ISSN 2218-4333 (online)

# World Journal of *Clinical Oncology*

*World J Clin Oncol* 2023 November 24; 14(11): 445-548

Published by Baishideng Publishing Group Inc

World Journal of Clinical Oncology

Contents

Monthly Volume 14 Number 11 November 24, 2023

## ABOUT COVER



## AIMS AND SCOPE

The primary aim of *World Journal of Clinical Oncology* (*WJCO*, *World J Clin Oncol*) is to provide scholars and readers from various fields of oncology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

*WJCO* mainly publishes articles reporting research results and findings obtained in the field of oncology and covering a wide range of topics including art of oncology, biology of neoplasia, breast cancer, cancer prevention and control, cancer-related complications, diagnosis in oncology, gastrointestinal cancer, genetic testing for cancer, gynecologic cancer, head and neck cancer, hematologic malignancy, lung cancer, melanoma, molecular oncology, neurooncology, palliative and supportive care, pediatric oncology, surgical oncology, translational oncology, and urologic oncology.

## INDEXING/ABSTRACTING

The *WJCO* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJCO* as 2.8; IF without journal self cites: 2.8; 5-year IF: 3.0; Journal Citation Indicator: 0.36.

## RESPONSIBLE EDITORS FOR THIS ISSUE

**Production Editor:** *Xiang-Di Zhang*; **Production Department Director:** *Xu Guo*; **Editorial Office Director:** *Xu Guo*.

**NAME OF JOURNAL**
*World Journal of Clinical Oncology*

**ISSN**
ISSN 2218-4333 (online)

**LAUNCH DATE**
November 10, 2010

**FREQUENCY**
Monthly

**EDITORS-IN-CHIEF**
Hiten RH Patel, Stephen Safe, Jian-Hua Mao, Ken H Young

**EDITORIAL BOARD MEMBERS**
https://www.wjgnet.com/2218-4333/editorialboard.htm

**PUBLICATION DATE**
November 24, 2023



**INSTRUCTIONS TO AUTHORS**
https://www.wjgnet.com/bpg/gerinfo/204

**GUIDELINES FOR ETHICS DOCUMENTS**
https://www.wjgnet.com/bpg/GerInfo/287

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**
https://www.wjgnet.com/bpg/gerinfo/240

**PUBLICATION ETHICS**
https://www.wjgnet.com/bpg/GerInfo/288

**PUBLICATION MISCONDUCT**
https://www.wjgnet.com/bpg/gerinfo/208

**ARTICLE PROCESSING CHARGE**
https://www.wjgnet.com/bpg/gerinfo/242

**STEPS FOR SUBMITTING MANUSCRIPTS**
https://www.wjgnet.com/bpg/GerInfo/239

**ONLINE SUBMISSION**
https://www.f6publishing.com

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

*World J Clin Oncol* 2023 November 24; 14(11): 445-458

DOI: 10.5306/wjco.v14.i11.445

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

# Analysis of clinicopathological features and prognostic factors of breast cancer brain metastasis

Yu-Rui Chen, Zu-Xin Xu, Li-Xin Jiang, Zhi-Wei Dong, Peng-Fei Yu, Zhi Zhang, Guo-Li Gu

**Specialty type:** Oncology

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

**P-Reviewer:** King MC, United States; Skarping I, Sweden

**Received:** September 7, 2023
**Peer-review started:** September 7, 2023
**First decision:** September 20, 2023
**Revised:** September 29, 2023
**Accepted:** October 16, 2023
**Article in press:** October 16, 2023
**Published online:** November 24, 2023

**Yu-Rui Chen, Li-Xin Jiang,** Department of General Surgery, Air Force Clinical College, China Medical University, Beijing 100142, China

**Zu-Xin Xu, Guo-Li Gu,** Department of General Surgery, Fifth Clinical College (Air Force Clinical College) of Anhui Medical University, Beijing 100142, China

**Zhi-Wei Dong, Peng-Fei Yu, Zhi Zhang, Guo-Li Gu,** Department of General Surgery, Air Force Medical Center, Air Force Clinical College of China Medical University, Beijing 100142, China

**Corresponding author:** Guo-Li Gu, Doctor, MD, Chief Doctor, Department of General Surgery, Air Force Medical Center, Air Force Clinical College of China Medical University, No. 30 Fucheng Road, Haidian District, Beijing 100142, China. kzggl@163.com

## Abstract

*BACKGROUND*

Breast cancer (BC) has become the most common malignancy in women. The incidence and detection rates of BC brain metastasis (BCBM) have increased with the progress of imaging, multidisciplinary treatment techniques and the extension of survival time of BC patients. BM seriously affects the quality of life and survival prognosis of BC patients. Therefore, clinical research on the clinicopathological features and prognostic factors of BCBM is valuable. By analyzing the clinicopathological parameters of BCBM patients, and assessing the risk factors and prognostic indicators, we can perform hierarchical diagnosis and treatment on the high-risk population of BCBM, and achieve clinical benefits of early diagnosis and treatment.

*AIM*

To explore the clinicopathological features and prognostic factors of BCBM, and provide references for diagnosis, treatment and management of BCBM.

*METHODS*

The clinicopathological data of 68 BCBM patients admitted to the Air Force Medical Center, Chinese People's Liberation Army (formerly Air Force General Hospital) from 2000 to 2022 were collected. Another 136 BC patients without BM were matched at a ratio of 1:2 based on the age and site of onset for retrospective analysis. Categorical data were subjected to $\chi^2$ test or Fisher's exact probability test, and the variables with $P < 0.05$ in the univariate Cox proportional hazards

model were incorporated into the multivariate model to identify high-risk factors and independent prognostic factors of BCBM, with a hazard ratio (HR) > 1 suggesting poor prognostic factors. The survival time of patients was estimated by the Kaplan–Meier method, and overall survival was compared between groups by log-rank test.

***RESULTS***

Multivariate Cox regression analysis showed that patients with stage III/IV tumor at initial diagnosis [HR: 5.58, 95% confidence interval (CI): 1.99–15.68], lung metastasis (HR: 24.18, 95%CI: 6.40–91.43), human epidermal growth factor receptor 2 (HER2)-overexpressing BC and triple-negative BC were more prone to BM. As can be seen from the prognostic data, 52 of the 68 BCBM patients had died by the end of follow-up, and the median time from diagnosis of BC to the occurrence of BM and from the occurrence of BM to death or last follow-up was 33.5 and 14 mo, respectively. It was confirmed by multivariate Cox regression analysis that patients with neurological symptoms (HR: 1.923, 95%CI: 1.005–3.680), with bone metastasis (HR: 2.011, 95%CI: 1.056-3.831), and BM of HER2-overexpressing and triple-negative BC had shorter survival time.

***CONCLUSION***

HER2-overexpressing, triple-negative BC, late tumor stage and lung metastasis are risk factors of BM. The presence of neurological symptoms, bone metastasis, and molecular type are influencing prognosis factors of BCBM.

**Key Words:** Breast cancer; Brain metastasis; Clinicopathological features; High-risk factors; Prognostic analysis



**Core Tip:** We aimed to identify the high-risk factors of breast cancer brain metastasis (BCBM) and conducted prognostic analyses. Sixty-eight BCBM patients diagnosed and treated in the Air Force Medical Center in 2000–2022 were enrolled. Patients with human epidermal growth factor receptor 2 overexpressing and triple-negative breast cancer were more prone to BM and had shorter survival time. Late tumor stage and lung metastasis were independent risk factors for BM. The presence or absence of neurological symptoms and bone metastasis, and molecular type were independent prognostic factors for BCBM. Early screening of high-risk patients for BM helps improve survival rate.

**Citation:** Chen YR, Xu ZX, Jiang LX, Dong ZW, Yu PF, Zhang Z, Gu GL. Analysis of clinicopathological features and prognostic factors of breast cancer brain metastasis. *World J Clin Oncol* 2023; 14(11): 445-458
**URL:** https://www.wjgnet.com/2218-4333/full/v14/i11/445.htm
**DOI:** https://dx.doi.org/10.5306/wjco.v14.i11.445

## INTRODUCTION

Breast cancer (BC) has become the malignancy with the highest morbidity rate in women[1]. The overall survival (OS) of BC patients has been prolonged with the popularization of universal screening and advances in treatment and management. The proportion of patients with BC brain metastasis (BM) (BCBM) is about 15%[2], which increases with the extension of OS[3]. BM seriously threatens the life expectancy and quality of life of BC patients, and leads to poor prognosis, with a median OS of only 7.4 mo[4]. At present, medical surveillance of the brain is not regarded as a routine follow-up item for BC patients in China and globally. BM has an insidious onset, and most patients are not given targeted diagnosis and treatment of the brain until clinical symptoms emerge, thus losing the best opportunity of diagnosis and treatment and, affecting the survival rate. Therefore, clinical research on the clinicopathological features and prognostic factors of BCBM is required. To identify the clinicopathological features and prognostic factors of BCBM, guide targeted medical monitoring and intervention and raise the survival rate of BCBM patients, 68 BCBM patients were screened from 2238 BC patients admitted to our center from 2000 to 2022, and their clinical data were retrospectively studied.

## MATERIALS AND METHODS

### *Inclusion and exclusion criteria*

Inclusion criteria: patients pathologically diagnosed with BC in the Air Force Medical Center (formerly Air Force General Hospital) from 2000 to 2022 were retrospectively collected. Patients with BM (including brain parenchymal metastasis and meningeal metastasis) identified by imaging, cytology or histology were selected.

Exclusion criteria: (1) Patients with concomitant malignancy or with a history of malignancy of other origin; (2) patients with incomplete clinical data; (3) patients diagnosed with other neurological diseases; (4) patients complicated with serious fatal clinical diseases; and (5) male patients with BC.

Enrolled cases and follow-up: 2238 BC patients were admitted to the Air Force Medical Center between 2000 to 2022, of whom, 101 (4.5%) developed BM. After ineligible cases were excluded, 68 patients were enrolled. Another 136 BC patients without BM (control group) were matched at a ratio of 1:2 based on the age and tumor site at initial diagnosis. Follow-up was performed by telephone interview, outpatient re-examination and inpatient examination until April 2023.

### Indicators and parameters

Clinicopathological parameters of BC patients were statistically analyzed. Univariate Cox proportional hazards model analysis was performed on age (at diagnosis of BC; menstrual status (at diagnosis of BC); family history of tumors; lymph node stage; primary tumor size; tumor stage at initial diagnosis; estrogen receptor (ER) and progesterone receptor (PR) status; molecular and pathological type; presence or absence of liver, lung and bone metastasis; number of liver, lung and bone metastases; presence or absence of metastasis to other sites; number of metastases to other sites; and whether or not surgical treatment was performed. Covariates with statistical significance were further incorporated into a multivariate Cox proportional hazards model for analysis.

Prognostic indicators: age at diagnosis of BM; time from diagnosis of BC to occurrence of BM; menstrual status at diagnosis of BM; molecular type; presence or absence of liver, lung and bone metastasis; number of liver, lung and bone metastases; presence or absence of metastasis to other sites; number of metastases to other sites; tumor stage at initial diagnosis; lymph node stage; pathological type; presence or absence of symptoms at diagnosis of BM; size and number of BMs; and treatment means for BM (systemic or local therapy).

Parameters of local therapy: surgical resection and radiotherapy [mainly including whole brain radiation therapy (WBRT) and stereotactic radiotherapy (SRT) of brain tumor].

Multidisciplinary treatment (MDT) was defined as systemic therapy combined with radiotherapy or surgery. The presence or absence of symptoms was determined according to whether abnormal vision, ataxia, symptoms of intracranial hypertension (headache, nausea and vomiting, lethargy, *etc*.), motor dysfunction, and paresthesia occurred in patients diagnosed previously with BC.

Parameters of hormone receptor: hormone receptor and human epidermal growth factor receptor 2 (HER2) status was determined by immunohistochemistry. HER2 positive was defined as HER2(3+), or HER2(2+) was positive in *in situ* hybridization test, and HER2 negative was defined as HER2(-), HER2(+), or HER2(2+) was negative in *in situ* hybridization test. The positive threshold of ER and PR in immunohistochemistry was ≥ 1%. Molecular typing of BC was performed according to hormone receptor status and HER2 expression: luminal A type (ER- and/or PR-positive, and HER2-negative); luminal B type (ER- and/or PR-positive, and HER2-positive); HER2-overexpressing type (ER- and PR-negative, and HER2-positive); and triple-negative type (ER-, PR- and HER2-negative). Tumor–node–metastasis (TNM) staging was carried out based on the American Joint Committee on Cancer 8$^{th}$ Edition Staging System.

### Statistical analysis

Numerical data were compared between the two groups by $\chi^2$ test or Fisher's exact probability test. The risk factors associated with BM at initial diagnosis of BC were first subjected to univariate Cox proportional hazards model analysis, and then covariates with $P < 0.05$ (selected by the backward conditional method) were incorporated into the multivariate Cox proportional hazards model. OS, defined as the time from the initial diagnosis of BM to death from any cause, or last follow-up, was compared between the groups using the log-rank test, and the survival time of patients was estimated by the Kaplan–Meier method. In the prognostic analysis of BCBM patients, univariate Cox proportional hazards model analysis was first performed, and then covariates with $P < 0.05$ (selected by the forward conditional method) were incorporated into the multivariate Cox proportional hazards model to identify the covariates related to survival outcomes, with a 95% confidence interval (95%CI). $P < 0.05$ was considered statistically significant. All statistical analyses were conducted with SPSS version 27.0 software (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Tumor characteristics of BCBM patients

Among the 2238 patients with BC, BM was found at the initial diagnosis in two cases (0.089%) and during follow-up in 99 cases (4.42%). In the BCBM group, the median age at diagnosis of BC was 47 (29–69) years; 35 cases (51.5%) were postmenopausal, eight (11.8%) had a family history of malignancy, and most patients (51.5%) had stage III/IV tumors. In terms of molecular type, there were 13 cases (19.1%) of luminal A BC, 22 (32.4%) of luminal B BC, 14 (20.6%) of HER2-overexpressing BC, and 14 (20.6%) of triple-negative BC. Fifty-one cases (75.0%) were pathologically classified as invasive ductal carcinoma. Bone metastasis was the most common (55.9%), followed by lung and liver metastasis. Ten cases (14.7%) developed liver, lung and bone metastasis and BM during the course of disease. Modified radical mastectomy dominated in both groups, and the proportion of patients undergoing neoadjuvant chemotherapy, radiotherapy and HER2-targeted therapy in the BCBM group was higher than that in the non-BCBM group (Table 1).

### Risk factors of BM

Sixty-eight BCBM patients were matched at a ratio of 1:2 with 136 BC patients of the same age and tumor site at initial diagnosis. The median time from the diagnosis of BC to the occurrence of BM was 33.5 (0–181) mo in BCBM patients. The risk factors of BCBM are shown in Table 2. In univariate Cox analysis, lymph node stage; tumor stage at the initial diagnosis; ER status; PR status; molecular type; presence or absence of bone metastasis, liver metastasis and lung

**Table 1 Clinicopathological features and tumor characteristics of patients**

| Item | Non-BCBM group (*n* = 136) [*n* (%)] | BCBM group (*n* = 68) [*n* (%)] | *P* |
|---|---|---|---|
| Age at diagnosis of BC (yr) | | | 0.009 |
| ≤ 35 | 9 (6.6) | 12 (17.6) | |
| 35-55 | 79 (58.1) | 43 (63.2) | |
| > 55 | 48 (35.3) | 13 (19.2) | |
| Menstrual status at diagnosis of BC | | | 0.77 |
| Premenopause | 69 (50.7) | 33 (48.5) | |
| Menopause | 67 (49.3) | 35 (51.5) | |
| Family history of cancer | | | 0.12 |
| None | 112 (82.4) | 60 (88.2) | |
| Other malignancies | 16 (11.8) | 8 (11.8) | |
| BC | 8 (5.9) | 0 (0.0) | |
| Lymph node stage | | | 0.01 |
| N0 | 56 (41.2) | 19 (27.9) | |
| N1 | 43 (31.6) | 14 (20.6) | |
| N2 | 25 (18.4) | 17 (25.0) | |
| N3 | 12 (8.8) | 15 (22.1) | |
| Missing | 0 (0.0) | 3 (4.4) | |
| Tumor size | | | 0.17 |
| T1 | 53 (39.0) | 18 (26.5) | |
| T2 | 67 (49.3) | 37 (54.4) | |
| T3 | 13 (9.6) | 11 (16.2) | |
| T4 | 1 (0.7) | 0 (0.0) | |
| Missing | 2 (1.5) | 2 (2.9) | |
| Tumor stage at the initial diagnosis | | | < 0.001 |
| IA | 29 (21.3) | 3 (4.4) | |
| IIA | 39 (28.7) | 19 (27.9) | |
| IIB | 29 (21.3) | 7 (10.3) | |
| IIIA | 22 (16.2) | 17 (25.0) | |
| IIIB | 2 (1.5) | 0 (0.0) | |
| IIIC | 11 (8.1) | 11 (16.2) | |
| IV | 3 (2.2) | 7 (10.3) | |
| Missing | 1 (0.7) | 4 (5.9) | |
| ER | | | 0.009 |
| Negative | 42 (30.9) | 32 (47.1) | |
| Positive | 94 (69.1) | 32 (47.1) | |
| Missing | 0 (0.0) | 4 (5.9) | |
| PR | | | 0.003 |
| Negative | 52 (38.2) | 39 (57.4) | |
| Positive | 84 (61.8) | 25 (36.8) | |
| Missing | 0 (0.0) | 4 (5.9) | |
| Molecular type | | | < 0.001 |

| | | | |
|---|---|---|---|
| Luminal A type | 71 (52.2) | 13 (19.1) | |
| Luminal B type | 28 (20.6) | 22 (32.4) | |
| HER2-overexpressing type | 18 (13.2) | 14 (20.6) | |
| Triple-negative type | 19 (14.0) | 14 (20.6) | |
| Missing | 0 (0.0) | 5 (7.4) | |
| Pathological type | | | 0.42 |
| Noninvasive carcinoma | 10 (7.4) | 2 (2.9) | |
| Invasive ductal carcinoma | 95 (69.9) | 51 (75.0) | |
| Invasive lobular carcinoma | 5 (3.7) | 2 (2.9) | |
| Others | 26 (19.1) | 8 (11.8) | |
| Missing | 0 (0.0) | 5 (7.4) | |
| Metastasis | | | |
| Bone metastasis | | | < 0.001 |
| Yes | 12 (8.8) | 38 (55.9) | |
| No | 124 (91.2) | 30 (44.1) | |
| Liver metastasis | | | < 0.001 |
| Yes | 7 (5.1) | 20 (29.4) | |
| No | 129 (94.9) | 48 (70.6) | |
| Lung metastasis | | | < 0.001 |
| Yes | 8 (5.9) | 35 (51.5) | |
| No | 128 (94.1) | 33 (48.5) | |
| Number of liver, lung and bone metastases | | | < 0.001 |
| 0 | 123 (90.4) | 21 (30.9) | |
| 1 | 8 (5.9) | 19 (27.9) | |
| 2 | 3 (2.2) | 18 (26.5) | |
| 3 | 2 (1.5) | 10 (14.7) | |
| Number of metastases to other sites | | | < 0.001 |
| 0 | 118 (86.8) | 33 (48.5) | |
| 1 | 15 (11.0) | 24 (35.3) | |
| 2 | 2 (1.5) | 8 (11.8) | |
| 3 | 1 (0.7) | 3 (4.4) | |
| Surgical treatment | | | < 0.001 |
| No | 3 (2.2) | 7 (10.3) | |
| Breast-conserving surgery | 20 (14.7) | 5 (7.4) | |
| Modified radical mastectomy | 106 (77.9) | 43 (63.2) | |
| Others | 7 (5.1) | 13 (19.1) | |
| Radiotherapy | | | 0.006 |
| No | 76 (55.9) | 24 (35.3) | |
| Yes | 60 (44.1) | 44 (64.7) | |
| Chemotherapy | | | |
| Neoadjuvant chemotherapy | | | 0.21 |
| No | 119 (87.5) | 55 (80.9) | |
| Yes | 17 (12.5) | 13 (19.1) | |

| | | | |
|---|---|---|---|
| Anthracyclines | | | < 0.001 |
| No | 28 (20.6) | 35 (51.5) | |
| Yes | 107 (78.7) | 32 (47.1) | |
| Missing | 1 (0.7) | 1 (1.5) | |
| Taxane | | | 0.35 |
| No | 35 (25.7) | 22 (32.4) | |
| Yes | 101 (74.3) | 46 (67.6) | |
| HER2 targeted therapy | | | < 0.001 |
| No | 118 (86.8) | 36 (52.9) | |
| Yes | 18 (13.2) | 32 (47.1) | |

BC: Breast cancer; BCBM: Breast cancer brain metastasis; ER: Estrogen receptor; PR: Progesterone receptor; HER2: Human epidermal growth factor receptor 2.

metastasis; number of bone, liver and lung metastases; number of metastases to other sites; and surgical mode were statistically significant. The above factors were incorporated into multivariate Cox analysis, and it was found that patients with stage III/IV tumor at initial diagnosis [hazard ratio (HR): 5.58, 95%CI: 1.99–15.68], lung metastasis (HR: 24.18, 95%CI: 6.40–91.43), and HER2-overexpressing and triple-negative BC were more prone to BM.

