Original paper

A study on expression of programmed death ligand-1 in small cell lung carcinoma and correlation with clinicopathological parameters

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Small cell lung carcinoma (SCLC) is characterized by rapid growth and an aggressive clinical course. Standard therapy regimes have limited effects on disease course; therefore the prognosis of SCLC is poor. In the current study, the frequency of programmed death ligand 1 (PD-L1) expression in SCLC and its correlation with clinico-pathological features were evaluated.

The study included 100 cases of SCLC wherein testing for PD-L1 was done with the SP263 clone on the Ventana benchmark XT system. Cases with > 1% PD-L1 expression in tumour cells or immune cells were categorized as positive.

PD-L1 expression was identified in 14% of cases using the cut-off of ≥ 1%. The tumour proportion score was 10% and the immune proportion score was 9.78% using a cut-off of ≥ 1%. PD-L1 positive expression was more frequent in the male population with age > 40 years. All the patients with positive PD-L1 expression were smokers. In the PD-L1 positive group, presence of necrosis was identified in 71.4% of cases and when compared with the PD-L1 negative subgroup this finding was statistically significant ($p = 0.010$).

Personalized targeted therapy for cases of SCLC is still under evaluation. The use of immunotherapeutic targets, such as PD-L1, may help to define a new treatment strategy for SCLC. Development of new treatment strategies may improve prognosis and survival.

Key words: immunotherapy, small cell lung carcinoma, programmed death ligand-1.

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide, with an estimated 2.1 million new cancer cases and 1.8 million cancer-related deaths [1]. Lung cancer is broadly categorized into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [2, 3]. Small cell lung cancer is an aggressive neuroendocrine tumour with early dissemination and poor prognosis [4]. The treatment protocols for both limited-stage and extensive-stage SCLC are concurrent chemotherapy and radiation therapy with platinum-based agents [2, 5]. Due to the high growth fraction of the disease, early dissemination with widespread metastases and early development of drug resistance, the treatment of SCLC remains dismal [5].

Programmed cell death ligand 1 (PD-L1) is a predictive biomarker for immunotherapy in

several solid tumours, but its role in treating SCLC is not well defined [6]. The PD-1/PD-L1 pathway controls the induction and maintenance of immune tolerance within the tumour microenvironment [7]. Immune checkpoint inhibition has shown promising advances in cancer immunotherapy. Unlike NSCLC, no driver genes are yet identified that can be used for targeted therapy in SCLC. Immunotherapeutic agents and monoclonal antibodies that target PD-1/PD-L1 are currently being evaluated for their potential use in SCLC [8]. As *per* the 2018 recommendations of the International Association for the Study of Lung Cancer and the US Food and Drug Association guidelines in 2019, anti-PD-L1 immunotherapy can now be combined with chemo-radiotherapy in cases of SCLC [6, 9]. The current study was undertaken with the following objectives: to assess the frequency of PD-L1 expression in SCLC and to correlate the expression of PD-L1 with clinico-pathological parameters. As personalized, targeted therapy in SCLC is still in the evaluation phase, the study of the immunotherapeutic target PD-L1 may help define a new treatment strategy for SCLC [9].

Material and methods

The current study was a retrospective and prospective case series that included 100 cases of SCLC. The study protocol was approved by the Institutional Ethics Committee. Adequate clinical details were documented including age, sex, presenting clinical features, site/side of biopsy, pertinent radiological findings and tumour stage. The survival data for all the cases were obtained during clinical follow-up visits or by telephone. Formalin-fixed paraffin-embedded (FFPE) tissue specimens were evaluated for morphology in haematoxylin and eosin staining. All the cases with the presence of adequate tumour were assessed. The sections were evaluated for the presence or absence of tumour necrosis. The presence of immune cells around the tumour was also assessed in all the cases. The cases were diagnosed as SCLC with the aid of immunohistochemistry (IHC). The immunohistochemical panel used included pan-cytokeratin and neuroendocrine markers, namely chromogranin, synaptophysin, and insulinoma-associated protein 1 (INSM-1), along with thyroid transcription factor-1 (TTF-1). The testing for PD-L1 was done on FFPE tissue blocks using the rabbit monoclonal anti-PD-L1 antibody, clone SP263 on the Ventana Benchmark XT automated staining system (USA). A positive control (human placenta tissue) and negative control (by omitting the primary antibody) were run with every batch. The cases was analysed for the presence of membranous staining for PD-L1 in both tumour cells and immune cells. The percentage and staining intensity were assessed. The staining intensity was categorized between 1+ to 3+, wherein 3+ indicated staining identified at 10× magnification and 1+ indicated staining identified at 40× magnification. Based on the recommendations of the International Association for Study of Lung Cancer (ISALC), cases with more than 1% staining in either tumour cells or immune cells were categorized as positive. PD-L1 was also assessed using the cut-offs of ≥ 1%, ≥ 10%, ≥ 25% and ≥ 50%. The tumour proportion score and the immune proportion score (IPS) were determined. The combined positive score (CPS) that combines the PD-L1 expressing tumour cells and the PD-L1 positive immune cells was also determined.

