Differences of Expression of PD-L1 in lung adenocarcinoma and lung squamous cell carcinoma

Rini Syahrani Harahap[*], Delyuzar[**], Soekimin[**]

[*] Resident in Department of Anatomical Pathology of Medical Faculty of Universitas Sumatera Utara, Medan, Indonesia
[**] Consultant in Resident in Department of Anatomical Pathology of Medical Faculty of Universitas Sumatera Utara, Medan, Indonesia

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Background : Most patients with NSCLC are diagnosed in advanced stage. Several investigators evaluated the possibility to modulate the immune system for treating lung cancer. PD-L1 expression on tumour cells leads to the inhibition of immune responses against cancer. The aim of this study is to differentiate expression PD-L1 in lung adenocarcinoma and lung squamous cell carcinoma.
Materials and Methods : We obtained 40 paraffin blocks of the lung adenocarcinoma and squamous cell carcinoma tumor specimens and assessed PD-L1 expression by immunohistochemistry. Clinicopathological characteristics of the patients were taken from the medical reports in Haji Adam Malik General hospital. Results were analyzed using H-Score to cut-off values of ≥ 30%.
Results : in our study we found PD-L1 expression was significantly higher in lung squamous cell carcinoma and in advanced stage.

keywords : lung adenocarcinoma, squamous cell carcinoma, PD-L1**,** imunohistochemistry**.**

I. INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide.[1] Despite significant advances in multidisciplinary cancer therapies, the overall prognosis for lung cancer patients remains poor. Immunotherapy with checkpoint inhibitors (CPIs) is becoming a new standard of treatment for non-small cell lung cancer (NSCLC) patients.
Lung cancer is the leading cause of cancer death worldwide.[1] According to Persahabatan hospital, Jakarta, Indonesia, found 151 (90,4%) patients with the histopathology of adenocarcinoma, and follwed by *squamous cell carcinoma* 11 patients (6,6%).[2] Soeroso showed the study in Haji Adam Malik General hospital in Medan, Sumatera Utara, Indonesia, found from January 2010 until May 2012, there were 167 lung cancer patients. The cytology / histopathology found was adenocarcinoma (56.8%) dan Stage III B found in about (54.8%) in one hundred sixty seven patients.[3] Asrul HH et al. found 72 patients of lung adenocarcinoma from cytology and histopathology preparations in same hospital.[4] Accoding to the data, in Medan there are many lung cancer patients, especially lung adenocarcinoma and lung squamous cell carcinoma. Immunotherapy with checkpoint inhibitors (CPIs), such as the programmed cell death-ligand 1 (PD-1) is becoming a new standard of treatment for non-small cell lung cancer (NSCLC) patients. Thus far, three agents, namely nivolumab, pembrolizumab and atezolizumab, have proven antitumor efficacy in terms of improved response rates and overall survival compared to standard chemotherapy in the second-line setting.[1,5-8] Several studies have research PD-L1 expression on lung cancer and clinicopathological characteristics of the patients.[9-12] So that we want to know how differ PD-L1 expression between patients of lung adenocarcinoma (LAC) and lung squamous cell carcinoma (LSCC) in Haji Adam Malik General Hospital in Medan, North Sumatera, Indonesia.

II. MATERIAL AND METHODS

A. Patients and specimens

This is cross sectional study was conducted on biopsy (35 cases) and surgical specimens (5 cases) which diagnosed lung adenocarcinoma (LAC) and lung squamous cell carcinoma (LSCC) were included. The specimens were collected by random sampling from the unit of anatomical pathology and the clinicopathological characteristic of those patients were taken from the medical reports in same location, Haji Adam Malik General Hospital in Medan, North sumatera, Indonesia. This study has recieved permission from Health Research Ethical Medical Faculty of Universitas Sumatera Utara / H. Adam Malik General Hospital (no 697/TGL/KEPK FK USU-RSUP HAM/2018)

