

JURNAL RESPIROLOGI INDONESIA

Majalah Resmi Perhimpunan Dokter Paru Indonesia
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Expression of Immune Checkpoint Marker PD-L1 in Surgical Lung Cancer Specimens

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Abstract

Background: Currently, immune checkpoint pathways of PD1-PD-L1 are being used to treat lung cancer and PD-L1 serves as a predictive biomarker. Investigations of PD-L1 expression as targeted immunotherapy in lung cancer specimens in Indonesia are needed. This study evaluated PD-L1 expression in resected lung cancer specimens using immunohistochemistry techniques.

Methods: Thirty surgically resected samples from lung cancer patients were obtained. The whole specimens were stained using immunohistochemistry techniques automatically in BOND-MAX Autostainer (Leica, Germany). PD-L1 polyclonal antibody (Genetex) at 1:500 dilution was applied to the immunohistochemistry staining procedure. Clinicopathological characteristics were acquired from the hospital registry database.

Result: A total of 30 surgical specimens were assessed from lung cancer patients. Twenty-four (80%) of them had the histological type of adenocarcinoma (AC), 1 (3,33%) were adenosquamous carcinoma, 2 (6,67%) squamous cell carcinoma (SSC), 1 (3,33%) large cell carcinoma, 1 (3,33%) neuroendocrine carcinoma and 1 (3,33%) adenoid cystic carcinoma. The expression of PDL-1 positive reactivity were detected in 16 of 30 (53.3%) specimens. Samples were categorized into strong positivity (>50%) in 7 specimens, medium (50%) in 0 specimen and low positivity (<50%) in 9 specimens.

Conclusion: PD-L1 expression could be detected in lung cancer specimens using polyclonal antibody. Further investigation is needed to determine the clinical correlation between this examination and lung cancer. (*J Respir Indones* 2022; 42 (2): 136-40)

Keywords: non-small cell lung cancer, PD-L1 expression, polyclonal antibody

Ekspresi Petanda Hayati “Immune Check Point” PD-L1 pada Sediaan Jaringan Bedah Kanker Paru

Abstrak

Latar belakang: Imunoterapi dengan intervensi “check point system” seperti PD1-PD-L1 saat ini sudah menjadi salah satu pilihan terapi kanker paru. Ekspresi PD-L1 merupakan salah satu petanda hayati kanker paru. Penelitian ekspresi molekul PD-L1 pada kanker paru di Indonesia saat ini masih jarang, terutama yang bersumber dari jaringan bedah. Oleh karena itu, penelitian ini bertujuan menilai ekspresi PD-L1 pada jaringan reseksi kanker paru menggunakan teknik imunohistokimia.

Metode: 30 jaringan reseksi kanker paru di RS Persahabatan Jakarta dilakukan pemeriksaan imunohistokimia menggunakan sistem BOND-MAX Autostainer (Leica, Germany). Antibodi yang digunakan adalah PD-L1 polyclonal antibody (Genetex) dengan pengenceran 1:500. Karakteristik klinis subjek dianalisis lebih lanjut.

Hasil: Dari 30 sampel jaringan reseksi kanker paru, 24 kasus (80%) merupakan adenokarsinoma (AC), 1 kasus (3,33%) adenoskuamosa, 2 kasus (6,67%) karsinoma sel skuamosa serta masing-masing 1 kasus (3,33%) karsinoma sel besar, karsinoma neuroendokrin dan karsinoma adenoid kistik. Ekspresi PD-L1 ditemukan pada 16 dari 30 spesimen (53,3%) dan dikategorikan menjadi positif kuat (>50%), medium (50%) dan positif rendah (<50%) dengan komposisi masing-masing 7, 0 dan 9 spesimen. Selain itu, ditemukan 14 spesimen tanpa ekspresi PD-L1.

Kesimpulan: Ekspresi PD-L1 dapat ditemukan pada sediaan kanker paru dengan menggunakan pemeriksaan imunohistokimia. Korelasi klinis pemeriksaan ini pada kanker paru perlu diteliti lebih lanjut. (*J Respir Indones* 2022; 42 (2): 136-40)

Kata kunci: KPKBSK, ekspresi PD-L1, antibodi poliklonal.

INTRODUCTION

Lung cancer is one of the leading cause of cancer related death in the world. Based on data from WHO, it was estimated that 9.6 million cases of death in 2018 were caused by cancer and 2.09 million of them were attributed to lung cancer. On 2012, WHO categorized Indonesia's lung cancer incidence as medium. Lung cancer incidence rate in Indonesian males was 25.8 with mortality rate of 23.2, meanwhile in female was 8.1 with mortality rate of 7.3.¹⁻³

Improvements in lung cancer management have been achieved in many ways. Radiotherapy, chemotherapy and surgery all contribute greatly to patients' survival, but more advanced and novel therapy is needed to increase quality of life and survival. Nowadays, targeted therapy based on specific genes is known to improve patient survival and has become a standard therapy in lung cancer. But newer therapeutic option such as immunotherapy that targets immune checkpoint mechanism may also have potential benefit in lung cancer management. Data from clinical trials have shown proven antitumor efficacy that resulted in better survival and durable response compared to standard therapy.^{4,5}