Statistical analysis

Statistical software (SPSS for Windows, version 16.0) was used for the analysis. Categorical data are reported as numbers and percentages and continuous data as the median and range. The frequency of PD-L1 expression was assessed in both the tumour cells and immune cells. The tumour proportion score, IPS and CPS were calculated. The correlation of PD-L1 expression with the clinico-pathological features was evaluated using tests of significance, namely the Fisher exact test or c^2 test or the unpaired Student *t*-test, and a *p*-value of < 0.05 was considered as significant while a *p*-value of < 0.01 was considered as highly significant. The survival data were analysed using the Kaplan-Meier estimator.

Results

Clinico-pathological parameters

The current study included 100 cases of SCLC wherein the age range of the patients was 24–82 years. In the current study, the M : F ratio was 6.1 : 1. The most common clinical feature was cough, which was documented in 88% ($n = 88$) of cases, followed by breathlessness, present in 84% ($n = 84$) of cases. Among the included patients, 86% ($n = 86$) were either current or ex-smokers while 14% ($n = 14$) were non-smokers. Expression of synaptophysin was present in 90% of cases while chromogranin was positive in 89% of cases. Immunostaining for INSM-1 was performed in 31 cases and 93.5% of cases ($n = 29$) had positive nuclear staining. Based on the histological analysis, the presence of necrosis was documented in 40% of cases (Fig. 1). Regarding disease stage, 42% ($n = 42$) of cases were of stage T3, while stage T4 was present in 11% ($n = 11$) of cases and 35% ($n = 35$) cases had the nodal status of N3. The evidence of distant metastasis (M1) was documented in 29% of cases ($n = 29$). The survival duration varied 0.5–