B. Immunohistochemistry and interpretations

Immunohistochemistry was performed in 40 paraffin blocks using formalin-fixed and paraffin-embedded tumor tissue sections according to the previously described PD-L1 immunohistochemistry protocol. The primary antibody was an anti-human PD-L1 rabbit monoclonal antibody (clone MD21R, ready to use, Medaysis, CA).[14] Immunohistochemical staining for PD-L1 was detected at the membrane or in the cytoplasm (or both) of tumor cells.[9] The PD-L1 expression of tumour cells staining level was counted using a semiquantitative evaluation, with cut off 30%.[9] PD-L1 expression score (H score) was calculated for each case according to the following formula : PD-L1 expression score (H score) (range, 0–300) =0×% of non-stained tumour cells +1×% of weakly stained tumour cells +2×% of moderately stained tumour cells +3×% of strongly stained tumour cells.[14,15] The specimens were describe by three researchers, including two pathologists using double blind.

Chi-square or Fisher's exact test was used to assess correlations between different immunoreactivity and clinicopathologic variables.

III. RESULTS

Based on clinical data obtained from medical records, it was found that the sample of this study had an average age of 58.4 (± 10.7) years, with the youngest age being 36 years and the oldest being 80 years old. Thirty-three specimens were male (82.5%) and women were 7 (17.5%). Of the 40 specimens, 23 were LAC and 17 were LSCC. Most of the 34 patients were smokers (85%) and 6 nonsmokers (15%). About the location tumor, 21 cases (52,5%) in central area and 17 (42,5%) in peripheral. There 2 difficult cases to define the exactly location, because of the massive pleural effusion.

One patients (2,5%), six (15%), 18 (45%), and 15 (37,5%) had stage I, II, III and IV disease, respectively.

A total of 40 tumor specimens were examined, 23 of them (57,5%) were AC and 17 (42,5%) were SCC. The growth pattern of lung adenocarcinoma was classified into lepidic (3 tumors), acinar (19), papillary (1). There were 22 patient (55%) with high PD-L1 expression, and 18 (45%) with low expression. (Table 1).

Table 1. Clinicopathological characteristic

Characteristic	(n)	Persentase (%)
Age, mean ± SD, year	57,5 ± 10,7	
Sex		
Male	33	82,5
Female	7	17,5
Smoking tatus		
Non-smoker	6	15
Smoker	34	85
Tumour Location		
Central	21	52,5
Peripheral	17	42,5
Unknown (pleural effusion)	2	5
Staging		
Staging I	1	2,5
Staging II	6	15
Staging III	18	45,0
Staging IV	15	37,5
Type of histopathology		
Adenocarcinoma	23	57,5
Lepidic	3	13,04
Acinar	19	82,6
Papillary	1	4,34
Micropapillary	0	0
Solid	0	0
PD-L1 expression		
> 30% (higher)	22	55
≤ 30% (lower)	18	45

From data in this study, we found there was no significant relationship between PD-L1 expression of smoking status and primary tumor location.

Stage I and II in this study, showed only 1 person (14.3%) with high PD-L1 expression and 6 persons (85.7%) with low PD-L1 expression. For stage III and IV, found that there were 21 persons (63.4%) with high PD-L1 expression and 12 people (36.4%) with low PD-L1 expression. There is a significant relationship between the expression of PD-L1 and the clinical stage, where stage III and IV have a tendency of 4,455 times to express PD-L1 compared to stages I and II.

Table 2. Distribution of PD-L1 expression according to staging

Staging	PD-L1 expression				P	PR (CI95%)
	High		Low			
	n	%	n	%		
Staging I & II	1	14,3	6	85,7	0,033	4,455 (0,713-27,847)
Staging III & IV	21	63,4	12	36,4		
Total	22		18			

We found 13 patients LSCC (76,5%) showing high PD-L1 exspression and 4 patients LSCC (23,5%) with low PD-L1 exspression. There were 9 patients LAC (39,1%) showing high PD-L1 exspression and 14 patients LAC (60,9%) with low PD-L1 exspression. There is differences of LSCC compared to LAC. LSCC has a tendency 1,954 times to express PD-L1 compared to LAC. (Table 3)

Table 3. Distribution of PD-L1 expression according to type of histopathology

Type of histopathology	PD-L1 expression				P	PR (CI95%)
	High		Low			
	n	%	n	%		
LSCC	13	76,5	4	23,5	0,043	1,954 (1,101-3,469)
LAC	9	39,1	14	60,9		
Total	22		18			

IV. DISCUSSIONS

In this study, in this study there are differences in PD-L1 expression in LSCC compared to LAC and PD-L1 expression was higher in advanced stage (stage III and IV) compared to low stage (I and II).

