Evaluation of proinflammatory cytokines and biochemical parameters in patients with advanced-stage lung cancer: A comparative analysis of different therapeutic approaches

TABARAK J. TALAB[1], FARHA A. ALI SHAFI[1] and SABA M. JASIM[2]

[1]Department of Biology, College of Science, Mustansiriyah University, Baghdad 10061, Iraq;
[2]Oncology Teaching Hospital, Medical City Center, Ministry of Health, Baghdad 10015, Iraq

Received April 24, 2025; Accepted July 9, 2025

DOI: 10.3892/wasj.2025.377

Abstract. Immunological and biochemical markers play a key role in assessing the therapeutic response of patients with lung cancer and recognizing the adverse effects of cancer therapy on these markers. The present study aimed to elucidate variations within certain proinflammatory cytokines and biochemical levels across several therapeutic approaches at both pre- and post-treatment, providing useful insight that may facilitate the development of more effective and tailored therapy strategies. The present study recruited 120 patients with advanced-stage lung cancer, who were divided into three groups based on the treatment they received (immunotherapy, chemotherapy or combination therapy). Differences in the levels of certain proinflammatory cytokines (IL-18 and IFN-γ) and biochemical parameters [alanine aminotransferase (ALT), aspartate aminotransferase, total serum bilirubin (TSB), creatinine, urea, random blood sugar (RBS)] were evaluated between the pre-and post-treatment time points. The results demonstrated that, when comparing between pre- and post-treatment changes, there were a significant elevation in the levels of proinflammatory cytokines in the immunotherapy group (IL-18, P=0.0090; and IFN-γ, P<0.0001), with parallel increases observed in the combination group (IL-18, P=0.0251; and IFN-γ, P=0.0012). However, in the chemotherapy group, the increase was not significant. By contrast, the level of ALT was significantly decreased in the chemotherapy group (P=0.0133), whilst the TSB level was significantly increased in the combination group post-treatment, compared with the other groups (P=0.0035). Furthermore, there were significant increases in the levels of creatinine, urea and RBS post-treatment in the immunotherapy group compared with the other groups (P=0.0019, P=0.0115 and P=0.0088, respectively). On the whole, in the complex field of several therapies designed to eliminate cancer and alleviate its consequences in patients with lung cancer, there is a necessity for a predictive marker to differentiate the varying implications of these treatments on the immunological profile and biochemical parameter levels of patients. The present study highlights the need of evaluating these parameter levels due to their vital involvement in anti-tumoral effects and potential side-effects.

Correspondence to: Dr Tabarak J. Talab, Department of Biology, College of Science, Mustansiriyah University, Albaladiat, Highway 6, Baghdad 10061, Iraq
E-mail: tabarak.jalil96@uomustansiriyah.edu.iq

Key words: lung cancer, immunotherapy, chemotherapy, programmed death-ligand 1, cytokine

Introduction

The prevalence of cancer is increasing globally, and lung cancer is the most commonly diagnosed type of cancer, constituting 11.6% of the total number of cancer cases. Lung cancer is the primary cause of global cancer-related mortality, constituting 18.4% of all cancer-related deaths, imposing notable societal and economic burdens (1). Therapeutic modalities for lung cancer encompass surgery, radiation therapy and chemotherapy; nevertheless, medical management is frequently associated with the emergence of treatment resistance, resulting in relapse (2). Consequently, an enhanced comprehension of lung cancer biology has resulted in the advancement of immunotherapy (3).

Immunotherapies, notably characterized by the use of immune checkpoint inhibitors (ICIs), enable T-cells to eliminate cancer cells by obstructing programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) signaling. This markedly transforms the treatment and outlook of patients with lung cancer who lack targetable genetic changes (4). The anti-PD-1 antibody, pembrolizumab, and the anti-PD-L1 antibody, atezolizumab, have demonstrated notable clinical efficacy in a number of solid tumors, leading to its US Food and Drug Administration (FDA) approval for patients with non-small cell lung cancer (NSCLC) (5,6). As cancer rates increase, immunotherapy has become an increasingly prevalent treatment option as a monotherapy or adjuvant therapy to chemotherapy (7).

The equilibrium of the immune system is regulated by cytokines; these are soluble proteins facilitating cell-to-cell communications (8). Cytokines are generated by innate and adaptive immune cells in reaction to tumor antigens and

pathogens. Each cytokine exerts a distinct effect on the immune system, contingent upon several factors, including local cytokine concentrations, cytokine receptor expression profiles, and the interplay of diverse pathways within the immune response (9). Cytokines can either stimulate tumor growth (oncogenic cytokines) or prevent it (antitumor cytokines) by altering variables associated with growth, proliferation, metastasis and apoptosis (10).

Interleukin (IL)-18 is an immunostimulatory cytokine that is part of the IL-1 family. It can modulate both innate and adaptive immune responses by influencing natural killer (NK) cells, monocytes, dendritic cells, T-cells and B-cells. It can also function synergistically with other pro-inflammatory cytokines to enhance interferon (IFN)-γ production by NK cells, T-cells and potentially, other cell types. IFN-γ is the only identified member of the type II IFN family, discovered ~60 years ago, and the paradoxical pathological and biological effects of IFN-γ continue to be a focal point of research literature (11). According to the current understanding, host-derived IFN-γ can exert both anti-tumorigenic and pro-tumorigenic effects due to its complex role in immunoediting (12). To diminish lung cancer mortality, the optimal approach is to enhance cancer preventive efficacy and use screening technologies for risk evaluation and early-stage diagnosis in lung cancer treatment (10).

The side-effects of cancer treatments can be evaluated through the assessment of alterations in the levels of biochemical parameters. Chemotherapy serves as a systemic intervention that inhibits the proliferation of cancer cells, which grow and divide abnormally and rapidly. However, it is unable to selectively differentiate between healthy and cancerous cells. Therefore, chemotherapy-induced adverse effects arise from damage to healthy cells (13). Moreover, as immunotherapy becomes increasingly prevalent in cancer treatment, it is crucial to recognize the potential rare, yet notable immune-related adverse effects associated with these medicines (14).

The present study aimed to assess the serum levels of several proinflammatory cytokines and biochemical parameters prior to and during immunotherapy or chemotherapy, to compare the impact of these treatments on these markers in patients with advanced NSCLC. The present study underscores the pivotal significance of cytokine and biomarker levels in assessing and enhancing lung cancer therapies, providing essential insight that may facilitate the development of more effective and specific treatment strategies.

