Original Article

PD-L1 Immunoexpression on Cytology and its Paired Comparison with Histopathology in Lung Carcinoma

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Abstract

Background

Cell blocks are being increasingly used as a less invasive diagnostic procedure for various ancillary tests like immunohistochemistry. The assays programmed death ligand-1 (PD-L1) is one such companion diagnostic test playing a role in progression of lung cancer. The present study was carried out to examine efficacy of cell blocks for PD-L1 expression in lung cancer patients.

Methods

Over the course of a year, our pathology department conducted this prospective study and included newly diagnosed lung carcinoma cases on cytopathological examination. Tumor Proportion Score (TPS) was used for grading cell membrane PD-L1 positivity. Excel sheet was used for compiling and analysing the data using the IBM SPSS software version 28 (Statistical Package for Social Sciences) Chicago, USA.

Result

The study included 36 cases in which cell blocks and paired histopathological sections were studied, of which 1 cell block was inadequate for reporting. PD-L1 expression was negative in 57%, low grade in 40% and high grade in 3% cases. It was seen in 40% squamous cell carcinoma, 40% adenocarcinoma and 20% small cell carcinoma. Among them, 11 were true positive, 5 were false positive, 3 were false negative and 16 were true negative. With a 78.5% sensitivity, 76% specificity, 68.7% positive predictive value and 84.2% negative predictive value, PD-L1 on cell blocks correlated with paired histopathological sections in 77% of cases in our study.

Conclusion: Cell blocks can be utilized for evaluating PD-L1 expression in lung carcinoma and act as an efficient and trustworthy substitute to routine histopathological techniques.

Keywords:

Cell Block, Immunohistochemistry, Lung Carcinoma, Programmed Death Ligand-1

Introduction

Lung carcinoma inflicts a heavy toll on global and Indian population with an incidence of 11.4% and 5.5% respectively.[1] Cell

blocks are being increasingly used as a less invasive diagnostic procedure for various ancillary tests like immunohistochemistry (IHC). Numerous targeted therapies are being developed in respect to mutations in the genome in lung carcinoma cases.[2] Programmed Death Ligand 1 is one such molecular receptor which dampens the immune system thus playing a role in progression of lung cancer.[3] Current guidelines require measuring of PD-L1 expression levels of a given tumor by IHC, and the cut-off points for each immunotherapeutic drug are clearly defined. Biopsies are historically preferred for this measurement of PD-L1 expression however cell blocks prepared from cytological samples can also serve the same role. PD-L1 expression may certainly differ from case to case and an ideal study should compare cytologic and tissue samples from the same patients, a task which we tried to accomplish in our setting. The present study was carried out to examine efficacy of cell blocks for PD-L1 expression in lung cancer patients.

Materials and Methods

Over the course of a year, our department conducted this prospective study and included newly diagnosed lung carcinoma cases on cytopathological examination. 36 cases were included in our study for preparation of cell blocks and associated histopathological examination. Cytological material was obtained by broncho-alveolar lavage (BAL), bronchial brushings, transbronchial needle aspiration (TBNA) and computed topography (CT) guided FNA with cell block preparation along with paired histopathology and immunohistochemistry. Cell blocks were prepared after centrifugation of cytological specimens at 2500 rpm for 15 minutes followed by mixing with 1:1 pooled plasma and 1:2 uniplastin. The resultant clot was handled for processing and embedding as formalin-fixed paraffin-embedded (FFPE). 100 viable tumor cells in cell block were considered adequate whereas <20 malignant cells, cytological smears with <5 clusters of 6-8 epithelial cells or containing only necrosis, hemorrhagic or mucoid material in the cell blocks were excluded from the study. Every case was extensively followed up with relevant clinical and radiological details and the WHO classification was used to classify the lung tumours.[4]

In lung cancer cases, PD-L1 was examined in cell blocks and paired histopathology sections. PD-L1 (405.9A11 isotype IgG1, Kappa) Mouse monoclonal antibody marker of BIOGENIX Company (USA) was used with sections from placenta as a control. Positive and negative controls were run with every IHC lot and external quality was maintained with other accredited labs as well. Tumor Proportion Score (TPS) was used for grading cell membrane PD-L1 positivity wherein the total number of viable tumour cells was multiplied by 100% after the number of positive tumour cells was split by that number. This membrane positivity was graded as negative (<1%), low grade (1–49%), and high grade (=/>50%).[5] Proportion of PD-L1 TPS positivity among sample types were compared using $\chi 2$ Independence test. The TPS scores in histology and cytology in paired samples were compared with paired samples t test. All data were compiled in an Excel sheet and statistically analyzed using the IBM SPSS software version 28 (Statistical Package for Social Sciences) Chicago, USA. The statistical significance threshold was accepted as p value <0.05. This study was self-funded by the authors and written informed consent was obtained from all the patients. All patients underwent follow ups and monthly inquiries, either at their hospital visit, or if not, by phone confirmation of their records, until 1 year. Parameters such as hemoptysis, dyspnoea, weight loss, reduced appetite etc. were asked from patients individually. A single high resonance computed topography (HRCT) at 3 months followed up by three monthly chest X-rays was used to keep a track on disease status as a part of treatment protocol. Additionally, in patients receiving chemotherapy and radiation therapy, tests such a complete hemogram, a serum creatinine level, and a liver function analysis were performed. The study was approved by the institutional research committee vide letter no. SRHU/HIMS/RC/2022/90.

