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RESEARCH ARTICLE

Evaluation of IL-22, IL-33, and IL-38 in Small-Cell and Non-Small-Cell Lung Cancer and Their Association with Histopathological and Immunohistochemical Markers

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Abstract

Introduction: Lung cancer is the leading cause of cancer deaths worldwide. Lung cancer is divided into two categories, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), apart from different cellular origin, their biological behavior, therapeutic response and clinical outcome also vary. Interleukins like IL-22, IL-33, and IL-38 were recently reported to play an important role in inflammation and tumor progression. The purpose of this study was to measure the serum levels of these interleukins in lung cancer patients according to the histological type and treatment status and to correlate with the clinicopathological parameters. **Materials and Methods:** A case–control study was conducted at the Oncology Teaching Hospital in Baghdad from December 2024 to June 2025. The study included 180 participants: 120 patients with histologically confirmed lung cancer 24 with SCLC and 96 with NSCLC and 60 healthy controls. Among patients, 48 were newly diagnosed and untreated, while 72 had received treatment. Serum concentrations of IL-22, IL-33, and IL-38 were measured using enzyme-linked immunosorbent assay and were correlated with histological subtypes and immunohistochemical markers, including thyroid transcription factor-1, Cytokeratin-7 (CK-7), Kiel 67 antigen (Ki-67), and synaptophysin. **Results:** IL-22 showed the highest levels in treated NSCLC, particularly in squamous cell carcinoma. IL-33 showed the highest levels in untreated adenocarcinoma. IL-38 showed a distinct pattern in squamous cell carcinoma. **Conclusion:** Different levels of IL-22, IL-33, and IL-38 in lung cancer subtypes and groups indicate that these may be immunological biomarkers for monitoring these cancers or response to treatment.

Keywords: IL-22, IL-33, IL-38, lung cancer, biomarker

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Introduction

Lung cancer is one of the most prevalent and fatal malignancies across the globe. About 18% of deaths associated with cancers occurs due to lung cancer. It has high morbidity and mortality. Further, lung cancer became a serious threat to public health worldwide. Histologically, lung cancer is classified into SCLC 13% and NSCLC 83% [1]. The three main types of NSCLC categorized by the World Health Organization are adenocarcinoma (40%), squamous cell carcinoma (25–30%), and large cell carcinoma (5–10%) [2].

Late diagnosis contributes to poor outcomes and limited treatment success [3].

Smoking remains the primary etiological factor, while exposure to radon, asbestos, heavy metals, and radioactive elements further increases risk Radon and its decay products, originating from uranium, significantly enhance carcinogenic risk, particularly among smokers [4].

IL-22 is a member of the IL-10 cytokine family and is secreted by Th17, NKT, γδ T, and ILC3 cells, as well as by innate immune cells such as macrophages and dendritic cells. Its production is mainly induced by IL-23, while IL-1β, IL-6, and TNF can act independently or synergistically to enhance its expression [5].

IL-22 signals through the IL-22R1/IL-10R2 receptor

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complex expressed on epithelial and fibroblast cells in the lung, liver, skin, and gastrointestinal tract, serving as a communication link between immune and tissue compartments .Activation of IL-22 triggers the JAK/STAT3 and AKT/mTOR pathways, promoting cell proliferation and anti-apoptotic responses [6].

The IL-22 gene is located on chromosome 12q15, adjacent to the IFN-γ gene, and its functions can be either immunoregulatory or pro-inflammatory, depending on the disease stage [7].

IL-33 is a member of the IL-1 cytokine family and shares structural similarity with IL-18. When cellular injury or necrosis occurs, the mature form of IL-33 is released and acts as a cytokine that triggers immune activation, functioning as an alarmin that signals tissue danger.

IL-33/ST2 pathway comprises IL-33, ST2 receptor, and accessory protein IL-1RAcP; it exerts double action in lung cancer, either pro-tumorigenic or regulatory depending on the tumor microenvironment [8]. According to another study [9], IL-33 was shown to enhance the migration and invasion of lung cancer cells through the activation of the AKT signaling pathway. Hence, IL-33 has potential as a therapeutic target for lung cancer.

IL-38, discovered in 2001, is part of the interleukin-1 (IL-1) cytokine family. It shows anti-inflammatory effects in many inflammatory diseases. The previous findings reported an unfavorable prognosis due to increased expression of IL-38 in lung adenocarcinoma tumor cells. The accurate role of it in the tumor microenvironment is, however, not clear [10].

