Annals of Biology **39** (2) : 441-446, 2023

Impact Score : 0.32 (Scopus)

A Clinical Analysis of Liver and Renal Function Tests as Predictive Markers in Lung Adenocarcinoma

IMAN ABDULLAH RAMAZHAN, LUQMAN RAHMAN SULAIMAN[1] AND ABBAS SALIHI*

Department of Biology, College of Science, Salahaddin University-Erbil, 44002 Erbil, Kurdistan Region, Iraq
**(e-mail: abbas.salihi@su.edu.krd; Mobile: 964 75042 06970)*

(Received: April 2, 2023; Accepted: May 7, 2023)

ABSTRACT

The aim of this research study was to examine and test the prognostic value of liver and renal functions as prospective prognostic markers in lung adenocarcinoma (LUAD). Serum was extracted from blood samples collected from 20 LUAD patients and 20 healthy controls for biochemical analysis of liver and renal function tests. In addition, 110 LUAD samples were examined for epidermal growth factor receptor (EGFR) mutations. Indicating liver injury, the results revealed significant differences between LUAD patients and healthy controls for Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels. In addition, the concentrations of urea and creatinine were substantially higher in LUAD patients compared to healthy controls, indicating renal impairment. In addition, the rate of EGFR mutations was 40%, and there was a strong association between EGFR mutations and liver function tests. The study indicated that liver and renal function tests may serve as valuable prognostic markers for LUAD. If these tests could serve as reliable markers, clinicians might be able to identify patients with a high risk of adverse outcomes and modify treatment accordingly. In addition, the findings suggest that novel therapies that target hepatic and renal problems may improve patient outcomes.

Key words: LUAD patients, liver function test, renal function test

INTRODUCTION

Lung cancer (LC) is the most common cause of cancer incidence and mortality worldwide, accounting for an estimated two million new cases and 1.8 million deaths annually (Thandra *et al.*, 2021). Lung adenocarcinoma (LUAD) is the most prevalent kind of lung cancer, accounting for around 40% of all occurrences (Kleczko *et al.*, 2019; Naranjo *et al.*, 2022). Advances in targeted treatments and immunotherapy have improved survival results for individuals with advanced lung cancer in recent years (Lahiri *et al.*, 2023; Wang *et al.*, 2023). However, early detection and accurate prognosis remain significant challenges in the treatment of this disease (Li *et al.*, 2022; Ning *et al.*, 2021).

Several studies have examined potential prognostic markers for LUAD, such as tumor stage, histology, molecular biomarkers, and imaging characteristics (Liu *et al.*, 2019; Šutic *et al.*, 2021; Fan *et al.*, 2022). However, hepatic and renal function tests have not been extensively investigated as potential prognostic markers in the overall picture of LUAD. Chemotherapy-induced liver and renal damage may have a major influence on treatment results and patient survival because the liver and kidneys play critical roles in the metabolism and removal of chemotherapeutic drugs (Santos *et al.*, 2020; Mudd and Guddati, 2021). Tests for liver and renal function are also helpful in predicting survival from breast (Evans *et al.*, 2020; Cai *et al.*, 2021), colorectal (Jin *et al.*, 2019; Kitsel *et al.*, 2023) and pancreatic cancers (Qader *et al.*, 2021; Gu *et al.*, 2022).

Therefore, the purpose of this research project was to examine the usefulness of liver and renal function tests as prospective predictors of LUAD. Prior to treatment, patients with LUAD who underwent liver and renal function tests were analyzed retrospectively. This study's findings have major implications for the development of individualized treatment strategies for LUAD patients. If liver and renal function tests serve as useful prognostic markers, clinicians might be able to identify patients with a higher risk of adverse outcomes and modify their treatment accordingly. Furthermore, by better

[1]Department of Medicine, College of Medicine, Hawler Medical University, Erbil, Kurdistan Region, Iraq.

understanding the relationship between liver and renal function and LUAD, the developers may be able to discover novel treatments that target these co-morbidities and improve patient outcomes.