### *Prognostic analysis*

The median age in 68 BCBM patients at diagnosis of BM was 50.5 (30–70) years. Presence or absence of bone metastasis, molecular type, and presence or absence of neurological symptoms at initial diagnosis of BCBM were significant covariates in multivariate Cox analysis. The median time from initial diagnosis of BM to death from any cause or last follow-up was 14 (2–138) mo in the 68 BCBM patients. The survival time of BCBM patients with different molecular types is shown in Figure 1. Of the 68 BCBM patients, 44 (64.7%) were diagnosed with BM due to neurological symptoms; mainly dizziness, which was the initial symptom of 23 (52.3%) patients with BCBM. In addition, typical symptoms of BM included headache (19 cases), nausea and vomiting (10 cases), walking instability (7 cases), blurred vision (5 cases), memory loss (4 cases) and slow response (3 cases), and they often occurred simultaneously. The median survival time was 12 mo among BCBM patients with neurological symptoms, 30 mo among asymptomatic patients (Figure 2), 14 mo among BCBM patients with bone metastasis, and 23 mo among those without bone metastasis (Figure 3). The relevant results are presented in Table 3.

Of the 68 BCBM patients, four (5.9%) underwent no treatment, 27 (39.7%) underwent MDT with local therapy plus systemic medication, 30 (44.1%) were given local therapy only, and seven (10.3%) were given medication only. The median survival time of patients receiving MDT was 21 mo, which was superior to that of patients receiving medication or local therapy alone. Tumor resection was performed in 11 cases and all of them were treated with postoperative radiotherapy. After BM, 16 patients received HER2-targeted therapy, including trastuzumab single targeted therapy for five cases, trastuzumab plus tyrosine kinase inhibitors (TKIs) for two cases, and capecitabine plus TKI for seven cases, and their median survival time was 17, 23 and 54 mo, respectively. The remaining two patients received trastuzumab + pertuzumab dual-targeted therapy, and they were still alive as of the follow-up endpoint (Table 4).

## DISCUSSION

In this study, the median time from the diagnosis of BC to the occurrence of BCBM was 33.5 (0–181) mo. The risk of BM varied among patients with different molecular subtypes of BC. HER2-overexpressing and triple-negative BC had a high tendency to BM, consistent with previous reports[5]. Patients with advanced stage and lung metastasis were also at high risk of BM. Due to the specificity of the physiological structure of the brain (such as the presence of the blood–brain barrier), there is still a lack of effective intervention means, and BM predicts poor survival outcomes. The results of descriptive statistics, univariate and multivariate Cox proportional hazards model analysis showed that the molecular type, and presence or absence of neurological symptoms and bone metastasis at diagnosis of BM were independent prognosis factors of patients with BM.

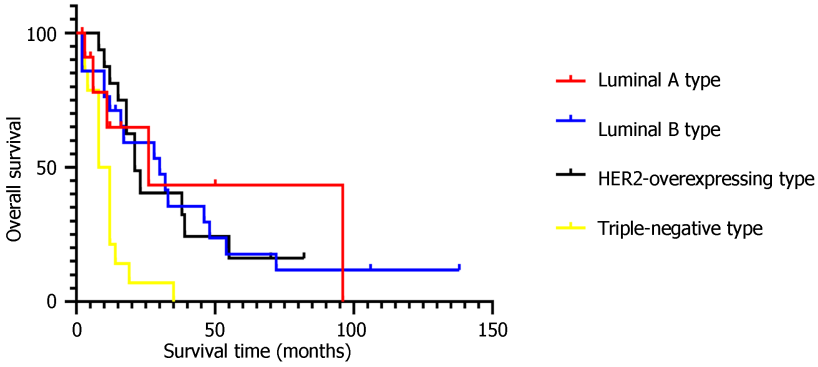
In this study, it was found that HER2-overexpressing and triple-negative types were high-risk molecular types of BCBM. Patients with HER2-overexpressing and triple-negative BC accounted for 20.6% of BM patients, in line with research findings that the incidence rate of BM in HER2-overexpressing type and triple-negative BC is 20%–30%[6]. Sixty-nine studies involving 28 countries on risk factors of BCBM concluded that young age, ER-negative, HER2 overexpression, later tumor stage, histological grade, tumor size, and lymph node metastasis are all independent risk factors of BCBM[7]. Univariate analysis of this study showed that lung, liver and bone metastasis, the number of liver, lung and bone metastases, and the number of metastases to other sites were associated with an increased risk of BM in BC patients. In multivariate analysis, only lung metastasis was statistically significant (HR: 24.18, 95%CI: 6.40–91.43). As shown in

**Table 2 High-risk factors of breast cancer brain metastasis**

| Item | Univariate Cox | | Multivariate Cox | |
|---|---|---|---|---|
| | HR (95%CI) | *P* | HR (95%CI) | *P* |
| Age at diagnosis of BC (yr) | | | | |
| ≤ 35 | Reference | | | |
| > 35 | 0.008 (0-1.89) | 0.083 | | |
| Menstrual status at diagnosis of BC | | | | |
| Premenopause | Reference | | | |
| Menopause | 1.29 (0.52-3.20) | 0.59 | | |
| Family history of cancer | | | | |
| None | Reference | | | |
| Yes | 0.62 (0.26-1.44) | 0.26 | | |
| Lymph node stage | | | | |
| N0-N1 | Reference | | | |
| N2-N3 | 2.77 (1.40-5.48) | 0.004 | NS | |
| Tumor size | | | | |
| T1-T2 | Reference | | | |
| T3-T4 | 1.44 (0.65-3.15) | 0.37 | | |
| Tumor stage at the initial diagnosis | | | | |
| I-II | Reference | | | |
| III-IV | 3.84 (1.89-7.78) | < 0.001 | 5.58 (1.99-15.68) | 0.001 |
| ER | | | | |
| Negative | Reference | | | |
| Positive | 0.49 (0.27-0.87) | 0.015 | NS | |
| PR | | | | |
| Negative | Reference | | | |
| Positive | 0.36 (0.18-0.69) | 0.002 | NS | |
| Molecular type | | | | |
| Luminal A type | Reference | | | |
| Luminal B type | 3.95 (1.71-9.14) | 0.001 | 5.36 (1.61-17.76) | 0.006 |
| HER2-overexpression type | 4.01 (1.61-9.96) | 0.003 | 5.0 (1.30-19.25) | 0.019 |
| Triple-negative type | 4.34 (1.55-12.11) | 0.005 | NS | NS |
| Pathological type | | | | |
| Others | Reference | | | |
| Invasive ductal carcinoma | 1.83 (0.83-4.05) | 0.14 | | |
| Invasive lobular carcinoma | 1.31 (0.22-7.69) | 0. 77 | | |
| Surgical treatment | | | | |
| Others | Reference | | | |
| Modified radical mastectomy | 0.48 (0.26-0.90) | 0.021 | NS | |
| Bone metastasis | | | | |
| No | Reference | | | |
| Yes | 7.19 (3.13-16.52) | < 0.001 | NS | |
| Liver metastasis | | | | |

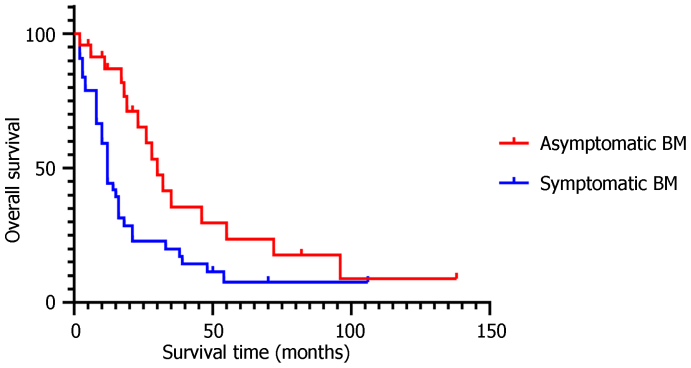
| | | | | |
|---|---|---|---|---|
| No | Reference | | | |
| Yes | 7.44 (2.77-20.01) | < 0.001 | NS | |
| Lung metastasis | | | | |
| No | Reference | | | |
| Yes | 15.87 (5.61-44.89) | < 0.001 | 24.18 (6.40-91.43) | < 0.001 |
| Number of liver, lung and bone metastases | | | | |
| < 2 | Reference | | | |
| ≥ 2 | 17.78 (5.38-58.73) | < 0.001 | NS | |
| Number of metastases to other sites | | | | |
| < 2 | Reference | | | |
| ≥ 2 | 7.33 (2.05-26.27) | 0.002 | NS | |

95%CI: 95% confidence interval; NS: Not statistically significant; HR: Hazard ratio; ER: Estrogen receptor; PR: Progesterone receptor; BC: Breast Cancer; HER2: Human epidermal growth factor receptor 2.




**DOI**: 10.5306/wjco.v14.i11.445 **Copyright** ©The Author(s) 2023.

**Figure 1 Survival analysis of patients with different molecular types.**




**DOI**: 10.5306/wjco.v14.i11.445 **Copyright** ©The Author(s) 2023.

**Figure 2 Survival analysis of patients with or without symptoms.**

previous studies, cyclooxygenase 2 and epidermal growth factor receptor ligand can serve as mediators of cancer cells passing through the blood–brain barrier[8], and they are associated with lung cancer infiltration, which may account for the predisposition of patients with lung metastases to BM[9]. Lymph node status and age at diagnosis of BC have been verified to be associated with the risk of BM[10], but no clear association was observed in this study. The later tumor stage often corresponds to later seeking of treatment, greater tumor burden, greater lymph node infiltration, and increased risk of metastasis and recurrence, including BM[11].

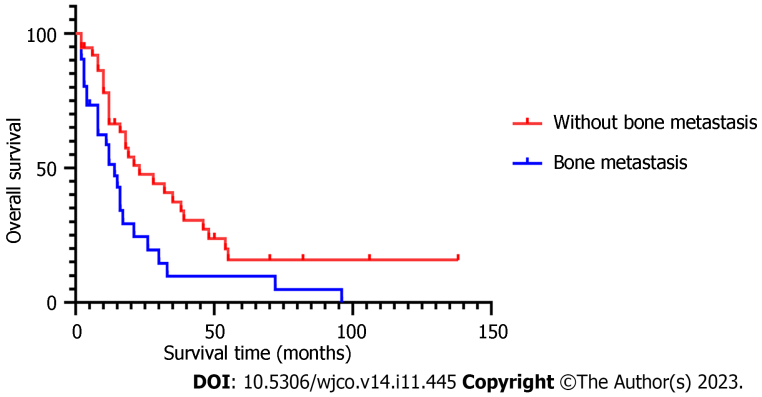
Clinically, the treatment regimen is often selected based on the number, location and size of BMs, the patient's physical condition, extracranial metastasis, and the possible benefits of treatment. SRT is mainly applied to BM patients with < 4

**Table 3 Prognostic factors of breast cancer brain metastasis**

| Item | Univariate Cox | | Multivariate Cox | |
|---|---|---|---|---|
| | HR (95%CI) | *P* | HR (95%CI) | *P* |
| Age at diagnosis of BM (yr) | | | | |
| ≤ 35 | Reference | | | |
| > 35 | 1.033 (0.315-3.385) | 0.957 | | |
| Time from BC to BM (mo) | | | | |
| ≤ 24 | Reference | | | |
| > 24 | 0.896 (0.482-1.664) | 0.727 | | |
| Menstrual status at diagnosis of BM | | | | |
| Premenopause | Reference | | | |
| Menopause | 0.685 (0.331-1.416) | 0.307 | | |
| Molecular type | | | | |
| Luminal A type | 0.253 (0.087-0.730) | 0.011 | 0.227 (0.074-0.693) | 0.009 |
| Luminal B type | 0.279 (0.128-0.607) | 0.001 | 0.293 (0.129-0.663) | 0.003 |
| HER2-overexpressing type | 0.274 (0.121-0.618) | 0.002 | 0.319 (0.135-0.754) | 0.009 |
| Triple-negative type | Reference | | | |
| Bone metastasis | | | | |
| No | Reference | | | |
| Yes | 1.980 (1.135-3.453) | 0.016 | 2.011 (1.056-3.831) | 0.034 |
| Liver metastasis | | | | |
| No | Reference | | | |
| Yes | 1.121 (0.626-2.007) | 0.701 | | |
| Lung metastasis | | | | |
| No | Reference | | | |
| Yes | 1.616 (0.929-2.810) | 0.089 | | |
| Number of liver, lung and bone metastases | | | | |
| ≤ 2 | Reference | | | |
| > 2 | 1.548 (0.894-2.682) | 0.119 | | |
| Number of metastases to other sites | | | | |
| < 2 | Reference | | | |
| ≥ 2 | 1.425 (0.711-2.858) | 0.318 | | |
| Tumor stage | | | | |
| I-II | Reference | | | |
| III-IV | 0.813 (0.466-1.419) | 0.466 | | |
| Lymph node stage | | | | |
| N0-N1 | Reference | | | |
| N2-N3 | 0.843 (0.486-1.464) | 0.545 | | |
| Pathological type | | | | |
| Others | 0.287 (0.073-1.133) | 0.075 | | |
| Invasive lobular carcinoma | Reference | | | |
| Invasive ductal carcinoma | 0.208 (0.060-0.725) | 0.014 | NS | |
| Symptoms | | | | |

| | | | | |
|---|---|---|---|---|
| No | Reference | | | |
| Yes | 2.171 (1.191-3.959) | 0.011 | 1.923 (1.005-3.680) | 0.048 |
| Size of BM (cm) | | | | |
| ≤ 3 | Reference | | | |
| > 3 | 0.803 (0.449-1.439) | 0.462 | | |
| Number of BM | | | | |
| ≤ 3 | Reference | | | |
| > 3 | 1.248 (0.721-2.160) | 0.428 | | |
| Surgery for BM | | | | |
| No | Reference | | | |
| Yes | 0.744 (0.348-1.591) | 0.446 | | |
| Treatment after BM | | | | |
| Local therapy | Reference | | | |
| Medication alone | 1.531 (0.638-3.674) | 0.064 | | |
| Systemic therapy | 0.748 (0.317-1.765) | 0.507 | | |
| Local therapy | | | | |
| SRT | 1.350 (0.513-3.549) | 0.543 | | |
| WBRT | 1.600 (0.633-4.043) | 0.320 | | |
| Both | 1.935 (0.680-5.506) | 0.216 | | |
| Neither | Reference | | | |

95%CI: 95% confidence interval; NS: Not statistically significant; HR: Hazard ratio; BC: Breast Cancer; BM□Brain Metastasis; WBRT: Whole brain radiation therapy; SRT: Stereotactic radiotherapy; HER2: Human epidermal growth factor receptor 2.




**DOI**: 10.5306/wjco.v14.i11.445 **Copyright** ©The Author(s) 2023.

**Figure 3 Survival analysis of patients with or without bone metastasis.**

tumor lesions and brain tumor < 3 cm. In this study, the median survival time was 12 mo among patients treated with WBRT alone, 16 mo among patients treated with SRT alone, 18 mo among patients treated with WBRT + SRT, and 18 mo among patients undergoing surgery for brain tumors. Consistent with this study, a study showed that WBRT produces no OS benefit but significant neurocognitive decline[12]. Of the 68 patients, 24 had BMs ≤ 3 cm, and 22 patients (91.7%) underwent SRT. SRT has become the first-line treatment for BC patients with small brain metastases[13].

Despite advances in early diagnosis and effective treatment, distant metastasis remains an important factor threatening the survival of BC patients[14]. In this study, the median survival time of patients with luminal A, luminal B, HER2-overexpressing and triple-negative BC was 26, 30, 21 and 8 mo, respectively. The prognosis of HER2-overexpressing and triple-negative BC patients was poor. It is difficult for most drugs to reach effective blood concentration in the brain due to the presence of the blood–brain barrier. With the progress made in novel targeted drugs in the past decade, breakthroughs have been made in the treatment of HER2-positive BC. Trastuzumab, pertuzumab, antibody-drug conjugate and TKIs (lapatinib, pyrotinib, *etc*.) have been marketed successively, extending the OS of HER2-positive patients. Studies have revealed that lapatinib + capecitabine can delay the time of WBRT[15]. The PERMEATE study

**Table 4 Treatment methods of brain metastasis**

| Item | Patients with BM (*n* = 68) | Median survival time (mo) |
|---|---|---|
| Medication | 7 | 12 |
| MDT | 27 | 21 |
| Local therapy alone | 30 | 15 |
| Neither | 4 | |
| Local therapy | *n* = 57 | |
| SRT alone | 19 | 16 |
| Surgery + SRT | 6 | 19 |
| SRT + WBRT | 10 | 18 |
| Surgery + WBRT | 3 | 30 |
| WBRT | 17 | 12 |
| Surgery + SRT + WBRT | 2 | 21 |
| HER2 targeted therapy | *n* = 16 | |
| Trastuzumab | 5 | 17 |
| Trastuzumab + pertuzumab | 2 | |
| Trastuzumab + TKI | 2 | 23 |
| Capecitabine + TKI | 7 | 54 |

MDT: Multidisciplinary treatment; WBRT: Whole brain radiation therapy; SRT: Stereotactic radiotherapy; TKI: Trastuzumab plus tyrosine kinase inhibitors; BM: Brain Metastasis; HER2: Human epidermal growth factor receptor 2.

explored the efficacy of lapatinib plus capecitabine in the treatment of patients with HER2-positive BM, and found that the objective response rate of brain can reach 74.6% in patients undergoing the initial neurological radiotherapy[16]. In this study, the median survival time of patients given capecitabine plus TKIs was 54 mo. As of April 2023, the median survival time of HER2-positive BCBM patients enrolled in PERMEATE is up to 31.5 mo[16]. In this study, the patients treated from 2000 to 2022 were enrolled, and trastuzumab was marketed in China since 2002, so some patients did not undergo targeted therapy due to early drug shortage and high treatment cost, which may be one of the reasons for poor prognosis of HER2-overexpressing BC patients. In view of the current effective treatment, HER2-positive patients should be more active in undergoing brain examination and timely treatment.

The results of multivariate Cox regression analysis showed that the HR value of BM patients with neurological symptoms was 1.923 times (95%CI: 1.005–3.680) that of asymptomatic patients ($P$ = 0.048), suggesting that the presence of neurological symptoms at diagnosis is associated with a poor prognosis. The median survival time was 30 mo for asymptomatic patients and 12 mo for symptomatic patients. A study involving long-term survivors of BCBM showed that asymptomatic BCBM patients are more likely to achieve long-term survival of > 15 mo[17]. In this study, asymptomatic patients with BM had a smaller diameter of brain tumor (≤ 3 cm: 95.8% *vs* 75.0%, $P$ = 0.031), fewer brain metastases (≤ 3: 58.3% *vs* 43.2%, $P$ = 0.232), and younger age at diagnosis of BC (≤ 35 years: 95.8% *vs* 75%, $P$ = 0.031) than symptomatic patients, consistent with the characteristics (young age, small diameter of brain tumor, small number of brain tumors, and good physical status without neurological burden) of asymptomatic patients[18]. Due to smaller size and number of brain tumors of asymptomatic patients, a wider range of treatment options is available, and SRT is preferred, which is associated with milder neurological impairment[17]. A prospective study on 1196 asymptomatic patients with BCBM treated with SRT also confirmed that compared with symptomatic patients, asymptomatic BM patients have good neurological status and reduced neurological mortality[18]. A previous study showed that early detection of BM is associated with longer OS as compared to symptomatic BM[19]. Considering the health economic benefits, however, brain screening has not been utilized as routine follow-up for BC patients in China and globally. According to an American study, regular head magnetic resonance imaging screening can save an average of USD 1326 in treatment costs for each BCBM patient. Although there are differences in the medical system between the USA and China, some references are still provided for the formulation of follow-up plans for BC patients in China[20].

