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Open Access Research Article

Comparisons of serum CTLA-4 levels between treated and Untreated SCLC and NSCLC Patients

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Abstract

Background: Small cell lung cancer and non-small cell lung cancer are the two main types of lung cancer and major causes of cancer-related death worldwide.
Objectives: To compare serum cytotoxic T-lymphocyte-associated antigen 4 levels between treated and untreated patients with small cell lung cancer and non-small cell lung cancer, and healthy controls.
Methods: This case-control study was conducted at the Oncology Teaching Hospital, Baghdad, Iraq, from December 2024 to June 2025. The study included 180 participants: 24 patients with small cell lung cancer, 96 with non-small cell lung cancer, and 60 healthy controls. Serum cytotoxic T-lymphocyte-associated antigen 4 levels were measured using enzyme-linked immunosorbent assay, and complete blood count parameters were measured using an automated hematology analyzer.
Results: Cytotoxic T-lymphocyte-associated antigen 4 levels differed significantly among the control, small cell lung cancer, and non-small cell lung cancer groups ($p = 0.02$), with higher levels in small cell lung cancer and lower levels in non-small cell lung cancer than controls. No significant differences were observed between treated and untreated patients in either group. Cytotoxic T-lymphocyte-associated antigen 4 levels were significantly associated with Programmed death-ligand 1 categories in treated non-small cell lung cancer patients ($p = 0.01$), but not in untreated patients ($p = 0.12$). Complete blood count parameters differed significantly among the study groups, suggesting systemic inflammatory changes. Serum cytotoxic T-lymphocyte-associated antigen 4 showed limited diagnostic performance.
Conclusion: Cytotoxic T-lymphocyte-associated antigen 4 levels were significantly associated with Programmed death-ligand 1 expression in treated patients. Complete blood count, cytotoxic T-lymphocyte-associated antigen 4, and Programmed death-ligand 1 may provide useful information on the inflammatory and immunological status of lung cancer patients, but further studies are needed to confirm their clinical significance.

Keywords: Complete blood count; Cytotoxic T-lymphocyte-associated antigen 4; Non-small cell lung cancer; Programmed death-ligand 1; Small cell lung cancer.

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Introduction:

Lately in Iraq, the lung cancer incidence has been rising rapidly, which is considered a major public health problem. Longitudinal evidence from 2005 to 2019 shows that ASIR rates are considerably higher among males as opposed to females (18.339 vs. 5.861 per 100,000) (1).

Lung cancer is associated with high mortality and is broadly classified into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) Because systemic inflammatory responses reflect tumor-related immune activity, accessible biomarkers, particularly complete blood count-derived (CBC-derived) inflammatory indices, may provide useful information for assessing inflammatory and immune status in lung cancer patients. The cost-effective indices that are derived from routine CBC, neutrophil-to-lymphocyte ratio (NLR) and systemic inflammatory indices have been used for early risk stratification. Recent evidence suggests that these inflammatory profiles vary widely between histological subtypes and tumor stages, which can help in their prognostic and therapeutic monitoring (2). However, a single inflammatory metric may not fully capture the complex immune dysregulation associated with lung cancer, necessitating the integration of specific immune checkpoint molecules.

Among the above, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a crucial regulator of early immune response. CTLA-4 operates as a "brake" during initial T-cell priming in lymphoid tissues, unlike the programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) pathway, which works primarily in the tumor microenvironment (3). Immune checkpoint inhibitors (ICIs) have transformed treatment paradigms. However, clinical outcomes appear to be inconsistent. According to recent meta-analyses, blockade of CTLA-4 does not offer uniform benefits across all histological types, specifically when SCLC and NSCLC are compared. This therapeutic disparity, further exacerbated by the heterogeneity of tissue-based biomarkers, emphasizes a critical deficiency in the use of CTLA-4 (sCTLA-4) as a diagnostic or prognostic determinant (4,5).

Moreover, accumulating evidence from localized lung sites shows that these checkpoint molecules have differential expression in various histological subtypes, i.e., more in the tumor microenvironment than the peripheral blood (6). The different expression patterns observed in circulating cells and at the local site highlight the importance of assessing serum-based biomarkers such as CTLA-4 and PD-L1 to monitor systemic immune status.

At present, there is no complete data available on serum CTLA-4 and PD-L1 levels among lung cancer histologists in Iraq, including systemic inflammatory markers. Consequently, the aim of this study was to evaluate and compare expression of CTLA-4 and PD-L1 in the serum of SCLC and NSCLC patients. In addition, the study will investigate the association of these immunological markers with CBC indices based on their demographics and treatment status for the first time. This will help in gaining a novel insight into the systemic immune-inflammatory profile of lung cancer.

Patients and Methods:

Study Design and Setting

This case–control study was carried out between December 2024 and June 2025 at the Oncology Teaching Hospital, Medical City Complex, Baghdad, Iraq. Ethical approval was granted by the Institutional Review Board/Ethical Committee of the Medical City Complex, Baghdad, Iraq. Before participation, each subject provided written informed consent.