MATERIALS AND METHODS

Blood samples were collected from both LUAD patients and healthy controls. For each patient or volunteer, 6 ml of venous blood was collected. Collected blood samples were placed in gel containing tubes. Serum was extracted after centrifugation of blood sample and extracted serum was stored at -80°C. Twenty serum samples of LUAD patients from Nanakaly hospital, and twenty healthy individual serum samples from Naznaw and Zina Al Dnya medical labs in Erbil city, Kurdistan Region of Iraq were collected. All participants were of Kurdish nationality, 75% males and 25% of females of 45-75 years age were selected. For this purpose, the ethical and regulatory issues related to human specimen collection for research purposes have been performed in compliance with the Declaration of Helsinki; approved by the Human Ethics Research Committee in the reference number (4/S/337) of the College of Science, Salahaddin University, Erbil. All patients were provided signed informed consent declaring to investigate the tissue materials. Before the biochemistry test, serum specimens were visually confirmed, then serum samples analyzed by Cobas 411 machine in Nobel medical lab in Erbil city, Kurdistan Region of Iraq.

For the EGFR mutation test, 110 FFPE samples of Kurdish nationality LUAD patients were used. Of these patients, 77 (70%) were men and 33 (30%) were women, respectively, with the following ages: 13 (11.82%) of the participants were under the age of fifty, followed by 29 (26.36%) in the 51-60 age range, 34 (30.91%) in the 61-70 age range, and 34 (30.91%) over the age of seventy.

EGFR mutation was examined using the therascreen EGFR PCR kit (Qiagen, Inc., Tokyo, Japan) on the Rotor-Gene Q real-time instrument (Corbett Research, Qiagen GmbH) in accordance with the directions in the package insert and as described by Hwang *et al.* (2022). For testing on the Rotor-Gene Q instrument, eight master mixes were created for each specimen. In exon 19 and exon 21, the real-time kit checks for deletions (it detected the presence of deletions but did not distinguish between them). In addition to the mutation reactions, a control gene reaction was included in the kit to assess specimen integrity and quantity. Testing using RT-PCR was completed in approximately 4 h. The EGFR gene analysis methods were previously validated in the laboratory, and negative and positive tissue controls were analyzed routinely for all reactions.

The unpaired t-test was used for statistical analysis of serological data and comparison of LUAD and control groups. All data were subjected to normality tests (D'Agostino and Pearson omnibus normality test, Shapiro-Wilk normality test, and KS normality test). The receiver operating characteristic (ROC) curve was used for calculating the area under the curves (AUC). All values were expressed as mean±SE, and a statistically significant difference was defined at $P<0.05$. GraphPad Prism 9.0 software (GraphPad Software, Inc.) was used for all statistical analyses, calculations and graphic design.

RESULTS AND DISCUSSION

The activity of Alkaline phosphatase (ALP) (U/L), aspartate aminotransferase (AST) (U/L), and Alanine aminotransferase (ALT) (U/L), and the serum concentration of urea (mg/dl) and creatinine (mg/dl) were significantly higher in LUAD patients than in healthy individuals (Fig. 1-3A and 5-6A, and Table 1). In addition, the serum concentration of total bilirubin (TB) (mg/dL) did not differ between LUAD and control patients (Fig. 4). In addition, ALP, AST, and ALT were the most effective biomarkers for the early detection of LUAD, as shown in (Fig. 1-6B and Table 1), with AUC values of 0.948, 0.993 and 1, respectively. Furthermore, with AUC values of 0.719, 0.771 and 0.821, TB, urea, and creatinine were considered moderate indicators for detecting LUAD.