The presence of extracranial metastases was identified as an independent prognostic factor in the 2020 version of the Breast Graded Prognostic Assessment. Bone metastasis is the most common mode of metastasis, accounting for 60%–70% of metastatic BC[21]. In this study, 38 (55.9%) patients with BM had bone metastasis. The prognosis of BC bone metastases is better than that of other distant metastases, with a median survival time of 36 mo[22], and the survival rate significantly declines when complicated with metastasis to other sites[23]. In a cohort of 1330 triple-negative BC patients with BM, the median OS was 13 mo (95%CI: 11.5–14.5 mo) for bone metastasis alone and 8 mo (95%CI: 6.3–9.7 mo) for bone metastasis + metastasis to other sites[24]. In this study, the median survival time was 14 mo for patients with BM + bone metastasis

and 23 mo for patients without bone metastasis, and the HR value in multivariate analysis was 2.011 times that of patients without bone metastasis (95%CI: 1.056–3.831) ($P$ = 0.034). Common skeletal-related events in patients with bone metastasis include pathological fracture, spinal cord compression, hypercalcemia, and bone pain[21], resulting in limited daily activity and reduced quality of life. In this study, the median age of 68 BCBM patients was 50.5 (30–70) years. The mean menopausal age of Chinese women is 49.5 years, and menopausal women are prone to osteoporosis as well as an increased risk of pathological fracture when complicated with bone metastasis. Fracture-induced immobilization, decrease of physical performance score, and long-term complications related to immobilization (thromboembolism, respiratory tract infection, *etc*.) are all reasons for the decrease in survival rate. In the case of paralysis caused by spinal cord compression, the survival rate declines further, with a 1-year survival rate of only 17.6%[25].

There were some limitations to this study, such as its small sample size and single-center, retrospective nature, which inevitably introduced selection and recall bias. The clinical data collected from 2000 to 2022 may have had missing follow-up data. Notably, the absence of pathological results and imaging data for some patients diagnosed before 2010 may have influenced the analytical outcomes. Moreover, shifts in clinical guidelines, the introduction of new medications, and advances in the healthcare economy during this period have altered the diagnostic criteria, treatment modalities, and patient management approaches. Other potential confounding factors like the genotype of BC patients were not extensively analyzed due to their low detection rate. The lack of prospective studies introduced uncertainty in pinpointing the precise onset time for patients with asymptomatic BM. Currently, the efficacy of early screening for BCBM, its potential for early intervention, and whether early detection enhances survival rate, necessitate validation through multicenter prospective studies.

## CONCLUSION

Stage III/IV, lung metastasis, and HER2-overexpressing and triple-negative types are high-risk factors of BCBM, and aggressive monitoring of BM is required. It is recommended that BC patients undergo regular brain examinations since detecting and treating BC before neurological symptoms emerge may produce better outcomes. Patients with central nervous system symptoms, HER2-overexpressing and triple-negative BC, and bone metastasis have poor prognosis.

## ARTICLE HIGHLIGHTS

### *Research background*

Breast cancer (BC) brain metastasis (BCBM) is an important influencing factor of the long-term prognosis of BC patients. Triple-negative type is a known risk factor of BCBM, suggesting that patients with different clinicopathological types have differences in survival time.

### *Research motivation*

To explore the influencing factors of the occurrence, development, and prognostic survival of BCBM to provide references for the diagnosis, treatment and management of patients with BM.

### *Research objectives*

To perform more aggressive screening of high-risk patients of BCBM, benefiting patients from early diagnosis and treatment, and producing better outcomes.

### *Research methods*

Clinicopathological data of 68 BCBM patients admitted to the Air Force Medical Center (formerly Air Force General Hospital) between 2000 and 2022 and another 136 matched BC patients were retrospectively analyzed. The high-risk factors and prognostic factors of BCBM patients were analyzed by univariate and multivariate Cox regression analyses, the survival time of patients was estimated by the Kaplan–Meier method, and the overall survival was compared between two groups by log-rank test.

### *Research results*

Stage III/IV, lung metastasis, and human epidermal growth factor receptor 2 (HER2)-overexpressing and triple-negative types were high-risk factors of BCBM. Patients with neurological symptoms, bone metastasis, and HER2-overexpressing and triple-negative BC had poor prognosis, requiring more effective treatment to improve the survival rate of these patients.

### *Research conclusions*

The prognosis of BCBM is poor. Active follow-up and screening of the brain should be performed for patients with late stage at initial treatment, lung metastasis, and HER2-overexpressing and triple-negative BC. The median survival time of patients with neurological symptoms, bone metastasis, and HER2-overexpressing and triple-negative BC significantly decreases.

### *Research perspectives*

More multicenter large studies on BCBM are required to provide references for the management of high-risk patients, and more effective treatment is needed to raise the survival rate of patients with poor prognosis.

## ACKNOWLEDGEMENTS

We thank all medical staff and technicians of dialysis centers who agreed to participate in this study.

## FOOTNOTES

**Co-first authors:** Yu-Rui Chen and Zu-Xin Xu.

**Author contributions:** Chen YR and Xu ZX contributed equally to this study, and share joint first authorship; Gu GL designed the research; Chen YR and Xu ZX conceived the study, developed the methodology, collected data, analyzed and interpreted data, and wrote the manuscript; Jiang LX, Zhang Z, Yu PF and Dong ZW collected and analyzed the clinical data; Gu GL and Dong ZW provided the material support, and revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

**Supported by** Outstanding Young Talents Program of Air Force Medical Center, PLA, No. 22BJQN004; Clinical Program of Air Force Medical University, No. Xiaoke2022-07.

**Institutional review board statement:** The study was approved by the ethics committee of the Air Force Medical Center, Chinese People's Liberation Army (Beijing, China).

**Informed consent statement:** Consent was obtained from patients or their relatives for publication of this report.

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author kzggl@163.com.



**Country/Territory of origin:** China

**ORCID number:** Yu-Rui Chen 0000-0002-2101-9435; Zu-Xin Xu 0000-0002-5068-9024; Li-Xin Jiang 0000-0002-8361-4280; Zhi-Wei Dong 0000-0001-7009-9331; Peng-Fei Yu 0000-0002-0528-1839; Zhi Zhang 0000-0001-5870-1940; Guo-Li Gu 0000-0002-9998-047X.

**S-Editor:** Lin C
**L-Editor:** Kerr C
**P-Editor:** Chen YX

## REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **DeSantis CE**, Ma J, Gaudet MM, Newman LA, Miller KD, Goding Sauer A, Jemal A, Siegel RL. Breast cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 438-451 [PMID: 31577379 DOI: 10.3322/caac.21583]

3 **Bollig-Fischer A**, Michelhaugh S, Ali-Fehmi R, Mittal S. The molecular genomics of metastatic brain tumours. *OA Mol Oncol* 2013; **1** [PMID: 25400938 DOI: 10.13172/2052-9635-1-1-759]

4 **Witzel I**, Laakmann E, Weide R, Neunhöffer T, Park-Simon TJ, Schmidt M, Fasching PA, Hesse T, Polasik A, Mohrmann S, Würschmidt F, Schem C, Bechtner C, Würstlein R, Fehm T, Möbus V, Burchardi N, Loibl S, Müller V. Treatment and outcomes of patients in the Brain Metastases in Breast Cancer Network Registry. *Eur J Cancer* 2018; **102**: 1-9 [PMID: 30099223 DOI: 10.1016/j.ejca.2018.07.004]

5 **Kennecke H**, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, Nielsen TO, Gelmon K. Metastatic behavior of breast cancer subtypes. *J Clin Oncol* 2010; **28**: 3271-3277 [PMID: 20498394 DOI: 10.1200/JCO.2009.25.9820]

6 **Kuksis M**, Gao Y, Tran W, Hoey C, Kiss A, Komorowski AS, Dhaliwal AJ, Sahgal A, Das S, Chan KK, Jerzak KJ. The incidence of brain metastases among patients with metastatic breast cancer: a systematic review and meta-analysis. *Neuro Oncol* 2021; **23**: 894-904 [PMID: 33367836 DOI: 10.1093/neuonc/noaa285]

7 **Koniali L**, Hadjisavvas A, Constantinidou A, Christodoulou K, Christou Y, Demetriou C, Panayides AS, Pitris C, Pattichis CS, Zamba-Papanicolaou E, Kyriacou K. Risk factors for breast cancer brain metastases: a systematic review. *Oncotarget* 2020; **11**: 650-669 [PMID: 32110283 DOI: 10.18632/oncotarget.27453]

8 **Bos PD**, Zhang XH, Nadal C, Shu W, Gomis RR, Nguyen DX, Minn AJ, van de Vijver MJ, Gerald WL, Foekens JA, Massagué J. Genes that mediate breast cancer metastasis to the brain. *Nature* 2009; **459**: 1005-1009 [PMID: 19421193 DOI: 10.1038/nature08021]

9 **Gupta GP**, Nguyen DX, Chiang AC, Bos PD, Kim JY, Nadal C, Gomis RR, Manova-Todorova K, Massagué J. Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature* 2007; **446**: 765-770 [PMID: 17429393 DOI: 10.1038/nature05760]

10 **Aversa C**, Rossi V, Geuna E, Martinello R, Milani A, Redana S, Valabrega G, Aglietta M, Montemurro F. Metastatic breast cancer subtypes and central nervous system metastases. *Breast* 2014; **23**: 623-628 [PMID: 24993072 DOI: 10.1016/j.breast.2014.06.009]

11 **Azim HA**, Abdel-Malek R, Kassem L. Predicting Brain Metastasis in Breast Cancer Patients: Stage Versus Biology. *Clin Breast Cancer* 2018; **18**: e187-e195 [PMID: 28888580 DOI: 10.1016/j.clbc.2017.08.004]

12 **Yamamoto M**, Serizawa T, Shuto T, Akabane A, Higuchi Y, Kawagishi J, Yamanaka K, Sato Y, Jokura H, Yomo S, Nagano O, Kenai H, Moriki A, Suzuki S, Kida Y, Iwai Y, Hayashi M, Onishi H, Gondo M, Sato M, Akimitsu T, Kubo K, Kikuchi Y, Shibasaki T, Goto T, Takanashi M, Mori Y, Takakura K, Saeki N, Kunieda E, Aoyama H, Momoshima S, Tsuchiya K. Stereotactic radiosurgery for patients with multiple brain metastases (JLGK0901): a multi-institutional prospective observational study. *Lancet Oncol* 2014; **15**: 387-395 [PMID: 24621620 DOI: 10.1016/S1470-2045(14)70061-0]

13 **Churilla TM**, Ballman KV, Brown PD, Twohy EL, Jaeckle K, Farace E, Cerhan JH, Anderson SK, Carrero XW, Garces YI, Barker FG 2nd, Deming R, Dixon JG, Burri SH, Chung C, Ménard C, Stieber VW, Pollock BE, Galanis E, Buckner JC, Asher AL. Stereotactic Radiosurgery With or Without Whole-Brain Radiation Therapy for Limited Brain Metastases: A Secondary Analysis of the North Central Cancer Treatment Group N0574 (Alliance) Randomized Controlled Trial. *Int J Radiat Oncol Biol Phys* 2017; **99**: 1173-1178 [PMID: 28939223 DOI: 10.1016/j.ijrobp.2017.07.045]

14 **Liang Y**, Zhang H, Song X, Yang Q. Metastatic heterogeneity of breast cancer: Molecular mechanism and potential therapeutic targets. *Semin Cancer Biol* 2020; **60**: 14-27 [PMID: 31421262 DOI: 10.1016/j.semcancer.2019.08.012]

15 **Petrelli F**, Ghidini M, Lonati V, Tomasello G, Borgonovo K, Ghilardi M, Cabiddu M, Barni S. The efficacy of lapatinib and capecitabine in HER-2 positive breast cancer with brain metastases: A systematic review and pooled analysis. *Eur J Cancer* 2017; **84**: 141-148 [PMID: 28810186 DOI: 10.1016/j.ejca.2017.07.024]

16 **Yan M**, Ouyang Q, Sun T, Niu L, Yang J, Li L, Song Y, Hao C, Chen Z, Orlandi A, Ishii N, Takabe K, Franceschini G, Ricci F, Verschraegen C, Liu Z, Zhang M, Lv H, Liu L, Yang X, Xiao H, Gao Z, Li X, Dong F, Chen X, Qiao J, Zhang G. Pyrotinib plus capecitabine for patients with human epidermal growth factor receptor 2-positive breast cancer and brain metastases (PERMEATE): a multicentre, single-arm, two-cohort, phase 2 trial. *Lancet Oncol* 2022; **23**: 353-361 [PMID: 35085506 DOI: 10.1016/S1470-2045(21)00716-6]

17 **Riecke K**, Müller V, Neunhöffer T, Park-Simon TW, Weide R, Polasik A, Schmidt M, Puppe J, Mundhenke C, Lübbe K, Hesse T, Thill M, Wuerstlein R, Denkert C, Decker T, Fehm T, Nekljudova V, Rey J, Loibl S, Laakmann E, Witzel I. Long-term survival of breast cancer patients with brain metastases: subanalysis of the BMBC registry. *ESMO Open* 2023; **8**: 101213 [PMID: 37075697 DOI: 10.1016/j.esmoop.2023.101213]

18 **Gao YK**, Kuksis M, Id Said B, Chehade R, Kiss A, Tran W, Sickandar F, Sahgal A, Warner E, Soliman H, Jerzak KJ. Treatment Patterns and Outcomes of Women with Symptomatic and Asymptomatic Breast Cancer Brain Metastases: A Single-Center Retrospective Study. *Oncologist* 2021; **26**: e1951-e1961 [PMID: 34506676 DOI: 10.1002/onco.13965]

19 **Laakmann E**, Witzel I, Neunhöffer T, Weide R, Schmidt M, Park-Simon TW, Möbus V, Mundhenke C, Polasik A, Lübbe K, Hesse T, Riecke K, Thill M, Fasching PA, Denkert C, Fehm T, Nekljudova V, Rey J, Loibl S, Müller V. Characteristics and Clinical Outcome of Breast Cancer Patients with Asymptomatic Brain Metastases. *Cancers (Basel)* 2020; **12** [PMID: 32998430 DOI: 10.3390/cancers12102787]

20 **Lester SC**, Taksler GB, Kuremsky JG, Lucas JT Jr, Ayala-Peacock DN, Randolph DM 2nd, Bourland JD, Laxton AW, Tatter SB, Chan MD. Clinical and economic outcomes of patients with brain metastases based on symptoms: an argument for routine brain screening of those treated with upfront radiosurgery. *Cancer* 2014; **120**: 433-441 [PMID: 24452675 DOI: 10.1002/cncr.28422]

21 **Xiong Z**, Deng G, Huang X, Li X, Xie X, Wang J, Shuang Z, Wang X. Bone metastasis pattern in initial metastatic breast cancer: a population-based study. *Cancer Manag Res* 2018; **10**: 287-295 [PMID: 29467583 DOI: 10.2147/CMAR.S155524]

22 **Wang R**, Zhu Y, Liu X, Liao X, He J, Niu L. The Clinicopathological features and survival outcomes of patients with different metastatic sites in stage IV breast cancer. *BMC Cancer* 2019; **19**: 1091 [PMID: 31718602 DOI: 10.1186/s12885-019-6311-z]

23 **Plunkett TA**, Smith P, Rubens RD. Risk of complications from bone metastases in breast cancer. implications for management. *Eur J Cancer* 2000; **36**: 476-482 [PMID: 10717523 DOI: 10.1016/s0959-8049(99)00331-7]

24 **Yao Y**, Chu Y, Xu B, Hu Q, Song Q. Risk factors for distant metastasis of patients with primary triple-negative breast cancer. *Biosci Rep* 2019; **39** [PMID: 31113872 DOI: 10.1042/BSR20190288]

25 **Oka H**, Kondoh T, Seichi A, Hozumi T, Nakamura K. Incidence and prognostic factors of Japanese breast cancer patients with bone metastasis. *J Orthop Sci* 2006; **11**: 13-19 [PMID: 16437343 DOI: 10.1007/s00776-005-0966-9]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

*World J Clin Oncol* 2023 November 24; 14(11): 459-470

DOI: 10.5306/wjco.v14.i11.459

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Retrospective Study

# Clinical study of standard residual liver volume and transient elastography in predicting poor prognosis of patients after hemihepatectomy

Zhi-Qiang Yue, Peng Zhang, Shuai Yan, Lin-Ling Ju, Hui-Xuan Wang, Liu-Xia Yuan, Lin Chen, Jin-Zhu Wu, Ya-Li Cao

**Specialty type:** Oncology

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

**P-Reviewer:** Sahin TT, Turkey

**Received:** September 4, 2023
**Peer-review started:** September 4, 2023
**First decision:** September 19, 2023
**Revised:** September 28, 2023
**Accepted:** October 16, 2023
**Article in press:** October 16, 2023
**Published online:** November 24, 2023

**Zhi-Qiang Yue, Shuai Yan,** Medical School of Nantong University, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, Nantong 226000, Jiangsu Province, China

**Peng Zhang, Jin-Zhu Wu,** Department of Hepatobiliary Surgery, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, Nantong 226000, Jiangsu Province, China

**Lin-Ling Ju, Hui-Xuan Wang, Liu-Xia Yuan, Lin Chen,** Nantong Institute of Liver Disease, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, Nantong 226000, Jiangsu Province, China

**Ya-Li Cao,** Preventive Health Department, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, Nantong 226000, Jiangsu Province, China

**Corresponding author:** Jin-Zhu Wu, MMed, Associate Chief Physician, Department of Hepatobiliary Surgery, Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University, No. 60 Qingnian Middle Road, Chongchuan District, Nantong 226000, Jiangsu Province, China. 39235978@qq.com

## Abstract

*BACKGROUND*

Liver cancer resection, especially in patients with hemihepatectomy or extended hemihepatectomy, often leads to poor prognosis, such as liver insufficiency and even liver failure and death, because the standard residual liver volume (SRLV) cannot be fully compensated after surgery.

*AIM*

To explore the risk factors of poor prognosis after hemihepatectomy for hepatocellular carcinoma and evaluate the application value of related prognostic approaches.

*METHODS*

The clinical data of 35 patients with primary liver cancer in Nantong Third People's Hospital from February 2016 to July 2020 were retrospectively analyzed.

The receiver operating characteristic curve was created using medcac19.0.4 to compare the critical values of the SRLV in different stages of liver fibrosis after hemihepatectomy with those of liver dysfunction after hemihepatectomy. It was constructed by combining the Child-Pugh score to evaluate its application value in predicting liver function compensation.

*RESULTS*

The liver stiffness measure (LSM) value and SRLV were associated with liver dysfunction after hemihepatectomy. Logistic regression analysis showed that an LSM value ≥ 25 kPa [odds ratio (OR) = 6.254, $P < 0.05$] and SRLV ≤ 0.290 L/m$^2$ (OR = 5.686, $P < 0.05$) were independent risk factors for postoperative liver dysfunction. The accuracy of the new liver reserve evaluation model for predicting postoperative liver function was higher than that of the Child-Pugh score ($P < 0.05$).

*CONCLUSION*

SRLV and LSM values can be used to evaluate the safety of hemihepatectomy. The new liver reserve evaluation model has good application potential in the evaluation of liver reserve function after hemihepatectomy.

**Key Words:** Hepatocellular carcinoma; Hemihepatectomy; Prognosis; Standard residual liver volume; Liver stiffness measure value



**Core Tip:** To explore the risk factors and predictive methods of poor prognosis after hemihepatectomy for hepatocellular carcinoma and evaluate its application value. The clinical data of 35 patients with primary liver cancer were retrospectively analyzed. The critical values of standard residual liver volume (SRLV) in different stages of liver fibrosis after hemihepatectomy were compared with those of liver dysfunction after hemihepatectomy. We found that SRLV and liver stiffness measure values can be used to evaluate the safety of hemihepatectomy.

**Citation:** Yue ZQ, Zhang P, Yan S, Ju LL, Wang HX, Yuan LX, Chen L, Wu JZ, Cao YL. Clinical study of standard residual liver volume and transient elastography in predicting poor prognosis of patients after hemihepatectomy. *World J Clin Oncol* 2023; 14(11): 459-470

**URL:** https://www.wjgnet.com/2218-4333/full/v14/i11/459.htm

**DOI:** https://dx.doi.org/10.5306/wjco.v14.i11.459

## INTRODUCTION

Liver cancer is a malignant tumor associated with high mortality worldwide[1-3]. Hepatocellular carcinoma (HCC), one of the main types of liver cancer, is often found in advanced stages and cannot be cured[4-6]. As a highly heterogeneous disease, HCC mostly develops as a result of hepatitis B cirrhosis[7]. China has the largest number of hepatitis B virus infections in the world; therefore, the number of HCC patients accounts for more than half of the total number of HCCs worldwide[8]. To date, surgical resection and liver transplantation are still effective treatments for HCC; however, due to the shortage of liver sources, the main treatment for HCC is surgery[9]. Liver cancer resection, especially in patients with hemihepatectomy or extended hemihepatectomy, often leads to poor prognosis, such as liver insufficiency and even liver failure and death, because the standard residual liver volume (SRLV) cannot be fully compensated after surgery[10]. Research suggests that preoperative liver fibrosis and cirrhosis are the main causes[11]. In recent years, an increasing number of studies have shown that the liver stiffness measure (LSM) value is significantly related to the degree of cirrhosis, which can reflect the degree of liver inflammation and fibrosis[12,13]. Accordingly, the purpose of this study was to investigate the risk factors and predictive methods of poor prognosis after hemihepatectomy for HCC and verify whether the changes in liver structure can be reflected by the LSM value and SRLV to assess the liver's compensatory capacity. Finally, we established a liver reserve function evaluation model by combining the LSM value and Child-Pugh scores and evaluated its application value.