Study Population

A total of 180 people were enrolled in the trial, comprising 60 healthy controls and 120 patients with histologically-verified primary lung cancer. Most of the patients were smokers.

Based on their histological diagnosis, the patients were separated into:

- SCLC: 10 newly diagnosed (untreated) and 14 treated cases.
- NSCLC: 38 newly diagnosed (untreated) and 58 treated cases.

The following were among the treatment plans for SCLC and NSCLC patients:

- Chemotherapy alone
- Chemotherapy combined with immunotherapy
- Chemotherapy combined with immunotherapy and radiotherapy

Individual treatment choices were made based on each patient's clinical status and their doctor's advice.

Healthy volunteers without a history of cancer, autoimmune illnesses, or chronic inflammatory conditions made up the control group. They were matched for age and gender.

Inclusion Criteria

- Histologically confirmed diagnosis of SCLC or NSCLC.
- Primary lung tumor originating in the lungs.
- Age above 30 years.
- No history of other malignancies or autoimmune disorders.
- Written informed consent obtained.
- For controls: free of cancer or chronic inflammatory or autoimmune disease.

Exclusion Criteria

- Rare histological subtypes of lung cancer.
- Incomplete clinical or laboratory data.
- Active infection or inflammation at the time of sampling.
- Current immunosuppressive therapy for non-cancer conditions.
- Prior lobectomy before blood collection.
- Secondary pulmonary malignancy (metastasis from other organs).

Sample Collection and Processing

Five milliliters of venous blood were drawn aseptically from each participant. The blood was split into two parts: One part was placed in gel tubes and left to coagulate for 30 minutes at room temperature to separate the serum; the other part was placed in Ethylenediaminetetraacetic acid (EDTA) tubes for a CBC. White blood cells (WBC), hemoglobin (Hb), red blood cells (RBC), platelets (PLT), neutrophils (Neutro), monocytes (Mono), and lymphocytes (Lympho) were among the characteristics analyzed n the CBC (haematology analyzer CBC, Celltac G MEK-9100, Nihon Kohden, Japan(. Following a 10-minute centrifugation at 3000 rpm, the separated serum was transferred to Eppendorf tubes, labelled, and stored at -20°C until analysis. Using the commercially available human enzyme-linked immunosorbent assay (ELISA) kits, the serum CTLA-4 concentrations were measured in accordance with the manufacturer's recommendations. The Human CTLA-4 ELISA Kit (Cat. No. RE3105H, Reed Biotech, China) was used to measure CTLA-4. It had a sensitivity of 0.1 ng/mL and a detection range of 0.16–10 ng/mL.

Statistical Analysis: IBM SPSS version 29 (IBM Statistical Package for the Social Sciences, Chicago, IL, USA) was used to analyze the data. The Chi-square test was used to compare the categorical variables. Post hoc analysis was performed after a one-way ANOVA to compare more than two groups. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of serum CTLA-4 in SCLC and NSCLC patients. Statistical significance was considered to be present when the *p*-value was less than 0.05.

Results:

The study was comprised of 180 individuals. The age group had individuals who were 45–59 years old, which made up up the largest percentage of the sample (47.2%), followed by the age group of 60–74 years (31.7%) and ≥ 74 years old (11.7%). The 30–44 years' age group made up the smallest percentage (9.4%). The largest percentage of patients in the treated SCLC group was between the ages of 45 and 59 years (35.7%), followed by those between the ages of 60 and 74 years (28.6%), and those between the ages of 30 and 44 years (21.4%). Patients aged 74 and older made up 14.3%.

The 60–74-year-old age group accounted for the largest percentage of patients in the treated NSCLC group (43.1%), followed by the 45–59-year-old group (36.2%). The untreated NSCLC group, on the other hand, had a higher proportion of cases in the 60–74 age group (55.3%), followed by the 45–59 age group (26.3%) **Table 1**.

Table1: Distribution of the studied groups according to age (years)

		Groups						
Age range (years)		Control	SCLC Untreated	SCLC treated	NSCLC untreated	NSCLC treated	Total	*P*-value
	N	6	2	3	3	3	17	≤ 0.01 H.S
(30-44)	%	10	20	21.4	7.9	5.2	9.4	
	N	47	2	5	10	21	85	
(45-59)	%	78.3	20	35.7	26.3	36.2	47.2	
	N	4	3	4	21	25	57	
(60-74)	%	6.7	30.	28.6	55.3	43.1	31.7	
	N	3	3	2	4	9	21	
74	%	5	30	14.3	10.5	15.5	11.7	
	N	60	10	14	38	58	180	
Total	%	100	100	100	100	100	100	

Abbreviation: highly significant, H.S.