The liver is a vital organ in the human body that conducts several vital biological processes such as protein synthesis, detoxification and biochemical digestion of food (Schulze *et al.*, 2019). Serum ALT, AST and ALP are liver enzymes, while bilirubin is a byproduct of heme metabolism that travels through the liver before being eliminated from the body

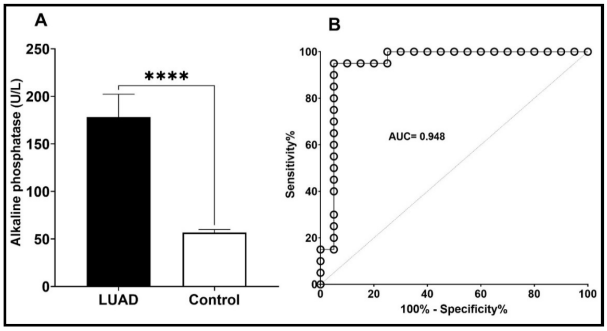

Fig. 1. ALP activity in patients with LUAD. (A) A significant increase in ALP activity in LUAD patients as compared to healthy controls and (B) The ROC curve for ALP activity in LUAD patients.

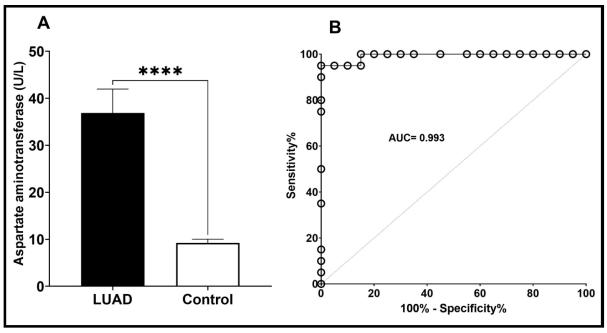

Fig. 2. AST activity in patients with LUAD. (A) A significant increase in AST activity in LUAD patients as compared to healthy controls and (B) The ROC curve for AST activity in LUAD patients.

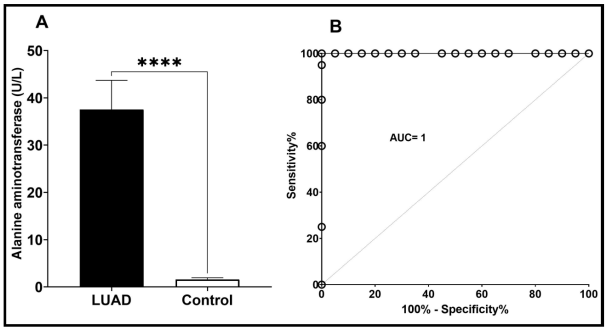

Fig. 3. ALT activity in patients with LUAD. (A) A significant increase in ALT activity in LUAD patients as compared to healthy controls and (B) The ROC curve for ALT activity in LUAD patients.

(Newsome *et al.*, 2018). These findings suggest that ALP, AST and ALT activity levels might be used as a predictor and indications of LUAD.

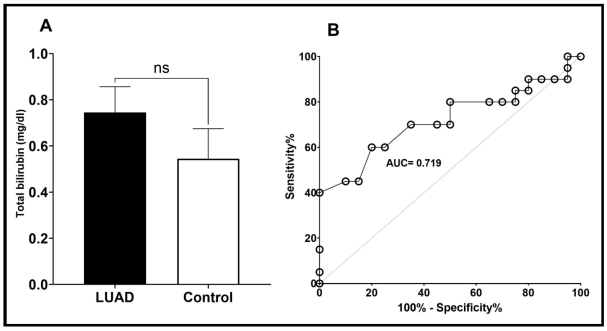

Fig. 4. The TB concentration in LUAD patients. (A) TB levels differ significantly between LUAD patients and healthy controls and (B) The ROC curve for TB levels in LUAD patients.

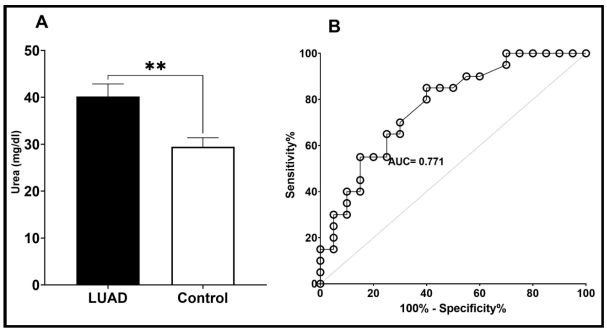

Fig. 5. The urea concentration in LUAD patients. (A) LUAD patients have a significantly greater urea level than healthy controls and (B) The ROC curve for urea level in LUAD patients.