## MATERIALS AND METHODS

### *Patient characteristics*

The clinical case data were obtained from 35 HCC patients undergoing hemihepatectomy in the Nantong Third People's Hospital between February 2016 and July 2020, and all patients met the inclusion criteria for this study. The study was approved by the Ethics Committee of the Nantong Third People's Hospital Affiliated with Nantong University. Written informed consent was obtained from all patients before being enrolled in the study. The inclusion criteria were as follows:

(1) According to the China liver cancer staging for the diagnosis and treatment standard of primary liver cancer (2019 edition), the stage of liver cancer was stage Ia, Ib or IIa, the tumor was located only in the left or right half of the liver, and hemihepatectomy was needed; (2) all patients were positive for HBsAg before the operation, and HCC was confirmed by pathology after surgery; (3) liver enhancement computed tomography (CT) was performed before the operation; (4) the LSM value was detected by transient elastography (Fibro Touch) before the operation; and (5) patients had more complete clinical case data. The exclusion criteria were as follows: (1) The patient did not have a standard hemihepatectomy; (2) the postoperative pathology of the patients was confirmed as cholangiocarcinoma or metastatic carcinoma; (3) the patient had a preoperative intervention, ablation, or chemoradiotherapy; (4) secondary operation; (5) other complications affecting liver function before the operation, such as hepatic encephalopathy, abdominal dropsy, and other conditions; and (6) the presence of other malignant tumors or serious diseases.

### Surgical procedure

The patient was placed in a supine position with a soft pad on the high right lumbar back (no pad height was required for left hemihepatectomy), an oblique incision was made at the right abdominal costal margin, approximately 30 cm in length, layer by layer into the abdominal cavity; adhesions were separated, and each connective tissue and ligament of the liver were cut to fully expose the liver. The texture and morphology of the liver and spleen were observed, the completely free left or right lobe of the liver was selected according to the location of the tumor, the lesions that had not been detected before the operation were examined by intraoperative ultrasound, and the abdominal cavity was explored for the presence or absence of tumor implantation and metastasis. Subsequently, the liver hilum was selectively blocked, and the left or right hemiliver was resected, along with the gallbladder removal and extended hemihepatectomy according to the preoperative conditions, with surgical margins generally larger than 1 cm from the tumor margin. The operation area was carefully checked for the presence or absence of bleeding and biliary fistula, an abdominal drainage tube was placed, and each layer of the abdominal wall was closed layer by layer.

### Determination of SRLV

Preoperatively, Philips brilliance CT was used to perform a routine double-phase scan of the patient's liver with a thickness of 1.25 mm. Then, portal vein stage tomography was selected, and rapid liver volume measurement software was used to draw the liver boundary layer-by-layer (the inferior vena cava and gallbladder were avoided) and calculate the total liver volume (TLV). The volume of half of the liver was measured by drainage after the liver was isolated. The body surface area (BSA) was calculated according to the literature[14,15]. Finally, postoperative remnant liver volume (RLV) = TLV-the volume of half of the liver, and SRLV = RLV/BSA.

### Determination of LSM values

According to the measurement requirements of the American Association for the Study of Liver Diseases[16], we measured the LSM value using a liver FibroTouch (FT) device developed by Haskell Medical Technology Company. In detail, the LSM value was measured 10 consecutive times for each patient, the quartile spacing was specified to be less than 30% as the effective measurement, and the median was chosen as the LSM value. All operations were performed by the same physician with extensive experience in the diagnosis of hepatobiliary diseases using ultrasound. A schematic illustration of the measurement results is shown in Figure 1. Then, a new model of liver reserve assessment was constructed according to the combination of the Child-Pugh score and the LSM value (Table 1).

### Evaluation of postoperative hepatic fibrosis

By observing the paraffin sections of the liver under the microscope, we phased fibrosis according to the Scheuer scoring system as follows[17]: S0 stage, no liver fibrosis; S1 stage, liver fibrosis limited to the portal region; S2 stage, liver fibrosis extending to the portal region or portal interval, but the vascular relationship was normal; S3 stage, liver fibrosis with structural changes but not obvious cirrhosis; and S4 stage, cirrhosis (Figure 2).

### Evaluation of postoperative liver function

According to the definition of liver dysfunction after hepatectomy from the International Study Group of Liver Surgery [18], we defined liver dysfunction as the results of a 5-d laboratory examination after hepatectomy that showed elevated international normalized ratio (INR) and total bilirubin (INR > 1.5; total bilirubin > 20.5 mmol/L); additionally, the patient was assessed for liver function, kidney function, respiratory function and the need for special assessment and special clinical treatment.

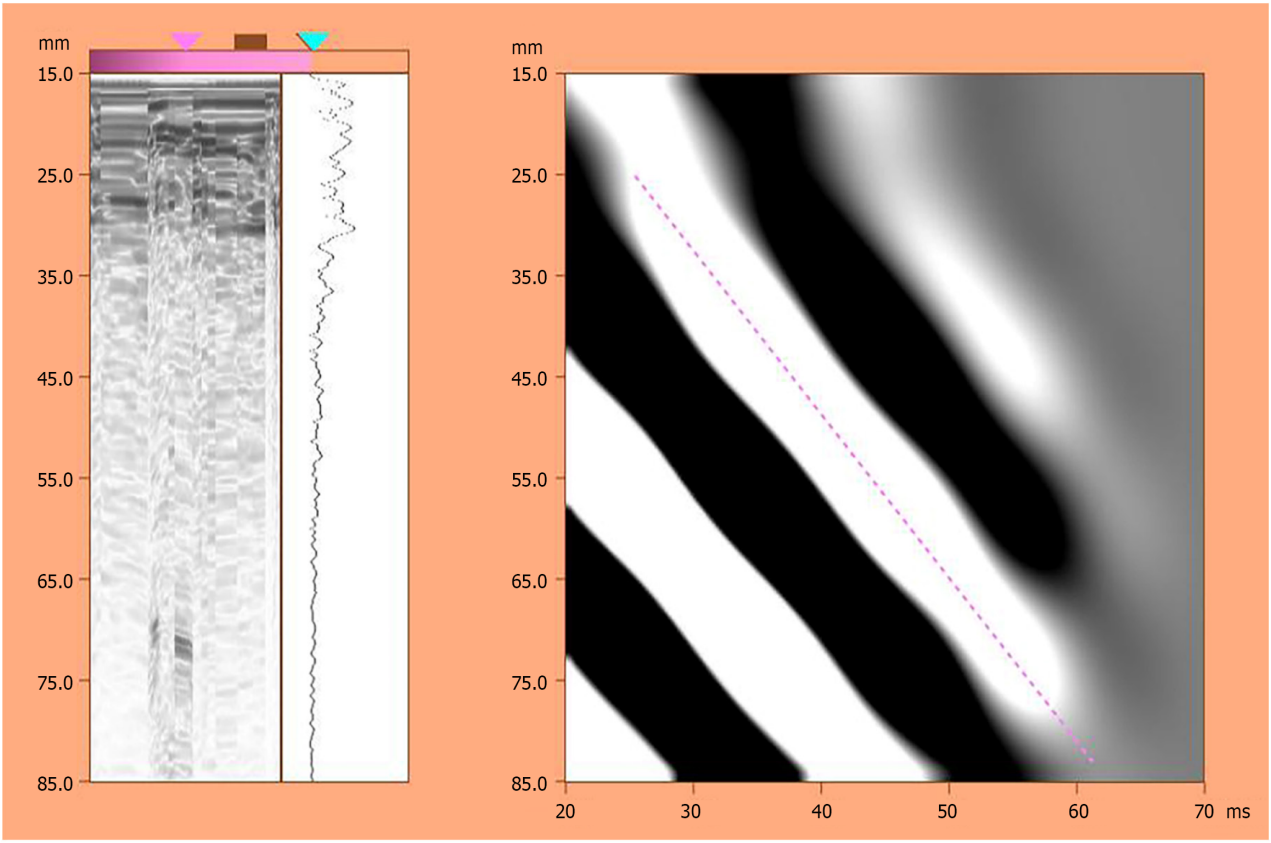
### Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 25.0, and the measurement data were compared using the *t* test or single-factor analysis of variance (ANOVA). The Wilcoxon rank-sum test was used when the variance was uneven, and the Chi square test was used for counting data. Analysis of independent risk factors was completed using unconditional logistic regression. We used medcalc19.0.4 to draw the receiver operating characteristic (ROC) curve of the subjects and analyzed the area under the ROC curve under different factors. $P < 0.05$ was considered statistically significant.

**Table 1 The new liver reserve assessment model**

| Score | 1 | 2 | 3 |
|---|---|---|---|
| HE | No | 1-2 | 3-4 |
| ABD | No | Mild | Moderate to severe |
| TIBL (μmol/L) | < 34 | 34-51 | > 51 |
| ALB (g/L) | > 35 | 28-35 | > 28 |
| Prothrombin Time (secprolonged) | < 15 | 15-17 | > 17 |
| LSM (kPa) | < 15 | 15-25 | > 25 |

Note-scoring criteria: A score less than 9 = grade I; a score between 9 and 12 = grade II; a score greater than 12 = grade III. HE: Hepatic encephalopathy; ABD: Abdominal dropsy; TIBL: Total bilirubin; ALB: Albumin; LSM: Liver stiffness measure.




**DOI**: 10.5306/wjco.v14.i11.459 **Copyright** ©The Author(s) 2023.

**Figure 1 Schematic diagram of transient elastography results of the liver.**

## RESULTS

### *Risk factors for liverdysfunction after hemihepatectomy*

The 35 patients in this study were grouped according to the presence or absence of liver dysfunction after surgery as follows: 12 patients had postoperative liver dysfunction, and 23 patients had no liver dysfunction. Then, the following 25 factors were analyzed. The results showed that the preoperative LSM value and SRLV were correlated with liver dysfunction after hemihepatectomy in HCC patients ($P < 0.05$, Table 2).

### *LSM value and SRLV are independent risk factors for liver dysfunction after hemihepatectomy*

The preoperative LSM value and SRLV were selected as independent variables, and regardless of whether liver dysfunction was selected as the dependent variable, a logistic regression model was developed for analysis. The results showed that the preoperative LSM value and SRLV were independent risk factors for liver dysfunction after hemihepatectomy ($P < 0.05$, Table 3).

**Table 2 Comparison of the clinical features of the surgical safety group and liver dysfunction group (mean ± SD)**

| Variables | Total | Postoperative liver function | | *P* value |
|---|---|---|---|---|
| | | No liver dysfunction | Liver dysfunction | |
| Sex | | | | |
| Male | 19 | 12 | 7 | 1.000 |
| Female | 16 | 11 | 5 | |
| Age (yr) | | | | |
| < 60 | 20 | 14 | 6 | 0.721 |
| ≥ 60 | 15 | 9 | 6 | |
| BMI | | | | |
| < 24 kg/m$^2$ | 21 | 13 | 8 | 0.721 |
| > 24 kg/m$^2$ | 14 | 10 | 4 | |
| BSA (m$^2$) | | 1.71 ± 0.18 | 1.75 ± 0.16 | 0.514 |
| WBC (× 10$^9$/L) | | 5.51 ± 2.39 | 5.19 ± 1.55 | 0.672 |
| RBC (10$^{12}$/L) | | 4.30 ± 0.65 | 4.61 ± 0.44 | 0.146 |
| PLT (× 10$^9$/L) | | 126.17 ± 53.74 | 149.33 ± 79.83 | 0.314 |
| ALB (g/L) | | 40.71 ± 4.23 | 39.63 ± 4.18 | 0.478 |
| Scr (μmol/L) | | 76.98 ± 39.07 | 64.87 ± 10.63 | 0.303 |
| ALT [U/L, M (QR)] | | 67.26 ± 94.41 | 46.58 ± 30.40 | 0.468 |
| AST [U/L, M (QR)] | | 45.61 ± 23.63 | 58.00 ± 68.68 | 0.436 |
| TB [umol/L, M (QR)] | | 15.63 ± 7.20 | 20.99 ± 8.36 | 0.056 |
| GGT [U/L, M (QR)] | | 115.17 ± 112.78 | 124.00 ± 145.78 | 0.844 |
| AFP [ng/mL, M (QR)] | | 9999.23 ± 25773.40 | 8545.66 ± 15372.77 | 0.859 |
| PIVKA-II [μg/L, M (QR)] | | 1799.54 ± 5017.78 | 3574.73 ± 6543.79 | 0.378 |
| PT (s) | | 12.59 ± 1.46 | 12.39 ± 1.04 | 0.682 |
| INR (s) | | 1.10 ± 0.12 | 1.05 ± 0.11 | 0.227 |
| LSM value (kPa) | | 20.34 ± 4.89 | 25.78 ± 5.38 | 0.005[a] |
| ICG R15 (%) | | 7.99 ± 5.13 | 11.96 ± 6.43 | 0.055 |
| SRLV (L/m$^2$) | | 0.349 ± 0.075 | 0.276 ± 0.036 | 0.003[a] |
| Tumor-localizing | | | | |
| Left half liver | 15 | 10 | 5 | 1.000 |
| Right half liver | 20 | 13 | 7 | |
| Tumor diameter [cm, M (QR)] | | 6.63 ± 3.86 | 7.81 ± 4.91 | 0.436 |
| Time of hepatic portal occlusion [min, M (QR)] | | 14.65 ± 19.42 | 15.75 ± 14.10 | 0.864 |
| Intraoperative bleeding [mL, M (QR)] | | 908.70 ± 818.76 | 1541.67 ± 1612.57 | 0.130 |
| Operation time (min) | | 176.30 ± 49.98 | 185.83 ± 72.89 | 0.651 |

[a]*P* < 0.01 *vs* group of safe operation (no liver dysfunction) and liver dysfunction. PIVKA-II was abnormal prothrombin. BMI: Body mass index; BSA: Body surface area; WBC: White blood cell; RBC: Red blood cell; PLT: Platelet; ALB: Albumin; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TB: Total bilirubin; GGT: γ-glutamyl transpeptidase; AFP: Alpha fetoprotein; PT: Prothrombin time; INR: International normalized ratio; LSM: Liver stiffness measure; ICG: Indocyanine green; SRLV: Standard residual liver volume.

### *The critical value of SRLV for different stages of postoperative fibrosis*

The staging results of postoperative liver fibrosis showed 0 cases in the S0 stage, 6 cases in the S1 stage, 14 cases in the S2-S3 stage, and 15 cases in the S4 stage. Then, we compared and analyzed the critical values of SRLV for different stages, and the results showed that the difference in SRLV among the three phases was statistically significant ($P < 0.05$, Table 4). ROC curve analysis showed that the area under the curve for the S2-S3 stage was 0.743, the sensitivity was 0.467, the specificity was 0.100, and the critical value of SRLV was 0.257 L/m$^2$; the area under the curve for the S4 phase was 0.861, the sensitivity was 0.857, the specificity was 0.762, and the critical value of SRLV was 0.311 L/m$^2$ (Figure 3).

### *The critical value of SRLV for postoperative liver dysfunction*

In 12 patients with postoperative liver dysfunction, the staging results of postoperative liver fibrosis showed 0 cases in the S0 stage, 1 case in the S1 stage, 7 cases in the S2-S3 stage, and 4 cases in the S4 stage. Additionally, the corresponding SRLVs were compared and analyzed, and the results showed that the difference in SRLV among the three phases was statistically significant ($P < 0.05$, Table 5). ROC curve analysis showed that the area under the curve for stage S2-S3 was 0.943, the sensitivity was 0.857, the specificity was 0.100, and the safety-critical value for SRLV was 0.285 L/m$^2$; the area under the curve for stage S4 was 0.938, the sensitivity was 0.100, the specificity was 0.750, and the safety-critical value of SRLV was 0.285 L/m$^2$ (Figure 4).

### *Application of a new assessment model in predicting liver dysfunction after hemihepatectomy*

We reviewed and analyzed the clinical data of 35 patients in this study and followed up with the patients. The results showed that there were no postoperative deaths, and all patients were discharged within 3 wk after the operation. Statistical analysis showed that with Child-Pugh score was grade A, the accuracy rate of predicting postoperative liver function compensation was 54.8%; the accuracy rate of grade B was 25.0%. The new model was classified as grade I, and the accuracy rate of predicting postoperative liver function compensation was 100.0%, which was higher than that of the Child-Pugh score ($\chi^2 = 7.452$, $P = 0.007$). Similarly, that of grade II was 91.3%, which was higher than the Child-Pugh score ($\chi^2 = 9.928$, $P = 0.013$). There was a significant difference between the two models in evaluating the prognosis after hemihepatectomy ($P < 0.05$, Table 6).

## DISCUSSION

HCC is one of the most common malignant tumors. With the improvement of the technical level of hepatectomy, the mortality rate after HCC resection has decreased significantly[19-21]. However, the mortality rate is still 5%-8%, especially in patients with hemihepatectomy[21]. The main cause of death after hemihepatectomy is liver failure[22]. The surgical resection range is so large such that the postoperative remnant liver cannot meet the needs of the body; more importantly, doctors lack a comprehensive understanding of the liver reserve function of patients before surgery. As a single evaluation indicator, indocyanine green (ICG) is better than many biochemical indicators. When many conventional liver function indicators have not yet become abnormal in value, the ICG retention rate at 15 min (ICG R15) can reflect liver function damage or occult liver disease in a timely manner[23]. However, ICG has certain limitations and is easily interfered with by factors such as the patient's cooperation ability, liver cell uptake capacity, liver blood flow, bile duct obstruction, bilirubin, *etc*[24,25]. SRLV is a reliable index of preoperative liver reserve function at home and abroad [26,27]. However, considering that HCC patients often have varying degrees of liver fibrosis before the operation, the liver reserve and regeneration function in such patients may vary depending on the extent of liver fibrosis, even if the SRLV is the same; therefore, it is not satisfactory to evaluate liver reserve function only in terms of liver volume. The diagnosis of preoperative liver fibrosis mainly depends on liver histopathological examination; however, because of invasive examination, a low positive rate, difficulty in follow-up, and dynamic detection, the need to consider the wishes of patients and other factors, scholars at home and abroad have explored the use of elastic techniques instead of liver biopsy to assess the extent of liver fibrosis or cirrhosis by measuring the LSM value[17]. Therefore, it is very important to evaluate the safety of hemihepatectomy by correctly staging the degree of liver fibrosis before surgery.

First, in this study, the factors that may be related to liver dysfunction in HCC patients after hemihepatectomy were statistically analyzed. The results showed that preoperative LSM and SRLV were associated with liver dysfunction in HCC patients after hemihepatectomy ($P < 0.05$). Multivariate logistic regression analysis showed that preoperative LSM and SRLV were independent risk factors for liver dysfunction in HCC patients after hemihepatectomy.

Then, according to the Scheuer score standard, we observed the degree of liver fibrosis using microscopy and analyzed the SRLV critical value of different stages of liver fibrosis in all patients and the SRLV critical value of different stages of liver fibrosis in postoperative liver insufficiency cases by ROC curve analysis. The results showed that the critical values of SRLV were 0.257 L/m$^2$ and 0.310 L/m$^2$ in patients with liver fibrosis in stages S2-S3 and S4, respectively, and 0.285 L/m$^2$ in patients with postoperative liver dysfunction. SRLV critical values were similar in both cases, suggesting that it is safe and feasible to predict the SRLV threshold of HCC patients undergoing hemihepatectomy by pathological stages of liver fibrosis. It is suggested that the operation is safe if SRLV > 0.310 L/m$^2$.

At present, the elastic technique has been used to evaluate the degree of liver fibrosis or cirrhosis. It has been widely accepted because of its simplicity, repeatability, noninvasiveness, low cost, and other factors. At present, studies have reported that the sensitivity and specificity of the LSM value to predict the degree of hepatitis cirrhosis are high, and the LSM value is confirmed to be related to complications after partial hepatectomy in patients[28]. However, there is no uniform standard for the patient's disease background, and the operation is limited to only partial or segmental hepatectomy. There is no study on the application of transient elastography to predict the degree of liver fibrosis and

**Table 3 Logistic regression analysis based on preoperative the liver stiffness measure value and standard residual liver volume**

| Independent variables | *P* value | OR | 95%CI |
|---|---|---|---|
| LSM ≥ 25 kPa | 0.032 | 6.254 | 1.172-33.374 |
| SRLV ≤ 290 ML/$m^2$ | 0.048 | 5.686 | 1.017-31.793 |

OR: Odds ratio; CI: Confidence interval; LSM: Liver stiffness measure; SRLV: Standard residual liver volume.