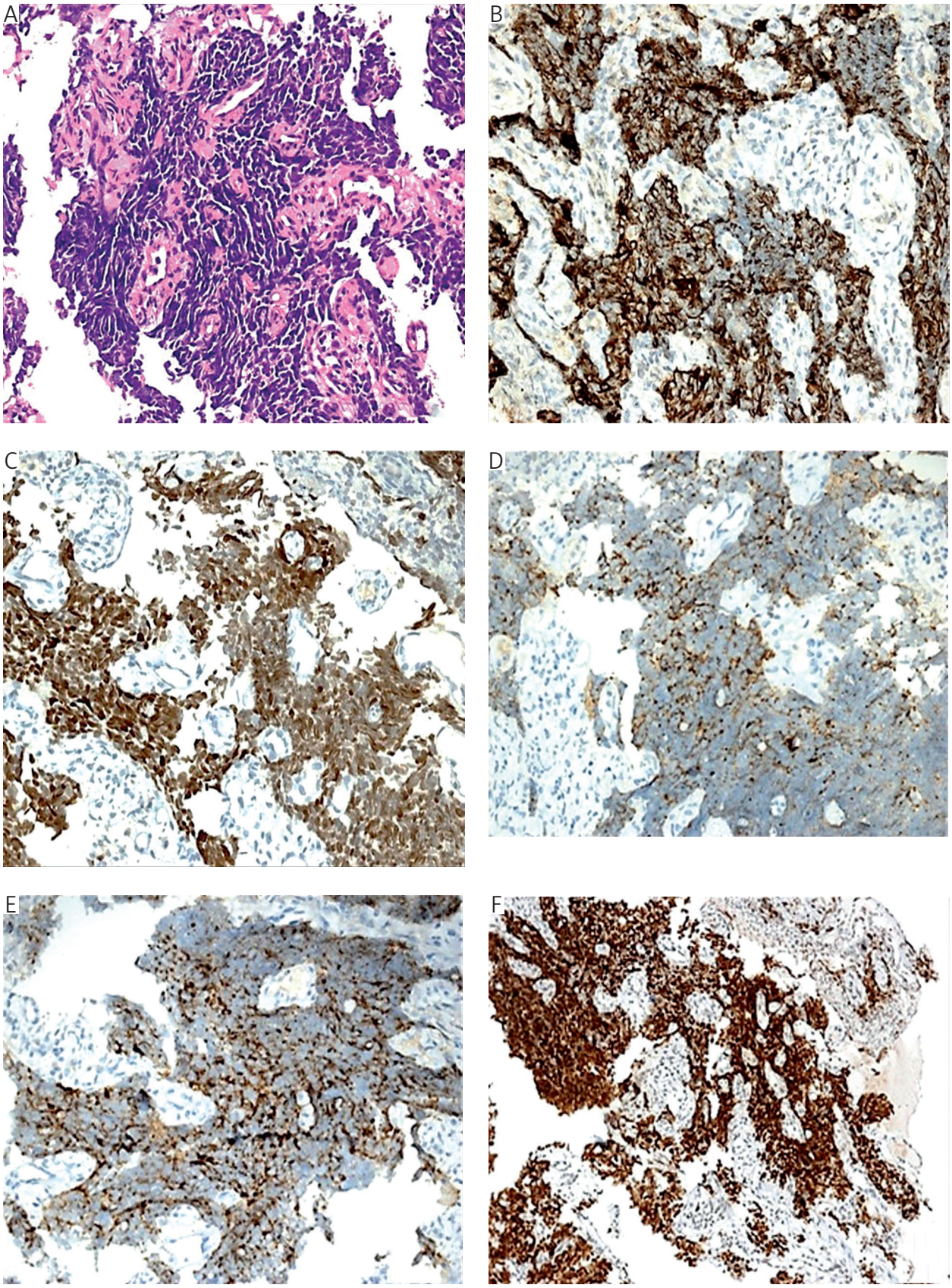

Fig. 1. A) Lung mass biopsy composed of sheets of small round cells with nuclear moulding. B) Positive staining for pan-cytokeratin. C) Strong nuclear positivity for TTF-1. D) Granular cytoplasmic staining for synaptophysin. E) Granular cytoplasmic staining for chromogranin. F) Strong nuclear positivity for INSM-1 (A – H and E 100×, B, C – DAB 100×, D, F – DAB 50×)

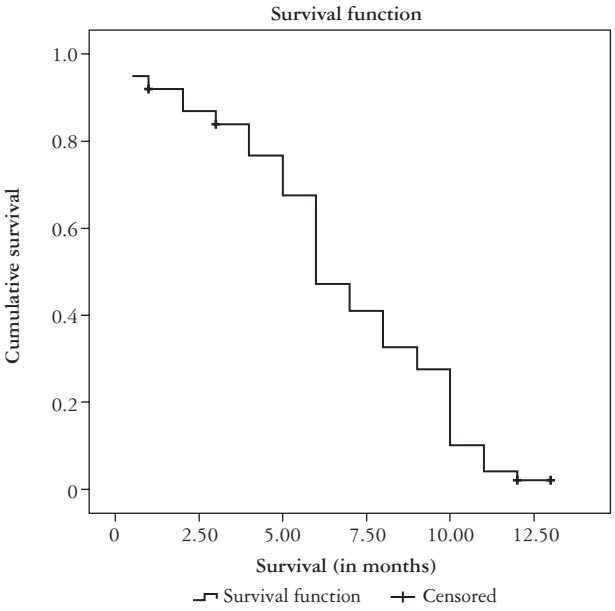

Fig. 2. Kaplan-Meier graph for survival of patients in the study

12 months and 4 patients were alive during survival documentation. In the majority of cases the survival duration was within 6–12 months (Fig. 2, Table I).

PD-L1 expression

The PD-L1 expression was identified in 14% of cases (n = 14) using the cut-off of ≥ 1%. The tumour proportion score was 10% using the cut-off of ≥ 1%. Using the cut-off of ≥ 10%, ≥ 25% and ≥ 50%, the PD-L1 expression in the tumour cells was 7%, 4% and 3%, respectively. The intensity of staining varied from 1+ to 3+. The intensity of staining was 1+ in 30% (n = 3/10) of cases, 2+ in 40% (n = 4/10) of cases and 3+ in 30% (n = 3/10) of cases.

The presence of immune cells could be assessed in 92 cases. The immune proportion score was 9.78% (n = 9/92) of cases using the cut-off of ≥ 1%. Using the cut-off of ≥ 10% and ≥ 25%, PD-L1 expression was identified in the immune cells in 6.52% (n = 6/92) and 1.09% (n = 1/92) of cases. However, using the cut-off of ≥ 50%, PD-L1 expression was not identified in any case in the immune cells. The intensity of staining for PD-L1 in the immune cells varied from 2+ to 3+. In 77.78% (n = 7/9) of cases the intensity was 2+ while in 22.22% (n = 2/9) cases. Among the 14 cases that harboured PD-L1 expression, in 5 cases (35.7%), both tumour and immune cells were positive. In 5 cases (35.7%), PD-L1 expression was identified only in tumour cells and in 4 (28.6%) cases, only the immune cells expressed PD-L1.

The combined proportion score at the cut-off of ≥ 1% was 10% (Fig. 3).