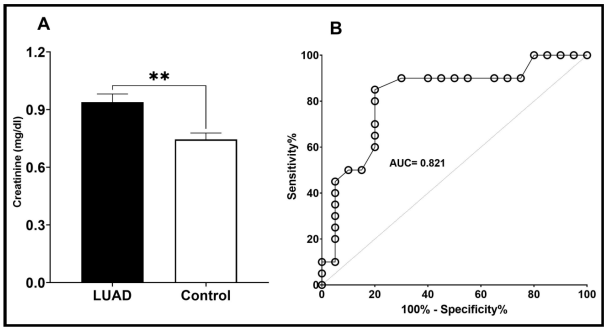

Fig. 6. The creatinine concentration in LUAD patients. (A) LUAD patients have a significantly greater creatinine level than healthy controls and (B) The ROC curve for creatinine level in LUAD patients.

The AST/ALT ratio is a promising biomarker for assessing health and long-term mortality, particularly in cancer patients (Chen *et al.*, 2022). An elevated preoperative serum AST

Table 1. Comparison of liver and renal function test serum levels and area under the curve between control and LUAD patients

		ALP	AST	ALT	TB	Urea	Creatinine
LUAD	Mean±SE	178.3±23.92	36.85±5.119	37.55±6.161	0.746±0.111	40.16±2.683	0.939±0.043
Control		56.6±3.315	9.2±0.82	1.6±0.343	0.545±0.13	29.45±1.934	0.746±0.033
Area under the curve		0.948	0.993	1	0.719	0.771	0.821

levels were associated with a good clinical outcome in non-small cell lung cancer (NSCLC) patients (Chen *et al.*, 2016) and the possibility of albumin-to-alkaline phosphatase ratio (AAPR) as a prognostic indicator for progression-free survival and overall survival in EGFR mutated advanced NSCLC patients treated with first-line first-generation EGFR-TKIs (Gan *et al.*, 2022). Meanwhile, Song *et al.* (2018) observed an inverse relationship between blood bilirubin levels and LC risk.

Kidneys are essential organs that aid in waste removal via blood filtration and homeostasis by controlling fluid balance in the body (Imenez Silva and Mohebbi, 2022). Urea and creatinine are byproducts of protein metabolism that are filtered in the kidneys and serve as an indication of renal function; hence, renal function tests are regarded as a critical step for LC patients (Peng *et al.*, 2021). In the current study, both urea and creatine concentrations increased in LUAD patients. The mortality prediction of LC patients with higher blood urea levels (Orwick *et al.*, 2023) and blood creatinine as a possible biomarker of skeletal muscle atrophy in NSCLC patients (das Neves *et al.*, 2021).

There were 14 (12.73%) samples with PD-L1 positive that expressed 50% or more PD-L1 protein among the 110 LUAD patients. While 8 (7.27%) of the samples had EGFR mutations, including two tumors with exon 19 deletions, two tumors with exon 19 point mutations, two tumors with exon 21 point mutation (c.2573T>G p.(Leu858Arg), and two tumors with exon 21 point mutation (p.L858R).

Lung adenocarcinoma with EGFR mutations account for 10-15% of cases, and these mutations are associated with heightened sensitivity to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib and osimertinib. EGFR TKIs have been demonstrated to increase overall survival and progression-free survival in LUAD patients with EGFR mutations. In order to guide treatment decisions and improve patient outcomes, EGFR mutation testing is essential (Wu *et al.*, 2019). Exons 18, 19, 20, and/or 21 are the most potential sites for EGFR mutations. Exon-19 deletion (Del19) and exon-21 mutation are the two most common activating mutations (Liu *et al.*, 2020). Only one study (Huang *et al.*, 2018) found that eNOS 894 G/T variants were significantly associated with EGFR mutation types of LUAD, specifically exon 19 in-frame deletion, and that this could be used to predict tumor invasiveness and response to therapy.