**Table 4 The standard residual liver volume of different stages of liver fibrosis in 35 patients after hemihepatectomy (mean ± SD)**

| Liver fibrosis stage | Number | SRLV (L/$m^2$) | *F* value | *P* value |
|---|---|---|---|---|
| S1 | 6 | 289.43 ± 22.36 | 8.164 | 0.001 |
| S2-S3 | 15 | 290.33 ± 56.70 | | |
| S4 | 14 | 375.53 ± 72.24 | | |

SRLV: Standard residual liver volume.

**Table 5 The standard residual liver volume of different liver fibrosis stages in 12 patients with liver insufficiency after hemihepatectomy**

| Liver fibrosis stage | Number | SRLV (L/$m^2$) | *F* value | *P* value |
|---|---|---|---|---|
| S1 | 1 | 234.20 | 4.768 | 0.039 |
| S2-S3 | 7 | 263.14 ± 31.28 | | |
| S4 | 4 | 308.98 ± 18.02 | | |

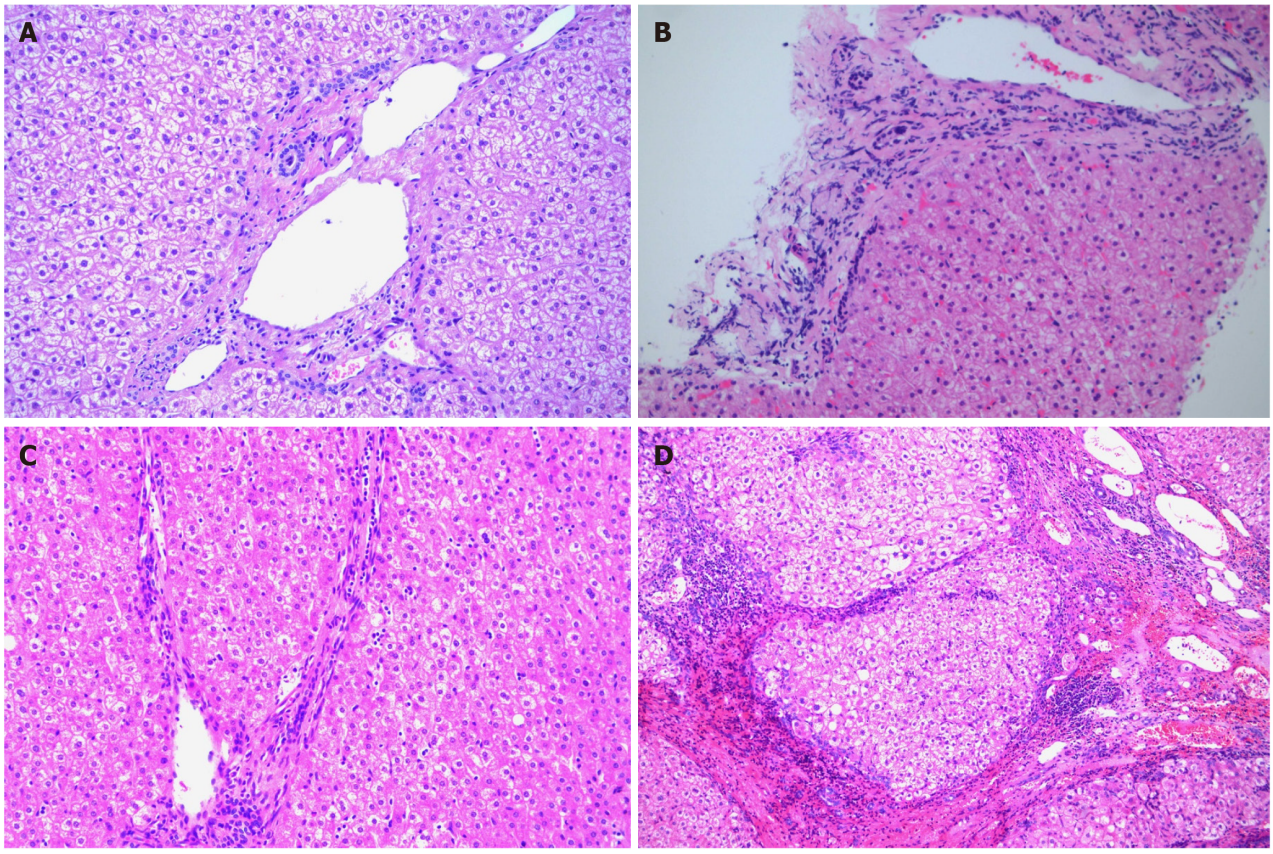
SRLV: Standard residual liver volume.

**Table 6 The comparison of two assessment methods, *n* (%)**

| Model | Total | Grade | Number | Grade 3 wk after surgery (cases) | | |
|---|---|---|---|---|---|---|
| | | | | A (I) | B (II) | C (III) |
| Child-Pugh score | 35 | A | 31 | 17 (54.8) | 14 (45.2) | 0 |
| | | B | 4 | 0 | 1 (25) | 3 (75) |
| | | C | 0 | 0 | 0 | 0 |
| The new evaluation model | 35 | I | 11 | 11 (100)[a] | 0 | 0 |
| | | II | 23 | 0 | 21 (91.3)[b] | 2 (18.7) |
| | | III | 1 | 0 | 0 | 1 |

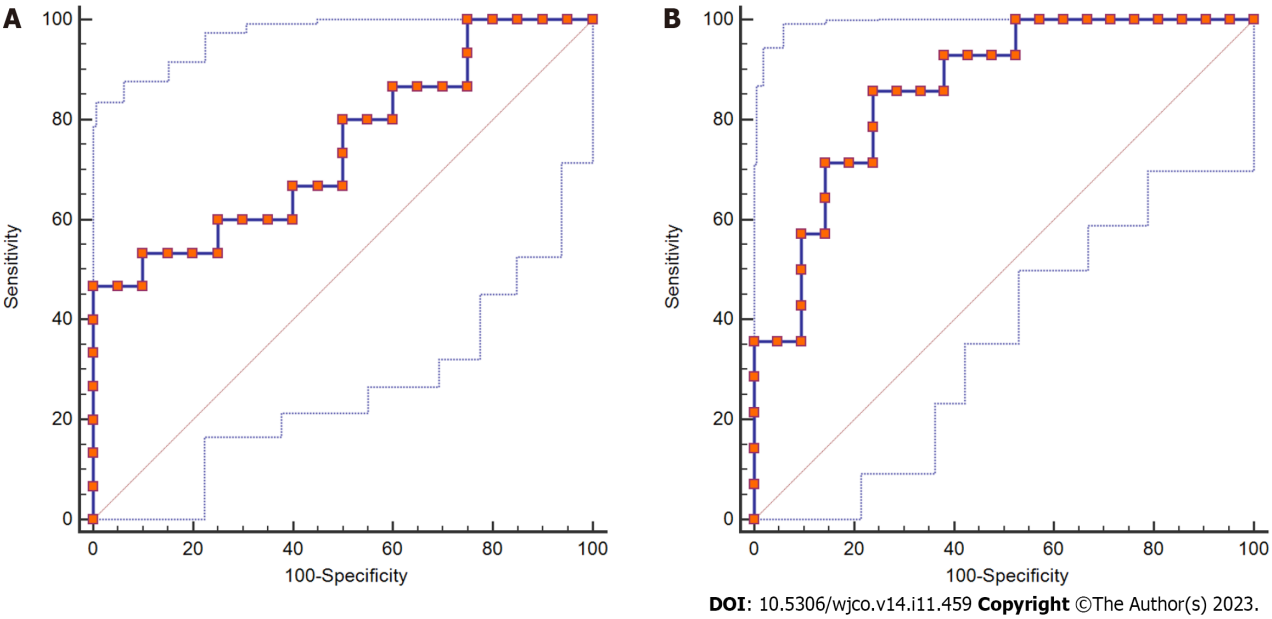
[a]$P < 0.05$ *vs* new evaluation model grade I and Child-Pugh grade A.
[b]$P < 0.05$ *vs* new evaluation model grade II and Child-Pugh grade B.

cirrhosis in hemihepatectomy, and there is no study on the LSM value in evaluating liver function reserve before hemihepatectomy. In addition, recent studies have shown that transient elastography cannot be used to accurately assess patients with obstructive jaundice. Therefore, more rigorous inclusion and exclusion criteria were adopted in this study. We used Fibro Touch elastic imaging equipment (FT-3.5R50) developed by Haskell Medical Technology Company and a two-dimensional ultrasonic probe to avoid the influence of liver tumors and large blood vessels inside and outside the liver on the measurement results. The measured LSM value was 22.20 ± 5.63 kPa, which is similar to that reported at home and abroad[12]. We established a new liver reserve assessment model based on the Child-Pugh score combined with the LSM value and observed its application in the evaluation of liver reserve function in patients with HCC undergoing hemihepatectomy. The results showed that the accuracy of the new evaluation model in predicting postoperative liver function compensation was 100.0% ($P < 0.05$), and the accuracy rate of predicting mildly poor liver function compensation after the operation was 91.3% ($P < 0.05$), which was higher than that of the Child-Pugh score.



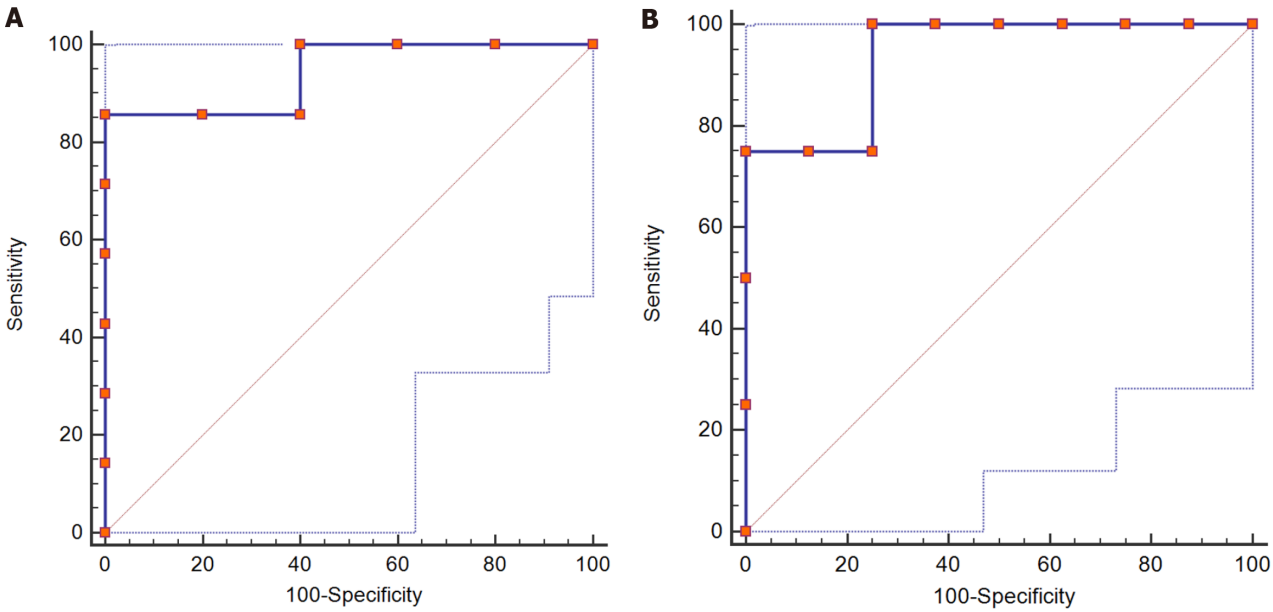

**DOI**: 10.5306/wjco.v14.i11.459 **Copyright** ©The Author(s) 2023.

**Figure 2 The different stages of liver fibrosis in 35 cases of hemihepatectomy (hepatic encephalopathy × 200).** A: Stage S1; B: Stage S2; C: Stage S3; D: Stage S4.




**DOI**: 10.5306/wjco.v14.i11.459 **Copyright** ©The Author(s) 2023.

**Figure 3 The receiver operating characteristic curve of the standard residual liver volume in different stages of liver fibrosis.** A: Stages S2-S3; B: Stage S4.




**DOI**: 10.5306/wjco.v14.i11.459 **Copyright** ©The Author(s) 2023.

**Figure 4 The receiver operating characteristic curve of the standard residual liver volume in different stages of liver fibrosis in patients with liver dysfunction.** A: Stage S2-S3; B: Stage S4.

Therefore, we believe that the new liver reserve assessment model can provide a reference for preoperative safety assessment of patients with liver cancer undergoing hemihepatectomy, which can increase patient safety during the perioperative period and reduce the incidence of liver failure after the operation. Additionally, it can provide a reference for patients with liver cancer who are expected to receive hemihepatectomy or extended hemihepatectomy.

## CONCLUSION

In summary, through this study, we found that for patients with moderate or severe liver fibrosis, when the predicted SRLV is greater than 0.310 L/m$^2$, the new evaluation model of liver function reserve predicts that the postoperative liver function compensation is good before the operation, and hemihepatectomy is safe; when the predicted SRLV is less than 0.285 L/m$^2$, the new liver reserve assessment model predicts poor liver function compensation after hepatectomy, and the probability of liver dysfunction after hemihepatectomy is higher. A blind operation should be avoided, and the operation should be evaluated after full liver protection. Patients in whom severe liver dysfunction is expected after surgery need to undergo antiviral treatment and undergo portal vein embolization or associated life partition and portal vein ligation for staged hepatectomy, and the values of SRLV and LSM should be reevaluated after liver regeneration. After contralateral liver regeneration, the SRLV and LSM values are reevaluated. It is expected that hemihepatectomy is still feasible for patients with well-compensated liver function. The LSM value combined with SRLV is safe and reliable.

However, the sample size involved in this study is too small and has no statistical significance in theory; nevertheless, the author believes that the LSM value and SRLV are useful safety indices for the evaluation of HCC hemihepatectomy. The new liver reserve evaluation model based on the Child-Pugh score combined with the LSM value can improve on the Child-Pugh score; it has important clinical guiding importance for the evaluation of liver reserve function in HCC patients with hemihepatectomy and provides a theoretical basis for further investigations conducted by our research group.

## ARTICLE HIGHLIGHTS

### *Research background*

Liver cancer resection often leads to poor prognosis, because the standard residual liver volume (SRLV) cannot be fully compensated after surgery.

### *Research motivation*

Hemihepatectomy or extended hemihepatectomy often leads to liver insufficiency and even liver failure.

### *Research objectives*
This study aimed to explore the risk factors of poor prognosis after hemihepatectomy for hepatocellular carcinoma and evaluate the application value of related prognostic approaches.

### *Research methods*
The clinical data of 35 patients with primary liver cancer were retrospectively analyzed. The critical values of SRLV in different stages of liver fibrosis after hemihepatectomy were compared with those of liver dysfunction after hemihepatectomy.

### *Research results*
Logistic regression analysis showed that the liver stiffness measure (LSM) value ≥ 25 kPa [odds ratio (OR) = 6.254, $P < 0.05$)] and SRLV ≤ 0.290 L/m$^2$ (OR = 5.686, $P < 0.05$) were independent risk factors for postoperative liver dysfunction. The accuracy of the new liver reserve evaluation model for predicting postoperative liver function was higher than that of the Child-Pugh score ($P < 0.05$).

### *Research conclusions*
LSM values and SRLV can be used to evaluate the safety of hemihepatectomy.

### *Research perspectives*
The new liver reserve evaluation model has good application potential in the evaluation of liver reserve function after hemihepatectomy.


## ACKNOWLEDGEMENTS

We appreciate teachers and classmates support and guidance through this process. Express respect and gratitude to patients who provide medical records.

## FOOTNOTES

**Co-first authors:** Zhi-Qiang Yue and Peng Zhang.

**Co-corresponding authors:** Jin-Zhu Wu and Ya-Li Cao.

**Author contributions:** Yue ZQ, Zhang P, Yan S, Yuan LX, Ju LL, Wang HX, Chen L, Cao YL and Wu JZ contributed equally to this work; Yue ZQ, Zhang P, Yan S, Yuan LX and Wu JZ were hepatobiliary surgeons; Ju LL, Wang HX, Yuan LX, Chen L and Cao YL were researchers in Nantong Institute of Liver Disease; Wu JZ, Chen L, Cao YL designed the research study; Yue ZQ, Zhang P, Yan S, Yuan LX, Ju LL and Wang HX performed the primary literature and data extraction; Yue ZQ, Zhang P analyzed the data and wrote the manuscript; Chen L, Cao YL and Wu JZ were responsible for revising the manuscript for important intellectual content; and all authors read and approved the final version. Yue ZQ and Zhang P have made equally significant contributions to this thesis. In accordance with the principles of rigorous and objective research and respect for the contributions of all team members, Zhang P is co-designated as the first author. Wu JZ (corresponding author) and Cao YL (co-corresponding author) provided financial support for this research. Wu JZ (corresponding author), as the research advisor of Yue ZQ (first author), was primarily responsible for the design and guidance of the paper. Meanwhile, Cao YL (co-corresponding author), as an authoritative expert, significantly contributed to the advancement of this study. Therefore, Cao YL is co-designated as the co-corresponding author.

**Supported by** Nantong Municipal Health Commission, No. MSZ2022036 and No. QN2022041; Nantong Science and Technology Bureau, No. JCZ2022036.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of the Nantong Third People's Hospital (approval NO. EK201628).

**Informed consent statement:** All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at 39235978@qq.com.



**Country/Territory of origin:** China

**ORCID number:** Zhi-Qiang Yue 0009-0001-0837-7414; Lin-Ling Ju 0000-0002-9541-0533; Lin Chen 0000-0002-1108-4735; Jin-Zhu Wu 0000-0002-7295-2030.