Characteristics of PD-L1 positive cases (n = 14)

In the current study, 85.7% of cases with PD-L1 positivity were > 40 years of age, with 78.6% of cases being male patients. In the cases that expressed PD-L1, 64.3% of biopsies were from the endobronchial region, while 35.7% were from lung/intrathoracic masses. The most common clinical feature identified in the PD-L1 positive cases was cough, documented in 92.9% of cases (n = 13), followed by breathlessness in 85.7% of cases. All the patients with positive expression of PD-L1 were smokers. In the cases with PD-L1 expression, 57.1% had tumour stage T2. The nodal metastasis stage was N1 in 35.7% of cases. Distant metastasis was identified in 21.4% of cases. In the PD-L1 positive group, chromogranin expression (92.9%) was higher than synaptophysin expression (78.6%). TTF-1 was positive in 63.6% of cases and INSM-1 was positive in 75% of cases. In the PD-L1 positive group, necrosis on histology was identified in 71.4% of cases.

PD-L1 positive vs. PD-L1 negative cases – correlation of clinico-pathological parameters

In the PD-L1 positive group, 85.7% of cases were more than 40 years of age (p = 0.197), and 78.6% were male (p = 0.388). In the cases that expressed PD-L1, the most common clinical feature was cough, documented in 92.9% of cases (n = 13), followed by breathlessness, present in 85.7% of cases. All the patients who were positive for PD-L1 expression were smokers (p = 0.104). In the PD-L1 positive group, chromogranin expression (92.9%) was higher than synaptophysin expression (78.6%). In the PD-L1 positive group, the presence of necrosis on histology was identified in 71.4% of cases. This finding was statistically significant when compared to the PD-L1 negative group, with a p-value of 0.010. In the cases with PD-L1 expression, 57.1% had tumour stage T2 (p = 0.575). The nodal metastasis stage was N1 in 35.7% of cases (p = 0657); distant metastasis was identified in 21.4% of cases (p = 0.501).

The comparison of the survival outcome between the PD-L1 positive and the PD-L1 negative cases was not statistically significant (p = 0.458), indicating that expression of PD-L1 may not have any effect on survival (Table II).

Discussion

In the current study, PD-L1 expression was identified in 14% of cases using the cut-off of ≥ 1%. The tumour proportion score was 10% and the IPS was 9.78% using the cut-off of ≥ 1%. The recommended cut-offs for PD-L1 detection using the SP263 clone as *per* the ISAAC recommendations is staining in at least

Table I. Comparison of characteristics of PD-L1 positive versus PD-L1 negative cases

Parameters	PD-L1 positive cases, $N = 14$ (%)	PD-L1 negative cases, $N = 86$ (%)	*p*-value
Age			
Less than 40 years	2 (14.3)	4 (4.7)	0.197
More than 40 years	12 (85.7)	82 (95.3%)	
Gender			
Male	11 (78.6)	75 (87.2)	0.388
Female	3 (21.4)	11 (12.8)	
Site of biopsy			
Endobronchial biopsy	9 (64.3)	46 (53.5)	0.676
Lung mass or intra-thoracic mass biopsy	5 (35.7)	38 (44.2)	
Pleural biopsy	0	2 (2.3)	
Laterality			
Right	7 (50)	40 (46.5)	0.808
Left	7 (50)	46 (53.5)	
Clinical feature – chest pain			
Present	10 (71.4)	65 (75.6)	0.739
Absent	4 (28.5)	21 (24.4)	
Clinical feature – haemoptysis			
Present	11 (78.6)	60 (69.8)	0.501
Absent	3 (21.4)	26 (30.2)	
Clinical feature – breathlessness			
Present	12 (85.7)	72 (83.7)	0.850
Absent	2 (14.3)	14 (16.3)	
Clinical feature – cough			
Present	13 (92.8)	75 (87.2)	0.546
Absent	1 (7.14)	11 (12.8)	
Clinical feature – weight loss			
Present	6 (42.85)	37 (43)	0.991
Absent	8 (57.2)	49 (57)	
Smoking history			
Present	14 (100)	72 (83.7)	0.104
Absent	0	14 (16.3)	
Tumour stage			
T1	1 (7.14)	5 (5.8)	0.575
T2	8 (57.2)	33 (38.4)	
T3	4 (28.5)	38 (44.2)	
T4	1 (7.14)	10 (11.6)	
Nodal status			
N0	4 (28.5)	24 (27.9)	0.657
N1	5 (35.71)	21 (24.4)	
N2	2 (14.3)	9 (10.5)	
N3	3 (21.4)	32 (37.2)	

Table I. Cont.

