, and Aebersold R. On the dependency of cellular protein levels on mRNA abundance. Cell 2016; 165(3): 535-50.
19. Hao D, Wang G, Yang W, Gong J, Li X, Xiao M, et al. Reactive versus constitutive: reconcile the controversial results about the prognostic value of PD-L1 expression in cancer. Int J Biol Sci 2019; 15(9): 1933-41.
20. Yi M, Niu M, Xu L, Luo S, Wu K. Regulation of PD-L1 expression in the tumor microenvironment. J Hematol Oncol 2021; 14(1): 10.
21. Hsu JM, Li CW, Lai YJ, Hung MC. Posttranslational modifications of PD-L1 and their applications in cancer therapy. Cancer Res 2018; 78(22): 6349-53.
22. Bellmunt J, Mullane SA, Werner L, Fay AP, Callea M, Leow JJ, et al. Association of PD-L1 expression on tumor-infiltrating mononuclear cells and overall survival in patients with urothelial carcinoma. Ann Oncol 2015; 26(4): 812-7.
23. Pierconti F, Raspollini MR, Martini M, Larocca LM, Bassi PF, Bientinesi R, et al. PD-L1 expression in bladder primary in situ urothelial carcinoma: evaluation in BCG-unresponsive patients and BCG responders. Virchows Archiv 2020; 477(2): 269-77.
24. Kubon J, Sikic D, Eckstein M, Weyerer V, Stöhr R, Neumann A, et al. Analysis of CXCL9, PD1 and PD-L1 mRNA in stage T1 non-muscle invasive bladder cancer and their association with prognosis. Cancers 2020; 12(10): 1-13.