The IL-38 gene, which consists of five coding exons and a highly conserved intron arrangement, is located within the IL-1 family cluster on human chromosome 2q13–14.1 [11]. IL-38 is expressed in a wide range of tissues such as the heart, tonsil, skin, thymus, and placenta, particularly in keratinocytes and proliferating B cells [12].

Previous research indicates that elevated intra-tumoral IL-38 expression in NSCLC is linked to a poor clinical outcome, including reduced survival and more aggressive disease characteristics. Increased IL-38 levels are typically found in poorly differentiated tumors and are associated with deeper invasion, lymph node metastasis, and pleural or vascular infiltration. It has activity and dampening antitumor immune responses. been suggested that IL-38 may facilitate tumor progression by inhibiting IL-36 [13].

Despite numerous studies on the tumor tissue of these interleukins (IL-22, IL-33, IL-38), there are very few comparative studies on their serum levels and the relationship between their levels in lung cancer subtypes, treatment status, and diagnostic IHC markers. This research was designed to address this gap. Therefore, the present study aimed to evaluate the serum levels of IL-22, IL-33, and IL-38 in patients with SCLC and NSCLC and to investigate their association with treatment status and selected histopathological and immunohistochemical markers.

Materials and Methods

Study Design and Setting

This case-control investigation was leading at the Oncology Teaching Hospital. Medical City Complex, Baghdad, Iraq, from December 2024 to June 2025, with the ethical approval of the institutional review board, and written informed consent was obtained from all study group members before their enrollment.

Study Population

A total of 180 participants were enrolled, including 120 patients with histopathologically proven primary lung cancer and 60 healthy controls. Most of the patients were cigarette smokers.

Subdivided the patients based on histopathological diagnosis into:

• SCLC: 10 newly diagnosed (untreated) and 14 treated cases.

• NSCLC: 38 newly diagnosed (untreated) and 58 treated cases.

Therapeutic regimens included among treated patients (SCLC and NSCLC) :

• Chemotherapy alone

• Chemotherapy combined with immunotherapy

• Chemotherapy combined with immunotherapy and radiotherapy

Therapy under electronic monitoring as needed. Decisions on treatment were taken individually as per clinical status of each patient and recommendation of physician. The control group studied consisted of healthy volunteers of similar age and sex without any history of cancer, autoimmune, or chronic inflammatory diseases.

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 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

From each participant, 5 mL of venous blood was collected under aseptic conditions into gel tubes and allowed to clot at room temperature for 30 minutes. The samples were then centrifuged at 3000 rpm for 10 minutes, and the separated serum was transferred into labeled Eppendorf tubes and stored at –20°C until analysis.

Serum concentrations of interleukins IL-22, IL-33, and IL-38 were determined using commercially available human ELISA kits following the manufacturers' protocols.

• IL-22 was measured using the Human IL-22 ELISA Kit (Cat. No. RE1057H, Reed Biotech, China), with a detection range of 15.63–1000 pg/mL and sensitivity of 9.38 pg/mL

• IL-33 was quantified using the Human IL-33 ELISA Kit (Cat. No. RE2428H, Reed Biotech, China), with a detection range of 3.13–200 pg/mL and sensitivity of 0.33 pg/mL

• IL-38 was assayed using the Human IL-1F10 (IL-38) ELISA Kit (Cat. No. EH14717, FineTest, China), with a detection range of 15.625–1000 pg/mL and sensitivity of 9.375 pg/mL

Statistical Analysis

Analysis of data was carried out using the available statistical package of IBM SPSS-29 (IBM Statistical Packages for Social Sciences- version 29, Chicago, IL, USA). Data of current study were analyzed by Chi-sequare test or using fisher exact probability (F.E.P) test to compare between percentages (qualitative data). Pearson Correlation: to examine the degree of relation between variables. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation). One-way ANOVA with post hoc analysis was used to investigate differences between the studied groups (more than 2 groups). To assess the significance of differences between pairs of group means Tukey test were applied. The sensitivity and specificity of studıed parametres were evaluated by a receiver operating characteristic (ROC) curve. -The comparison of significant (p-value) in any test was considered as:

• P-Value of greater than 0.05 (P>0.05) was non-statistically significant (NS).

• P-Value of less than 0.05 (P<0.05) was statistically significant (S).

• P-Value of less than 0.01 (P<0.01) was highly statistically significant (HS).