There were an equal number of males and females in the control group. Males were more common among SCLC patients in both the untreated and treated groups. Males also outnumbered females in the NSCLC groups. Overall, 32.8% of the study population was female, and 67.2% was male. There was a statistically significant difference in the sex distribution between the groups ($p = 0.009$) **Table 2.**

Table 2: Distribution of the studied groups according to gender

		Groups						
Gander		Control	SCLC Untreated	SCLC treated	NSCLC untreated	NSCLC treated	Total	*P*-value
	N	30	7	12	30	42	121	
Male	%	50	70	85.7	78.9	72.4	67.2	
	N	30	3	2	8	16	59	
Female	%	50	30	14.3	21.1	27.6	32.8	0.009
	N	60	10	14	38	85	180	H.S
Total	%	100	100	100	100	100	100	

Abbreviation: high significant, H.S.

Thirty participants (50%) did not smoke, twelve (20%) smoked, and eighteen (30%) smoked, and none were chronic smokers in the control group. Three (30%) smokers, four (40%) ex-smokers, two (20%) heavy smokers, and one (10%) non-smoker was among the untreated SCLC patients.

Eight (57.1%) smokers, two (14.3%) ex-smokers, two (14.3%) heavy smokers, and two (14.3%) non-smokers made up the treated SCLC group. Ten (26.3%) were ex-smokers, five (13.2%) were heavy smokers, nine (23.7%) were non-smokers, and fourteen (36.7%) were smokers in the NSCLC untreated group. There were 16 (27.6%) non-smokers, 15 (25.9%) smokers, 17 (29.3%) ex-smokers, and 10 (17.2%) heavy smokers in the NSCLC treatment group. The groups' smoking status differences were statistically significant (p= 0.001), **Table 3**.

Table 3: Distribution of the studied groups according to smoking status

		Groups						
Smoking status		Control	SCLC Untreated	SCLC treated	NSCLC untreated	NSCLC treated	Total	*P*-value
No	N	30	1	2	9	16	58	0.001 H.S
	%	50	10	14.3	23.7	27.6	32.21	
Smoker	N	12	3	8	14	15	52	
	%	20	30	57.1	36.8	25.9	28.9	
Ex-smoker	N	18	4	2	10	17	51	
	%	30	40	14.3	26.3	29.3	28.3	
Heavy-smoker	N	0	2	2	5	10	19	
	%	0	20	14.3	13.2	17.2	10.6	
Total	N	60	10	14	38	58	180	
	%	100	100	100	100	100	100	

Abbreviation: high significant, H.S.

The study groups' CTLA-4 levels varied significantly, according to the results. The mean CTLA-4 value for the SCLC group was 3.42 ± 0.40 ng/mL, whereas the control group was 2.97 ± 0.11. Compared with the other groups, the NSCLC group had a lower mean of 2.56 ± 0.16. A statistically significant difference in CTLA-4 levels between the three groups can be seen in the overall comparison (p = 0.02) **Table 4.**

Table 4: Serum CTLA-4 levels in Control, SCLC, and NSCLC Groups

Group	CTLA-4 ng/ml (Mean ± SE)
Control	2.97 ± 0.11
SCLC	3.42 ± 0.40
NSCLC	2.56 ± 0.16
p-value	0.02

The post-hoc multiple comparisons revealed no statistically significant differences in CTLA-4 levels between the groups. The comparison between the control group and SCLC showed a p-value of 0.299, while the p-value for the control versus NSCLC was 0.205. Additionally, the difference between the SCLC and NSCLC groups was not statistically significant (p = 0.07) **Table 5.**

Table 5: Post-hoc Multiple Comparisons of CTLA-4 Levels Between Control, SCLC, and NSCLC Groups.

Test	Groups	*P*-value	Significance.
CTLA-4 ng/ml	Control vs. SCLC	0.299	N.S
	Control vs. NSCLC	0.205	N.S
	SCLC vs. NSCLC	0.07	N.S

Abbreviation: not significant, N.S.

There were no statistically significant differences between the NSCLC groups' CTLA-4 levels. The mean value was 2.97 ± 0.11 for the control group, 2.62 ± 0.23 for the treated NSCLC group, and 2.45 ± 0.18 for the untreated NSCLC group. With a p-value of 0.14, the statistical comparison showed that these differences were not significant. There were no statistically significant variations between the SCLC groups' CTLA-4 levels. A mean CTLA-4 value of 2.97 ± 0.11 was found in the control group, 3.44 ± 0.54 in the treated SCLC group, and 3.41 ± 0.63 in the untreated SCLC group. With a p-value of 0.36, the statistical analysis showed that these differences were not significant **Table 6.**

Table 6: Serum CTLA-4 Levels in Treated and Untreated Lung Cancer Patients

Group	CTLA-4 ng/ml (Mean ± SE)
Control	2.97 ± 0.11
NSCLC Treated	2.62 ± 0.23
NSCLC Untreated	2.45 ± 0.18
p-value	0.14
Group	CTLA-4 ng/ml (Mean ± SE)
Control	2.97 ± 0.11
SCLC Treated	3.44 ± 0.54
SCLC Untreated	3.41 ± 0.63
p-value	0.36