Results

Cell blocks and associated histopathological sections from 36 cases were included in the study. Male to Female ratio was 6:1 with a mean age of 62.64 ± 10.48 years. 94% cases suffering from lung carcinoma were smokers, amongst which 37% cases were smoking for duration of 20 to 29 years. Out of all the cytological investigations, bronchial brushings (87.1%) was the most common diagnostic mode performed. Predominant type of lung cancer in our study was squamous cell carcinoma (SCC) (40%) followed by adenocarcinoma (AC) (34%), small cell carcinoma (SmCC) (23%) and non-small cell carcinoma NOS (3%). Most of squamous cell carcinomas were moderately differentiated (57%) while most of adenocarcinomas (75%) were poorly differentiated. 97% of the cell blocks made were adequate with a single case showing inadequate material. In one case, diagnosis on cytology (cell block) was made as histopathology was inadequate for any opinion. Table-1 shows PD-L1 expression according to different cytopathological types of lung cancer cases. One case, among the initial 36 cases, was excluded as it showed inadequate material for reporting. The PD-L1 expression was negative in 57% (20/35), low grade in 40% (14/35) and high grade in 3% (1/35) cases (Figure-1).

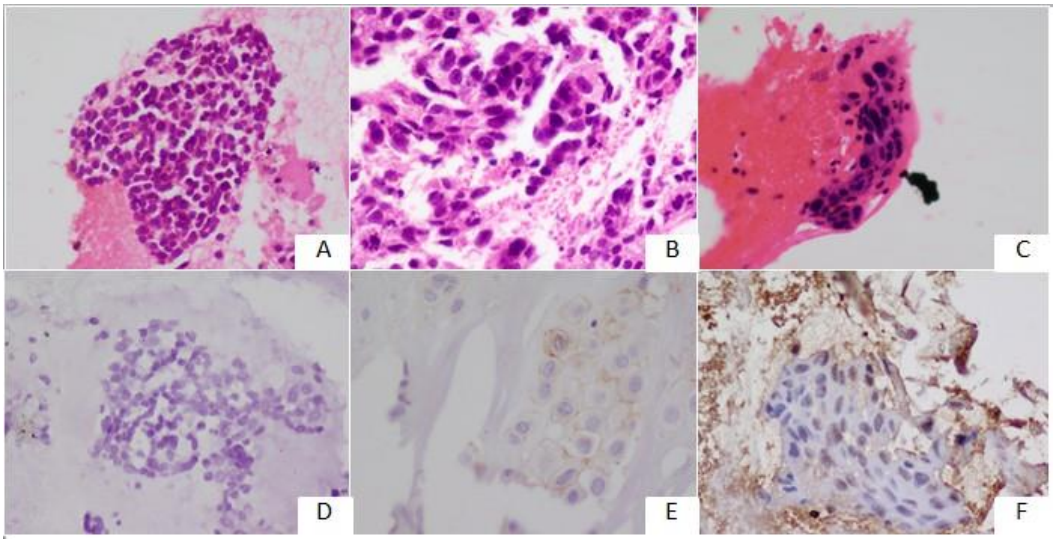

Figure 1: Cell block sections showing (A) H&E and (D) negative PD-L1 expression in small cell carcinoma; (B) H&E and (E) low grade PD-L1 expression in adenocarcinoma; (C) H&E and (F) high grade PD-L1 expression in squamous cell carcinoma (x400)

Table 1: Association of PD-L1 expression according to cell block diagnosis (n = 35)

Cell block diagnosis		<1% (Negative)	1% - 49% (Low)	≥50% (High)
Squamous Cell Carcinoma (n=14)	Moderately differentiated (Grade II) (n=8)	5 (38%)	2 (14.2%)	1 (7.1%)
	Poorly differentiated (Grade III) (n=6)	3 (21.4%)	3 (21.4%)	0 (0%)
Adenocarcinoma (n=12)	Moderately differentiated (Grade II) (n=3)	2 (16.7%)	1 (8.3%)	0 (0%)
	Poorly differentiated (Grade III) (n=9)	4 (33.3%)	5 (41.7%)	0 (0%)
Non-Small Cell Lung Carcinoma- NOS (n=1)		1 (100%)	0 (0%)	0 (0%)
Small Cell Carcinoma (n=8)		5 (62.5%)	3 (37.5%)	0 (0%)