Parameters	PD-L1 positive cases, $N = 14$ (%)	PD-L1 negative cases, $N = 86$ (%)	*p*-value
Metastasis			
M0	11 (78.57)	60 (69.8)	0.501
M1	3 (21.4)	26 (30.2)	
Clinical stage			
I	1 (7.14)	8 (9.3)	0.863
II	5 (35.71)	23 (26.7)	
III	5 (35.71)	29 (33.7)	
IV	3 (21.4)	26 (30.2)	
IHC – synaptophysin			
Positive	11 (78.57)	79 (91.9)	0.124
Negative	3 (21.4)	7 (8.1)	
IHC – chromo-granin			
Positive	13 (92.8)	76 (88.4)	0.619
Negative	1 (7.14)	10 (11.6)	
IHC – TTF-1			
Positive	7 (63.63)	49 (75.4)	0.413
Negative	4 (36.36)	16 (24.6)	
Histological feature – necrosis			
Present	10 (71.4)	30 (34.9)	0.010*
Absent	4 (28.5)	56 (65.1)	
Survival			
Expired	13 (92.8)	83 (96.5)	0.458
Alive	1 (7.14)	3 (3.5)	

IHC – immunohistochemistry, PD-L1 – programmed death ligand-1, TTF – thyroid transcription factor
Applied c2 test/Fisher exact test as appropriate.

25% of tumour cells for durvalumab therapy. In cases where nivolumab therapy is administered, the staining is sub-grouped as < 1%, 1%, 1–5%, 5–10% and 10% or greater [10]. The only study published from the Indian sub-continent, by Guleria *et al*., reported PD-L1 expression in 8.4% of cases. In cases of SCLC, the authors reported the positivity of PD-L1 as 2.9% in the tumour cells and 23.5% in the immune cells [11]. PD-L1 expression in SCLC is variable and the frequencies varied from as low as 5.8% to as high as 71.6% [12, 13]. Among the 14% of cases harbouring PD-L1 expression, 35.7% of both the tumour and immune cells were positive. In 35.7%, PD-L1 expression was identified only in the tumour cells and in 28.6% of cases, only the immune cells expressed PD-L1. In the study conducted by Wang *et al*. PD-L1 expression was identified in 45.3% of cases, including 5.7% of cases with PD-L1 expression in tumour cells, 28.9% of cases with PD-L1 expression in immune cells, and 10.7% of cases with PD-L1 positivity in both tumour and immune cells [14] (Table III).

In the present study, the intensity of staining varied from 1+ to 3+ in the tumour cells. The intensity of staining was 1+ in 30% of cases, 2+ in 40% of cases and 3+ in 30% ($n = 3/10$) of cases. The intensity of staining for PD-L1 in the immune cells varied from 2+ to 3+. In 77.78% of cases, the intensity was 2+ while in 22.22% of cases it was 3+. Yu *et al*. stated in their study that the staining intensity for PD-L1 was variable and varied from weak to strong. The majority of cases displayed a moderate intensity of staining [15].

The various commercially available IHC clones for PD-L1 include 5H1, E1L3N, E1J2J, SP142, 28–8, 22C3 and SP263. The analysis of staining intensity and percentage of cell staining (both tumour and immune cells) are variable for the various antibody clones. Amongst the various clones of PD-L1, SP263, 22C3 and 28–8 are validated for counting in tumour