Results

IL-22 levels showed a statistically significant increase in SCLC (269.64±38.22 pg/mL) and NSCLC (295.47±16.65 pg/mL) compared with controls (150.47±8.40 pg/mL) ($p \leq 0.01$). IL-33 levels showed a statistically significant increase in SCLC (89.11±8.18 pg/mL) and NSCLC (96.05±3.68 pg/mL) compared with controls (78.13±3.85 pg/mL) ($p = 0.008$). Similarly, IL-38 levels showed a statistically significant increase in SCLC (165.20±20.22 pg/mL) and NSCLC (184.18±7.12 pg/mL) compared with controls (154.21±8.88 pg/mL) ($p = 0.04$) Table 1.

IL-22 levels showed a statistically significant increase in treated NSCLC patients (317.05±24.40 pg/mL) and untreated patients (262.53±18.68 pg/mL) compared with controls (150.47±8.40 pg/mL) ($p \leq 0.01$). IL-33 levels showed a statistically significant increase in treated (94.96±5.52 pg/mL) and untreated (97.71±3.98 pg/mL) patients compared with controls (78.13±3.85 pg/mL) ($p = 0.006$). Similarly, IL-38 levels showed a statistically significant increase in treated (189.02±10.36 pg/mL) and untreated (176.78±8.61 pg/mL) patients compared with controls (154.21±8.88 pg/mL) ($p = 0.02$) Table 2.

IL-22 levels showed a statistically significant increase in squamous cell carcinoma (375.07±42.75 pg/mL) and adenocarcinoma (259.29±12.42 pg/mL) compared with controls (150.47±8.40 pg/mL) ($p \leq 0.01$). IL-33 levels showed a statistically significant increase in squamous cell carcinoma (93.69±6.37 pg/mL) and adenocarcinoma (97.12±4.52 pg/mL) compared with controls (78.13±3.85 pg/mL) ($p = 0.006$).Similarly, IL-38 levels showed a statistically significant increase in squamous cell carcinoma (203.36±14.08) and adenocarcinoma (175.46±7.99 pg/mL) compared with controls (154.21±8.88 pg/mL) ($p = 0.007$) Table 3.

In the squamous cell carcinoma group, IL-22 levels (422.22±77.12 pg/mL for none, 355.29±46.75 pg/mL for negative, and 183.50±0.04 pg/mL for positive TTF-1 categories) did not show a statistically significant difference across TTF-1 categories ($p = 0.38$). IL-33

Table 1. Comparison of Serum IL-22, IL-33, and IL-38 Levels between SCLC, NSCLC and Controls

	Studies group			
Test	Control (pg/mL)	SCLC (pg/mL)	NSCLC (pg/mL)	P-value
IL-22	150.47±8.40	269.64±38.22	295.47±16.65	≤0.01
IL-33	78.13±3.85	89.11±8.18	96.05±3.68	0.008
IL-38	154.21±8.88	165.20±20.22	184.18±7.12	0.04

SCLC: Small Cell Lung Cancer. NSCLC: Non-Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively.

Table 2. Comparison of Serum IL-22, IL-33, and IL-38 Levels between Treated and Untreated NSCLC Patients and Controls.

		Conc. (M±SE)		
Test	Control (pg/mL)	NSCLC-treated (pg/mL)	NSCLC-Untreated (pg/mL)	P-value
IL-22	150.47±8.40	317.05±24.40	262.53±18.68	≤0.01
IL-33	78.13±3.85	94.96±5.52	97.71±3.98	0.006
IL-38	154.21±8.88	189.02±10.36	176.78±8.61	0.02

NSCLC: Non-Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively.

levels (106.01±8.57 pg/mL for none, 85.54±9.73 pg/mL for negative, and 64.49±0.04 pg/mL for positive TTF-1 categories) did not show a statistically significant difference across TTF-1 categories (p = 0.14). IL-38 concentrations (212.33±25.68 pg/mL for none, 194.75±16.62 pg/mL for negative, and 200.79±0.10 pg/mL for positive TTF-1 categories) did not show a statistically significant difference across TTF-1 categories (p = 0.84)

In the adenocarcinoma group, IL-22 levels (247.16±13.20 pg/mL for none, 414.16±47.81 pg/mL for negative, and 239.85±24.66 pg/mL for positive TTF-1 categories) showed a statistically significant difference across TTF-1 categories (p = 0.002). IL-33 levels (104.14±4.98 pg/mL for none, 79.61±6.66 pg/mL for negative, and 91.19±10.92 pg/mL for positive TTF-1 categories) did not show a statistically significant difference across TTF-1 categories (p = 0.14). IL-38 concentrations (170.08±11.12 pg/mL for none, 201.81±9.34 pg/mL for negative, and 187.58±13.39 pg/mL for positive TTF-1 categories) did not show a statistically significant difference across TTF-1 categories (p = 0.23) Table 4.