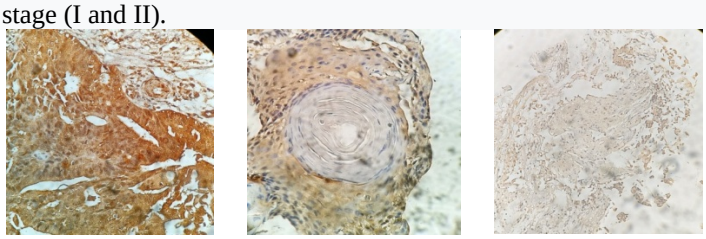

Figure 1. PD-L1 immunostaining intensity in the specimens with +3 (left figure), +2 (central figure) or +1 (right figure). PD-L1 expression was presented at the membrane or / and the cytoplasm.

Lin G et al. found a lower incidence of positive PD-L1 expression in their cohort of patients with early stage NSCLC and showed that the PD-L1 expression is more likely influenced by disease stage, which indicated that the induction of PD-L1 expression was not an initial event in the development of cancer. They also found squamous carcinoma tumors to be strongly associated with PD-L1 expression. The biological determinants

and potential clinical implications of these observations are unknown and require further study.[10]

Janzic et al. observed a significantly higher proportion of PD-L1 positivity among SCC than AC, when considering staining in the TC, whereas PD-L1 positivity in IC is quite high in both histological subtypes of NSCLC. SCC seems to be distinct from non-SCC. That reflects both in high PD-L1 positivity and in steady responses to immune checkpoint therapy across SCC subgroup of patients. One of the possible explanations could be high levels of acquired somatic mutations in SCC patients caused with carcinogens such as cigarette smoke, especially because most of the patients with SCC are smokers.[11]

D'Incecco et al. and Azuma et al. had different results. D'Incecco et al. analyzed PD-L1 expression in SCC and non-SCC was reported on TC positivity rate of 30% and 63%.[12] Azuma et al. analyzed that PD-L1 expression PD-L1 was significantly higher in adenocarcinoma, never smokers, and advanced stage.[9]

In this study, although PD-L1 expression is significantly different in LSCC compared to LAC, but there is no significant relationship in smoking status. We assumed that, there were several patients of lung adenocarcinoma with smoking status in our study, using different antibody and cut-off values. We also found that PD-L1 expression in advanced stage is significantly higher than low stage. And advanced stage is association with poor prognosis.

Based on the results of Azuma et al. and ours are consistent with previous studies showing that high expression of PD-L1 is associated with poor prognosis.[16-18]

V. CONCLUSION

Expression of PD-L1 was significantly different in lung adenocarcinoma than lung squamous cell carcinoma histology (P=0.027), and in those from advanced staging (III&IV) than in those from early staging (I&II) (P=0.033). No significant relationship was found between expression of PD-L1 and smoking status, or tumor location.

ABBREVIATIONS

PD-L1: programmed death ligand 1; NSCLC: non-small cell lung cancer; LAC: lung adenocarcinoma; LSCC: lung squamous cell carcinoma;CPI : Immunotherapy with checkpoint inhibitors; IHC: immunohistochemistry; CI: confidence interval..

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REFERENCES

[1] Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015; 373**:** 123-35. doi:10.1056/NEJMoa1504627.