CONCLUSION

EGFR mutation status is linked with liver and renal function parameters in LUAD patients. These findings suggest that liver and renal function tests could serve as valuable as predictors for LUAD, allowing clinicians to identify patients at a higher risk for poor outcomes and adapt their treatment accordingly. Furthermore, a better understanding of the connection between LUAD and hepatic and renal function could lead to the development of novel treatments that target these complications while improving the health of patients. More study is needed to validate these findings and determine their clinical relevance.

REFERENCES

Cai, J. H., Zheng, J. H., Lin, X. Q., Lin, W. X., Zou, J., Chen, Y. K., Li, Z. Y. and Chen, Y. X. (2021). Individualized treatment of breast cancer with chronic renal failure: A case report and review of literature. *World J. Clin. Cases* **9**: 10345-10354. *10.12998/wjcc.v9.i33.10345*.

Chen, S. L., Xue, N., Wu, M. T., Chen, H., He, X., Li, J. P., Liu, W. L. and Dai, S. Q. (2016). Influence of preoperative serum aspartate aminotransferase (AST) level on the prognosis of patients with non-small cell lung cancer. *Int. J. Mol. Sci.* **17**. *10.3390/ijms17091474*.

Chen, W., Wang, W., Zhou, L., Zhou, J., He, L., Li, J., Xu, X., Wang, J. and Wang, L. (2022). Elevated AST/ALT ratio is associated with all-cause mortality and cancer incident. *J. Clin. Lab. Anal.* **36**: e24356. *10.1002/jcla.24356.*

das Neves, W., Alves, C. R. R., de Souza, Borges A. P. and de Castro, G. Jr. (2021). Serum creatinine as a potential biomarker of skeletal muscle atrophy in non-small cell lung cancer patients. *Front. Physiol.* **12**: 625417. *10.3389/fphys.2021.625417.*

Evans, A., Petty, R. and Macaskill, J. (2020). Why is renal impairment associated with poorer cancer specific survival in breast cancer patients?: A comparison with patients with other comorbidities. *Int. J. Clin. Oncol.* **25**: 1786-1792. *10.1007/s10147-020-01733-7.*

Fan, J., DeFina, S. M. and Wang, H. (2022). Prognostic value of selected histologic features for lung squamous cell carcinoma. *Explor. Res. Hypothesis Med.* **7**: 165-168. *10.14218/erhm.2021.00071.*

Gan, Y., Ren, J., Xian J,., Yu, H., Jin, J., Li, D. and Li ,W. (2022). Prognostic value of albumin-to-alkaline phosphatase ratio for EGFR-mutated advanced non-small-cell lung cancer patients treated with first-line EGFR-TKIs: A large population-based study and literature review. *Int. J. Gen. Med.* **15**: 3405-3416. *10.2147/ijgm. S348912.*

Gu, X., Wu, J., Liu, X., Hong, Y., Wu, Y. and Tian, Y. (2022). Role of serum creatinine levels in prognostic risk stratification of prostate cancer patients. *Med. Sci. Monit.* **28**: *e937100. 10.12659/msm.937100.*

Huang, C. Y., Hsieh, M. J., Wu, W. J., Chiang, W. L., Liu, T. C., Yang, S. F. and Tsao, T. C. (2018). Association of endothelial nitric oxide synthase (eNOS) polymorphisms with EGFR-mutated lung adenocarcinoma in Taiwan. *J. Cancer.* **9**: 2518-2524. *10.7150/jca.25824.*

Hwang, C. C., Hsieh, T. Y., Yeh, K. Y., Chen, T. P., Hua, C. C., Chang, L. C. and Chen, J. R. (2022). A rare epidermal growth factor receptor (EGFR) gene mutation in small cell lung carcinoma patients. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.* **166**: 274-279. *10.5507/bp.2022. 007.*