Patients and methods

Patient selection. The present study prospectively enrolled a total of 120 patients diagnosed with stage IV NSCLC between January, 2024 and February, 2025 at the Oncology Teaching Hospital (Medical City Center, Baghdad, Iraq). The patients were divided into three groups based on the type of treatment they received and the level of PD-L1, as follows: i) Immunotherapy group (PD-L1 score, ≥50%); ii) chemotherapy group (PD-L1 score, 0%); and iii) combination therapy group (PD-L1 score, 1-49%). All treatments were administered as first line treatments. In the immunotherapy group, the patients were intravenously administered 1,200 mg atezolizumab or 200 mg pembrolizumab. In the chemotherapy group, the patients were administered 175 mg/m^2 paclitaxel with carboplatin or 60 mg/m^2 vinorelbine or carboplatin with 500 mg/m^2 pemetrexed. In the combination therapy group, patients received a combination of both immunotherapy and chemotherapy. These patients received treatment every 3 weeks until disease progression or unacceptable toxicities. Relevant clinical information and blood samples were collected before and after 6 cycles (equivalent to 4.2 months) of treatment. No delays were noted in the administration of treatment due to toxicity or other reasons. The Research Ethics Committee of Mustansiriyah University (Baghdad, Iraq) reviewed and approved the study (approval no. BCSMU/1024/0050Z). All patients who participated in this study provided written informed consent for the publication of their data. The consent form emphasized that participation was entirely voluntary, and the participants could withdraw at any time without facing any repercussions. Anonymity and confidentiality were safeguarded by assigning coded identifiers rather than names or medical record numbers.

Blood samples. venous blood was collected from 120 patients with stage IV lung cancer both at pre- and post-treatment to assess certain immunological and biochemical markers. Blood was collected in a gel tube and allowed to clot at room temperature for 30 min, after which the sera were separated by centrifugation for 10 min at 136,955 x g at 4˚C and divided into small, equal portions in Eppendorf tubes and preserved in a deep freeze at -20˚C until use. Each sample was designated by a serial number and the name of the patient. A total of 2 ml serum was used for the analysis of IL-18 and IFN-γ using ELISA kits for humans, according to the manufacturer's instructions for IL-18 (cat. no. RE1123H from Reed Biotech Ltd.) and IFN-γ (cat. no. RE1059H from Reed Biotech Ltd.). Moreover, 3 ml residual serum was used to analyze alanine aminotransferase (ALT), aspartate aminotransferase (AST), total serum bilirubin (TSB), creatinine, urea and random blood sugar (RBS) levels using Cobas® (Roche Diagnostics).

Statistical analysis. Statistical analysis was performed using GraphPad Prism version 9.2 (IBM Corp.). Quantitative parametric data were subjected to the Shapiro-Wilk test to confirm the normal distribution and are expressed as mean ± standard deviation. One-way analysis of variance and the Student's t-test were used to determine the significance of group variance. Tukey's post hoc test was performed to adjust for multiple comparisons. Fisher's exact test was employed to test count variances. A value of $P<0.05$ was considered to indicate a statistically significant difference.

Results

A total of 120 patients with advanced-stage NSCLC were enrolled in the present study. These patients were divided into three groups based on the type of treatment received: Immunotherapy, chemotherapy and a combination of both. The demographic and clinical characteristics of the patients were analyzed, including age, sex, NSCLC subtype, body mass index (BMI), family history and smoking status (Table I).

Table I. Characteristics of the patients in the present study.

Characteristic	Group			P-value
	Immunotherapy	Chemotherapy	Combination	
Sex, n (%)				
Male	39 (97.5%)	38 (95%)	37 (92.5%)	0.4716
Female	1 (2.5%)	2 (5%)	3 (7.5%)	
Total	40 (100%)	40 (100%)	40 (100%)	
Subtype, n (%)				
Adenocarcinoma	31 (77.5%)	20 (50%)	26 (65%)	0.0251
Squamous cell carcinoma	9 (22.5%)	20 (50%)	14 (25%)	
Total	40 (100%)	40 (100%)	40 (100%)	
Smoking status, n (%)				
Never	7 (17.5%)	5 (12.5%)	6 (15%)	0.8331
Current	17 (42.5%)	15 (37.5%)	16 (40%)	
Former	16 (40%)	20 (50%)	18 (45%)	
Total	40 (100%)	40 (100%)	40 (100%)	
Family history of any cancer type, n (%)				
Yes	1 (2.5%)	4 (10%)	1 (2.5%)	0.2043
No	39 (97.5%)	36 (90%)	39 (97.5%)	
Total	40 (100%)	40 (100%)	40 (100%)	
PMH, n (%)				
Yes	7 (17.5%)	4 (10%)	5 (12.5%)	0.5014
No	33 (82.5%)	36 (90%)	35 (87.5%)	
Total	40 (100%)	40 (100%)	40 (100%)	
Treatment, n (%)				
Pembrolizumab	20 (50%)		20 (50%)	>0.9999
Atezolizumab	20 (50%)		20 (50%)	
Total	40 (100%)		40 (100%)	
BMI, mean ± SD	24.2±3.14	22.62±3.1	23.02±3.14	0.3282[a]
Age in years, mean ± SD	65.41±4.2	63.54±9.2	61.33±11.24	0.6572[a]

Data were analyzed using Fisher's exact test or one-way ANOVA. [a]The reported P-value corresponds to the overall comparison between all groups. PMH, past medical history; BMI, body mass index.

The patients in the immunotherapy group (n=40) had a mean age of 65.41 years, with 39 (97.5%) male and 1 (2.5%) female patients. In total, 77.5% of patients were diagnosed with adenocarcinoma and 22.5% with squamous cell carcinoma. Furthermore, 17.5% of the patients never smoked, 42.5% were current smokers and 40% were former smokers. The mean BMI of the patients was 24.2 kg/m^2. Patients received anti-PD-1/PD-L1 treatments. Atezolizumab was administered to 20/40 (50%) patients, and pembrolizumab was administered to the remaining 20/40 (50%) patients, as a first-line treatment. All the patients in the immunotherapy group were positive for PD-L1 expression, with a tumor proportion score ≥50%.

In the chemotherapy group (n=40), the mean BMI was 22.62 kg/m^2 and the mean age was 63.54 years, with 38 (95%) male and 2 (5%) female patients. In total, 37.5% of the patients were current smokers. In the combined therapy group (n=40), the mean age of the patients was 61.33 years, with 37 (92.5%) male and 3 (7.5%) female patients. A total of 40% were current smokers. The mean BMI was 23.02 kg/m^2. In this group, 20/40 (50%) patients received chemotherapy in combination with atezolizumab and 20/40 (50%) received chemotherapy in combination with pembrolizumab, as a first-line treatment. Patients were monitored both prior to initiating treatment and six cycles post-treatment (4.2 months) to assess changes in the levels of proinflammatory cytokines and biochemical parameters.

The IL-18 and IFN-γ levels were measured both before and after treatment for each therapeutic group. In the immunotherapy group, there was a significant elevation in the level of IL-18 (P=0.0090) and IFN-γ (P<0.0001), with parallel increases observed in the combination group for IL-18 (P=0.0251) and IFN-γ (P=0.0012) (Table II).

Liver function test levels (ALT, AST and TSB) were assessed before and after treatment for each therapeutic group. In the immunotherapy group, there were no significant differences between pre- and post-treatment levels. However, a significant difference in ALT levels was found in the chemotherapy group (P=0.133). Furthermore, in the combination

Table II. Comparison of IL-18 and IFN-γ levels before and after treatment across three different therapeutic groups.

A, Immunotherapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
IL-18 (pg/ml)	42.37±14.2	58.61±16.3	0.0090
IFN-γ (pg/ml)	81.32±25.4	140.6±44.72	<0.0001
B, Chemotherapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
IL-18 (pg/ml)	54.49±21.25	43.17±13.6	0.1597
IFN-γ (pg/ml)	119.2±49.3	131.4±30.3	0.1873
C, Combination therapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
IL-18 (pg/ml)	45.87±17.2	67.56±18.12	0.0251
IFN-γ (pg/ml)	94.78±58.4	170.8±62.24	0.0012

Table III. Comparison of ALT, AST, TSB levels before and after treatment across three different therapeutic groups.

A, Immunotherapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
ALT (U/l)	24.13±10.9	21.14±6.7	0.4319
AST (U/l)	24.04±7.6	20.76±2.43	0.2215
TSB (mg/dl)	0.3746±0.2	0.4672±0.46	0.5789
B, Chemotherapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
ALT (U/l)	33.41±19.4	17.30±14.2	0.0133
AST (U/l)	36.00±19.4	27.81±18.4	0.4335
TSB (mg/dl)	0.7972±0.2	0.8895±0.3	0.2723
C, Combination therapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
ALT (U/l)	23.42±4.81	25.71±2.3	0.3891
AST (U/l)	26.47±2.5	20.37±3.4	0.0957
TSB (mg/dl)	0.4298±0.3	0.7891±0.13	0.0035

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TSB, total serum bilirubin.

therapy group, there was a significant difference in TSB levels between pre- and post-treatment (P=0.0035) (Table III).

Renal function tests (creatinine and urea) were performed both before and after treatment for each therapeutic group. In the immunotherapy group, there were significant differences between pre- and post-treatment levels for both creatinine and urea (P=0.0019 and 0.0115, respectively). However, in the chemotherapy and combination therapy groups there were no significant differences in these parameters (Table IV).

RBS levels were measured both before and after treatment for each therapeutic group. In the immunotherapy group, there was a significant difference between pre- and post-treatment

Table IV. Comparison of creatinine and urea levels before and after treatment across three different therapeutic groups.

A, Immunotherapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
Urea (mg/dl)	39.18±3.19	49.65±12.9	0.0115
Creatinine (mg/dl)	0.9092±0.2	1.162±0.1	0.0019
B, Chemotherapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
Urea (mg/dl)	39.21±5.4	46.11±15.3	0.1254
Creatinine (mg/dl)	0.9945±0.23	1.031±0.4	0.6545
C, Combination therapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
Urea (mg/dl)	41.64±11.9	46.73±20.8	0.2793
Creatinine (mg/dl)	0.9821±0.2	1.272±0.6	0.2186

Table V. Comparison of random blood sugar levels before and after treatment across three different therapeutic groups.

A, Immunotherapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
RBS (mg/dl)	127.9±15.56	159.3±60.51	0.0088
B, Chemotherapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
RBS (mg/dl)	147.5±32.01	158.7±52.7	0.5363
C, Combination therapy group			
Parameter	Pre-treatment (mean ± SD)	Post-treatment (mean ± SD)	P-value
RBS (mg/dl)	116.4±16.35	110.7±23.2	0.7816

RBS, random blood sugar.

levels (P=0.0088). However, no significant differences were found in both the chemotherapy and combination therapy groups (Table V).

Discussion

Generally, scientific studies tend to assess a solitary immune system parameter within a single treatment group, predominantly focusing on immunotherapy as a monotherapy, which may inadequately reflect the immunological signature associated with the emergence of a suitable antitumor response. Moreover, they are unable to compare this response across other treatment groups (15-17). The present study followed-up patients with a confirmed diagnosis of advanced-stage NSCLC that received immunotherapy, chemotherapy or combination therapy, to evaluate the changes in the levels of certain proinflammatory cytokines and biochemical parameters before and after treatment.

The results of the present study demonstrated that serum IL-18 levels were significantly increased in the immunotherapy and combination therapy groups following treatment compared with prior to treatment (P=0.0090 and P=0.0251, respectively). However, in the chemotherapy group, this increase was not significant (P=0.1597) (Table II). A previous study reported

that during the first cycle of anti-PD-L1 immunotherapy, the IL-18 levels increased in the serum of patients with NSCLC, which was associated with the reactivation and cytotoxicity of antitumor effector cells, specifically the production of IFN-γ (18). Preclinical research has indicated that IL-18 is essential for the suppression of tumor development (19). An independent investigation emphasized the potential of IL-18 as a viable biomarker for response to anti-PD-1 treatment in lung cancer (20). Similar findings have been reported in pancreatic, metastatic and non-metastatic melanoma mouse models, wherein the *in vivo* administration of IL-18 was shown to inhibit tumor proliferation by enhancing the activation and cytotoxicity of $CD4^+$ and $CD8^+$ T-cells, as well as NK cells (21), along with their interaction with tumor cells and the vigorous production of memory T-cells (8,22). Furthermore, in a previous study, elevated levels of IL-18 were detected in the plasma and tissue of patients with NSCLC treated with immunotherapy, and were associated with an anticancer immune gene profile (23), and a reduced tumor burden (18,20). Upon recognition of pathogenic stressors by innate immune cells, inflammasomes are assembled, leading to the activation of caspase-1, which converts pro-IL-18 into mature IL-18. Due to the expression of IL-18 receptors on NK cells, IL-18 may contribute to the immunological functions of these cells (24). Notably, studies using preclinical animal models of lung malignancies have reported that IL-18 enhances the sensitivity of lung tumors to anti-PD-1 immunotherapy (21,25). Moreover, in patients with NSCLC receiving atezolizumab, IL-18 has been shown to exhibit a dose-exposure association that is associated with tumor size (18). Subsequently, IL-18 is a crucial element in initiating cellular immunity, thereby promoting an efficient antitumor immune response (25).

Chemotherapy is one of the most effective available therapies for malignant tumors; however, it is protracted and non-specific, killing tumor cells whilst injuring normal tissues to varied degrees, in which the most notable is the phased loss of lymphocytes, inhibiting normal immune function (26). In the present study, the levels of IL-18 in the chemotherapy group were not altered when compared with the other groups, and the increased level was very slight (P=0.1597). This is consistent with the findings of previous studies which reported that a non-significant increase in the levels of IL-18 in the chemotherapy group (27,28). These findings correspond with previous findings that report that elevated IL-18 levels in the serum and tissue of patients with ovarian cancer are inactive due to the absence of IFN-γ production (29).