In NSCLC-squamous cell carcinoma, IL-22 levels (436.92±59.47 pg/mL for none, 286.79±84.89 pg/mL for CK7-negative, and 274.59±77.19 pg/mL for CK7-positive cases) did not show a statistically significant difference across CK7 categories (p = 0.254).IL-33 levels (112.82±5.91 pg/mL for none, 67.41±13.98 pg/mL for CK7-negative, and 69.39±6.75 pg/mL for CK7-positive cases) showed a statistically significant difference across CK7 categories (p = 0.006).IL-38 concentrations (213.17±20.20 pg/mL for none, 192.15±13.93 pg/mL for CK7-negative, and 154.64±21.79 pg/mL for CK7-positive cases) did not show a statistically significant difference across CK7 categories (p = 0.23).

In NSCLC-adenocarcinoma, IL-22 levels (255.27±22.20 pg/mL for none, 210.36±6.36 pg/mL for CK7-negative, and 267.22±16.64 pg/mL for CK7-positive cases) did not show a statistically significant difference across CK7 categories (p = 0.67). IL-33 levels (104.60±7.80 pg/mL for none, 97.34±1.93 pg/mL for CK7-negative, and 91.61±5.93 pg/mL for CK7-positive cases) did not show a statistically significant difference across CK7 categories (p = 0.19).IL-38 concentrations (165.01±13.79 pg/mL for none, 173.74±3.31 pg/mL for CK7-negative, and 178.69±10.89 pg/mL for CK7-positive cases) did not show a statistically significant difference across CK7 categories (p = 0.77) Table 5.

IL-22 levels showed a statistically significant difference in treated SCLC patients (293.30 ± 57.69 pg/mL) and untreated SCLC patients (236.52 ± 44.97 pg/mL) compared with controls (150.47 ± 8.40 pg/mL) ($p \leq 0.01$).

Table 3. Comparison of Serum IL-22, IL-33, and IL-38 in NSCLC Subtypes (Squamous and Adenocarcinoma) and Controls

		NSCLC groups-(M±SE)		
Test	Control (pg/mL)	Squamous (pg/mL)	Adenocarcinoma (pg/mL)	P-value
IL-22	150.47±8.40	375.07±42.75	259.29±12.42	≤0.01
IL-33	78.13±3.85	93.69±6.37	97.12±4.52	0.006
IL-38	154.21±8.88	203.36±14.08	175.46±7.99	0.007

NSCLC: Non-Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively.

Table 4. Comparison of Serum IL-22, IL-33, and IL-38 in NSCLC Subtypes (Squamous and Adenocarcinoma) According to TTF-1 Status.

Ttf1 categories	NSCLC-squamous (pg/mL)			NSCLC-Adenocarcinoma (pg/mL)		
	IL-22	IL-33	IL-38	IL-22	IL-33	IL-38
None	422.22±77.12	106.01±8.57	212.33±25.68	247.16±13.20	104.14±4.98	170.08±11.12
Negative	355.29±46.75	85.54±9.73	194.75±16.62	414.16±47.81	79.61±6.66	201.81±9.34
Positive	183.50±0.04	64.49±0.04	200.79±0.10	239.85±24.66	91.19±10.92	187.58±13.39
P-value	0.38	0.14	0.84	0.002	0.14	0.23

NSCLC: Non-Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively. TTF-1: Thyroid Transcription Factor-1.

Table 5. Comparison of Serum IL-22, IL-33, and IL-38 in NSCLC Subtypes (Squamous and Adenocarcinoma) According to CK7 Status

CK7 categories	NSCLC-squamous (pg/mL)			NSCLC-Adenocarcinoma (pg/mL)		
	IL-22	IL-33	IL-38	IL-22	IL-33	IL-38
None	436.92±59.47	112.82±5.91	213.17±20.20	255.27±22.20	104.60±7.80	165.01±13.79
Negative	286.79±84.89	67.41±13.98	192.15±13.93	210.36±6.36	97.34±1.93	173.74±3.31
Positive	274.59±77.19	69.39±6.75	154.64±21.79	267.22±16.64	91.61±5.93	178.69±10.89
P-value	0.254	0.006	0.23	0.67	0.19	0.77

NSCLC: Non-Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively. CK-7: Cytokeratin-7.