Immune checkpoint is a protein located on the surface of immune cells, notably on cytotoxic T-cells. Upon binding to a specific ligand, it's capable of transmitting the stimulatory or inhibitory signal.⁶ The primary role of immune checkpoint is to protect tissue damage during severe active inflammation.⁷

One of the potential immune check point molecules is programmed cell death protein 1 (PD1) and its ligand (programmed death ligand 1/PD-L1). Tumor exploited this interaction between PD1 and PD-L1 by increasing their activation, thus limiting immune cells recognition, reducing immune cells activation and reducing elimination of tumor cells. Clinical trials using PD1 or PD-L1 inhibitor so far have shown durable response and better survival in lung cancer.⁸ PD1 or PD-L1 expression in lung cancer tissue are considered as potential biomarker, but further investigation is needed especially in Indonesia.

METHODS

Surgical specimens were obtained from 30 patients diagnosed with primary lung cancer at Department of Anatomical Pathology, Persahabatan Hospital, Jakarta. The specimens were collected and stored at the Laboratory of Anatomical Pathology at the same hospital. Clinical data were taken from the hospital medical records. This research was conducted according to the Ethics Committee of the Faculty of Medicine University of Indonesia No: 534/UN2.F1/ETIK/2017.

This study used formalin-fixed, paraffin embedded (FFPE) specimens of lung cancer. FFPE blocks were sliced at 4 µm sections. Immunohistochemistry (IHC) staining performed on the whole tissue used primary antibody PDL-1 Rabbit polyclonal GTX 104763 (Genetex, USA) at a dilution of 1:500. The IHC staining for PDL-1 antibody was performed automatically on BOND-MAX Autostainer M495401 (Leica, Germany).

Antigen retrieval and secondary antibody used Leica Detection System. The percentage of PD-L1 expression was evaluated and ranging from 0-100% as reviewed by pathologist. The staining result of PD-L1 showed positive reaction in cytoplasm. Villi was used as reactive positive control. The positive expression of PD-L1 in the specimens ranged from 0-100%. They were categorized into strong positivity (>50%), medium (50%), low (<50%) and negative (0%).

RESULT

A total of 30 specimens from lung cancer patients were examined. Among all of them, 24 (80%) had the histological type of adenocarcinoma (AC), 1 (3,33%) was adenosquamous carcinoma, 2 (6,67%) squamous cell carcinoma (SSC), 1 (3,33%) large cell carcinoma, 1 (3,33%) neuroendocrine carcinoma and 1 (3,33%) adenoid cystic carcinoma. The majority of samples came from male patients, amounting to 20 (66,67%), while the rest were female (10/33,3%).

We found that PD-L1 was tested positive in 16 of 30 samples (53.5%). Strong positivity of PD-L1 expression was found in 7 samples, consisting of 1

adenocarcinoma sample with 70% positivity, 3 adenosquamous carcinoma/SCC samples with 80% positivity and 3 adenocarcinoma samples with 90% positivity.

Table 1. The expression of PDL-1 positivity in surgically resected lung cancer specimens.

PDL1 expression	N	Histology
Strong positivity (>50%)	7	SCC, AdenoCa
Medium (50%)	0	-
Low positivity (1-49%)	9	adenoCA, SCC, LC-NEC, ACC
Negative (0%)	14	adenoCa

Note=SCC: squamous cell lung cancer; adenoCA: adenocarcinoma; LC-NEC: Large cells neuroendocrine cells; ACC: adenoid cystic carcinoma.

No medium positivity of PD-L1 were found. Low positivity of PD-L1 expression was found in 9 samples, consisting of 2 adenocarcinoma samples with 1% positivity, 1 adenocarcinoma with 2% positivity, 1 large cell carcinoma with 3% positivity, 1 adenocarcinoma with 5% positivity and each 2 samples of neuroendocrine carcinoma and adenocarcinoma with 20% positivity. PD-L1 expression was completely absent in 14 samples. The results are listed in on Table 1 and PD-L1 positive reaction can be seen in Figure 1.