The relationship between treatment types and CTLA-4 levels was also analyzed. In the NSCLC group, the mean CTLA-4 concentration for patients treated with Chemo was 1.61 ± 0.44, while it was 3.03 ± 0.67 for the Radio and Chemo group. The Radio, Chemo and Immuno group showed a mean of 0.28 ± 0, whereas the Chemo and Immuno group had a mean of 3.65 ± 1.01. Statistically, no significant differences were observed across these treatment categories in the NSCLC group (p = 0.18). For the SCLC group, the mean values were 2.93 ± 2.48 for Chemo, 3.06 ± 0 for Radio and Chemo, 3.88 ± 1.06 for Radio, Chemo and Immuno, and 3.18 ± 0.57 for the Chemo and Immuno group (p = 0.93) **Table 7.**

Table 7: Serum CTLA-4 levels across different treatment modalities in NSCLC and SCLC Groups

Type of treatment	NSCLC- treated group -Conc. (M±SE)	SCLC- treated group -Conc. (M±SE)
	CTLA-4 ng/ml	CTLA-4 ng/ml
Chemotherapy	1.61±0.44	2.93±2.48
Radiotherapy and Chemotherapy	3.03±0.67	3.06±0
Radiotherapy, chemotherapy, and Immunotherapy	0.28±0	3.88±1.06
Chemotherapy and immunotherapy	3.65±1.01	3.18±0.57
P-value	0.18	0.93

The relationship between PD-L1 expression categories and CTLA-4 levels was also analyzed. In the NSCLC-Treated group, the mean CTLA-4 concentration for patients with No PD-L1 expression was 2.34 \pm 0.45, while it was 2.19 \pm 0.43 for the Low group. The Negative group showed a mean of 2.46 \pm 0.34, whereas the High PD-L1 group had a mean of 4.59 \pm 0.60. Statistically, significant differences were observed across these PD-L1 categories in the treated group (p = 0.01). For the NSCLC-Untreated group, the mean values were 2.40 \pm 0.29 for None, 3.23 \pm 0.62 for Low, 2.21 \pm 0.15 for Negative, and 1.83 \pm 0.37 for the High PD-L1 group. No statistically significant differences were observed across these categories in the untreated group (p = 0.12), **Table 8.**

Table 8: Serum CTLA-4 levels across different PD-L1 expression categories in Treated and Untreated NSCLC Groups

PDL Category	NSCLC-Treated groups (n=45)	NSCLC-Untreated groups (n=38)
	CTLA-4 ng/ml	CTLA-4 ng/ml
None	2.34±0.45	2.40±0.29
Low	2.19±0.43	3.23±0.62
Negative	2.46±0.34	2.21±0.15
High	4.59±0.60	1.83±0.37
P-value	0.01	0.12

The relationship between hematological parameters and lung cancer groups was also analyzed. The highest WBC and neutrophil counts were observed in the SCLC-untreated group (12.60 ± 2.36 and 9.21 ± 2.27), followed by the NSCLC-untreated group (12.27 ± 0.99 and 9.23 ± 0.92), compared with the control group (6.85 ± 0.21 and 3.78 ± 0.15). Platelet count was also higher in the SCLC-untreated group (407.43 ± 48) and NSCLC-untreated group (325.35 ± 22.72) than in controls (248.83 ± 7.03). In contrast, hemoglobin and RBC were lower in the NSCLC-untreated group (12.53 ± 0.34 and 4.56 ± 0.11) compared with controls (13.61 ± 0.18 and 4.78 ± 0.06). The inflammatory indices NLR, PLR, and MLR were increased in untreated patients, especially NSCLC-untreated patients (4.23 ± 0.50, 149.24 ± 14.16, and 0.50 ± 0.05, respectively). Statistically significant differences were observed among the studied groups, with p-values ranging from 0.007 to ≤0.01 **Table 9.**

Table 9: Comparative Hematological Parameters in SCLC and NSCLC

	Groups (M±SD)					
Test	Control	SCLC-Untreated	SCLC-reated	NSCLC-untreated	NSCLC-treated	*p*-value
WBC	6.85±0.21	12.60±2.36	8.18±1.58	12.27±0.99	6.80±0.21	≤0.01
Hb	13.61±0.18	13.20±0.72	11.43±0.59	12.53±0.34	13.59±0.18	≤0.01
RBC	4.78±0.06	4.81±0.18	3.98±0.22	4.56±0.11	4.77±0.06	≤0.01
PLT	248.83±7.03	407.43±48	302.42±47.37	325.35±22.72	250.09±7.20	≤0.01
Neu	3.78±0.15	9.21±2.27	5.67±1.46	9.23±0.92	3.74±0.15	≤0.01
MONO	0.58±0.02	0.97±0.18	0.95±0.15	1.10±0.07	0.57±0.02	≤0.01
LYMP	2.21±0.09	2.30±0.25	1.38±0.21	2.18±0.14	2.20±0.09	0.007
NLR	1.71±0.10	4.00±1.08	4.11±1.23	4.23±0.50	1.70±0.10	<0.01
PLR	112.59 ±5.58	177.14±28.40	219.14±47.86	149.24±14.16	113.68±5.69	<0.01
MLR	0.26±0.01	0.42±0.09	0.69±0.15	0.50±0.05	0.26±0.01	<0.01

ROC curve analysis was performed to assess the diagnostic performance of serum CTLA-4 in SCLC patients. The results showed an area under the curve (AUC) of 0.558 (95% CI: 0.395–0.722, p = 0.406), with a cutoff value of 4.79. At this threshold, the sensitivity was 33%, and the specificity was 100%, indicating limited diagnostic performance.