IL-33 levels showed a statistically significant difference in treated SCLC patients (77.86 ± 8.05 pg/mL) and untreated SCLC patients (104.86 ± 15.25 pg/mL) compared with controls (78.13 ± 3.85 pg/mL) (p = 0.05). Similarly, IL-38 levels did not show a statistically significant difference in treated SCLC patients (167.02 ± 29.06 pg/mL) and untreated SCLC patients (162.66 ± 28.26 pg/mL) compared with controls (154.21 ± 8.88 pg/mL) (p = 0.84) Table 6.

IL-22 levels (218.29±43.95 pg/mL for none and 295.32±52.71 pg/mL for Ki-67 positive tumors) did not show a statistically significant difference between the groups (p = 0.35).IL-33 levels (64.12±9.97 pg/mL for none and 101.61±9.98 pg/mL for Ki-67 positive tumors) showed a statistically significant difference between the groups (p = 0.02).IL-38 concentrations (151.29±30.02 pg/mL for none and 172.16±26.84 pg/mL for Ki-67 positive tumors) did not show a statistically significant difference between the groups (p = 0.63) Table 7.

IL-22 levels (171.76±58.72 pg/mL for none, 259.46±73.63 pg/mL for TTF-1-negative tumors, and 308.23±55.47 pg/mL for TTF-1-positive tumors) did not show a statistically significant difference among the groups (p = 0.39).IL-33 levels (64.99±16.47 pg/mL for none, 125.08±16.51 pg/mL for TTF-1-negative tumors, and 84.89±9.52 pg/mL for TTF-1-positive tumors) showed a statistically significant difference among the groups (p = 0.04). IL-38 levels (176.45±42.88 pg/mL for none, 104.82±20.88 pg/mL for TTF-1-negative tumors, and 182.75±29.37 pg/mL for TTF-1-positive tumors) did not show a statistically significant difference among the groups (p = 0.32) Table 8.

IL-22 levels (243.07±55.81 pg/mL for none and 278.50±48.08 pg/mL for Synaptophysin-positive tumors) did not show a statistically significant difference between the groups (p = 0.69).IL-33 levels (67.48±13.29 pg/mL for none and 96.32±9.56 pg/mL for Synaptophysin-positive tumors) did not show a statistically significant difference between the groups (p = 0.13). IL-38 concentrations (167.57±38.34 pg/mL for none and 164.42±24.35 pg/mL for Synaptophysin-positive tumors) did not show a statistically significant difference between the groups (p = 0.94) (Table 9).

Discussion

Interleukin-22 (IL-22) in Lung Cancer

In the present study, serum IL-22 levels were significantly elevated in lung cancer patients compared with healthy controls, with the highest concentrations observed in NSCLC, followed by SCLC. This finding reinforces the role of IL-22 as a key mediator in lung tumorigenesis, particularly in NSCLC, where it enhances cancer cell proliferation, migration, and survival through the PI3K–Akt–mTOR and STAT3 signaling pathways [14] Consistent with this mechanism, IL-22R1 expression is markedly increased in NSCLC especially in squamous cell carcinoma and is associated with significantly poorer overall survival [15].

Serum IL-22 levels were notably higher in treated NSCLC compared with untreated cases, suggesting a potential involvement of the IL-22/IL-22R1 axis in

Table 6. Comparison of Serum IL-22, IL-33, and IL-38 in Treated and Untreated SCLC Patients and Controls

		Conc. (M±SE)		
Test	Control (pg/mL)	SCLC-treated (pg/mL)	SCLC-Untreated (pg/mL)	P-value
IL-22	150.47±8.40	293.30±57.69	236.52±44.97	≤0.01
IL-33	78.13±3.85	77.86±8.05	104.86±15.25	0.05
IL-38	154.21±8.88	167.02±29.06	162.66±28.26	0.84

SCLC: Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively.