Currently, chemotherapy is the primary treatment for advanced-stage NSCLC; however, the total survival duration for patients averages ~10 months, with a 2-year survival rate of ~10% (1). This may be associated with the deleterious side-effects induced by chemotherapy (30). The factors contributing to the unfavorable prognosis of patients encompass tumor invasion and the immunosuppressive effects of chemotherapy (31). Specifically, diminished levels of IL-18 are associated with metastasis and unfavorable outcomes (32,33). Consequently, elucidating the impact of chemotherapy on the organism and providing effective therapies are essential for enhancing patient prognosis (31). The immunosuppressive effects of chemotherapy may be associated with this event; however, limited information has been documented at this time, to the best our knowledge. Consequently, the combination of chemotherapy and immunotherapy may amplify the antitumor effect; however, the precise mechanism underlying this effect remains ambiguous. Nevertheless, the present study used several chemotherapy regimens (such as paclitaxel/carboplatin and carboplatin/pemetrexed), which matches real-world NSCLC treatment patterns where combination treatments are customized to individual patients. Notably, all analyses were stratified by treatment method and the consistent pattern of biomarker alterations across these groups indicated that the key conclusions were robust despite the variability of chemotherapy. However, future research utilizing standardized chemotherapy regimens is necessary to remove potential regimen-specific confounders and further confirm the findings of the present study.

Furthermore, in the present study, it was demonstrated that the level of IFN-γ was significantly increased in the immunotherapy and combination groups following treatment (P<0.0001 and 0.0012, respectively), whilst in the chemotherapy group, this increase was minimal and non-significant (P=0.1873) (Table II). A previous study reported that the increased production of IFN-γ was observed with high doses of IL-18 (34). This is consistent with the results of the present study, reflecting its reliability. The paradoxical biological and pathological effects of IFN-γ continue to be a focal point of research in the literature (11). According to current knowledge, host-derived IFN-γ can exert both anti-tumorigenic and pro-tumorigenic effects due to its complex role in immunoediting (12). The anti-tumoral effects of IFN-γ include the reduction of growth in several tumor cell lines. This is achieved by direct antitumor mechanisms and the enhancement of immune cell-mediated cytotoxicity, alongside the modulation of PD-L1 expression (35), or by blocking angiogenesis in neoplastic tissue, inducing regulatory T-cell death, and/or stimulating the activity of M1 proinflammatory macrophages to counteract tumor growth (8). Furthermore, another study reported that tumor cells can evade immune system regulation in the initial phases of tumor growth, either due to the reduced expression of IFN-γ or through modifications of its receptors (30). Moreover, IFN-γ may diminish immune responses by intensifying the activation of specific immunosuppressive pathways that facilitate cancer progression and metastasis (36). The study by Jorgovanovic *et al* (11) reported that the concentration of IFN-γ in the tumor microenvironment was associated with its function. Moreover, it was revealed that tumors treated with low-dose IFN-γ developed metastatic characteristics, whereas those administered high doses resulted in tumor regression (11). Deficiencies in the IFN signaling pathway are a primary route of resistance to immune checkpoint inhibition (37,38). Additionally, previous research reported that high doses of IFN-γ could trigger apoptosis in NSCLC cell-lines by activating JAK-STAT1-caspase signaling, which subsequently launched apoptotic processes in cancer cells (39). Moreover, some other research has examined baseline plasma cytokines, including IFN-γ, in individuals with advanced-stage NSCLC (40,41). In response to immunotherapy, IFN-γ levels increased in sera of patients with lung cancer (40). These results indicate that serum cytokine level analysis, which is non-invasive, cost-effective and reproducible, may serve a notable role in future integrated prediction

models. However, future large-scale research requires tumor cytokine analysis. Consequently, the findings of the present study may help to establish a basis for forthcoming extensive validation studies assessing temporal variations in serum cytokine levels and exploring their associations with tumor responses to cancer therapies.

As regards chemical biomarkers, previous studies have reported that immunomodulating agents targeting cytotoxic T-lymphocyte-associated protein 4, PD-1 or PD-L1 are associated with several immune-related adverse effects, including autoimmune pneumonitis, colitis, hepatitis, nephritis and several endocrinopathies (42-44). As immunotherapy becomes increasingly prevalent in cancer treatment, it is crucial to recognize the potential rare, yet severe immune-related adverse effects associated with these treatments (14). This novel oncological treatment has been reported to be a more effective and robust alternative to conventional chemotherapy, leading to extended remission durations and less side effects. However, as ICI enhances the immune system, subsequent autoimmune diseases may arise (7).

The incidence of hepatotoxicity in patients with lung cancer vary by cohort. Liver function tests serve as the principal baseline indicator of hepatic function and health in patients undergoing anticancer treatment, with elevations in liver function tests signifying a predominant portion of therapy-related hepatotoxicity (45,46). In the present study, ALT, AST and TSB levels demonstrated non-significant differences in the immunotherapy group; however, in the chemotherapy group, ALT levels exhibited a significant difference between pre- and post-treatment levels (P 0.0133). Moreover, in the combination group, there was a significant difference in TSB levels (Table III).

The fluctuation of ALT and AST levels due to chemotherapy has been commonly reported in previous clinical studies (47-49), which is in agreement with the results of the present study. Hepatic dysfunction under chemotherapy mainly consists of abnormal liver tests indicating changes in the levels of bilirubin and alkaline phosphatase, with or without abnormal levels of AST and/or ALT. The mechanism of liver injury following chemotherapy remains incompletely understood; however, enzymes involved in folate metabolism may be critical components. These enzymes are present in both hepatic and neoplastic cells, hence chemotherapy may affect hepatic cells (50,51). Chemotherapy-induced hepatotoxicity can generally be effectively controlled through careful monitoring for elevated liver function tests indicative of liver damage, dose adjustments and the cessation of the causative agent if liver function tests do not normalize despite dose reduction (45,52).

Furthermore, previous research has reported that, in comparison with chemotherapy alone, PD-1/PD-L1 inhibitors, whether administered with or without chemotherapy, increase the incidence of both all-grade and high-grade hepatitis; however, they do not elevate the risk of increased blood markers indicative of hepatotoxicity (53). This aligns with the results of the present study, in which the findings in the combination therapy group demonstrated that there was no elevation in ALT or AST levels, with a significant difference in TSB levels only. Moreover, previous research on breast cancer has reported that combination chemotherapy elevates liver toxicity (54). The incidence of hepatotoxicity associated with individual anticancer agents differs in clinical trials; nevertheless, the advent of combination chemotherapy complicates the current situation and necessitates further analysis.

The adverse impacts of certain ICIs on hepatic function remain contentious. Hepatotoxicity subsequent to ICI treatment is rare, although clinically significant (55). The majority of associated toxicities are immune-related side-effects, occurring in ≤5% of patients (56). Hepatocellular or mixed hepatitis with immunological characteristics typically manifests within 2-12 weeks following the commencement of ICI therapy; however, it can arise at any point during treatment, including ≤1 year post-initial dosage (57). In the immunotherapy group in the present study, there were no significant differences in the liver function parameters. This may be attributed to the short follow-up period, which is a limitation of the present study. However, the finding is consistent with the findings of previous studies (58,59), including the study by Guo *et al* (53), which reported a minimal or no difference in the risk of elevated blood indicators of hepatotoxicity for patients who received PD-1/PD-L1 inhibitor monotherapy, a PD-1/PD-L1 inhibitor plus chemotherapy, and chemotherapy alone. Future studies with large sample sizes and prolonged follow-up are essential to confirm the findings of the present study and assess long-term toxicity.