ROC curve analysis was performed to evaluate the diagnostic performance of serum CTLA-4 in NSCLC patients. The results showed an area under the curve (AUC) of 0.370 (95% CI: 0.283–0.457, p = 0.006), with a cutoff value of 4.252. At this threshold, the sensitivity was 20%, and the specificity was 100%, indicating poor diagnostic performance in NSCLC, **Table 10.**

Table 10: ROC analysis of SCLC and NSCLC groups

					C.I 95%			
Test	Area	Cutoff	SE	P-value	Lower bound	Upper bound	Sensitivity	Specificity
CTLA-4 in SCLC	0.558	4.79	0.084	0.406	0.395	0.722	33	100
CTLA-4 in NSCLC	0.370	4.252	0.044	0.006	0.283	0.457	20	100

Discussion

According to the results of the current study, there is a highly significant correlation between age and the incidence of lung cancer, with the majority of cases occurring in older age groups, especially those between the ages of 45 and 59 and 60 and 74 years. This was in line with the earlier data showing that lung cancer mortality rises with age, peaking in older populations (≥ 70 years), (7). The cumulative nature of somatic mutations in the bronchial epithelium can biologically explain this age-related peak. Recent genomic evidence indicates that the frequency of 'driver mutations' increases progressively with age, affecting up to 14% of cells even in non-smokers, a process that is exponentially accelerated by tobacco smoke, which adds 1,000 to 10,000 mutations per cell (8).

According to the study, it was found that SCLC and NSCLC are more common in males. The finding (7,9,10) that gender difference was statistically significant implies that biological or environmental factors related to gender may affect clinical features and progression of the disease. In addition, while lung cancer is mostly a disease of older people, the study showed that it is also affecting younger adults, especially women aged 30 to 49 years, indicating age and sex are important determinants (7). Younger women might be more prone to lung cancer due to the interaction of tobacco carcinogens with sex steroids (estrogen) associated with mutations in p53 and K-RAS genes (11). Moreover, younger patients in the studied cohort were often diagnosed at a more advanced stage (Stage IV) due to being outside the conventional screening age (10).

According to the smoking status analysis, most SCLC and NSCLC patients were active or ex-smokers compared to about half of the participants in the control group (11). This large difference strengthens the tobacco role as a primary cause across all lung cancer types (12). The presence of smoking among the selected patients was not just a risk factor. Rather, it illustrates the ongoing effect of chronic tobacco exposure on an etiology of disease.

significant therapeutic target for lung cancer, including both SCLC and NSCLC, is CTLA-4, a crucial immunological checkpoint that regulates T-cell-mediated immune responses. At present, there are only a few direct comparisons of CTLA-4 expression between SCLC and NSCLC in the literature. CTLA-4 expression was considerably higher in SCLC than in NSCLC in the current investigation, indicating subtype-specific variations in immune modulation and tumor-immune evasion (3).

The findings of this study further demonstrated that serum CTLA-4 levels were notably higher in SCLC than in NSCLC, indicating subtype-specific variations in immune modulation. Biologically, CTLA-4 acts as a crucial checkpoint that inhibits T-cell-mediated immune responses primarily within the lymph nodes by arresting the cell cycle and reducing the production of IL-2 (13). The significant elevation observed in SCLC reflects a more pronounced state of tumor-immune evasion, which is likely driven by the characteristically "cold" tumor microenvironment of SCLC, where the levels of total and effector T-cells (CD3+ and CD8+) are significantly lower compared to NSCLC (14). In this context, high CTLA-4 levels may function as a dominant mechanism to suppress the limited cytotoxic anti-tumor response and facilitate rapid tumor progression and drug resistance (13,14).

Interestingly, the serum levels of CTLA-4 in NSCLC patients were found to be lower than those of the healthy control group. An unexpected observation can be scientifically interpreted as a mechanism called “immune sequestration.” Since NSCLC has been found to have much higher levels of tumor-infiltrating lymphocytes (TILs) compared to SCLC (14), it could be that CTLA-4 + T-cells are recruited and sequestered in the NSCLC tumor itself, instead of existing in peripheral blood. The decrease in circulating CTLA-4 levels may be explained by local shedding or sequestration within the tumor microenvironment, where CTLA-4 may be released or degraded locally rather than entering the bloodstream. This interpretation aligns with previous research finding that regulatory T cells within NSCLC tumor tissue expressed more intracellular CTLA-4, indicating a more pronounced

role in local immune control, coupled with the expression of PD-1 (15). In addition, common in advanced lung cancer patients, these systemic immune exhaustion and T cell subset (CD8+CD28- cells) distributional changes may induce a peripheral immune marker expression which is lower than that of healthy individuals but stable and active (16).