**S-Editor:** Qu XL
**L-Editor:** A
**P-Editor:** Zhang XD

## REFERENCES

1 **Marrero JA**, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2018; **68**: 723-750 [PMID: 29624699 DOI: 10.1002/hep.29913]

2 **Liu Z**, Suo C, Mao X, Jiang Y, Jin L, Zhang T, Chen X. Global incidence trends in primary liver cancer by age at diagnosis, sex, region, and etiology, 1990-2017. *Cancer* 2020; **126**: 2267-2278 [PMID: 32201944 DOI: 10.1002/cncr.32789]

3 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

4 **Shen S**, Qiu Y, Yang X, Wang W. Remnant Liver-to-Standard Liver Volume Ratio Below 40% is Safe in Ex Vivo Liver Resection and Autotransplantation. *J Gastrointest Surg* 2019; **23**: 1964-1972 [PMID: 30374819 DOI: 10.1007/s11605-018-4022-4]

5 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin* 2019; **69**: 7-34 [PMID: 30620402 DOI: 10.3322/caac.21551]

6 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

7 **Liu PJ**, Harris JM, Marchi E, D'Arienzo V, Michler T, Wing PAC, Magri A, Ortega-Prieto AM, van de Klundert M, Wettengel J, Durantel D, Dorner M, Klenerman P, Protzer U, Giotis ES, McKeating JA. Hypoxic gene expression in chronic hepatitis B virus infected patients is not observed in state-of-the-art in vitro and mouse infection models. *Sci Rep* 2020; **10**: 14101 [PMID: 32839523 DOI: 10.1038/s41598-020-70865-7]

8 **Zheng Y**, Wu J, Ding C, Xu K, Yang S, Li L. Disease burden of chronic hepatitis B and complications in China from 2006 to 2050: an individual-based modeling study. *Virol J* 2020; **17**: 132 [PMID: 32859216 DOI: 10.1186/s12985-020-01393-z]

9 **Salimi S**, Pandya K, Sastry V, West C, Virtue S, Wells M, Crawford M, Pulitano C, McCaughan GW, Majumdar A, Strasser SI, Liu K. Impact of Having a Planned Additional Operation at Time of Liver Transplant on Graft and Patient Outcomes. *J Clin Med* 2020; **9** [PMID: 32102393 DOI: 10.3390/jcm9020608]

10 **Mai RY**, Lu HZ, Bai T, Liang R, Lin Y, Ma L, Xiang BD, Wu GB, Li LQ, Ye JZ. Artificial neural network model for preoperative prediction of severe liver failure after hemihepatectomy in patients with hepatocellular carcinoma. *Surgery* 2020; **168**: 643-652 [PMID: 32792098 DOI: 10.1016/j.surg.2020.06.031]

11 **Möller K**, Safai Zadeh E, Görg C, Dong Y, Cui X, Lim A, de Molo C, Serra C, Martín Algíbez A, Berzigotti A, Piscaglia F, Faiss S, Dietrich CF. Focal Liver Lesions other than Hepatocellular Carcinoma in Cirrhosis: Diagnostic Challenges. *J Transl Int Med* 2022; **10**: 308-327 [PMID: 36860624 DOI: 10.2478/jtim-2022-0068]

12 **Hwang JY**, Yoon HM, Kim JR, Lee JS, Jung AY, Kim KM, Cho YA. Diagnostic Performance of Transient Elastography for Liver Fibrosis in Children: A Systematic Review and Meta-Analysis. *AJR Am J Roentgenol* 2018; **211**: W257-W266 [PMID: 30106615 DOI: 10.2214/AJR.18.19535]

13 **Zhang Y**, Wang C, Li H, Ding Y. Decreased liver stiffness by transient elastography indicates lower incidence of hepatocellular carcinoma in patients with chronic hepatitis B. *Medicine (Baltimore)* 2019; **98**: e13929 [PMID: 30653099 DOI: 10.1097/MD.0000000000013929]

14 **Feng LM**, Wang PQ, Yu H, Chen RT, Wang J, Sheng X, Yuan ZL, Shi PM, Xie WF, Zeng X. New formula for predicting standard liver volume in Chinese adults. *World J Gastroenterol* 2017; **23**: 4968-4977 [PMID: 28785151 DOI: 10.3748/wjg.v23.i27.4968]

15 **van Wissen J**, Bakker N, Doodeman HJ, Jansma EP, Bonjer HJ, Houdijk AP. Preoperative Methods to Reduce Liver Volume in Bariatric Surgery: a Systematic Review. *Obes Surg* 2016; **26**: 251-256 [PMID: 26123526 DOI: 10.1007/s11695-015-1769-5]

16 **Furlan A**, Tublin ME, Yu L, Chopra KB, Lippello A, Behari J. Comparison of 2D Shear Wave Elastography, Transient Elastography, and MR Elastography for the Diagnosis of Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *AJR Am J Roentgenol* 2020; **214**: W20-W26 [PMID: 31714842 DOI: 10.2214/AJR.19.21267]

17 **Taneja S**, Tohra S, Duseja A, Dhiman RK, Chawla YK. Noninvasive Assessment of Liver Fibrosis By Transient Elastography and FIB4/APRI for Prediction of Treatment Response in Chronic Hepatitis C-An Experience from a Tertiary Care Hospital. *J Clin Exp Hepatol* 2016; **6**: 282-290 [PMID: 28003717 DOI: 10.1016/j.jceh.2016.08.001]

18 **Maruyama M**, Yoshizako T, Araki H, Yoshida R, Ando S, Nakamura M, Kitagaki H. Future Liver Remnant Indocyanine Green Plasma Clearance Rate as a Predictor of Post-hepatectomy Liver Failure After Portal Vein Embolization. *Cardiovasc Intervent Radiol* 2018; **41**: 1877-1884 [PMID: 30132102 DOI: 10.1007/s00270-018-2065-2]

19 **Bozkurt B**, Emek E, Arikan T, Ceyhan O, Yazici P, Sahin T, Mammadov E, Serin A, Gurcan NI, Yuzer Y, Tokat Y. Liver Graft Volume Estimation by Manual Volumetry and Software-Aided Interactive Volumetry: Which is Better? *Transplant Proc* 2019; **51**: 2387-2390 [PMID: 31324483 DOI: 10.1016/j.transproceed.2019.01.152]

20 **Endo Y**, Ohta M, Tada K, Nakanuma H, Saga K, Masuda T, Hirashita T, Iwashita Y, Ozeki Y, Masaki T, Inomata M. Improvement of non-alcoholic fatty liver disease after laparoscopic sleeve gastrectomy in Japanese obese patients. *Ann Gastroenterol Surg* 2019; **3**: 285-290 [PMID: 31131357 DOI: 10.1002/ags3.12234]

21 **Gong WF**, Zhong JH, Lu Z, Zhang QM, Zhang ZY, Chen CZ, Liu X, Ma L, Zhang ZM, Xiang BD, Li LQ. Evaluation of liver regeneration and post-hepatectomy liver failure after hemihepatectomy in patients with hepatocellular carcinoma. *Biosci Rep* 2019; **39** [PMID: 31383787 DOI: 10.1042/BSR20190088]

22 **Xue C**, Chu Q, Li L. Research Progress on the Role of Probiotics in Acute Liver Failure. *J Transl Int Med* 2022; **10**: 83-85 [PMID: 35959453 DOI: 10.2478/jtim-2021-0052]

23 **Zhang D**, Pan Y, Yang Z, Zeng H, Wang X, Chen J, Wang J, Zhang Y, Zhou Z, Chen M, Hu D. A Nomogram Based on Preoperative Lab Tests, BMI, ICG-R15, and EHBF for the Prediction of Post-Hepatectomy Liver Failure in Patients with Hepatocellular Carcinoma. *J Clin Med* 2022; **12** [PMID: 36615125 DOI: 10.3390/jcm12010324]

24 **Allaire M**, Goumard C, Lim C, Le Cleach A, Wagner M, Scatton O. New frontiers in liver resection for hepatocellular carcinoma. *JHEP Rep* 2020; **2**: 100134 [PMID: 32695968 DOI: 10.1016/j.jhepr.2020.100134]

25 **Kokudo T**, Hasegawa K, Shirata C, Tanimoto M, Ishizawa T, Kaneko J, Akamatsu N, Arita J, Demartines N, Uldry E, Kokudo N, Halkic N. Assessment of Preoperative Liver Function for Surgical Decision Making in Patients with Hepatocellular Carcinoma. *Liver Cancer* 2019; **8**: 447-456 [PMID: 31799202 DOI: 10.1159/000501368]

26 **Fitzpatrick JA**, Kim JU, Cobbold JF, McPhail MJ, Crossey MM, Bak-Bol AA, Zaky A, Taylor-Robinson SD. Changes in Liver Volume in Patients with Chronic Hepatitis C Undergoing Antiviral Therapy. *J Clin Exp Hepatol* 2016; **6**: 15-20 [PMID: 27194891 DOI: 10.1016/j.jceh.2015.11.004]

27 **Yang X**, Yang JD, Lee S, Hwang HP, Ahn S, Yu HC, You H. Estimation of Standard Liver Volume Using CT Volume, Body Composition, and Abdominal Geometry Measurements. *Yonsei Med J* 2018; **59**: 546-553 [PMID: 29749138 DOI: 10.3349/ymj.2018.59.4.546]

28 **Serra-Burriel M**, Graupera I, Torán P, Thiele M, Roulot D, Wai-Sun Wong V, Neil Guha I, Fabrellas N, Arslanow A, Expósito C, Hernández R, Lai-Hung Wong G, Harman D, Darwish Murad S, Krag A, Pera G, Angeli P, Galle P, Aithal GP, Caballeria L, Castera L, Ginès P, Lammert F; investigators of the LiverScreen Consortium. Transient elastography for screening of liver fibrosis: Cost-effectiveness analysis from six prospective cohorts in Europe and Asia. *J Hepatol* 2019; **71**: 1141-1151 [PMID: 31470067 DOI: 10.1016/j.jhep.2019.08.019]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

*World J Clin Oncol* 2023 November 24; 14(11): 471-478

DOI: 10.5306/wjco.v14.i11.471

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Observational Study

# System describing surgical field extension associated with flap reconstruction after resection of a superficial malignant soft tissue tumor

Akio Sakamoto, Takashi Noguchi, Shuichi Matsuda

**Specialty type:** Orthopedics

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

**P-Reviewer:** Gupta R, India

**Received:** August 29, 2023
**Peer-review started:** August 29, 2023
**First decision:** September 14, 2023
**Revised:** September 24, 2023
**Accepted:** October 13, 2023
**Article in press:** October 13, 2023
**Published online:** November 24, 2023

**Akio Sakamoto, Takashi Noguchi, Shuichi Matsuda,** Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan

**Corresponding author:** Akio Sakamoto, MD, PhD, Adjunct Professor, Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Shogoin, Kawahara-cho 54, Sakyo-ku, Kyoto 606-8507, Japan. akiosaka@kuhp.kyoto-u.ac.jp

## Abstract

***BACKGROUND***

Flap reconstruction after resection of a superficial malignant soft tissue tumor extends the surgical field and is an indicator for potential recurrence sites.

***AIM***

To describe a grading system for surgical field extension of soft tissue sarcomas.

***METHODS***

Grading system: CD-grading is a description system consisting of C and D values in the surgical field extension, which are related to the compartmental position of the flap beyond the nearby large joint and deeper extension for the pedicle, respectively. C1/D1 are positive values and C0/D0 are negative. With a known location, 1/0 values can be "p" (proximal), "d" (distal), and "b" (in the tumor bed), and the description method is as follows: flap type, CxDx [x = 0, 1, p, d or b].

***RESULTS***

Four representative patients with subcutaneous sarcomas who underwent reconstruction using fasciocutaneous flaps are presented. The cases involved a distal upper arm (elbow) synovial sarcoma reconstructed using a pedicled latissimus dorsi (pedicled flap: CpDp); a distal upper arm (elbow) pleomorphic rhabdomyosarcoma reconstructed using a transpositional flap from the forearm (transpositional flap: CdD0); an undifferentiated pleomorphic sarcoma in the buttocks reconstructed using a transpositional flap (transpositional flap: C0D0); and a myxofibrosarcoma in the buttocks reconstructed using a propeller flap from the thigh (pedicled flap: CdDd).

***CONCLUSION***

The reconstruction method is chosen by the surgeon based on size, location, and

other tumor characteristics; however, the final surgical field cannot be determined based on preoperative images alone. CD-grading is a description system consisting of C and D values in the surgical field extension that are related to the compartmental position of the flap beyond the nearby large joint and deeper extension for the pedicle, respectively. The CD-grading system gives a new perspective to the flap reconstruction classification. The CD-grading system also provides important information for follow-up imaging of a possible recurrence.

**Key Words:** Soft tissue; Sarcoma; Surgery; Sarcoma; Grading system; Surgical flap



**Core Tip:** Flap reconstruction after resection of a superficial malignant soft tissue tumor extends the surgical field and is an indicator for potential recurrence sites. CD-grading is a description system consisting of C and D values in the surgical field extension that are related to the compartmental position of the flap beyond the nearby large joint and deeper extension for the pedicle, respectively. C1/D1 and C0/D0 are positive and negative values, respectively. The CD-grading system gives a new perspective to flap reconstruction classification. The CD-grading system also provides important information for follow-up imaging of a possible recurrence.

**Citation:** Sakamoto A, Noguchi T, Matsuda S. System describing surgical field extension associated with flap reconstruction after resection of a superficial malignant soft tissue tumor. *World J Clin Oncol* 2023; 14(11): 471-478
**URL:** https://www.wjgnet.com/2218-4333/full/v14/i11/471.htm
**DOI:** https://dx.doi.org/10.5306/wjco.v14.i11.471

## INTRODUCTION

Soft tissue sarcomas comprise a group of rare heterogeneous neoplasms that account for < 1% of all cancers[1]. Soft tissue sarcomas can occur in any soft tissue, but are most common in the extremities. Wide resection of the sarcoma and the surrounding normal tissue is necessary to reduce the recurrence rate[2]. Flap reconstruction is used to repair soft tissue defects after resection of a soft tissue sarcoma, especially a superficial soft tissue sarcoma[3,4].

The term "tumor bed" refers to the area of tissue remaining after a malignant tumor is removed. The tumor bed includes the tumor and surrounding healthy tissues where cancer cells may exist[5]. Use of a reconstruction flap following soft tissue sarcoma resection can extend the surgical field or tumor bed because of flap elevation or dissection of recipient vessels.

The current Cancer Staging Manual of the American Joint Committee on Cancer (AJCC) of soft tissue sarcoma is widely used based upon tumor size, histologic grade, and the presence of metastasis[6]. AJCC supports the *R classification*, which categorizes surgical margins as negative (R0), microscopically positive (R1), or grossly positive (R2)[7,8]. Flaps can be classified based on several factors (pedicled, free, or the tissue type from which the flap is made). Classification of flaps according to clinical complications has also been reported[9]; however, there is no system describing surgical field extension related to flap reconstruction.

In the current report we propose a grading classification, the CD-grading system, to describe extension of the surgical field related to flap reconstruction after superficial soft tissue sarcoma resection. Representative cases are also presented.

## MATERIALS AND METHODS

### *Classification: CD-grading system for a superficial sarcoma in the extremities*

The new grading system (CD-grading system) was used herein for superficial soft tissue sarcomas with extremity resection reconstructed by fascio-(musculo)-cutaneous flaps. Upper extremity tumors are defined as lesions arising distal to the acromioclavicular joint and include tumors of the shoulder girdle and axilla. Lower extremity tumors are defined as lesions arising distal to the iliac crest, including tumors of the gluteal region[9]. Additional skin grafting does not affect the grade; the skin grafting cases were not excluded.

The CD-grading system consists of C- and D-values. The C-value indicates the "compartmental position of the flap beyond the nearby large joint " and when the flap crosses a nearby large joint, the C-value is positive (C1). When the flap is within the compartment, the C-value is negative (C0). Large joints include the shoulders, elbows, wrists, hips, knees, and ankles. If the location of a flap crossing the joint location is proximal, the C-value is Cp (p = proximal) and when crossing a distal large joint the C-value is Cd (d = distal).

D-value means "deeper extension for the pedicle." The pedicle is already exposed, and the negative D-value is D0. If dissection of the pedicle is necessary, the positive D-value is D1. When the dissected pedicle is located proximal to the surgical field, the D-value is Dp (p = proximal), when the dissected pedicle is located distally, the D-value is Dd (d = distal), and when the pedicle dissection is within the surgical bed, the D-value is Db (b = surgical bed; Tables 1 and 2).

**Table 1 C-value for flap location beyond the nearby large joint**

| | C-value | Description |
|---|---|---|
| Within the compartment | 0 | C0 |
| Extra compartment | 1: any | C1 |
| | p: proximal | Cp |
| | d: distal | Cd |

**Table 2 D-value for surgical field extension for the pedicle dissection**

| Pedicle | D-value | Description |
|---|---|---|
| Already exposed | 0 | D0 |
| Dissection of the pedicle | 1: Any | D1 |
| | p: Proximal | Dp |
| | d: Distal | Dd |
| | b: Within tumor bed | Db |

The flap type is described before the CD-values as "flap type, CxDx," in which x can be 0, 1, p, d, or b. There is no strict rule in the description of the flap type; however, an easy and understandable description, such as distinguishing between a local or free flap, would be required.

### *Transpositional fascial flap/propeller flap*

In cases involving transpositional fasciocutaneous or propeller flaps[10], the flap is located within the compartment, the C-value is C0, the D-value is D0, and the CD-grade is C0D0. When the flap is from the extra compartment across the large joint, the C-value is C1, the D-value is D0, and the CD-grade is C1D0. When the flap is obtained proximally and crosses a large joint, the CD-grade is CpD0, and when the flap is derived distally and crosses a large joint, the CD-grade is CdD0 (Tables 3 and 4).

### *Pedicled flap*

The C-value in the pedicle flap is the same as the transpositional flap. The flap is located within the compartment and the C-value is C0. When the flap comes from the extra compartment across the joint, the C-value is C1. C1 can be Cp or Cd depending on the flap location (proximal or distal). The D-value reflects the location of the pedicle. The pedicle flap needs extension of the surgical field to deeper tissues, therefore the D-value is always D1. When the pedicle is located proximal to the surgical field, D1 can be Dp (p = proximal), and when the pedicle is located distal to the surgical field, the D1 can be Dd (d = distal; Tables 3 and 5).

### *Free flap*

The donated area of the flap does not affect the surgical field in terms of tumor contamination, and the C-value in the free flap is always C0. When the pedicle is already exposed at the surgical field, the D-value is D0. When the pedicle is not exposed, and the pedicle needs to be exposed, then the D-value is D1. When the pedicle is located proximal to the surgical field, D1 can be Dp (p = proximal), and when the pedicle is located distal to the surgical field, the D1 can be Dd (d = distal). When the pedicle is exposed at the deeper tissues within the surgical field, the D-value is Db (Tables 3 and 6).

## RESULTS

Herein we present four cases of superficial soft tissue sarcomas. Two elbow soft tissue sarcomas and two buttock soft-tissue sarcomas are presented. One elbow soft tissue sarcoma patient was a 47-year-old female with a synovial sarcoma at the elbow (distal upper arm) reconstructed with a pedicled latissimus dorsi; the CD-grade was CpDp (pedicled flap, CpDp; Figure 1). The second elbow soft tissue sarcoma patient was an 85-year-old male with a pleomorphic rhabdomyosarcoma at the elbow (distal upper arm) reconstructed using a transpositional flap from the forearm; the CD-grade was CdD0 (transpositional flap, CdD0; Figure 2). The first patient with a buttock soft tissue sarcoma was a 65-year-old female with an undifferentiated pleomorphic sarcoma at the buttock reconstructed using a transpositional flap; the CD-grade was C0D0 (transpositional flap, C0D0[11]; Figure 3). The second patient with a buttock sarcoma was a 46-year-old male with a myxofibrosarcoma that was reconstructed using a propeller flap from the thigh; the CD-grade was CdDd (pedicled flap, CdDd; Figure 4).

**Table 3 Possible classification of flap reconstruction**

| CD-grade | Transpositional flap | Pedicled flap | Free flap |
|---|---|---|---|
| C0D0 | C0D0 | Not applied | C0D0 |
| C0D1 | NA | C0Dp, C0Dd | C0Dp, C0Dd, C0Db |
| C1D0 | CpD0, CdD0 | NA | NA |
| C1D1 | NA | CpDp, CdDd | NA |

NA: Not available.

**Table 4 Transpositional fasciocutaneous flap/propeller flap**

| Flap location | C-value | D-value | CD-grade |
|---|---|---|---|
| Within the compartment | 0 | 0 | C0D0 |
| Extra compartment: from any | 1 | 0 | C1D0 |
| From proximal | p | 0 | CpD0 |
| From distal | d | 0 | CdD0 |

**Table 5 Pedicled flap**

| Flap location | C-value | D-value (pedicle location) | CD-grade |
|---|---|---|---|
| Within the compartment | 0 | 1 (any) | C0D1 |
| From proximal | 0 | p (proximal) | C0Dp |
| From distal | 0 | d (distal) | C0Dd |
| Extra compartment | 1 | 1 (any) | C1D1 |
| From proximal | p | p (proximal) | CpDp |
| From distal | d | d (distal) | CdDd |

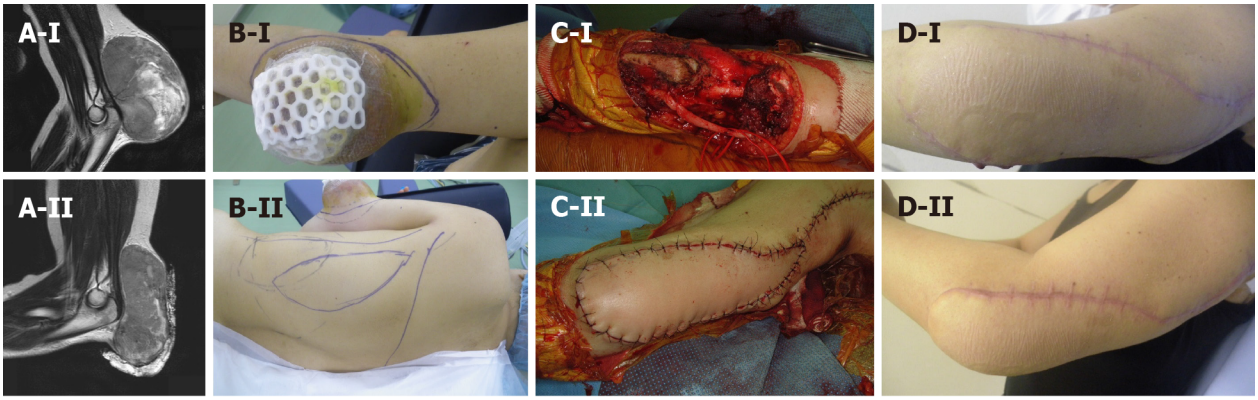
**Table 6 Free flap**

| Pedicle location | C-value | D-value (pedicle location) | CD-grade |
|---|---|---|---|
| Already exposed | 0 | 0 | C0D0 |
| Necessary for dissection | 0 | 1 (any) | C0D1 |
| From proximal | 0 | p (proximal) | C0Dp |
| From distal | 0 | d (distal) | C0Dd |
| Within tumor bed | 0 | b (within tumor bed) | C0Db |

## DISCUSSION

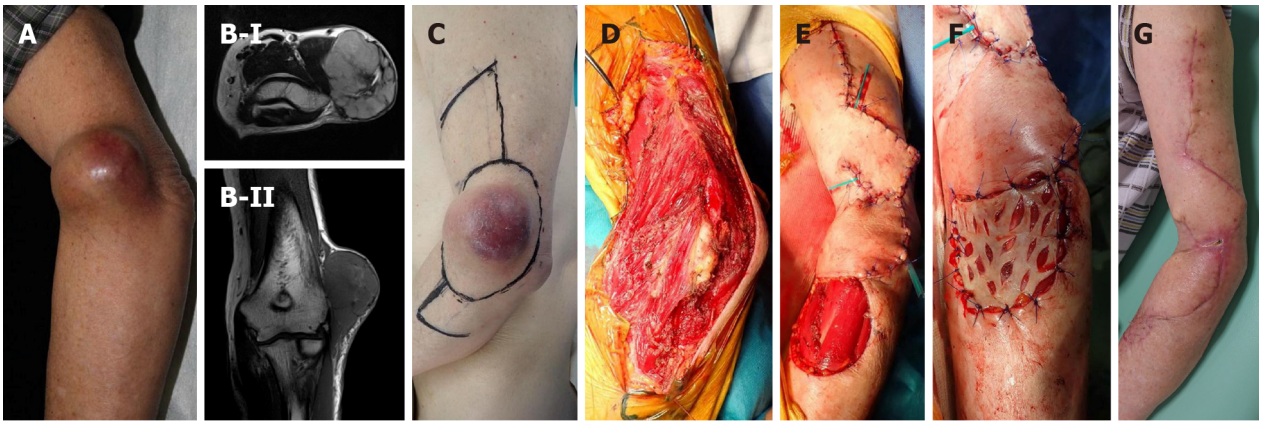
Soft tissue sarcomas require wide resection with healthy tissue margins[12,13]. Thus, the surgical field is wider than the tumor size. The extension of the tumor bed has the possibility of tumor contamination. Therefore, recognition of tumor bed extension is necessary. Discrepancies between the preoperative tumor burden and postoperative tumor bed contour have been identified after tumor burden replacement with a latissimus dorsi flap[14]. Flap reconstruction increases the surgical field during superficial soft tissue sarcoma resection[15].