Table 7. Comparison of Serum IL-22, IL-33, and IL-38 in SCLC Patients According to Ki-67 Status

	SCLC (pg/mL)		
Ki-67 categories	IL-22	IL-33	IL-38
None	218.29±43.95	64.12±9.97	151.29±30.02
Positive	295.32±52.71	101.61±9.98	172.16±26.84
P-value	0.35	0.02	0.63

SCLC: Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively. Ki-67: Kiel 67 antigen.

Table 8. Comparison of Serum IL-22, IL-33, and IL-38 in SCLC Patients According to TTF-1 Status

	SCLC (pg/mL)		
TTF1categories	IL-22	IL-33	IL-38
None	171.76±58.72	64.99±16.47	176.45±42.88
Negative	259.46±73.63	125.08±16.51	104.82±20.88
Positive	308.23±55.47	84.89±9.52	182.75±29.37
P-value	0.39	0.04	0.32

SCLC: Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively. TTF-1: Thyroid Transcription Factor-1.

therapy resistance. These results are consistent with previous findings [16] who reported that IL-22R1 is upregulated in chemotherapy-refractory lung cancer cells, contributing to a more aggressive phenotype.

Furthermore, IL-22 levels were significantly higher in squamous cell carcinoma compared with adenocarcinoma, supporting its role in tumor aggressiveness and progression in this histotype [15].

IL-22 levels were significantly higher in TTF-1–negative adenocarcinomas compared with TTF-1–positive cases, while no significant difference was observed in squamous tumors TTF-1 overexpression has been associated with favorable prognosis in adenocarcinoma [17]. Elevated IL-22 in TTF-1–negative adenocarcinomas may contribute to poorer clinical outcomes, potentially through activation of oncogenic pathways such as STAT3 and PI3K/AKT/mTOR, which promote tumor survival and proliferation [15].

IL-22 levels tended to be higher in CK7-negative squamous cell carcinomas compared with CK7-positive cases. Even though the difference was not statistically significant. In adenocarcinomas, IL-22 levels were similar between CK7-positive and CK7-negative cases.

Since CK7 negativity is often associated with poorly differentiated squamous cell carcinomas and worse prognosis.[18], this trend suggests that IL-22 upregulation may accompany loss of epithelial differentiation and more aggressive tumor behavior.

In the current study, serum IL-22 levels were markedly elevated in small-cell lung cancer (SCLC) patients compared with healthy controls, with higher concentrations in treated cases.

This suggests that IL-22 may contribute both to tumor progression and to therapy resistance.

Consistently, a previous study reported preferential IL-22 expression in SCLC (58% of cases) and showed that IL-22/IL-22R1 signaling activates STAT3-mediated proliferative pathways and is upregulated in cisplatin-resistant cells, supporting its role in an aggressive, therapy- refractory phenotype [16].

Serum IL-22 levels were higher in Ki-67-positive SCLC cases compared with those without available Ki-67 results, indicating a potential association between IL-22 activity and cellular proliferation in SCLC [19].

Regarding TTF-1, the current study showed a gradual, non-significant increase in IL-22 levels across expression categories, suggesting a link between IL-22/STAT3 signaling and neuroendocrine differentiation rather than epithelial maturation [16].

In the current study, IL-22 levels tended to be slightly higher in synaptophysin-positive SCLC cases. Consistently, previous studies suggest that IL-22/STAT3 activation may interact with the neuroendocrine program to modulate treatment sensitivity [20].

In NSCLC and SCLC, elevated serum IL-22 may be a biomarker of tumor aggressiveness and resistance to therapy. Measuring IL-22 may allow identification of patients at risk of a poor outcome and personalization of treatment. Targeting the IL-22/IL-22R1 axis may help overcome resistance and improve patient outcomes.

Interleukin-33 (IL-33)

In the present study, serum IL-33 levels were significantly elevated in lung cancer patients compared with healthy controls, with the highest concentrations observed in NSCLC, particularly in adenocarcinoma. This aligns with previous findings in NSCLC patients, which exhibited higher IL-33 levels correlating with increased tumor progression and worse prognosis [21]. Similarly, previous studies highlighted the involvement of IL-33 in tumor-related inflammation and remodeling of the tumor microenvironment, noting its dual role in cancer either promoting progression via chronic inflammation and angiogenesis or enhancing anti-tumor immunity through immune activation [22].

Additionally, higher serum IL-33 levels were observed in untreated NSCLC compared with treated cases, implying that IL-33 release is more active during early disease, reflecting tumor associated inflammation. The reduction after therapy may indicate tumor regression and decreased inflammatory activity, supporting its potential role as a dynamic biomarker of immune response and treatment modulation [21].