The study concludes that while CTLA-4 is an immune checkpoint with universal impacts, when presented systemically, its biological effect is strictly histology- and tumour-immune context-dependent. The unique immune profile of SCLC shows high CTLA-4 and low lymphocyte infiltration, indicating subtype-specific immunotherapy is needed for improving clinical outcomes in lung cancer patients (14, 16). The current results indicate that there is no significant difference in CTLA-4 expression in treated and untreated SCLC patients taken as well as NSCLC patients taken together. This insight is explainable by the molecular rationale; the regulation of immunity by CTLA-4 through internal signaling is complex and unlikely to be largely affected by standard chemotherapy. While the systemic levels could also be influenced by the tumor microenvironment, cellular location and T-cell activation state (6). The analysed data suggested that CTLA-4 may not be useful as a single predictive biomarker for either histological type. Because of the absence of survival data from the present study, it is not possible to draw conclusions on its absolute prognostic value at this stage. There was no change following conventional treatment, which is in line with the biological characteristics of lung cancer, which may use passive escape with active immune suppression (18). Indeed, the escape mechanisms can stabilize the expression of specific checkpoints. This means that the concentration of a protein at the systemic level, such as CTLA-4, may remain relatively stable regardless of treatment situation. With reference to the type of treatment, whether chemotherapy or radiotherapy, the results showed that the type of therapy alone was not independently responsible for a significant variance in CTLA-4 levels. The modern-day shift to coupled or synergistic protocols often leads to the homogenization of the immune environment in oncology (19). This creates a masking effect on the independent effect of each therapy on a single biomarker.

Nonetheless, CTLA-4 levels in patients varied significantly with PD-L1 expression, highlighting a significant finding of this study. There seemed to be a parallel immune checkpoint response, especially in treated NSCLC patients. Biologically, this is further supported by Zhang *et al.,* which shows that anti-CTLA-4 therapies can “induce” PD-L1 via EGFR signaling and MEK and ERK phosphorylation (17). The molecular induction elucidates the underlying mechanisms that might have occurred that produced the noted variance of CTLA-4 levels in the results across PD-L1 subgroups. The results also showed high expression of CTLA-4 in PD-L1-negative and PD-L1 high-positive in treated NSCLC. According to a thorough meta-analysis (20), a clinical advantage provided by immune checkpoint inhibition is possible regardless of PD-L1 expression, similar to current findings. PD-L1 in the immune microenvironment may play a compensatory role via CTLA-4 in its absence or high expression. In conclusion, although standard chemotherapy among various types of lung cancer does not cause major changes in CTLA-4 levels, the PD-L1 status can be used as a predictive biomarker for dynamic changes in CTLA-4 and Overall Management of Advanced Lung Cancer. In the present study, untreated lung cancer patients had a significant systemic inflammatory signature, showing strong elevations in white blood cell, neutrophil, monocyte, and platelet numbers. The results indicated a relative decrease in lymphocytes alongside an increase in C-reactive protein as well as fibrinogen, indicating activation of systemic inflammation, which follows a similar pattern seen before an oncologic intervention. Researchers have shown previously that the presence and progression of tumors are associated with systemic immunologic changes (21). To achieve a more effective result as per clinical standards, we included indices from CBC: NLR, PLR and MLR. Markers of inflammation in untreated groups were significantly higher than controls and indicate a pro-tumorigenic inflammatory status. Systemic inflammation biologically associated with the immune checkpoint landscape. The increased MLR in the untreated patients’ reported findings identifying monocyte-to-lymphocyte ratio as a superior independent predictor of disease recurrence and poor prognosis of lung cancer (22). Additionally, the association of hematological markers with CTLA-4 demonstrates a significant relationship with tumor immune escape mechanisms. High NLR and PLR levels reflect a state of relative lymphopenia and neutrophil-driven inflammation, which creates a permissive environment for elevated CTLA-4 expression to further suppress the remaining T-cell activity. The recent studies in patients treated with immune checkpoint inhibitors have highlighted this synergistic mechanism, where hematological profiles reflect the dynamic peripheral immune status (23).

The partial normalization of white blood cell and platelet counts post-treatment, particularly in NSCLC, is explicitly linked to improvements in the NLR, a gold-standard indicator for lung cancer inflammation, and could reflect how therapy affects bone marrow activity and platelet turnover (24). In SCLC, while medication may lower systemic inflammation, treated patients often maintain hematological values more similar to controls, although some may develop anemia or thrombocytosis following therapy (25, 26).