Moreover, as regards renal adverse events in the present study, the levels of creatinine and urea were significantly elevated in immunotherapy group (P=0.0019 and P=0.0115, respectively), whilst in the chemotherapy and combination therapy groups, the differences were not significant (Table IV). These results are agreement with the findings of previous studies (60,61), which reported a marked association between immunotherapy and increased creatinine levels, potentially resulting in renal toxicity that may be risky if not managed. Generally, the inhibition of immunological checkpoints augments the ability of the immune cells of the body to identify foreign cells, which concurrently increases the likelihood of misidentifying its own cells as foreign. This underlies the adverse effects that may arise during therapy with ICIs, potentially affecting almost every system of the human body (62). Renal toxicity with ICIs is considered an uncommon consequence; however it may be underreported (63,64).

In a previous study by Xipell *et al* (61), following the administration of ICIs to patients, blood test results indicated an increase in the serum creatinine level to 5.6 mg/dl, compared with a previous level of ~1.2 mg/dl. Furthermore, Izzedine *et al* (60), defined a series of renal adverse events by high creatinine levels in patients treated with pembrolizumab. These results are in agreement with the findings of the present study. The precise mechanism causing systemic or organ damage during or following therapy with ICIs remains ambiguous and requires additional research (65). However, one suggested mechanism suggests that the kidneys have immunological checkpoint molecules, such as PD-L1, which may shield healthy tissue from T-cell cytotoxicity and invasion. Consequently, tissue damage may arise from PD-L1 inhibition (66,67). Another mechanism involves activated T-cells, which can enter tumors or normal tissue and use T-cell receptors (TCRs) to identify antigens. A TCR attaches itself to antigens that are expressed on healthy cells and whose

sequences are sufficiently similar to those of neoantigens in the first instance (66). Additionally, ICIs can reactivate drug-specific T-cells that have been primed by nephritogenic medications. As a result, memory T cells are activated against the drug due to a lack of tolerance (60). Finally, a large number of pro-inflammatory cytokines are produced following renal damage. Another mechanistic theory suggests that ICIs may be involved in the production of certain autoantibodies that harm healthy organs (66). Finally, a large number of pro-inflammatory cytokines are produced following renal damage (68); however, it remains unknown whether elevated serum levels of cytokines induce or are result of tissue damage. This finding necessitates more thorough research. Both the European Society for Medical Oncology and American Society of Clinical Oncology incorporate suggestions on managing renal toxicities in the guidelines regarding immunotherapy due to the increasing usage of ICIs in the treatment of cancer and the growing understanding of the potential renal side effects of the therapy (69,70). The noted elevation in creatinine (P-value 0.0019) and urea (P-value 0.0115) in the present study may indicate modified renal function during immunotherapy. While these alterations were often insufficient to warrant the cessation of treatment in the majority of cases, they highlight the critical need of regular renal monitoring to identify early indicators of nephrotoxicity. According to the ASCO/ESMO guidelines, the accurate monitoring of hepatic and renal functions (including ALT, AST, TSB, urea and creatinine) should be performed at baseline and every 3-4 weeks during the treatment of patients with lung cancer undergoing immunotherapy or on combination therapies (69,70).

In the present study, the RBS level was significantly increased in the immunotherapy group following treatment ($P<0.0088$) (Table V). This result is in agreement with the findings of previous research, which reported that type 1 diabetes mellitus (T1DM) developed in conjunction with ICI therapies (71,72). The FDA has issued a warning that pembrolizumab may induce immune-mediated endocrinopathies (56).

However, the mechanism linking immunotherapy and the endocrine system is inadequately understood. It is hypothesized that the PD-1 receptors on T-cells facilitate the binding and activation of PD-L1. The interaction between PD-1 and PD-L1 generates an inhibitory signal that modulates activated and autoreactive T-cells, hence suppressing the immune system. Consequently, PD-1 inhibitors result in an active immune system characterized by elevated quantities of functional T-cells. Moreover, β-cells in the pancreas express PD-1, and the elevation of T-cells due to PD-1 inhibitors suggests that β-cells may respond maladaptively to increased autoreactive T-cells, ultimately leading to their inability to produce insulin and resulting in diabetes (73).

Furthermore, in the present study, the elevation in RBS levels within the chemotherapy and combination therapy groups were non-significant ($P=0.5363$ and $P=0.7816$, respectively). This finding is in agreement with the findings of previous studies (74,75). The results of the study by Nagy *et al* (7) demonstrate that checkpoint inhibitors are the most frequently associated with new-onset diabetes compared with conventional chemotherapy. Moreover, Chae *et al* (14) reported that the platinum and taxane types of chemotherapy were not associated with the onset of T1DM. With the increasing incidence of immunotherapy-induced diabetes, the ASCO has developed clinical recommendations for the management of endocrine problems resulting from ICIs (76). The guidelines advise that fasting or random blood glucose and HbA1c tests should be conducted prior to and following the administration of ICIs (77). According to the results of the present study, careful glucose monitoring and rapid dietary or pharmacological therapies are recommended to avoid the onset of diabetes. However, further research is waranted to elucidate the underlying process, particularly with checkpoint inhibitors, which constitute the primary class associated with autoimmune diabetes. Until the pathogenic process is thoroughly understood, healthcare personnel must endeavor to avert the emergence of life-threatening endocrinopathies (7).

Finally, the present study performed a power analysis test which confirmed that the sample size (n=40/group) provided adequate statistical power (99.9%) to detect clinically meaningful effects for the primary endpoints, based on an effect size of 0.5 and $\alpha=0.05$. Whilst this sample size was sufficient for the main outcomes, larger cohort studies are required to confirm whether the identified changes in immunological and biochemical variables can function as predictive indicators, and to reduce immune-related adverse events whilst maintaining treatment efficacy.

In conclusion, in the complex field of several therapies designed to eliminate cancer and alleviate its consequences in patients with lung cancer, there is a critical necessity for predictive markers to differentiate the varying implications of these treatments on immunological profiles and biochemical parameter levels. The present study assessed several proinflammatory cytokine and biochemical levels in patients with NSCLC before and after treatments to identify differences among several treatment groups, and the results highlight the need of evaluating these parameter levels due to their vital involvement in anti-tumoral effects and potential side-effects.