Our study revealed a significant difference between NSCLC subtypes, with higher IL-33 levels in adenocarcinoma than in squamous cell carcinoma. This agrees with previous findings, which showed that while IL-33 and its receptor ST2 were generally downregulated in lung cancer tissues, higher IL-33 mRNA expression in adenocarcinoma correlated with longer survival, suggesting an immunoprotective role [23]. Conversely, other studies reported that high IL-33 expression in stage II–III squamous cell carcinoma was linked to shorter survival, identifying IL-33 as an independent marker of poor prognosis [24]. These observations highlight the dual function of IL-33 in lung cancer it may exert a protective immune-regulatory effect in adenocarcinoma but act as a pro-inflammatory, tumor-promoting factor in squamous cell carcinoma [21-24].

No significant correlation was observed between TTF-1 expression and serum IL-33 levels in either adenocarcinoma or squamous cell carcinoma. A slight decline in IL-33 with higher TTF-1 expression was

Table 9. Comparison of Serum IL-22, IL-33, and IL-38 in SCLC Patients According to Synaptophysin Status

	SCLC (pg/mL)		
Synaptophysin categories	IL-22	IL-33	IL-38
None	243.07±55.81	67.48±13.29	167.57±38.34
Positive	278.50±48.08	96.32±9.56	164.42±24.35
P-value	0.69	0.13	0.94

SCLC: Small Cell Lung Cancer. IL-22, IL-33, IL-38: Interleukins 22, 33, and 38, respectively.

observed in squamous tumors, while the pattern was variable in adenocarcinoma. These results suggest that IL-33 secretion is independent of TTF-1 status, likely reflecting differences in inflammatory activity or cellular differentiation between histological subtypes. Thus, TTF-1 and IL-33 may function as independent biomarkers with distinct biological roles in NSCLC.

A significant correlation was observed between CK7 expression and serum IL-33 levels in squamous cell carcinoma, showing reduced IL-33 levels in CK7-negative compared to CK7- positive tumors, suggesting that IL-33 variation may reflect differences in epithelial differentiation. No significant correlation was observed in adenocarcinoma. These results emphasize the context-dependent role of IL-33 in NSCLC, influenced by histological differentiation [21, 22].

In SCLC, serum IL-33 levels were slightly higher in untreated patients than in treated ones ،suggesting that elevated IL-33 reflects tumor-associated inflammation in active disease. The post-treatment decline may indicate suppression of inflammatory pathways and reduced tumor burden following chemotherapy. Similar findings in breast cancer showed higher IL-33 expression in chemotherapy-naïve cases, implying that cytotoxic therapy can downregulate IL-33 and lessen tumor aggressiveness [25]. These observations support IL-33 as a dynamic biomarker linking tumor activity and treatment response.

In SCLC, a significant positive correlation was found between serum IL-33 and Ki-67 expression, indicating that higher IL-33 levels are associated with enhanced tumor

proliferation. Given that Ki-67 reflects proliferative and aggressive tumor behavior, this finding suggests that IL-33 may promote tumor growth through activation of proliferative and inflammatory pathways. These findings align with the Ki-67 prognostic study, which linked high Ki-67 expression to advanced stage, lymph node involvement, and poor prognosis in SCLC [19].

In SCLC, serum IL-33 levels were significantly higher in TTF-1–negative tumors than in positive ones, suggesting that loss of TTF-1 expression, associated with tumor progression and dedifferentiation, coincides with increased IL-33 secretion and a more aggressive, inflammatory phenotype. Previous immunohistochemical studies likewise linked reduced TTF-1 to advanced stages and poor differentiation in SCLC. This trend suggests a possible link between IL-33 activity and the neuroendocrine phenotype of SCLC. Histopathological studies have shown that synaptophysin, along with TTF-1, CD56, and CgA, is commonly expressed in SCLC and reflects tumor progression and phenotypic diversity. Hence, elevated IL-33 in synaptophysin-positive tumors may indicate its role in sustaining neuroendocrine differentiation and inflammatory signaling [18].

Our results show that serum IL-33 levels are significantly higher in Ki-67–positive SCLC patients compared to those without available Ki-67 results, indicating that IL-33 may serve as a reliable biomarker of tumor proliferative activity. Monitoring IL-33 could help identify patients with more aggressive tumors and guide treatment strategies. Furthermore, targeting the IL-33/ST2 signaling pathway may offer a potential therapeutic approach in SCLC.