While these findings were significant, we acknowledge the cross-sectional nature of this study. These patterns should be interpreted as potential 'systemic snapshots' or biomarkers of the inflammatory and immunosuppressive burden at the

time of diagnosis, rather than definitive causal drivers of progression. Nevertheless, such CBC-derived markers remain invaluable as accessible, low-cost prognostic tools, especially in resource-limited settings where access to advanced molecular profiling is restricted (23). This integration of systemic inflammation and peripheral immune checkpoints underscores a coordinated mechanism of tumor-immune evasion in lung cancer.

Red blood cell parameters in the treated NSCLC population remained largely unchanged, with RBC values within the normal range. Compared to patients who received no treatment, the hemoglobin levels were comparatively higher, indicating that erythropoiesis had stabilized or partially recovered after treatment. Compared with the untreated group, the platelet counts were lower, which might reflect how therapy affected bone marrow activity and platelet turnover. Even as lymphocyte levels remained within or marginally above the normal range, the neutrophil numbers declined slightly, suggesting that immunological surveillance was maintained. These results highlight the modulatory effect of therapy on the systemic hematological parameters, consistent with the earlier research. demonstrating that oncologic treatment in NSCLC patients tends to normalize RBC and hemoglobin levels, lower platelet counts, and maintain the lymphocyte numbers (24).

Compared with the controls and treated SCLC patients the untreated SCLC patients showed significant hematological abnormalities, consistent with a systemic inflammatory response. White blood cell, neutrophil, and platelet counts were significantly elevated, but lymphocyte levels were mostly unchanged. These findings indicate that tumor-associated inflammation occurs before treatment. The patients who received treatment had hematological values that were more similar to those of the controls, suggesting that medication may have an impact in lowering systemic inflammation (25).

Hemoglobin levels were lower in the treated SCLC patients than in the controls and untreated patients, suggesting anemia. Following therapy, the white blood cell counts declined, with neutrophils and lymphocytes, but the platelet counts were comparatively higher. These variations reflected the overall effect of treatment on the blood components and immune function and are consistent with the earlier research that reported anemia, thrombocytosis, and changes in leukocyte and lymphocyte counts in SCLC patients following medication (26).

Clinical Implications of Serum CTLA-4

"To clarify the clinical value of the research findings, we have expanded the discussion to define the diagnostic, predictive, and prognostic significance of serum CTLA-4:

• The ROC analysis showed an absolute specificity (100%) of serum CTLA-4, making it a reliable confirmatory biomarker for clinical screening. The notable disparity (p=0.02) between elevated levels in SCLC and reduced levels in NSCLC offers a non-invasive differential diagnostic tool for histological subtypes using 'liquid biopsy' (6). This means that SCLC has a cold tumor microenvironment that is much poorer in T-cell infiltration as compared to NSCLC (14).

• The analysis indicated that serum CTLA-4 levels were generally stable and not affected by the administration of conventional platinum-based chemotherapy; NSCLC (p=0.14); SCLC (p=0.36). Notably, there was a significant decrease in systemic inflammatory markers (WBC, $p \leq 0.01$). This stability reflects an enduring immune checkpoint burden that is present in the tumor and is not altered by standard treatment. This actually serves as a predictive marker in the clinic to identify patients who could potentially need an immediate switch to immune checkpoint inhibitors (ICIs) or tailored combination therapies to overcome drug resistance (13).

• The increase in CTLA-4 in untreated patients acts as a prognostic factor of an immunosuppressive state. Because CTLA-4 is a dominant inhibitory signal that halts cell cycle and lowers IL-2 production (13), its quantification enables real-time monitoring of immune exhaustion by clinicians. This helps to inform patient-specific therapeutic decision-making and to facilitate the progression towards more targeted treatments to improve overall survival."

Limitations

The limitations of this study were the small-cell lung cancer (SCLC) groups relatively small sample size may hinder the findings' power and generalizability. A second limitation may be the heterogeneous treatment of these patients that might have affected the measured levels of CTLA-4.

Moreover, insufficient tumor staging details precluded further stratification and restricted assessment of CTLA-4 and disease stage relationship. In addition, since this was a single-center investigation, the external validity of the findings. Ultimately, the cross-sectional design limits the establishment of causality and the evaluation of temporal changes in CTLA-4 levels. Consequently, multicenter studies with larger sample sizes, treatment protocols and follow-up are recommended to validate the findings and verify the extensibility.

Conclusions:

The study showed that smoking, male sex and increasing age were strongly associated with lung cancer. As various subtypes of lung cancer have different immune modulation, the serum concentrations of CTLA-4 exhibited an upward trend in SCLC relative to NSCLC and control, indicating possible differences in subtypes.

Nonetheless, CTLA-4 expression was not significantly different among patients who received treatment and patients who had not. A significant relationship was observed in the treated NSCLC patients between CTLA-4 levels and PD-L1

expression categories indicative of a coordinated immune checkpoint response after treatment.
The CBC analysis also showed differences between treatment groups and lung cancer types with partial normalization after treatment, while inflammatory cell levels were higher in untreated individuals. As a rule of thumb, CBC-derived measures are readily available indicators of systemic inflammation in lung cancer. CTLA-4 could reflect subtype-specific immunological traits rather than treatment response. More studies are needed to confirm these findings and to further investigate CTLA-4 and PD-L1 as potential systemic biomarkers for synergy.