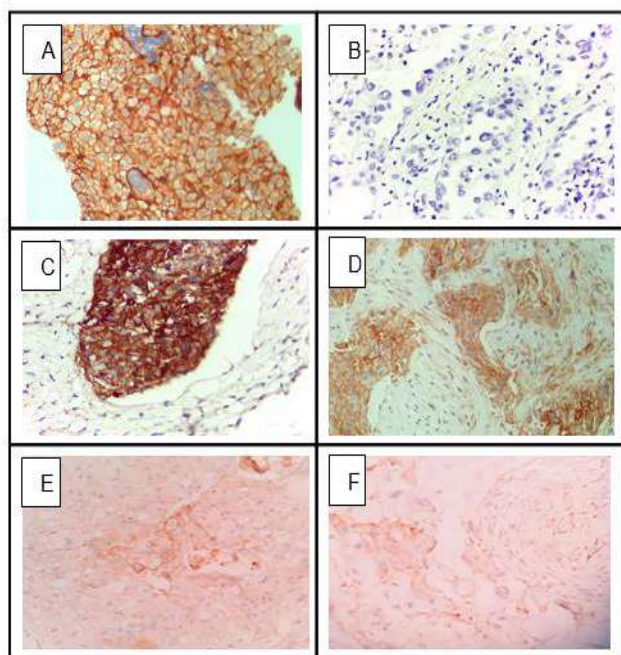


Figure 1. Representative images of PD-L1 staining. A) positive control from villi tissue showed strong membrane staining of PD-L1; B) Negative control showed no cells with PD-L1 staining; C) Strong positive expression of PD-L1 (90% of tumor cells were stained) in adenocarcinoma; D) Strong positive expression of PD-L1 (70% of tumor cells were stained); E) Low positive expression of PD-L1 (5% of tumor were stained) in adenocarcinoma; F) Low positive expression of PD-L1 (3% of tumor cells were stained) in large cell carcinoma

DISCUSSION

PD-L1 is an important immune checkpoint inhibitor expressed by the tumor cells (TCs) or tumor-infiltrating immune cells (ICs) and determined as the major membrane inhibitory ligand.^{6,7} PD-L1 binds to PD1 as a key immune checkpoint receptor activated by the T cells. This PD1 and PD-L1 interaction decreases the ability of the activated T cells to produce an effective immune response and inhibit the immune system to destroy the tumor. This interaction has been studied as a target for lung cancer treatment.⁷

PD-L1 expression in lung cancer is varied according to the histological type of the specimen and the selection of the tumor sample location, which mainly influence the positivity rate of PD-L1 expression in the specimens.^{9,10} The prevalence of PD-L1 expression in the population of patients with non-small cell lung cancer (NSCLC) ranges from 24% to 60%, even with a cutoff for positivity set at 5%.^{11,12} Surgical specimen from lung cancer were used in this study.

Expression of PD-L1 also ranged widely in the NSCLC sample because of the variation of antibody and platform.⁹ PD-L1 has a dynamic expression related to the heterogeneity in many tumors.¹⁰ The evaluation of PD-L1 using IHC techniques also needs more validation regarding the protocol, since different ones might also affect its positivity. The positivity indicates immune active tumor that could be sensitive to anti PD-1 therapy and serves as predictive biomarker. In some clinical trials, high positivity of the PD-L1 expression, i.e., >50% correlates with better clinical outcomes during anti-PD-1 treatment.^{12,13}

There are various methods and platforms to evaluate PD-L1 expression in lung cancer. The commonly used clones are 22C3, SP263, SP142, 28-8, E1L3N, E1J2J and 5H1 with DAKO, Benchmark and BONDMAX as the platforms.⁸ This study used BOND-MAX Autosteiner platform from Leica with PD-L1 Rabbit polyclonal antibody GTX 104763 (Genetex, USA) at a dilution of 1:500.

This study shows that PD-L1 was dominantly expressed in non-small cell lung carcinoma

(NSCLC). High positivity of PD-L1 was majorly found in SCC and adenocarcinoma. From 30 samples included in this study, low positivity of PD-L1 mostly came from neuroendocrine carcinoma, large cell carcinoma and adenoid cystic carcinoma. Meanwhile, the lowest positivity was found in adenocarcinoma. A study from Heymann et al showed that out of 102 samples from surgical lung cancer resection, 26% had high positive staining (>50% of tumor cells) using IHC-based, 22C3 pharmDx assay.¹⁴ Another study by McLaughlin et al using conventional chromogenic IHC with E1L3N and SP142CV antibody in histological lung cancer specimen showed variability/heterogeneity of result.¹⁵

As stated previously, this study showed conflicting result of PD-L1 staining which might be attributed to the small sample size, different protocols of IHC application and different PD-L1 antibodies.⁸ Hence, no precise standard for FFPE unstained slide cut-off could be applied in the IHC staining procedure. Despite the vast differences with other studies, this IHC protocol proved to be feasible, but its correlation with the clinical value warrant further investigation.

CONCLUSION

New therapy to treat lung cancer is currently being developed and immunotherapy is one promising approach to be investigated to reach the goals of better survival. As more treatment options are available, evaluating PD-L1 expression in lung cancer will become more relevant and served as prognostic marker. Characterizing tumor and immune cells via PD-L1 protein by IHC may be helpful to identify the patients who potentially benefit with anti PD1 or anti PD-L1 agents. PD-L1 is a predictive biomarker in lung cancer and its positivity presents an opportunity to administer the agents that prevent PD-1 and PD-L1 pathway interaction either in advanced lung cancer or metastatic lung cancer.

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