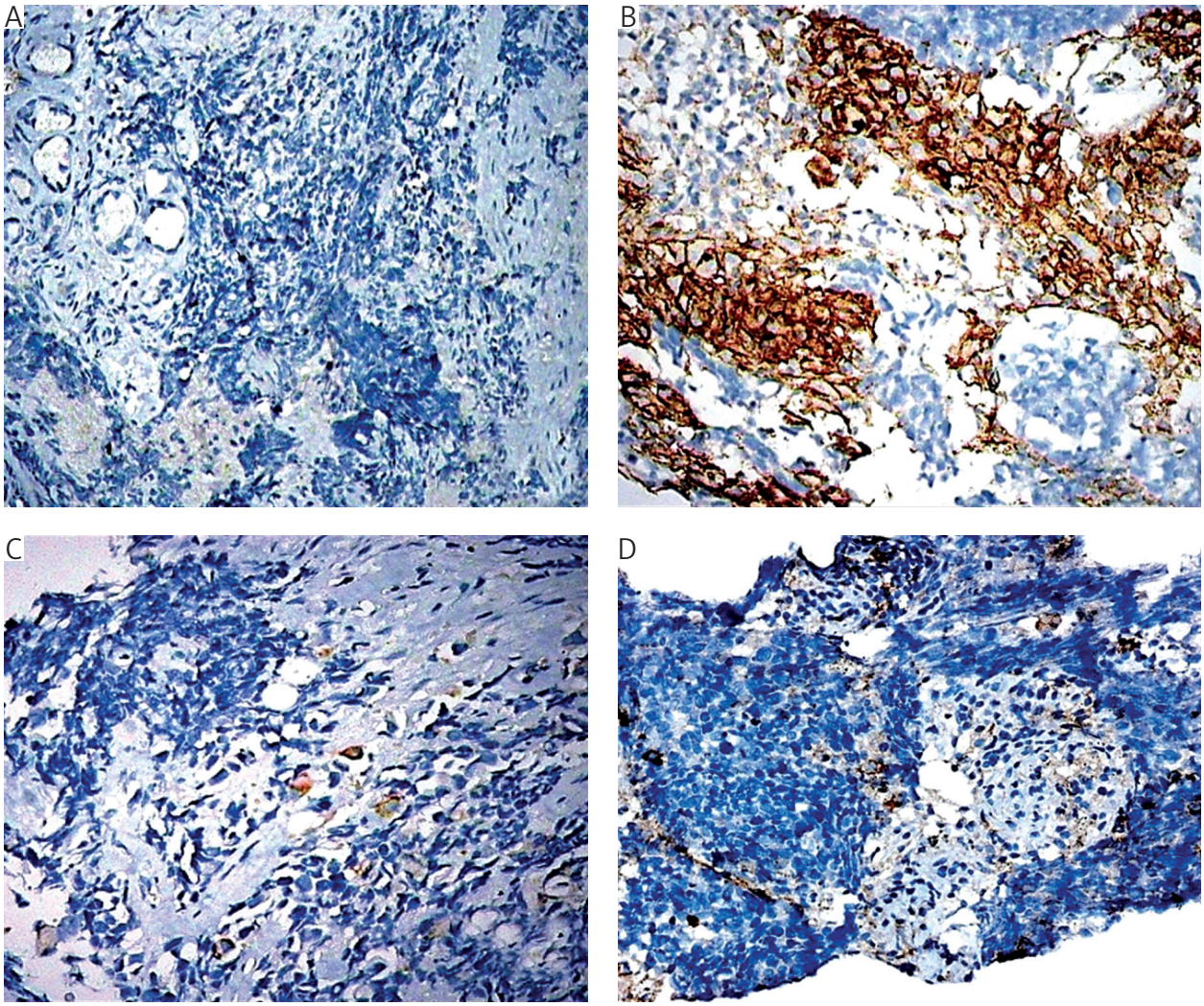

Fig. 3. A) A case of small cell lung carcinoma with absence of staining for PD-L1 in both tumour cells and immune cells. B) A case of small cell lung carcinoma with positive of staining for PD-L1 in tumour cells; more than 90% tumour cells have 3+ intensity of staining for PD-L1. The immune cells are negative. C) PD-L1 expression in immune cells – a case of small cell lung carcinoma with positive staining for PD-L1 in immune cells; the tumour cells are negative. D) PD-L1 expression in tumour cells and immune cells – a case of small cell lung carcinoma with positive staining for PD-L1 in tumour cells and in immune cells (A – DAB 100×, B–D – DAB 200×)

cells while the SP142 clone has been standardized for counting the immune cells (ICs). The current guidelines for assessing PD-L1 in tumour cells/ICs or both have not been universally standardized [30]. In the present study, the SP263 clone of PD-L1 was used to detect PD-L1 expression in SCLC. The SP263 clone stains the tumour cells better than the other clones; however, its efficacy to stain the ICs has not been validated [31]. Amongst the various studies published in the literature wherein PD-L1 expression was assessed in SCLC using the SP263 clone, the frequency of PD-L1 varied from 2.9 to 5% in the tumour cells [15–17].

In the present study, a comparison of the characteristics of the PD-L1 positive vs. the PD-L1 negative cases was performed. In the current study, 85.7% of patients with PD-L1 positivity were > 40 years of age, with 78.6% of patients being male. These findings indicate that PD-L1 positivity is likely in elderly males and is concordant with the findings of Wang *et al*. and Inamura *et al*. [14, 23]. In the study conducted by Yu *et al*. and Fan *et al*.,

Table II. Duration of survival of cases in the study

Parameters	Outcome, *n* (%)		Total, *N* (%)
	Expired	Alive	
Survivals			
Up to 3 months	13 (13.5)	1 (25.0)	14 (14.00)
3 to < 6 months	19 (19.8)	1 (25.00)	20 (20.0)
6 to < 9 months	34 (35.4)	0 (00)	34 (34)
9–12 months	30 (31.2)	1 (25.00)	31 (31)
Above 12 months	0 (0)	1 (25.00)	1 (1)
Total	96 (100.0)	4 (100.00)	100 (100)

Table III. Programmed death ligand-1expression in small cell lung carcinoma and neuroendocrine tumours