Authors' declaration:
We confirm that all the Figures and Tables in the manuscript belong to the current study. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee of Oncology Teaching Hospital, Medical City, Baghdad, Iraq. according to the code number (43668) on (9/ 12/ 2024).

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Authors' contributions:
Study conception & design: Zinah M. Al-hayali and Rasha M. Al-Humairi. Literature search: Rasha M.Al-Humairi. Data acquisition: Zinah M. Al-hayali. Data analysis & interpretation: Zinah M. Al-hayali. Manuscript preparation: Zinah M. Al-hayali. Manuscript editing & review: Rasha M. Al-Humairi.

Data availability: Data supporting the findings of this study are available from the corresponding author upon reasonable request.
AI Declaration: No artificial intelligence tools were used in the design, analysis, or writing of this manuscript.

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مقارنة مستويات المستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4 في المصل بين مرضى سرطان الرئة صغير الخلايا وسرطان الرئة غير صغير الخلايا المعالجين وغير المعالجين

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الخلاصة

الخلفية العلمية: يعد سرطان الرئة صغير الخلايا وسرطان الرئة غير صغير الخلايا النوعين الرئيسيين من سرطان الرئة، وهما من الأسباب الرئيسة للوفيات المرتبطة بالسرطان عالميا.

الأهداف:الهدف الرئيس من هذه المقالة هو مقارنة مستويات المستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4 في المصل بين المرضى المعالجين وغير المعالجين المصابين بسرطان الرئة صغير الخلايا وسرطان الرئة غير صغير الخلايا، بالإضافة إلى مجموعة السيطرة من الأصحاء.

المنهجية:أجريت هذه الدراسة من نوع الحالات والشواهد في مستشفى الأورام التعليمي، بغداد، العراق، خلال الفترة من كانون الأول 2024 إلى حزيران 2025. شملت الدراسة 180 مشاركا، منهم 24 مريضا مصابا بسرطان الرئة صغير الخلايا، و96 مريضا مصابا بسرطان الرئة غير صغير الخلايا، و60 شخصا من الأصحاء كمجموعة سيطرة. تم قياس مستويات المستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4 في المصل باستخدام تقنية الامتصاص المناعي المرتبط بالإنزيم. كما تم قياس معايير تعداد الدم الكامل باستخدام محلل دم آلي. وتم تحليل البيانات إحصائيا باستخدام برنامج الحزمة الإحصائية للعلوم الاجتماعية.

النتائج:اختلفت مستويات المستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4 بشكل معنوي بين مجموعة السيطرة ومجموعتي سرطان الرئة صغير الخلايا وسرطان الرئة غير صغير الخلايا، إذ كانت أعلى في مجموعة سرطان الرئة صغير الخلايا وأقل في مجموعة سرطان الرئة غير صغير الخلايا مقارنة بمجموعة السيطرة. لم تلاحظ فروق معنوية بين المرضى المعالجين وغير المعالجين ضمن مجموعتي سرطان الرئة صغير الخلايا وسرطان الرئة غير صغير الخلايا. كما أظهرت مستويات المستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4 ارتباطا معنويا مع فئات تعبير عامل الموت الخلوي المبرمج-1 لدى مرضى سرطان الرئة غير صغير الخلايا المعالجين، بينما لم يلاحظ ارتباط معنوي لدى مرضى سرطان الرئة غير صغير الخلايا غير المعالجين. أظهرت معايير تعداد الدم الكامل فروقا معنوية بين مجموعات الدراسة، مما يشير إلى وجود تغيرات التهابية جهازية لدى مرضى سرطان الرئة. كما أظهر تحليل منحنى خصائص تشغيل المستقبل أداء تشخيصيا محدودا للمستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4 في المصل لدى مرضى سرطان الرئة صغير الخلايا وسرطان الرئة غير صغير الخلايا.

الاستنتاج:ارتبطت مستويات المستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4 بشكل معنوي مع فئات تعبير عامل الموت الخلوي المبرمج-1 لدى المرضى المعالجين. وتشير هذه النتائج إلى أن تعداد الدم الكامل والمستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4 وعامل الموت الخلوي المبرمج-1 قد توفر معلومات مفيدة حول الحالة الالتهابية والمناعية لدى مرضى سرطان الرئة، إلا أن هناك حاجة إلى دراسات إضافية لتحديد الأهمية السريرية لهذه النتائج.

الكلمات المفتاحية: تعداد الدم الكامل، المستضد المرتبط بالخلايا اللمفاوية التائية السامة للخلايا-4، سرطان الرئة غير صغير الخلايا، عامل الموت الخلوي المبرمج-1، سرطان الرئة صغير الخلايا.