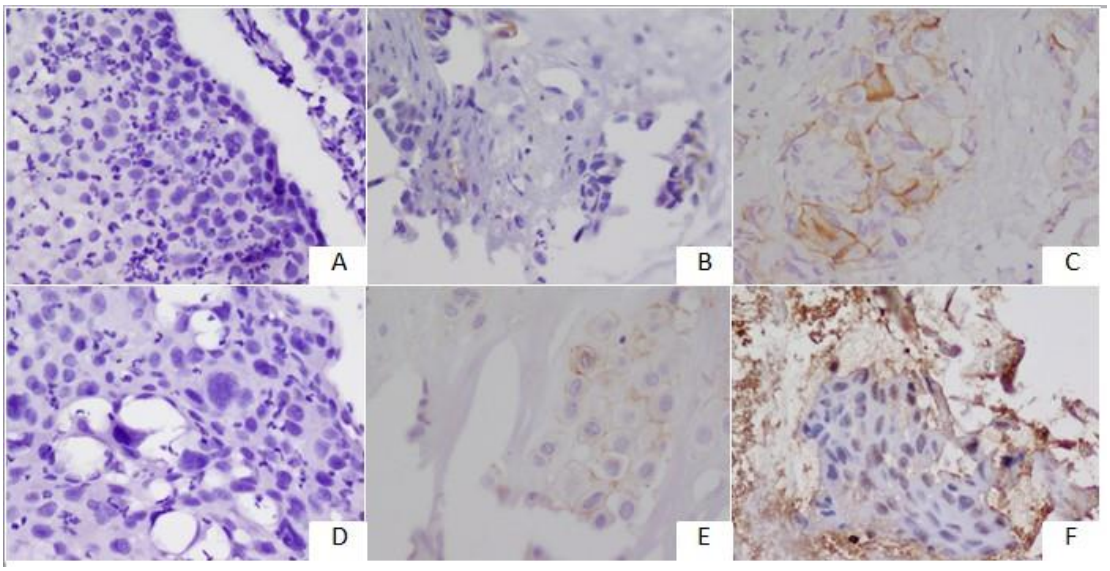

Figure 2: Concordance between negative PD-L1 expression in (A) HPE and (D) cell block section of SCC; low grade in (B) HPE and (E) cell block section of AC; high grade in (C) HPE and (F) cell block section of SCC (x400)

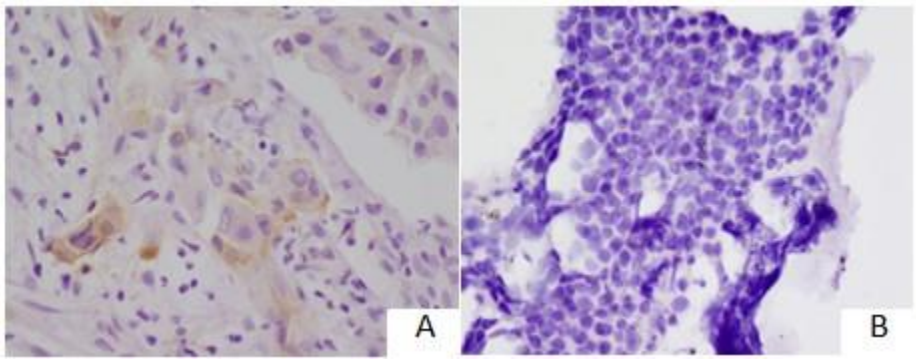

Figure 3: Discordance between (A) low grade PD-L1 expression in histopathological section and (B) negative PD-L1 expression in cell block section of poorly differentiated squamous cell carcinoma(x400)

Out of the total positive cases (15), PD-L1 expression was seen in 40% (6/15) squamous cell carcinoma, 40% (6/15) adenocarcinoma and 20% (3/15) small cell carcinoma. Among these 35 cases, 11 were true positive, 5 were false positive, 3 were false negative and 16 were true negative. With a 78.5% sensitivity, 76% specificity, 68.7% positive predictive value (PPV) and 84.2% negative predictive value (NPV), PD-L1 on cell blocks correlated with paired histopathological sections in 77% of cases in our study (Figure-2, 3).