Author	Number of cases	Histology	Clone of PD-L1 used	Cut-off used (%)	TPS (%)	IPS (%)
Present study	100	SCLC	SP-263	≥ 1	10	9.78
Yu *et al*. [15]	142	SCLC	22C3	≥ 1	19.7	41.5
Guleria *et al*. [11]	85	SCLC = 70 LCNEC = 11 Mixed = 4	SP-263	≥ 1	8.4 For SCLC: 2.9	60.2 For SCLC: 23.5
Yoshimura *et al*. [16]	44	SCLC	28-8, 22C3, SP-263	≥ 1 for 28-8 and 22C3 25 for SP 263	25	Not assessed
Oizumi *et al*. [17]	933	SCLC = 101 NSCLC = 832	22C3, 28-8, SP263 and SP142	≥ 1	For SCLC: 18 using 22C3 17 using 28-8 11 using SP263 8 using SP142	Not assessed
Kim *et al*. [18]	192	SCLC = 120 NET = 72	B7-H1/PD-L1	≥ 1	15.1	31.1
Wang *et al*. [14]	159	SCLC = 94 NET = 65	SP142	≥ 5 in TCs ≥ 1 in ICs	5.7	28.9
Eichhorn *et al*. [19]	76	LCNEC	SP263	≥ 1	22.4	36.8
Kasajima *et al*. [20]	242	SCLC = 127 LCNEC = 58 Typical carcinoid = 39 Atypical carcinoid = 18	22C3	≥ 1	12	39
Ohtaki *et al*. [21]	95	SCLC = 94 LCNEC = 28 Typical carcinoid = 35 Atypical carcinoid = 2	SP142	≥ 5 in TCs ≥ 1 in ICs	16.3	39.6
Yasuda *et al*. [22]	39	SCLC	22C3	≥ 1	2.5	Not assessed
Inamura *et al*. [23]	115	SCLC = 74 LCNEC = 41	E1L3N	≥ 5 in TC	21	Not assessed
Tsuruoka *et al*. [12]	227	SCLC = 69 LCNEC = 106 Typical carcinoid = 46 Atypical carcinoid = 6	E1L3N	1	7	Not assessed

Table III. Cont.

Author	Number of cases	Histology	Clone of PD-L1 used	Cut-off used (%)	TPS (%)	IPS (%)
Miao *et al*. [24]	83	SCLC	SP66 (Springer-bio, USA)	> 5	21	Not assessed
Takada *et al*. [25]	40	SCLC	E1L3N, 28-8, SP142	Allred score > 1 > 5	22.5–35 20–32.5 15	42.5–50 40–52.5 37.5–40
Yu *et al*. [26]	194	SCLC	SP142, 28-8	> 1	20.6	43.3
Fan *et al*. [27]	80	SCLC = 48 Carcinoids = 22	Abcam	≥ 3	58.8	Not assessed
George *et al*. [28]	210	SCLC	E1L3N	> 3	1.90	Not assessed
Schultheis *et al*. [29]	94	SCLC	5H1 E1L3N	Not mentioned	Nil	18.5
Ishii *et al*. [13]	102	SCLC	Abcam	> 5	71.6	Not assessed

ICs – immune cells, IPS – immune proportion score, LCNEC – large cell neuroendocrine carcinoma, NET – neuroendocrine tumour, NSCLC – non-small cell lung carcinoma, SCLC – small cell lung carcinoma, TPS – tumour proportion score

the authors documented a female predominance in the cases with PD-L1 positivity [15, 27]. A comparison between the PD-L1 positive vs. the negative sub-groups regarding age and gender was not statistically significant, with *p*-values of 0.197 and 0.388, respectively. In the current study, cases that expressed PD-L1 included 64.3% of biopsies from the endobronchial region, while 35.7% were from lung/intrathoracic masses. Fan *et al*. reported PD-L1 expression to be significantly present in the central type of tumours that involved the segmental or more proximal bronchi compared to the peripheral tumours that involved the sub-segmental or more distal bronchi [27]. This finding may imply that PD-L1 expression is more frequent in tumours that involve the endobronchial or central regions. The preferential expression of PD-L1 in tumours of these regions may additionally affect the therapeutic response [15]. The most common clinical feature identified in the PD-L1 positive cases in the present study was cough, documented in 92.9% of cases, followed by breathlessness in 85.7% of cases. All the patients with positive PD-L1 expression were smokers. Smoking is a significant carcinogen responsible for the development of lung malignancies, in particular SCLC. Smoking leads to the development of multiple mutations and field carcinogenic effects. Mutations in p53, retinoblastoma and the *KRAS* gene play an important role in carcinogenesis [32–34]. In addition, the carcinogens in smoke possibly lead to activation of the PD-1/PD-L1 pathway. Smoking leads to activation of the inflammatory cascade with the secretion of cytokines in addition to activation of tumorigenesis [33]. This likely explains the high frequency of PD-L1 expression in association with smoking. This finding is in concordance with the results reported by Tsuruoka *et al*. [12]. However, a comparison of the PD-L1 positive vs. the PD-L1 negative categories regarding smoking was not statistically significant, with a *p*-value of 0.104. In the current study in the PD-L1 positive group, chromogranin expression (92.9%) was higher than synaptophysin expression (78.6%) and INSM-1 was positive in 75% of cases. This finding contrasts with the entire study group in which the expression of synaptophysin was higher than that of chromogranin. Chromogranin A is very specific but has limited sensitivity in the diagnosis of SCLC [35, 36]. This finding is concordant with the results of the study conducted by Yu *et al*. wherein the authors identified higher expression of chromogranin in the PD-L1 positive category, whereas the overall study group had a higher expression of synaptophysin [15]. TTF-1 was positive in 63.6% of cases in the PD-L1 positive category. The high expression of PD-L1 in the present study was in concordance with the findings of Yu *et al*., where the authors reported higher PD-L1 positivity in SCLCs expressing