Imenez Silva, P. H. and Mohebbi, N. (2022). Kidney metabolism and acid-base control: Back to the basics. *Pflugers Arch.* **474**: 919-934. *10.1007/s00424-022-02696-6.*

Jin, L. J., Chen, W. B., Zhang, X. Y., Bai, J., Zhao, H. C. and Wang, Z. Y. (2019). Analysis of factors potentially predicting prognosis of colorectal cancer. *World J. Gastrointest. Oncol.* **11**: 1206-1217. *10.4251/wjgo.v11. i12.1206.*

Kitsel, Y., Cooke, T., Sotirchos, V. and Sofocleous, C. T. (2023). Colorectal cancer liver metastases: Genomics and biomarkers with focus on local therapies. *Cancers* **15**: 1679. *10.3390/cancers15061679.*

Kleczko, E. K., Kwak, J. W., Schenk, E. L. and Nemenoff, R. A. (2019). Targeting the complement pathway as a therapeutic strategy in lung cancer. *Front. Immunol.* **10**: 954. *10.3389/fimmu.2019.00954.*

Lahiri, A., Maji, A., Potdar, P. D., Singh, N., Parikh, P., Bisht, B., Mukherjee, A. and Paul, M. K. (2023). Lung cancer immunotherapy: Progress, pitfalls and promises. *Mol. Cancer* **22**: 40. *10.1186/s12943-023-01740-y.*

Li, C., Wang, H., Jiang, Y., Fu, W., Liu, X., Zhong, R., Cheng, B., Zhu, F., Xiang, Y., He, J. and Liang, W. (2022). Advances in lung cancer screening and early detection. *Cancer Biol. Med.* **19**: 591-608. *10.20892/ j.issn.2095-3941.2021.0690.*

Liu, C., Huang, Q., Ma, W., Qi, L., Wang, Y., Qu, T., Sun, L., Sun, B., Meng, B. and Cao, W. (2019). A combination of tumor and molecular markers predicts a poor prognosis in lung adenocarcinoma. *Int. J. Clin. Exp. Pathol.* **12**: 1690-1701.

Liu, G., Xu, Z., Ge, Y., Jiang, B., Groen, H., Vliegenthart, R. and Xie, X. (2020). 3D radiomics predicts EGFR mutation, exon-19 deletion and exon-21 L858R mutation in lung adenocarcinoma. *Transl. Lung Cancer Res.* **9**: 1212-1224. *10.21037/tlcr-20-122.*

Mudd, T. W. and Guddati, A. K. (2021). Management of hepatotoxicity of chemotherapy and targeted agents. *Am. J. Cancer Res.* **11**: 3461-3474.

Naranjo, S., Cabana, C. M., LaFave, L. M., Romero, R., Shanahan, S. L., Bhutkar, A., Westcott, P. M. K., Schenkel, J. M., Ghosh, A., Liao, L. Z., Del Priore, I., Yang, D. and Jacks, T. (2022). Modelling diverse genetic subtypes of lung adenocarcinoma with a next-generation alveolar type 2 organoid platform. *Genes Dev.* **36**: 936-949. *10.1101/ gad.349659.122.*

Newsome, P. N., Cramb, R., Davison, S. M., Dillon, J. F., Foulerton, M., Godfrey, E. M., Hall, R., Harrower, U., Hudson, M., Langford, A., Mackie, A., Mitchell-Thain, R., Sennett, K., Sheron, N. C., Verne, J., Walmsley, M. and Yeoman, A. (2018). Guidelines on the management of abnormal liver blood tests. *Gut* **67**: 06-19. *10.1136/gutjnl-2017-314924.*

Ning, J., Ge T., Jiang, M., Jia, K., Wang, L., Li, W., Chen, B., Liu, Y., Wang, H., Zhao, S. and He, Y. (2021). Early diagnosis of lung cancer: Which is the optimal choice? *Aging (Albany NY)* **13**: 6214-6227. *10.18632/ aging.202504.*