Interleukin-38 (IL-38)

The present study revealed a significant elevation of serum IL-38 levels in both SCLC and NSCLC patients compared with healthy controls, with a more marked increase in NSCLC.

Previous study that higher IL-38 expression in lung adenocarcinoma correlates with advanced stage and poor prognosis, suggesting that IL-38 contributes to an immunomodulatory and pro-tumor microenvironment that facilitates tumor progression and immune evasion [26].

Treated NSCLC patients showed higher IL-38 levels than untreated ones, indicating that therapy may modulate cytokine regulation in response to tumor burden. To date, no prior studies have compared IL-38 levels by treatment status, making this a novel observation that enhances understanding of its immunological dynamics during therapy.

IL-38 levels were significantly elevated in squamous cell carcinoma compared to adenocarcinoma, suggesting distinct immune regulation between NSCLC subtypes. As no prior studies have examined this variation, this finding highlights a novel aspect of NSCLC immunopathological heterogeneity.

No significant correlations were observed between IL-38 and TTF-1 or CK7, indicating that its expression is influenced by the tumor immune microenvironment rather than differentiation markers. This supports the view that IL-38 functions as an immunomodulatory cytokine, promoting immune suppression and tumor persistence.

Conversely, no significant differences in serum IL-38 levels were observed between treated and untreated SCLC patients, indicating that this cytokine may not be markedly influenced by therapeutic intervention in small-cell lung cancer. Similarly, IL-38 expression did not show significant associations with tumor proliferation index (Ki-67), TTF-1, or synaptophysin expression, suggesting that its regulation in SCLC might be independent of proliferative or neuroendocrine activity.

To date, no published studies have specifically investigated IL-38 dynamics in SCLC, and therefore, the observations regarding the lack of significant differences between treated and untreated patients, as well as the absence of associations with Ki-67, TTF-1, or synaptophysin, represent novel findings of the present study."

Serum IL-38 not currently being significantly associated with Ki-67 and TTF-1, synaptophysin may be owing to a lack of sample size and IL-38 may be acting locally inside the tumor microenvironment rather than systemically. Also, IL-38 may function independently of the proliferative and neuroendocrine markers indicating a distinctive immunomodulatory role for SCLC. In general, IL-22, IL-33, and IL-38 show distinct biological patterns in lung cancer. While IL-22 is linked with tumor growth and therapy resistance, IL-33 reflects inflammatory and proliferative activity, especially in SCLC. Furthermore, IL-38 appears to have an immunomodulatory role that

is context-dependent and varies by subtype. Together, these interleukins illustrate the heterogeneous immune landscape of lung cancer and support them potential as complementary biomarkers and therapeutic targets.

Limitations

Various regimens were used to treat patients: chemotherapy alone, chemotherapy plus immunotherapy, or radiation. Blood samples from patients who were given treatment were collected at varying time points after varying cycles. The timing varied in different patients. The study's limitation may be the effect of this variability on cytokine levels. Due to the small sample size, we were unable to perform the subgroup analysis of immunotherapy. Not computing 95% confidence intervals may restrict the precision of the estimates.

Declarations

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Clinical trial registration

Not applicable.

Conflicts of interest/Competing interests

The authors declare that they have no conflicts of interest.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

Zinah M. Al-hayali designed the study, performed the experiments, analyzed the data, and drafted the manuscript. She was responsible for participant selection based on disease type, stage, and treatment regimen, as well as determining the sample size, conducting laboratory tests, and documenting clinical progression. Rasha M. Al-humairi conducted the literature review, critically evaluated the scientific content, suggested revisions, and assisted with manuscript formatting. All authors discussed the results, provided feedback, and approved the final version of the manuscript for submission.

Ethics approval

This study was approved by the Ethical Committee of the Medical City Complex, Baghdad, Iraq (Approval No.: 43668, dated 9 December 2024). The authors also thank the Oncology Teaching Hospital / Medical City in Baghdad for their cooperation.

Consent to participate

Written informed consent was obtained from all participants, and the trial was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Written informed consent was obtained from all participants, and the trial was conducted in accordance with the Declaration of Helsinki.

Originality Declaration for Figures

Not applicable. This manuscript does not contain any figures.

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Declaration on generative AI and AI-assisted technologies in the writing process

The authors used AI-assisted tools solely for language polishing, grammar correction, and stylistic improvements.

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