Acknowledgements

The authors would like to thank Dr Hiba Q. Mahmoud at the College of Science, Mustansiriyah University (Baghdad, Iraq), Dr Hussein I. Hayal at Al-Kindy College of Medicine, Baghdad University (Baghdad, Iraq), and Dr Ali Z. Al-Saffar at the College of Biotechnology, Al-Nahrain University (Baghdad, Iraq), for providing academic assistance and guidance.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

TJT contributed to the conception and design of the study, and wrote and edited the manuscript. SMJ collected and analyzed data. FAAS reviewed and edited the manuscript, and was also involved in data curation, and supervised the study. All authors

have read and approved the final manuscript. TJT and SMJ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was reviewed and approved by the Research Ethics Committee of Mustansiriyah University, Baghdad, Iraq (approval No. 1024/0050Z) and subsequently authorized by THE Oncology Teaching Hospital/Medical City administration per standard institutional collaboration procedures. All patients who participated in the study provided written informed consent for the publication of their data. The consent form emphasized that participation was entirely voluntary, and the participants could withdraw at any time without facing any repercussions. Anonymity and confidentiality were safeguarded by assigning coded identifiers rather than names or medical record numbers. In adherence to international ethical guidelines, including the Declaration of Helsinki, the study maintained strict ethical standards. A statement of consent for publication was obtained from the patient according to the principles of the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2019. CA Cancer J Clin 69: 7-34, 2019.
2. Lahiri A, Maji A, Potdar PD, Singh N, Parikh P, Bisht B, Mukherjee A and Paul MK: Lung cancer immunotherapy: Progress, pitfalls, and promises. Mol Cancer 22: 40, 2023.
3. Mamdani H, Matosevic S, Khalid AB, Durm G and Jalal SI: Immunotherapy in lung cancer: Current landscape and future directions. Front Immunol 13: 823618, 2022.
4. Mino-Kenudson M, Schalper K, Cooper W, Dacic S, Hirsch FR, Jain D, Lopez-Rios F, Tsao MS, Yatabe Y, Beasley MB, *et al*: Predictive biomarkers for immunotherapy in lung cancer: Perspective from the international association for the study of lung cancer pathology committee. J Thorac Oncol 17: 1335-1354, 2022.
5. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, *et al*: Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 372: 2018-2028, 2015.
6. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, Von Pawel J, Gadgeel SM, Hida T, Kowalski DM, Dols MC, *et al*: Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): A phase 3, open-label, multicentre randomised controlled trial. Lancet 389: 255-265, 2017.
7. Nagy S, Demory Beckler M, Hussein A and Kesselman MM: The development of diabetes and diabetic ketoacidosis following immunotherapy treatment: A systematic review of case reports. Cureus 16: e57894, 2024.
8. Berraondo P, Sanmamed MF, Ochoa MC, Etxeberria I, Aznar MA, Pérez-Gracia JL, Rodríguez-Ruiz ME, Ponz-Sarvise M, Castañón E and Melero I: Cytokines in clinical cancer immunotherapy. Br J Cancer 120: 6-15, 2019.
9. Chaplin DD: Overview of the immune response. J Allergy Clin Immunol 125 (2 Suppl 2): S3-S23, 2010.
10. Lan T, Chen L and Wei X: Inflammatory cytokines in cancer: Comprehensive understanding and clinical progress in gene therapy. Cells 10: 100, 2021.
11. Jorgovanovic D, Song M, Wang L and Zhang Y: Roles of IFN-γ in tumor progression and regression: A review. Biomark Res 8: 49, 2020.
12. Alspach E, Lussier DM and Schreiber RD: Interferon γ and its important roles in promoting and inhibiting spontaneous and therapeutic cancer immunity. Cold Spring Harb Perspect Biol 11: a028480, 2019.
13. PDQ Supportive and Palliative Care Editorial Board: Family Caregivers in Cancer: Roles and Challenges (PDQ®). In: PDQ Cancer Information Summaries. National Cancer Institute, Bethesda, 2010.
14. Chae YK, Chiec L, Mohindra N, Gentzler R, Patel J and Giles F: A case of pembrolizumab-induced type-1 diabetes mellitus and discussion of immune checkpoint inhibitor-induced type 1 diabetes. Cancer Immunol Immunother 66: 25-32, 2017.
15. Zhao N, Yi Y, Cao W, Fu X, Mei N and Li C: Serum cytokine levels for predicting immune-related adverse events and the clinical response in lung cancer treated with immunotherapy. Front Oncol 12: 923531, 2022.
16. Zhang C, Mo H, Li M, Wang S, Dou X and Zhang X: The effects of postoperative targeted immunotherapy on peripheral blood cytokines and immune cell profile in lung cancer patients. Front Oncol 14: 1342624, 2024.
17. Sanmamed MF, Perez-Gracia JL, Schalper KA, Fusco JP, Gonzalez A, Rodriguez-Ruiz ME, Oñate C, Perez G, Alfaro C, Martín-Algarra S, *et al*: Changes in serum interleukin-8 (IL-8) levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients. Ann Oncol 28: 1988-1995, 2017.
18. Netterberg I, Li C, Molinero L, Budha N, Sukumaran S, Stroh M, Jonsson EN and Friberg LE: A PK/PD analysis of circulating biomarkers and their relationship to tumor response in atezolizumab-treated non-small cell lung cancer patients. Clin Pharmacol Ther 105: 486-495, 2019.
19. Janho Dit Hreich S, Hofman P and Vouret-Craviari V: The role of IL-18 in P2RX7-mediated antitumor immunity. Int J Mol Sci 24: 9235, 2023.
20. Wang Y, Chen H, Zhang T, Yang X, Zhong J, Wang Y, Chi Y, Wu M, An T, Li J, *et al*: Plasma cytokines interleukin-18 and CXC motif chemokine ligand 10 are indicative of the anti-programmed cell death protein-1 treatment response in lung cancer patients. Ann Transl Med 9: 33, 2021.
21. Zhou T, Damsky W, Weizman OE, McGeary MK, Hartmann KP, Rosen CE, Fischer S, Jackson R, Flavell RA, Wang J, *et al*: IL-18BP is a secreted immune checkpoint and barrier to IL-18 immunotherapy. Nature 583: 609-614, 2020.
22. Savid-Frontera C, Viano ME, Baez NS, Lidon NL, Fontaine Q, Young HA, Vimeux L, Donnadieu E and Rodriguez-Galan MC: Exploring the immunomodulatory role of virtual memory CD8+ T cells: Role of IFN gamma in tumor growth control. Front Immunol 13: 971001, 2022.
23. Chen B, Yang M, Li K, Li J, Xu L, Xu F, Xu Y, Ren D, Zhang J and Liu L: Immune-related genes and gene sets for predicting the response to anti-programmed death 1 therapy in patients with primary or metastatic non-small cell lung cancer. Oncol Lett 22: 540, 2021.
24. Senju H, Kumagai A, Nakamura Y, Yamaguchi H, Nakatomi K, Fukami S, Shiraishi K, Harada Y, Nakamura M, Okamura H, *et al*: Effect of IL-18 on the expansion and phenotype of human natural killer cells: Application to cancer immunotherapy. Int J Biol Sci 14: 331-340, 2018.
25. Janho Dit Hreich S, Humbert O, Pacé-Loscos T, Schiappa R, Juhel T, Ilié M, Ferrari V, Benzaquen J, Hofman P and Vouret-Craviari V: Plasmatic inactive IL-18 predicts a worse overall survival for advanced non-small-cell lung cancer with early metabolic progression after immunotherapy initiation. Cancers (Basel) 16: 2226, 2024.
26. Jiao J, Li WW, Shang YH, Li XF and Jiao M: Clinical effects of Chemotherapy combined with Immunotherapy in patients with advanced NSCLC and the effect on their nutritional status and immune function. Pakistan J Med Sci 39: 404-408, 2023.
27. Schoenfeld DA, Djureinovic D, Su DG, Zhang L, Lu BY, Kamga L, Mann JE, Huck JD, Hurwitz M, Braun DA, *et al*: Decoy-resistant IL-18 reshapes the tumor microenvironment and enhances rejection by anti-CTLA-4 in renal cell carcinoma. JCI insight 10: e184545, 2024.
28. Kessel C, Rossig C and Abken H: Weal and woe of interleukin-18 in the T cell therapy of cancer. J Immunother Cancer 13: e010545, 2025.