Orwick, A., Sears, S. M., Sharp, C. N., Doll, M. A., Shah, P. P., Beverly, L. J. and Siskind, L. J. (2023). Lung cancer-kidney cross talk induces kidney injury, interstitial fibrosis, and enhances cisplatin-induced nephrotoxicity. *Am. J. Physiol.-Renal Physiol.* **324**: F287-F300. *10.1152/ ajprenal.00317.2022.*

Peng, X., Huang, Y., Fu, H., Zhang, Z., He, A. and Luo, R. (2021). Prognostic value of blood urea nitrogen to serum albumin ratio in intensive care unit patients with lung cancer. *Int. J. Gen. Med.* **14**: 7349-7359. *10.2147/ijgm.s337822.*

Qader, G., Aali, M., Smail, S. W., Mahmood, K., Hasan, B., K M. A., Rahman, D. B., Qadir, F. A., Mohammad, D. K., Najmuldeen, H. H., Rahman, F. M., Ahmad, S. I., Salih, N. S., Khdhr, Z. M., Mohammed, B. A., Majeed, A. M., Hasan, X. M., Khidhir, B. H., Muhammad, E. S., Muhamadsalih, B. A., Hasan, S. K., Hamad, A. J., Esmail, Z. K., Ismael, C. M., Husaen, S. M., Abdulla, C. A., Hussen, B. M., Housein, Z., Shekha, M. and Salihi, A. (2021). Cardiac, hepatic and renal dysfunction and IL-18 polymorphism in breast, colorectal and prostate cancer patients. *Asian Pac. J. Cancer Prev.* **22**: 131-137. *10.31557/apjcp.2021.22.1.131.*

Santos, M. L. C., de Brito, B. B., da Silva, F. A. F., Botelho, A. and de Melo, F. F. (2020). Nephrotoxicity in cancer treatment: An overview. *World J. Clin. Oncol.* **11**: 190-204. *10.5306/wjco.v11.i4.190.*

Schulze, R. J., Schott, M. B., Casey, C. A., Tuma, P. L. and McNiven, M. A. (2019). The cell biology of the hepatocyte: A membrane trafficking machine. *J. Cell Biol.* **218**: 2096-2112. *10.1083/jcb.201903090.*

Song, Y. J., Gao, X. H., Hong, Y. Q. and Wang, L. X. (2018). Direct bilirubin levels are prognostic in non-small cell lung cancer. *Oncotarget* **9**: 892-900. *10.18632/oncotarget.23184.*

Šutic, M., Vukic, A., Baranašic, J., Försti, A., Džubur, F., Samaržija, M., Jakopovic, M., Brcic, L. and Kneževic, J. (2021). Diagnostic, predictive and prognostic biomarkers in non-small cell lung cancer (NSCLC) management. *J. Pers. Med.* **11**: 1102. *10.3390/jpm11111102.*

Thandra, K. C., Barsouk, A., Saginala, K., Aluru, J. S. and Barsouk, A. (2021). Epidemiology of lung cancer. *Contemp. Oncol. (Pozn)* **25**: 45-52. *10.5114/wo.2021.103829.*

Wang, Q., Su, C. and Zhou, C. (2023). Recent advances in immunotherapy for lung cancer. *Cancer Innovation* **2**: 18-24. *https://doi.org/10.1002/cai2.55.*

Wu, Y. L., Planchard, D., Lu, S., Sun, H., Yamamoto, N., Kim, D. W., Tan, D. S. W., Yang, J. C., Azrif, M., Mitsudomi, T., Park, K., Soo, R. A., Chang, J. W. C., Alip, A., Peters, S. and Douillard, J. Y. (2019). Pan-Asian adapted clinical practice guidelines for the management of patients with metastatic non-small-cell lung cancer: A CSCO-ESMO initiative endorsed by JSMO, KSMO, MOS, SSO and TOS. *Ann. Oncol.* **30**: 171-210. *10.1093/annonc/mdy554.*