A flap is applied to the defect after resection of a soft tissue sarcoma, especially a superficial soft tissue sarcoma. The choice of flap is often determined by the surgeon's preference, as well as the location of the tumor. The tumor bed after resection of soft tissue sarcomas cannot be predicted solely based on preoperative imaging. If amputation is necessary in the case of a re-occurrence, the level of amputation is important. Extension of the tumor bed due to flap reconstruction carries the risk of tumor contamination and may require more proximal amputation. The C-value gives information that indicates the likelihood of tumor contamination across the greater joint.




**DOI**: 10.5306/wjco.v14.i11.471 **Copyright** ©The Author(s) 2023.

**Figure 1 Synovial sarcoma at the distal upper arm (elbow) reconstructed by a pedicled latissimus dorsi (pedicled flap, CpDp).** A: A 47-year-old female with a synovial sarcoma at the elbow (distal upper arm). Magnetic resonance imaging showed a tumor with heterogenous low-to-high signal intensity on the T2-weighted image. Before (A-I) and after (A-II) chemotherapy of doxorubicin and ifosfamide, the tumor size was reduced. B-D: A wide surgical resection was performed with a pedicled latissimus dorsi. The CD-grade was CpDp (pedicled flap, CpDp).

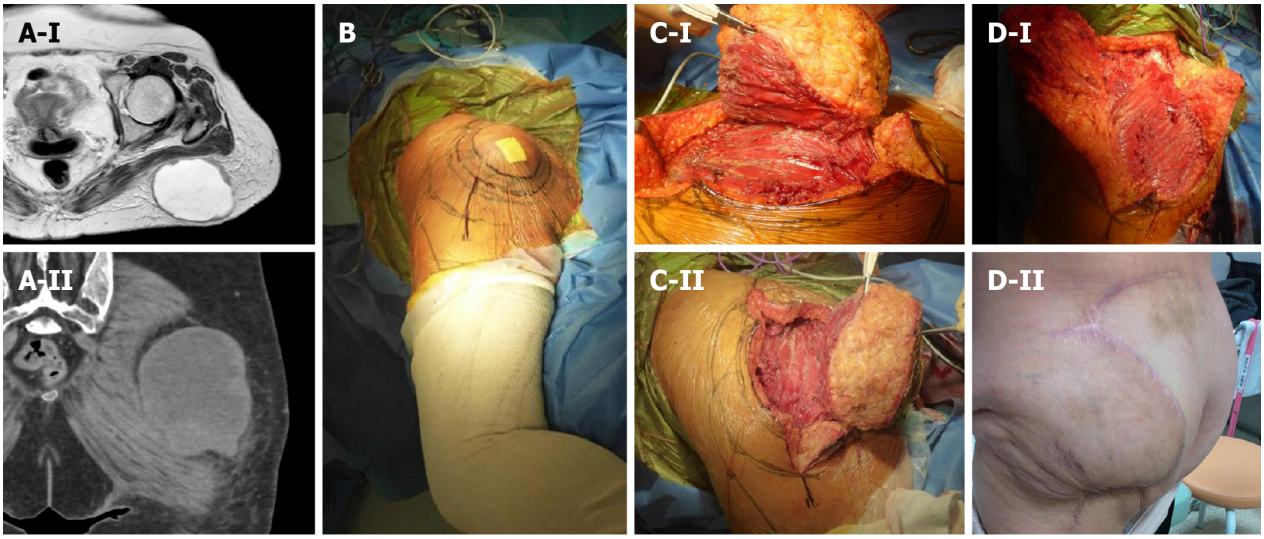



**DOI**: 10.5306/wjco.v14.i11.471 **Copyright** ©The Author(s) 2023.

**Figure 2 Pleomorphic rhabdomyosarcoma at the distal upper arm (elbow) reconstructed by transpositional flap (transpositional flap, CdD0).** A: An 85-year-old male with a pleomorphic rhabdomyosarcoma at the elbow (distal upper arm); B: Magnetic resonance imaging showed a tumor with homogenous high-signal intensity on T2-weighted images (B-I) and low-signal intensity on T1-weighted images (B-II). A wide surgical resection was performed. The transpositional flap was obtained from the upper arm and forearm; C-G: Skin grafting was performed at the forearm. The CD-grade was CdD0 (transpositional flap, CdD0).

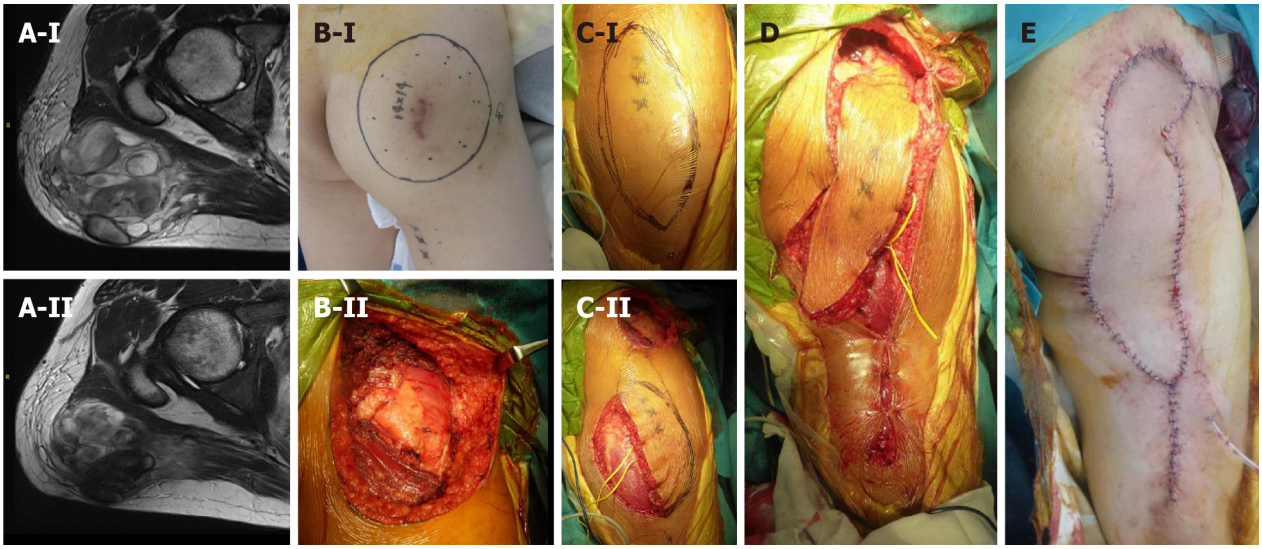
There is a risk of tumor contamination if deep tissues are created in the surgical field. The D-value represents the location of the pedicle. Dissection of the pedicle and recipient vessels requires dissection to the deeper layers, resulting in extension of the surgical field, with a D-value of D1. Transposition flaps in the compartment do not require exposure of the donor vessels, therefore less deep tissue exposure is advantageous in terms of reducing the potential for tumor recurrence, with a D-value of D0. Similarly, even in cases of a free flap requiring microsurgery, if the recipient vessels are already exposed, the D-value is D0 because deeper tissue dissection is not necessary.

The AJCC Staging of Soft Tissue Sarcomas (eighth edition) is based upon the tumor size, histologic grade, and the presence of metastasis. Tumor size is classified into four categories with border values of 5, 10, and 15 cm. The notation regarding tumor depth (superficial or deep from the superficial fascia) has been eliminated from the seventh edition of the AJCC Staging of Soft Tissue Sarcomas[6]. The surgical staging of musculoskeletal sarcomas has 4 types of surgical margins [intralesional, marginal, wide, and radical (compartmental)], as proposed by Enneking *et al*[16]. A 2-3 cm surgical margin provides reasonable local control of soft tissue sarcomas[17]. The AJCC supports the *R classification,* which categorizes margins as negative (R0), microscopically positive (R1), or grossly positive (R2)[7,8]. Furthermore, the Union Against Cancer (UICC) proposed a *R + 1 mm classification* that requires 1 mm of healthy tissue between the tumor and margin to define a negative margin (R0)[18,19], thus resulting in more resections being considered microscopically positive (R1). Radiation therapy can be performed as adjuvant therapy, especially if cancer cells remain after the resection. Radiation has a role in reducing the risk of recurrence in soft tissue sarcoma resection[5]. The term, tumor bed, refers to the area of tissue remaining after a malignant tumor is removed. The tumor bed may have cancer cells[5]. Recognition of tumor bed extension is necessary for postoperative radiation. Without flap reconstruction following soft tissue sarcoma resection, the tumor bed can largely be predicted with the preoperative staging based upon the images




**DOI**: 10.5306/wjco.v14.i11.471 **Copyright** ©The Author(s) 2023.

**Figure 3 Undifferentiated pleomorphic sarcoma at the buttock reconstructed by a transpositional flap (transpositional flap, C0D0).** A: A 65-year-old female with an undifferentiated pleomorphic sarcoma at the buttock. Magnetic resonance imaging revealed a subcutaneous tumor. The tumor had a cystic appearance and contained liquid with slightly high signal intensity on the T2-weighted image. The periphery of the cystic wall was thick with a solid neoplastic lesion and intermediate signal intensity on T2-weighted images (A-I). Computed tomography showed that the lesion is located at the buttock (A-II); B: A resection of the tumor was designed; C and D: The tumor was resected and the defect was reconstructed with a transpositional flap donated from the lateral abdomen. The CD-grade was C0D0 (transpositional flap, C0D0).




**DOI**: 10.5306/wjco.v14.i11.471 **Copyright** ©The Author(s) 2023.

**Figure 4 Myxofibrosarcoma at the buttock reconstructed by a propeller flap (pedicled flap, CdDd).** A: A 46-year-old male with a myxofibrosarcoma at the buttock. Magnetic resonance imaging revealed that the tumor showed heterogenous low-to-high signal intensity on the T2-weighted image. Before (A-I) and after (A-II) chemotherapy of doxorubicin and ifosfamide, the tumor size was reduced; B: The resection of the tumor was designed (B-I) and performed (B-II); C and E: A propeller flap from the thigh was designed (C-I) and the pedicle was preserved (C-II) and performed. The CD-grade was CdDd (pedicled flap, CdDd).

and the histologic findings. With flap reconstruction, tumor bed prediction is difficult without the surgical method information. Indeed, the new grading system can give information of surgical field extension associated with flap reconstruction.

There are several limitations in the new grading system. First, this classification is completely new and still theoretical. Clinical use of the assembled clinical data would be necessary, and some modification may be required for improvement. Second, the new grading system was used for superficial soft tissue sarcomas resected in the extremities and fascio-(musculo)-cutaneous flaps, in which hands and feet were not included. The new grading system might be modified for any part of bones and soft tissue sarcomas. Third, flap type description is not strictly defined in the new grading system, which may result in ambiguity; however, according to the flap technique improvement, description of the flap would be diverse. Therefore, no flap description restrictions were used in the new grading system. Finally, the new grading system cannot describe the length or area required for postoperative radiation. Excessive information in the grading system,

however, would make the new grading system difficult for clinical use.

## CONCLUSION

We have proposed a method to describe extension of the surgical field in reconstruction after superficial soft tissue sarcoma resection. The method described can give values for tumor bed extension after flap reconstruction. The description of whether or not the operative field has been extended due to reconstruction is considered to be important information for image evaluation of recurrence.

## ARTICLE HIGHLIGHTS

### *Research background*

Flap reconstruction can extend the surgical field or tumor bed because of flap elevation or dissection of recipient vessels during resection of superficial soft tissue sarcomas. There is currently no method describing extension of the surgical field.

### *Research motivation*

Extension of the surgical field cannot be predicted based on preoperative images for flap reconstruction after superficial soft tissue sarcoma resection. Knowledge of the surgical field extension is important information for evaluation of recurrence images or possible postoperative radiation.

### *Research objectives*

A theoretical CD-grading system was developed consisting of C and D values in the surgical field extension. The C-value represents the flap beyond the nearby large joint and the D-value pertains to a deeper extension.

### *Research methods*

C1/D1 and C0/D0 are positive and negative values, respectively. With a known location, C values are "p" (proximal), "d" (distal), and "b" (in the tumor bed). The description method is as follows: flap type, CxDx [x = 0, 1, p, d or b].

### *Research results*

Classification and possible values are shown in the tables (transpositional fascial flap/propeller, pedicled, and free flaps). Four representative patients with subcutaneous sarcomas who underwent reconstruction using fasciocutaneous flaps are presented.

### *Research conclusions*

The new grading system can give values for tumor bed extension after flap reconstruction following superficial soft tissue sarcoma resection. The description of whether or not the operative field has been extended due to reconstruction is thought to be important information for evaluation of recurrence images.

### *Research perspectives*

Clinical use of assembled clinical data would be necessary and some modification may be required for improvement, especially if the new grading system is modified for any part of bone and soft tissue sarcomas.

## FOOTNOTES

**Author contributions:** Sakamoto A developed the classification and drafted the manuscript; Noguchi T and Matsuda S participated in the study design; All authors read and approved the final manuscript.

**Institutional review board statement:** Analysis of clinical data accumulation in patients with bone and soft tissue tumors. Retrospective study.

**Informed consent statement:** The patients represented in this study were informed that the data from the case would be de-identified and used in a journal publication. There is a specific signed document because the analysis used anonymous clinical data that were obtained after each patient had been notified at the Kyoto University home page that the data could be used for a clinical study.

**Conflict-of-interest statement:** We, the authors, declare no conflicts of interest regarding our manuscript entitled "A system describing surgical field extension associated with flap reconstruction after resection of a malignant soft tissue tumor."

**Data sharing statement:** No additional data are available.

**STROBE statement:** The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.



**Country/Territory of origin:** Japan

**ORCID number:** Akio Sakamoto 0000-0002-7518-1606; Takashi Noguchi 0000-0003-2965-4772; Shuichi Matsuda 0000-0003-0802-1255.

**S-Editor:** Liu JH
**L-Editor:** A
**P-Editor:** Liu JH

## REFERENCES

1 **Nijhuis PH**, Schaapveld M, Otter R, Molenaar WM, van der Graaf WT, Hoekstra HJ. Epidemiological aspects of soft tissue sarcomas (STS)--consequences for the design of clinical STS trials. *Eur J Cancer* 1999; **35**: 1705-1710 [PMID: 10674017 DOI: 10.1016/s0959-8049(99)00152-5]
2 **Samà L**, Binder JP, Darrigues L, Couturaud B, Boura B, Helfre S, Chiche L, Nicolas N, Tzanis D, Bouhadiba T, Gentile D, Perlbarg-Samson J, Bonvalot S. Safe-margin surgery by plastic reconstruction in extremities or parietal trunk soft tissue sarcoma: A tertiary single centre experience. *Eur J Surg Oncol* 2022; **48**: 526-532 [PMID: 34702592 DOI: 10.1016/j.ejso.2021.10.010]
3 **Slump J**, Hofer SOP, Ferguson PC, Wunder JS, Griffin AM, Hoekstra HJ, Bastiaannet E, O'Neill AC. Flap choice does not affect complication rates or functional outcomes following extremity soft tissue sarcoma reconstruction. *J Plast Reconstr Aesthet Surg* 2018; **71**: 989-996 [PMID: 29706552 DOI: 10.1016/j.bjps.2018.04.002]
4 **Koulaxouzidis G**, Schlagnitweit P, Anderl C, Braig D, Märdian S. Microsurgical Reconstruction in Orthopedic Tumor Resections as Part of a Multidisciplinary Surgical Approach for Sarcomas of the Extremities. *Life (Basel)* 2022; **12** [PMID: 36362956 DOI: 10.3390/life12111801]
5 **Brinkmann EJ**, Ahmed SK, Houdek MT. Extremity Soft Tissue Sarcoma: Role of Local Control. *Curr Treat Options Oncol* 2020; **21**: 13 [PMID: 32025823 DOI: 10.1007/s11864-020-0703-9]
6 **Tanaka K**, Ozaki T. New TNM classification (AJCC eighth edition) of bone and soft tissue sarcomas: JCOG Bone and Soft Tissue Tumor Study Group. *Jpn J Clin Oncol* 2019; **49**: 103-107 [PMID: 30423153 DOI: 10.1093/jjco/hyy157]
7 **Hermanek P**, Wittekind C. The pathologist and the residual tumor (R) classification. *Pathol Res Pract* 1994; **190**: 115-123 [PMID: 8058567 DOI: 10.1016/S0344-0338(11)80700-4]
8 **Hoang K**, Gao Y, Miller BJ. The Variability in Surgical Margin Reporting in Limb Salvage Surgery for Sarcoma. *Iowa Orthop J* 2015; **35**: 181-186 [PMID: 26361463]
9 **O'Neill AC**, Roy M, Boucher A, Fitzpatrick AM, Griffin AM, Tsoi K, Ferguson PC, Wunder JS, Hofer SOP. The Toronto Sarcoma Flap Score: A Validated Wound Complication Classification System for Extremity Soft Tissue Sarcoma Flap Reconstruction. *Ann Surg Oncol* 2021; **28**: 3345-3353 [PMID: 33005992 DOI: 10.1245/s10434-020-09166-2]
10 **Pignatti M**, Ogawa R, Mateev M, Georgescu AV, Balakrishnan G, Ono S, Cubison T, Pinto V, D'Arpa S, Koshima I, Hyakusoku H, Hallock GG. Our Definition of Propeller Flaps and Their Classification. *Semin Plast Surg* 2020; **34**: 139-144 [PMID: 33041682 DOI: 10.1055/s-0040-1715158]
11 **Sakamoto A**, Noguchi T, Matsuda S. A Transposition Flap Reconstruction after Resection of a Soft-Tissue Sarcoma in the Buttock. *J Orthop Case Rep* 2021; **11**: 38-40 [PMID: 35415100 DOI: 10.13107/jocr.2021.v11.i10.2458]
12 **Garreffa E**, Hughes-Davies L, Russell S, Lightowlers S, Agrawal A. Definition of Tumor Bed Boost in Oncoplastic Breast Surgery: An Understanding and Approach. *Clin Breast Cancer* 2020; **20**: e510-e515 [PMID: 32284305 DOI: 10.1016/j.clbc.2020.03.003]
13 **Tse T**, Knowles S, Bélec J, Caudrelier JM, Lock M, Brackstone M, Arnaout A. Consensus statement on tumour bed localization for radiation after oncoplastic breast surgery. *Curr Oncol* 2020; **27**: e326-e331 [PMID: 32669940 DOI: 10.3747/co.27.5977]
14 **Alço G**, Igdem S, Okkan S, Dincer M, Sarsenov D, Ilgun AS, Agacayak F, Elbüken F, Ercan T, Selamoglu D, Ozmen V. Replacement of the tumor bed following oncoplastic breast-conserving surgery with immediate latissimus dorsi mini-flap. *Mol Clin Oncol* 2016; **5**: 365-371 [PMID: 27699027 DOI: 10.3892/mco.2016.984]
15 **Pu LL**. Reconstruction of a large gluteal soft-tissue defect with the double-opposing V-Y fasciocutaneous advancement flap. *Plast Reconstr Surg* 2007; **119**: 599-603 [PMID: 17230096 DOI: 10.1097/01.prs.0000246508.21562.f1]
16 **Enneking WF**, Spanier SS, Goodman MA. A system for the surgical staging of musculoskeletal sarcoma. *Clin Orthop Relat Res* 1980; 106-120 [PMID: 7449206]
17 **Sampo M**, Tarkkanen M, Huuhtanen R, Tukiainen E, Böhling T, Blomqvist C. Impact of the smallest surgical margin on local control in soft tissue sarcoma. *Br J Surg* 2008; **95**: 237-243 [PMID: 17703500 DOI: 10.1002/bjs.5906]
18 **Gronchi A**, Miceli R, Fiore M, Collini P, Lozza L, Grosso F, Mariani L, Casali PG. Extremity soft tissue sarcoma: adding to the prognostic meaning of local failure. *Ann Surg Oncol* 2007; **14**: 1583-1590 [PMID: 17260106 DOI: 10.1245/s10434-006-9325-0]
19 **Wittekind C**, Compton CC, Greene FL, Sobin LH. TNM residual tumor classification revisited. *Cancer* 2002; **94**: 2511-2516 [PMID: 12015777 DOI: 10.1002/cncr.10492]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

*World J Clin Oncol* 2023 November 24; 14(11): 479-503

DOI: 10.5306/wjco.v14.i11.479

ISSN 2218-4333 (online)

ORIGINAL ARTICLE

Basic Study

# Computational exploration of the significance of *COPS6* in cancer: Functional and clinical relevance across tumor types

Shi-Lin Wang, Guang-Zheng Zhuo, Li-Ping Wang, Xiang-Hu Jiang, Guo-Hong Liu, Yun-Bao Pan, Yi-Rong Li

**Specialty type:** Oncology

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

**P-Reviewer:** Martinou E, United Kingdom

**Received:** August 28, 2023
**Peer-review started:** August 28, 2023
**First decision:** September 14, 2023
**Revised:** September 20, 2023
**Accepted:** October 23, 2023
**Article in press:** October 23, 2023
**Published online:** November 24, 2023

**Shi-Lin Wang, Guang-Zheng Zhuo, Li-Ping Wang, Xiang-Hu Jiang, Yun-Bao Pan, Yi-Rong Li,** Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, Hubei Province, China

**Guo-Hong Liu,** Department of Radiology, Zhongnan Hospital of Wuhan University, Wuhan 430071, Hubei Province, China

**Corresponding author:** Yi-Rong Li, Professor, Department of Laboratory Medicine, Zhongnan Hospital of Wuhan University, No. 169 Donghu Road, Wuchang District, Wuhan 430071, Hubei Province, China. liyirong2021@126.com

## Abstract

***BACKGROUND***

The COP9 signalosome subunit 6 (*COPS6*) has been implicated in cancer progression, while its precise role in most types of cancer remains elusive.