[2] Syahruddin E, Pratama A, Arief N. A Retrospective Study : Clinical and Diagnostic Characteristics in Advanced Stage of Lung Cancer Patients with Pleural Effussion in Persahabatan Hospital 2004 – 2007. J Respir Indo. 2010;30:145-51

[3] Soeroso NN, Soeroso L, Syafiuddin T. Kadar Carcinoembroyogenic Antigen (CEA) Serum Penderita Kanker Paru Karsinoma Bukan Sel Kecil Di RSUP Adam Malik. J Respir Indo.2014;34:17-25

[4] Asrul HH, Delyuzar, Soekimin. Relationship of VArious Characteristics of Lung Adenocarcinoma PAtients with status of mutation of epidermal growth factor receptor in Haji Adam Malik Medan Hospital 2015-2017. IJCRGG, 2019, 12(2): 212-217.

[5] Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer : a randomised controlled trial. Lancet 2016; 387**:** 1540-50. doi:10.1016/S0140-6736(15)01281-7

[6] Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017; 389: 255-65. doi:10.1016/S0140-6736(16)32517-X

[7] Besse B, Johnson M, Janne PA, et al. Phase II, single-arm trial (BIRCH) of atezolizumab as first-line or subsequent therapy for locally advanced or metastatic PD-L1-selected non-small cell lung cancer (NSCLC). [abstract]. Eur J Cancer 2015; 51(Suppl 3): abstr 16LBA, S717-18. doi:10.1016/S0959-8049(16)31938-4

[8] Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. New Engl J Med 2016; 375: 1823-33. doi:10.1056/Nejmoa1606774.

[9] Azuma K, Ota K, Kawahara A, et al. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. 2014. Annals of Oncology 25: 1935–1940

[10] Lin G, Fan X, Zhu W, et al. Prognostic significance of PD-L1 expression and tumor infiltrating lymphocyte in surgically resectable non-small cell lung cancer. Oncotarget, 2017, Vol. 8, (No. 48), pp: 83986-83994

[11] Janzic U, Kern I, Janzic A, et al. PD-L1 expression in squamous-cell carcinoma and adenocarcinoma of the lung. Radiol Oncol 2017; 51(3): 357-362

[12] D'Incecco A, Andreozzi M, Ludovini V, et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. Br J Cancer 2015; 112: 95-102. doi:10.1038/bjc.2014.555

[13] Rabbit Anti-PD-L1/PDCD1-L1/CD274 [MD21R]: RM0324, RM0324RTU7. [Product Data Sheet]. Medaysis. 2017

[14] Igarashi T, Teramoto K, Ishida M, et al. Scoring of PD-L1 expression intensity on pulmonary adenocarcinomas and the correlations with clinicopathological factors. ESMO Open 2016;1:e000083. doi:10.1136/esmoopen-2016-000083.

[15] Wang X, Teng F, Kong L, Yu J. PD-L1 expression in human cancers and its association with clinical outcomes. OncoTargets and Therapy 2016:9 5023–5039

[16] Thompson RH, Kuntz SM, Leibovich BC et al. Tumor B7-1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res 2006; 66: 3381–3385.

[17] Hino R, Kabashima K, Kato Y et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. Cancer 2010;116: 1757–1766.

[18] Li YF, Ding JW, Liao LM, et al. Expression of programmed death ligand-1 predicts poor outcome in nasopharyngeal carcinoma. MOLECULAR AND CLINICAL ONCOLOGY 7: 378-382, 2017.

AUTHORS

First Author – Rini Syahrani Harahap, MD, Anatomical Pathology of Faculty Medicine in Universitas Sumatera Utara, Medan, Indonesia.
rinisyahraniharahap@yahoo.com
Second Author – Delyuzar, Pathologist Consultant, Department of Anatomical Pathology of Faculty Medicine in Universitas Sumatera Utara, Medan, Indonesia

Third Author – Soekimin, Pathologist Consultant, Department of Anatomical Pathology of Faculty Medicine in Universitas Sumatera Utara, Medan, Indonesia

Correspondence Author – Rini Syahrani Harahap, rinisyahraniharahap@yahoo.com
tiaraalthafi@gmail.com