Discussion

FNA is a simple, rapid, non-invasive test which allows cytomorphological evaluation of diagnostic elements in cytology specimens. The residual specimen is then recommended for preparation of cell blocks that further can be used for various ancillary tests like IHC and provides opportunity for evaluation of architectural features like papillary, acinar, duct like formations etc. in peritoneal/ serous cavity washings. This makes it easier to compare the histological characteristics of cell block sections with those of resection of related primary neoplasms.[6,7] Though histopathology is considered as a gold standard investigation and

has been widely used for molecular and immunohistochemical studies but lately cytopathological material has caught researcher's attention, in light of the accessibility and minimal invasion. Cell block comprises of concentrated diagnostic tumor cells without stromal contamination and can be used for various ancillary tests like IHC, as compared to FFPE tissue from resection specimens. With the continuous addition of new IHC markers and technical developments like multicolor IHC and the SCIP (subtractive coordinate immunoreactivity pattern) method, its role is expanding. High-quality cell-blocks made using improved techniques as opposed to core biopsies typically include concentrated diagnostic tumour cells needed for the molecular assays without considerable stromal contamination.

A new era in clinical oncology, particularly in the treatment of lung malignancies has ushered in without question by the growing number of checkpoint inhibitors currently being developed. Over the past decade, many drugs targeting the epidermal growth factor receptor (EGFR) for patients with EGFR mutations or anaplastic lymphoma kinase (ALK) translocations, such as gefitinib, erlotinib or afatinib, and ALK translocation, such as crizotinib, are offering signs of hope.[4] Over the years, many opportunities to modify the immune system to treat lung cancer have yielded disappointing results because of the ability of these tumours to escape immune activity, including endogenous immune checkpoints.[8]

The 40 kDa type 1 transmembrane protein known as programmed death ligand 1 (PD-L1) suppresses the adaptive immune system during pregnancy, tissue allografts, autoimmune disease, and disease states like hepatitis. It is found on antigen-presenting cells, myeloid dendritic cells, activated monocytes, and B cells.[9] It is an immune checkpoint inhibitor as PD-1:PD-L1 interaction inhibits cytokine production and thus may play a role in advancement of lung cancer therefore it has been studied in lung carcinoma for targeted therapy especially in EGFR/ALK/ROS1 wild type advanced non-small cell lung carcinoma (NSCLC), including squamous cell carcinoma.[10] Tumours expressing PD-L1 can often be aggressive, carrying a poor prognosis and PD-L1 targeted therapy has shown to benefit (15% - 25%) patients with NSCLC in overall increased life expectancy.[11]

The upcoming cell block technique is seen to enhance the overall yield by 9% of bronchial samplings as compared to conventional smears of bronchial washings as seen in various previous studies by Calabretto et al., Flint et al., Collins et al and Kakodkar et al.[12] Literature search pointed to a study done by Jain et al. in which a 88.4% concordance was found between cytopathology and matched histopathology advocating the fact that cytological material represent a potential resource for immunocytochemistry.[13] In an another study by Jug et al., PD-L1 sensitivity, specificity, PPV, NPV, and overall agreement for the cytology method was found to be 73.3%, 65.2%, 73.3%, 65.2%, and 69.8%, respectively which was seen in concordance with our study.[14] Wang et al. found that PD-L1 IHC performed well with cytology cell blocks when the TPS ≥50% was used as the end point. Thus, suggesting that cell blocks are a useful resource for PD-L1 testing in lung cancer and should be taken into consideration.[15]

Cell blocks, when prepared with robust care and proper methodology, mimics histopathological sections in classifying various neoplastic lesions and certain studies have also found better immunocytochemistry results when compared on respective histologic specimens.[16,17] Our literature search also landed us on certain studies like Kuempers et al. who reported a higher interobserver variability in PD-L1 expression on cytology when evaluating paired cyto-histological samples.[18] KulaÇ et al also found no statistical significance on comparison of PD-L1 positivity rates of only the small biopsies and cell blocks.[19] However on weighing the favoring and the contrasting studies, it can be inferred that a cell block can still prove to be useful and act as a surrogate for the histopathology techniques. The primary limitation of our study was the inability to remove blood contamination-related interference from the formalin fixed cytological specimens. Extensive studies with longer follow-ups may be conducted

to determine the precise function and effectiveness of cell blocks in lung cancer patients with PD-L1 expression.

Conclusion

Studying PD-L1 immunoexpression in lung cancer using cell blocks is efficient and trustworthy. They can be utilized as a substitute for PD-L1 expression in lung carcinoma and may be helpful in assessing the prognosis of lung carcinoma because they are equivalent to matched histopathological sections.

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Competing Interests: None declared

Statement of Informed consent: Informed consent taken, wherever applicable

Statement of Human and animal rights: Consent and care taken

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