29. Orengo AM, Fabbi M, Miglietta L, Andreani C, Bruzzone M, Puppo A, Cristoforoni P, Centurioni MG, Gualco M, Salvi S, *et al*: Interleukin (IL)-18, a biomarker of human ovarian carcinoma, is predominantly released as biologically inactive precursor. Int J cancer 129: 1116-1125, 2011.
30. Parker BS, Rautela J and Hertzog PJ: Antitumour actions of interferons: Implications for cancer therapy. Nat Rev Cancer 16: 131-144, 2016.
31. Zhang J, Song D, Dong Y, Bai L, Gao D, Zhang S, Guo Y, Li F, Ao M and Li Q: Effects of Chemotherapy on peripheral blood NK cell receptor NKG2D and related immune cytokines in patients with non-small cell lung cancer. J Cancer Ther 13: 631-639, 2022.
32. Feng X, Zhang Z, Sun P, Song G, Wang L, Sun Z, Yuan N, Wang Q and Lun L: Interleukin-18 is a prognostic marker and plays a tumor suppressive role in colon cancer. Dis Markers 2020: 6439614, 2020.
33. Jiang K, Lin B, Zhang Y, Lu K, Wu F and Luo D: A Novel Pyroptosis-Related Gene Signature for Prediction of Disease-Free Survival in Papillary Thyroid Carcinoma. J Pers Med 13: 85, 2022.
34. Nakanishi K: Unique action of interleukin-18 on T cells and other immune cells. Front Immunol 9: 763, 2018.
35. Gao Y, Yang J, Cai Y, Fu S, Zhang N, Fu X and Li L: IFN-γ-mediated inhibition of lung cancer correlates with PD-L1 expression and is regulated by PI3K-AKT signaling. Int J Cancer 143: 931-943, 2018.
36. Burke JD and Young HA: IFN-γ: A cytokine at the right time, is in the right place. Semin Immunol 43: 101280, 2019.
37. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, Torrejon DY, Abril-Rodriguez G, Sandoval S, Barthly L, *et al*: Mutations associated with acquired resistance to PD-1 blockade in Melanoma. N Engl J Med 375: 819-829, 2016.
38. Song M, Ping Y, Zhang K, Yang L, Li F, Zhang C, Cheng S, Yue D, Maimela NR, Qu J, *et al*: Low-dose IFNγ induces tumor cell stemness in tumor microenvironment of non-small cell lung cancer. Cancer Res 79: 3737-3748, 2019.
39. Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Hu-Lieskovan S, Kalbasi A, Grasso CS, Hugo W, Sandoval S, Torrejon DY, *et al*: Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. Cancer Discov 7: 188-201, 2017.
40. Boutsikou E, Domvri K, Hardavella G, Tsiouda D, Zarogoulidis K and Kontakiotis T: Tumour necrosis factor, interferon-gamma and interleukins as predictive markers of antiprogrammed cell-death protein-1 treatment in advanced non-small cell lung cancer: A pragmatic approach in clinical practice. Ther Adv Med Oncol 10: 1758835918768238, 2018.
41. Guo Q, Wu CY, Jiang N, Tong S, Wan JH, Xiao XY, Mei PY, Liu HS and Wang SH: Downregulation of T-cell cytotoxic marker IL18R1 promotes cancer proliferation and migration and is associated with dismal prognosis and immunity in lung squamous cell carcinoma. Front Immunol 13: 986447, 2022.
42. Talab TJ, Shafi FAA and Jasim SM: Association between immune-related adverse events and therapeutic outcomes in patients with advanced non-small cell lung cancer treated with pembrolizumab: A retrospective study. World Acad Sci J 7: 1-9, 2025.
43. Bajwa R, Cheema A, Khan T, Amirpour A, Paul A, Chaughtai S, Patel S, Patel T, Bramson J, Gupta V, *et al*: Adverse Effects of Immune Checkpoint Inhibitors (Programmed Death-1 Inhibitors and Cytotoxic T-Lymphocyte-Associated Protein-4 Inhibitors): Results of a Retrospective Study. J Clin Med Res 11: 225-236, 2019.
44. Casagrande S, Sopetto GB, Bertalot G, Bortolotti R, Racanelli V, Caffo O, Giometto B, Berti A and Veccia A: Immune-related adverse events due to cancer immunotherapy: Immune mechanisms and clinical manifestations. Cancers (Basel) 16: 1440, 2024.
45. Grigorian A and O'Brien CB: Hepatotoxicity secondary to chemotherapy. J Clin Transl Hepatol 2: 95-102, 2014.
46. European Association for the Study of the Liver: EASL clinical practice guidelines: Drug-induced liver injury. J Hepatol 70: 1222-1261, 2019.
47. Clarke SJ, Abratt R, Goedhals L, Boyer MJ, Millward MJ and Ackland SP: Phase II trial of pemetrexed disodium (ALIMTA, LY231514) in chemotherapy-naïve patients with advanced non-small-cell lung cancer. Ann Oncol 13: 737-741, 2002.
48. Ohe Y, Ichinose Y, Nakagawa K, Tamura T, Kubota K, Yamamoto N, Adachi S, Nambu Y, Fujimoto T, Nishiwaki Y, *et al*: Efficacy and safety of two doses of pemetrexed supplemented with folic acid and vitamin B12 in previously treated patients with non-small cell lung cancer. Clin Cancer Res 14: 4206-4212, 2008.
49. Smit EF, Mattson K, Von Pawel J, Manegold C, Clarke S and Postmus PE: ALIMTA (pemetrexed disodium) as second-line treatment ofnon-small-cell lung cancer: A phase II study. Ann Oncol 14: 455-460, 2003.
50. Sakamori Y, Kim YH, Yoshida H, Nakaoku T, Nagai H, Yagi Y, Ozasa H and Mishima M: Effect of liver toxicity on clinical outcome of patients with non-small-cell lung cancer treated with pemetrexed. Mol Clin Oncol 3: 334-340, 2015.
51. Mohammed Bakheet M, Mohssin Ali H and Jalil Talab T: Evaluation of some proinflammatory cytokines and biochemical parameters in pre and postmenopausal breast cancer women. Cytokine 179: 156632, 2024.
52. King PD and Perry MC: Hepatotoxicity of chemotherapy. Oncologist 6: 162-176, 2001.
53. Guo X, Li W, Hu J, Zhu EC and Su Q: Hepatotoxicity in patients with solid tumors treated with PD-1/PD-L1 inhibitors alone, PD-1/PD-L1 inhibitors plus chemotherapy, or chemotherapy alone: Systematic review and meta-analysis. Eur J Clin Pharmacol 76: 1345-1354, 2020.
54. Larroquette CA, Hortobagyi GN, Buzdar AU and Holmes FA: Subclinical hepatic toxicity during combination chemotherapy for breast cancer. JAMA 256: 2988-2990, 1986.
55. Huffman BM, Kottschade LA, Kamath PS and Markovic SN: Hepatotoxicity after immune checkpoint inhibitor therapy in Melanoma: Natural progression and management. Am J Clin Oncol 41: 760-765, 2018.
56. Kwok G, Yau TC, Chiu JW, Tse E and Kwong YL: Pembrolizumab (keytruda). Hum Vaccin Immunother 12: 2777-2789, 2016.
57. De Martin E, Michot JM, Papouin B, Champiat S, Mateus C, Lambotte O, Roche B, Antonini TM, Coilly A, Laghouati S, *et al*: Characterization of liver injury induced by cancer immunotherapy using immune checkpoint inhibitors. J Hepatol 68: 1181-1190, 2018.
58. Zhao P, Yu L, Ma W and Zhao T: Clinical Manifestations and Risk Factors of Liver Injury Induced by PD-1 Inhibitors in Patients with Malignancies: A Case-Control Study. Ther Clin Risk Manag 21: 309-320, 2025.
59. Tamaki N, Mori N, Takaki S, Tsuji K, Tada T, Nakamura S, Ochi H, Mashiba T, Doisaki M, Marusawa H, *et al*: Change in liver function in durvalumab plus tremelimumab treatment for unresectable hepatocellular carcinoma. Anticancer Res 44: 3913-3918, 2024.
60. Izzedine H, Mathian A, Champiat S, Picard C, Mateus C, Routier E, Varga A, Malka D, Leary A, Michels J, *et al*: Renal toxicities associated with pembrolizumab. Clin Kidney J 12: 81-88, 2019.
61. Xipell M, Victoria I, Hoffmann V, Villarreal J, Garcia-Herrera A, Reig O, Rodas L, Blasco M, Poch E, Mellado B and Quintana LF: Acute tubulointerstitial nephritis associated with atezolizumab, an anti-programmed death-ligand 1 (pd-l1) antibody therapy. Oncoimmunology 7: e1445952, 2018.
62. Esfahani K, Meti N, Miller WH Jr and Hudson M: Adverse events associated with immune checkpoint inhibitor treatment for cancer. CMAJ 191: E40-E46, 2019.
63. Espi M, Teuma C, Novel-Catin E, Maillet D, Souquet PJ, Dalle S, Koppe L and Fouque D: Renal adverse effects of immune checkpoints inhibitors in clinical practice: ImmuNoTox study. Eur J Cancer 147: 29-39, 2021.
64. Rassy EE, Kourie HR, Rizkallah J, Karak FE, Hanna C, Chelala DN and Ghosn M: Immune checkpoint inhibitors renal side effects and management. Immunotherapy 8: 1417-1425, 2016.
65. Borówka M, Łącki-Zynzeling S, Nicze M, Kozak S and Chudek J: Adverse renal effects of anticancer immunotherapy: A review. Cancers (Basel) 14: 4086, 2022.
66. Sury K, Perazella MA and Shirali AC: Cardiorenal complications of immune checkpoint inhibitors. Nat Rev Nephrol 14: 571-588, 2018.
67. Waeckerle-Men Y, Starke A and Wüthrich RP: PD-L1 partially protects renal tubular epithelial cells from the attack of CD8+ cytotoxic T cells. Nephrol Dial Transplant 22: 1527-1536, 2007.
68. Murakami N, Borges TJ, Yamashita M and Riella LV: Severe acute interstitial nephritis after combination immune-checkpoint inhibitor therapy for metastatic melanoma. Clin Kidney J 9: 411-417, 2016.