TTF-1 [15]. TTF-1 is the chief regulator for lung structure development and plays a role in neuroendocrine differentiation and tumour aggressiveness, particularly in cases of SCLC [37]. The high expression of PD-L1 in cases of SCLC that are TTF-1 positive may be due to the activation of oncogenic pathways mediated *via* TTF-1. The comparison of the PD-L1 positive vs. the PD-L1 negative groups in terms of TTF-1 expression was not statistically significant ($p = 0.413$).

In the present study, among the cases with PD-L1 expression, 57.1% of cases had a tumour stage of T2 followed by T3, which was present in 28.6% of cases. The nodal metastasis stage was N1 in 35.7% of cases and distant metastasis was identified in 21.4%. Clinical stages II and III together constituted 71.4% of the cases with PD-L1 positivity. In the study conducted by Inamura *et al*., 46% of cases with PD-L1 positivity had stage I disease [23]. Fan *et al*. reported the stage of the disease to be I/II in 77.8% of cases with PD-L1 expression, while distant metastasis was identified in 54.5% of cases [27]. In the study conducted by Wang *et al*. 63.6% of cases with PD-L1 expression had the clinical stage of I [14]. Compared to studies published in the literature, the cases with PD-L1 expression had a higher disease stage in the present study. In the PD-L1 positive group, the presence of necrosis on histology was identified in 71.4% of cases. A comparison with the PD-L1 negative subgroup in terms of necrosis was statistically significant with a *p*-value of 0.010. This finding is concordant with the study conducted by Wang *et al*., wherein the authors identified necrosis in 61.5% of tumours with PD-L1 expression [14]. In small cell lung cancer the tumours have a high mutation burden with a propensity of distant metastasis and an aggressive disease course. The occurrence of necrosis in histology implies that the tumour has a higher grade and poor differentiation. The presence of necrosis in cases with PD-L1 expression may imply that positivity is related to tumours with advanced disease stages [38].

The utility of PD-L1 as a prognostic marker in neuroendocrine tumours and SCLC is still not very clearly understood. The true prognostic potential of this immunotherapeutic marker is controversial [38].

Among the various studies published in the literature wherein PD-L1 expression has been assessed in neuroendocrine tumours and SCLCs, most studies have reported a better prognosis in tumours with PD-L1 positivity [12, 13, 21, 23, 24, 26]. Kim *et al*., Eichhorn *et al*. and Kasajima *et al*. concluded from their studies that PD-L1 expression in tumour cells was associated with poor prognosis; however, PD-L1 positivity in the immune cells was associated with longer survival and better prognosis [18–20]. Yu *et al*. and Fan *et al*. stated that tumours with PD-L1 positivity are generally associated with poor prognosis [15, 27].

In the present study, the survival analysis of the PD-L1 positive vs. the PD-L1 negative cases revealed no significant difference ($p = 0.458$). This finding is concordant with the conclusions derived from the studies conducted by Yoshimura *et al.*, Oizumi *et al.* and Wang *et al*., wherein the authors did not find any significant association between PD-L1 expression and survival [14, 16, 17].

In the studies where PD-L1 expression was explicitly assessed in SCLCs using the SP263 clone, no significant difference in survival was documented by Yoshimura *et al*. and Oizumi *et al*., which was concordant with the findings of the present study [16, 17]. However, Yu *et al*. reported that cases of SCLCs with PD-L1 expression had the worst prognosis [15].

The present study is a novel attempt to study the frequency of PD-L1 in SCLC; however, the study has certain limitations. Firstly, the study sample included only cases of SCLCs; therefore, the expression of PD-L1 across the spectrum of neuroendocrine tumours could not be assessed. Secondly, all samples included in the study were small biopsy specimens. PD-L1 expression is associated with tumour heterogeneity; therefore, including only small biopsies fails to address this aspect accurately. However, most patients presented with advanced disease or metastasis in the current study, which are contraindications for resections. Finally, clinical data in response to treatment involving standard chemotherapy/radiotherapy or anti-PD-L1 therapy are not available. However, treatment response was not included in the objectives of the study.

Conclusions

In cases of SCLC, due to the high mutation burden of the tumour and aggressive clinical course, the treatment regime includes chemotherapy and radiotherapy. Immunotherapeutic drugs constitute a novel potential treatment modality, and combination therapy may serve as a path-breaking phenomenon in SCLC management.

The authors declare no conflict of interest.

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