69. Haanen JBAG, Carbonnel F, Robert C, Kerr KM, Peters S, Larkin J and Jordan K; ESMO Guidelines Committee: Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 28 (suppl 4): iv119-iv142, 2017.
70. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, Chau I, Ernstoff MS, Gardner JM, Ginex P, *et al*: Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol 36: 1714-1768, 2018.
71. Hughes J, Vudattu N, Sznol M, Gettinger S, Kluger H, Lupsa B and Herold KC: Precipitation of autoimmune diabetes with anti-PD-1 immunotherapy. Diabetes Care 38: e55-e57, 2015.
72. Hansen E, Sahasrabudhe D and Sievert L: A case report of insulin-dependent diabetes as immune-related toxicity of pembrolizumab: Presentation, management and outcome. Cancer Immunol Immunother 65: 765-767, 2016.
73. Carrero JA, McCarthy DP, Ferris ST, Wan X, Hu H, Zinselmeyer BH, Vomund AN and Unanue ER: Resident macrophages of pancreatic islets have a seminal role in the initiation of autoimmune diabetes of NOD mice. Proc Natl Acad Sci USA 114: E10418-E10427, 2017.
74. Schnell PM, Kassem M, Miah A, Ramaswamy B, Williams N, Cherian M, Stover DG, Gatti-Mays ME, Pariser A, Wesolowski R, *et al*: Changes in glucose level during chemotherapy treatment in patients with stage I-IV breast cancer. Clin Breast Cancer: May 27, 2025 (Epub ahead of print).
75. Fang TZ, Wu XQ, Zhao TQ, Wang SS, Fu GM, Wu QL and Zhou CW: Influence of blood glucose fluctuations on chemotherapy efficacy and safety in type 2 diabetes mellitus patients complicated with lung carcinoma. World J Diabetes 15: 645-653, 2024.
76. Schneider BJ, Naidoo J, Santomasso BD, Lacchetti C, Adkins S, Anadkat M, Atkins MB, Brassil KJ, Caterino JM, Chau I, *et al*: Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: ASCO guideline update. J Clin Oncol 39: 4073-4126, 2021.
77. Inzucchi SE and Lupsa B: Clinical presentation, diagnosis, and initial evaluation of diabetes mellitus in adults-UpToDate. https://www.uptodate.com/contents/clinical-presentation-diagnosis-and-initial-evaluation-of-diabetes-mellitus-in-adults. 2021.