***AIM***

To investigate the functional and clinical relevance of *COPS6* across various tumor types using publicly available databases.

***METHODS***

We used R software and online analysis databases to analyze the differential expression, prognosis, mutation and related functions of *COPS6* in pan-cancer.

***RESULTS***

Differential expression analysis and survival analysis demonstrated that *COPS6* was highly expressed and associated with high-risk profiles in the majority of cancer types. Possible associations between *COPS6* expression level and prognostic outcomes were found using data from public databases. Mutational analysis revealed that missense mutations were the predominant type of *COPS6* mutation. Additionally, positive correlations were identified between *COPS6* expression level and tumor mutational burden and microsatellite instability in most types of cancer. Immune infiltration analysis demonstrated a negative correlation between *COPS6* expression level and CD8+ T cell infiltration in certain types of cancer. The correlation between *COPS6* expression level and cancer-associated fibroblast infiltration exhibited heterogeneity, in which a positive correlation was found in head and neck squamous cell carcinoma and tenosynovial giant cell tumor, and a negative correlation was identified in diffuse large

B-cell lymphoma and thymoma. The correlation between *COPS6* expression level and macrophage infiltration was closely related to macrophage type. Gene co-expression and enrichment analysis highlighted transcription elongation factor B polypeptide 2 and G protein pathway suppressor 1 were significantly and positively associated with *COPS6* expression level. These genes were predominantly involved in processes, such as ubiquitin-mediated proteolysis and human immunodeficiency virus 1 infection.

***CONCLUSION***

In conclusion, this study systematically explored the significance of *COPS6* across different tumor types, providing a solid foundation for considering *COPS6* as a novel biomarker in cancer research.

**Key Words:** *COPS6*; Biomarker; Tumor mutational burden; Immune infiltration; Prognostic analysis



**Core Tip:** The COP9 signalosome subunit 6 *COPS6* has been implicated in several cancer types. However, its precise role in most cancer types remains poorly understood. Therefore, we aimed to investigate the function of *COPS6* in various tumor types. Through our analysis, we discovered that *COPS6* is highly expressed and associated with high-risk profiles in most cancers. Meanwhile, *COPS6* expression was positively correlated with tumor mutation burden, microsatellite instability, and immune infiltration of the tumor microenvironment. Our findings suggest that *COPS6* could be a potential biomarker for cancer research. Our study contributes to the understanding of the role of *COPS6* in cancer progression and highlights the clinical applications.

**Citation:** Wang SL, Zhuo GZ, Wang LP, Jiang XH, Liu GH, Pan YB, Li YR. Computational exploration of the significance of *COPS6* in cancer: Functional and clinical relevance across tumor types. *World J Clin Oncol* 2023; 14(11): 479-503
**URL:** https://www.wjgnet.com/2218-4333/full/v14/i11/479.htm
**DOI:** https://dx.doi.org/10.5306/wjco.v14.i11.479

## INTRODUCTION

According to recent data from the American Cancer Society, it is projected that the United States will witness 1958310 new cancer cases and 609820 cancer-related deaths by 2023[1]. While there has been a 1.5% decrease in cancer mortality rates between 2019 and 2020, with an overall decline of 33% since 1991, the incidence of breast cancer, prostate cancer, and uterine cancer is on the rise, imposing potential challenges to the future progress. Furthermore, these types of cancer demonstrate significant disparities in mortality rates among different ethnic groups. Conversely, gastric cancer, esophageal cancer, and cervical cancer have shown downward mortality rates, while lung cancer, colorectal cancer (CRC), and female breast cancer continue to exhibit gradually upward mortality rates[2,3]. These trends underscore the persisting challenges caused by cancer. Hence, it is imperative to explore new targets for early diagnosis and personalized treatment, as emphasized by previous studies[4,5]. The identification and analysis of novel pan-cancer genes can provide valuable insights into the intricate process of tumorigenesis. Public databases and online analysis tools, such as The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) provide convenient access to comprehensive cancer-related functional genomics datasets across diverse cancer types, enabling in-depth pan-cancer analysis[6,7].

The COP9 signalosome (COPS) is a multiprotein complex involved in protein degradation, transcriptional activation, signal transduction, and tumor progression[8,9]. *COPS6*, together with its dimerization partner *COPS5*, plays a crucial role in the activation process of deneddylase activity by embedding into the core of the helical bundle[10]. While the literature has reported the mechanisms of *COPS6* in human malignancies, such as cervical cancer, papillary thyroid carcinoma (THCA), CRC, breast cancer, lung adenocarcinoma (LUAD), and glioblastoma, the available information is incomplete and the underlying mechanisms remain to be fully elucidated[11].

The present study aimed to comprehensively elucidate the involvement and clinical implications of *COPS6* in many diverse types of cancer. This involved an in-depth analysis of differential expression patterns, prognostic values, gene mutations, immune infiltration, correlation analysis, and functional enrichment assessment, utilizing publicly available databases.

## MATERIALS AND METHODS

### *Differential expression analysis*

GEPIA2 (http://gepia2.cancer-pku.cn/#degenes) was utilized for analysis of RNA-seq expression data collected from TCGA and Genotype-Tissue Expression (GTEx) projects, enabling differential analysis, correlation analysis, and survival analysis[12]. Genetic difference analysis was conducted using TIMER2 (http://timer.cistrome.org/)[13]. *COPS6*

expression level in tumor and normal samples was compared using R (ver. 4.0.3), TIMER2, and GEPIA2. Box plots were generated using the ggpubr R (ver. 4.0.3) package, and differential expression of *COPS6* in TCGA samples was determined using the Wilcoxon test. For cancers lacking normal controls in the TCGA database, the TIMER2 website was employed for differential analysis of the *COPS6* gene.

For protein analysis, UALCAN online portal (http://ualcan.path.uab.edu/analysis-prot.html) was utilized to examine gene, protein, methylation, and phosphorylation differences[14]. UALCAN facilitated the comparison of differential expression of *COPS6* protein between tumor and normal tissues. Age-differential expression data was obtained using the limma and ggpubr R packages, while clinical stage differential expression of *COPS6* was obtained from the GEPIA2 website.

### Survival analysis

To perform survival analysis for *COPS6*, the "Survival Map" feature of GEPIA2 was utilized. This facilitated plotting heatmaps representing overall survival (OS) and disease-free survival (DFS) using data from TCGA database. Forest plots, encompassing OS, progression-free interval (PFI), disease-specific survival (DSS), and disease-free interval (DFI), were generated using the survival and forestplot R packages in association with Cox analysis.

In March 2022, the pan-cancer data were downloaded from the TCGA database, including tumor stage, tumor grade, survival time, and mutation information. The raw data were preprocessed by the R programming language. The survminer R package was utilized to generate Kaplan-Meier survival curves for OS, PFI, DSS, and DFI, with a significance level set at $P < 0.05$.

### Genetic alteration analysis

For mutation analysis, cBioPortal (https://www.cbioportal.org/) was utilized[15]. In this study, the "Quick Search" feature of cBioPortal was employed to examine the mutation frequency, type, copy number alteration (CNA), and structural variants of TCGA tumors involving *COPS6*. Furthermore, information related to the specific mutation sites and three-dimensional (3D) structure of the *COPS6* protein was collected. To assess the impact of *COPS6* alterations on patient survival, "TCGA, PanCancer Atlas" and "Compassion/Survival" modes were utilized to plot OS, DSS, DFS, and progression-free survival curves for TCGA cases with and without *COPS6* alterations, respectively, using the log-rank test.

### Immune infiltration analysis

To investigate immune infiltration, TIMER2 web server was used. In the present study, the presence of CD8+ T cells, cancer-associated fibroblasts, natural killer (NK) cells, and macrophages was assessed using the "Immune" module. To explore the relationship between immune inflammatory cells and *COPS6* expression, multiple algorithms were utilized, including XCELL, EPIC, TIMER, MCPCOUNTER, CIBERSORT-ABS, TIDE, CIBERSORT, and QUANTISEQ. *P* values and partial correlation values were obtained using the purity-adjusted Spearman's rank correlation test to quantify the strength and significance of the observed correlations.

### Enrichment analysis and correlation analysis

The "Similar Gene Detection" module on the GEPIA2 website was utilized to identify the top 100 genes correlated with *COPS6*. Further analysis using the "correlation analysis" module on the same website narrowed down the selection to the top 5 genes with the highest correlation coefficients. Heatmap analysis of these genes was conducted using the "Gene_Corr" module available on the TIMER2 website. The purity-adjusted Spearman's rank correlation test was applied to obtain P-values and partial correlation values.

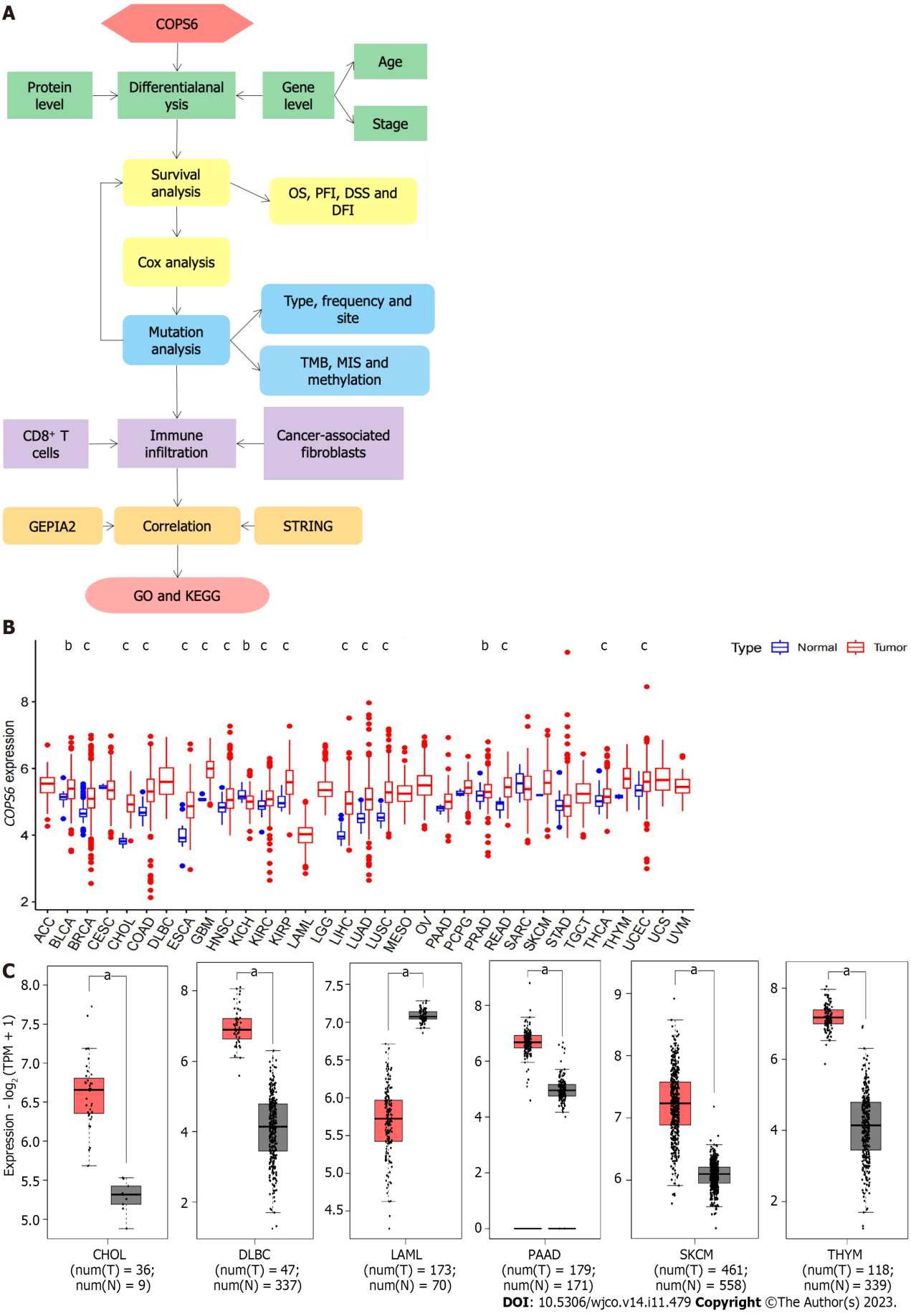
For protein-protein interaction (PPI) analysis, the STRING database (https://string-db.org/) was employed[16]. *COPS6* was submitted to the database to generate a PPI network with *Homo sapiens* as the reference organism. The network settings included a full network type, evidence-based network edges, experiments as active interaction sources, a minimum required interaction score of high confidence (0.7), and a maximum number of interactors shown in the 1st and 2nd shells.

To identify overlapping genes between the *COPS6*-related genes obtained from the GEPIA2 and STRING, a Venn diagram was generated using GraphPad Prism 9.0.0 software (GraphPad Software Inc., San Diego, CA, United States). The gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the DAVID database (https://david.ncifcrf.gov/home.jsp) with involvement of parameters, such as "OFFICIAL_GENE_SYMBOL", "Homo sapiens", and "functional annotation chart".

## RESULTS

### COPS6 expression level varied in various tumors

The overview of the pan-cancer analysis workflow is shown in Figure 1A. Differential expression analysis of *COPS6* was conducted on TCGA data using R programming language. Significant differential expression ($P < 0.05$) of *COPS6* was found between normal and tumor tissues in several cancer types, including bladder cancer (BLCA), breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC),



**DOI**: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

**Figure 1 Differential expression analysis of *COP9 signalosome subunit 6* in pan-cancer.** A: Overview of the pan-cancer analysis workflow; B:

Differential expression analysis of *COP9 signalosome subunit 6 (COPS6)* in the The Cancer Genome Atlas (TCGA) database using the Wilcoxon test in R software; C: Differential expression analysis of *COPS6* in matched TCGA normal and Genotype-Tissue Expression data using the GEPIA2 website with specific cutoff criteria. [b] *P* 0.01 *P* 0.001. CHOL: Cholangiocarcinoma; DFI: Disease-free interval; DLBC: Diffuse large B-cell; DSS: Disease-specific survival; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; LAML: Acute myeloid leukemia; MIS: Microsatellite instability; OS: Overall survival; PAAD: Pancreatic adenocarcinoma; PFI: Progression-free interval; SKCM: Skin cutaneous melanoma; THYM: Thymoma; TMB: Tumor mutational burden.

LUAD, lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), THCA, and uterine corpus endometrial carcinoma (UCEC) (Figure 1B). Differential expression analysis results of *COPS6* in these tumors and normal samples were obtained from the GEPIA2 website. Furthermore, *COPS6* expression level exhibited significant differences in skin cutaneous melanoma (SKCM), pancreatic adenocarcinoma (PAAD), thymoma (THYM), diffuse large B-cell lymphoma (DLBCL), acute myeloid leukemia (LAML), and CHOL (Figure 1C) compared with TCGA normal samples using GTEx data.

To compare protein expression level of *COPS6* among multiple types of cancer, protein expression differences between tumor and normal tissues from the CPTAC database on the UALCAN website were compared. The results revealed the elevated expression level of *COPS6* in hepatocellular carcinoma and clear cell renal cell carcinoma tissues (Figure 2A). Additionally, the relationship between *COPS6* expression level and clinical parameters was investigated using the GEPIA2 website, indicating the presence of association between *COPS6* expression level and clinical stages of LUAD, KICH, KIRP, and LIHC ($P < 0.05$) (Figure 2B). Furthermore, significantly upregulated *COPS6* expression level in ESCA, LUAD, and LUSC in cases who aged < 65-years-old in TCGA database was found, whereas *COPS6* expression level was reduced in KIRC ($P < 0.05$) (Figure 2C).

### *COPS6 expression level was associated with the prognosis of patients with diverse types of cancer*

To assess the relationship between *COPS6* expression level and patient prognosis across various tumors, patients were divided into high and low *COPS6* expression groups based on the median *COPS6* expression level. Utilizing the GEPIA2 website, it was revealed that high expression level of *COPS6* was significantly associated with poor OS in GBM ($P = 0.017$), KICH ($P = 0.031$), mesothelioma (MESO) ($P = 0.0026$), lower grade glioma (LGG) ($P = 0.007$), LIHC ($P = 0.011$), and LUAD ($P = 0.018$) (Figure 3A). Conversely, low expression level of *COPS6* was correlated with poor DFS in KIRP ($P = 0.042$), while high expression level of *COPS6* was associated with poor DFS in LGG ($P = 0.0013$), LIHC ($P = 0.024$), adrenocortical carcinoma (ACC) ($P = 0.034$), KIRC ($P = 0.009$), MESO ($P = 0.0027$), and stomach adenocarcinoma (STAD) ($P = 0.049$) (Figure 3B). Cox regression analysis indicated that *COPS6* was a high-risk gene for OS in HNSC, KICH, KIRC, LGG, LIHC, and MESO ($P < 0.05$), while it was appeared as a low-risk gene for OS in BRCA ($P < 0.05$). Additionally, *COPS6* was identified as a high-risk gene for DSS in KICH, KIRC, LGG, MESO, and READ ($P < 0.05$), as well as a low-risk gene for DSS in BRCA ($P < 0.05$). Moreover, *COPS6* was found as a high-risk gene for DFI in ACC, LGG, LIHC, and STAD ($P < 0.001$), as well as a high-risk gene for PFI in KICH, KIRC, LGG, MESO, and STAD ($P < 0.05$), while a low-risk gene for PFI in BRCA ($P < 0.05$). These findings were derived from TCGA database using the survival and forestplot R package (Figure 3C). The association between *COPS6* expression level and OS (Figure 4A), PFI (Figure 4B), DSS (Figure 4C), and DFI (Figure 4D) was further confirmed through Kaplan-Meier survival analysis in pan-cancer patients from TCGA database.

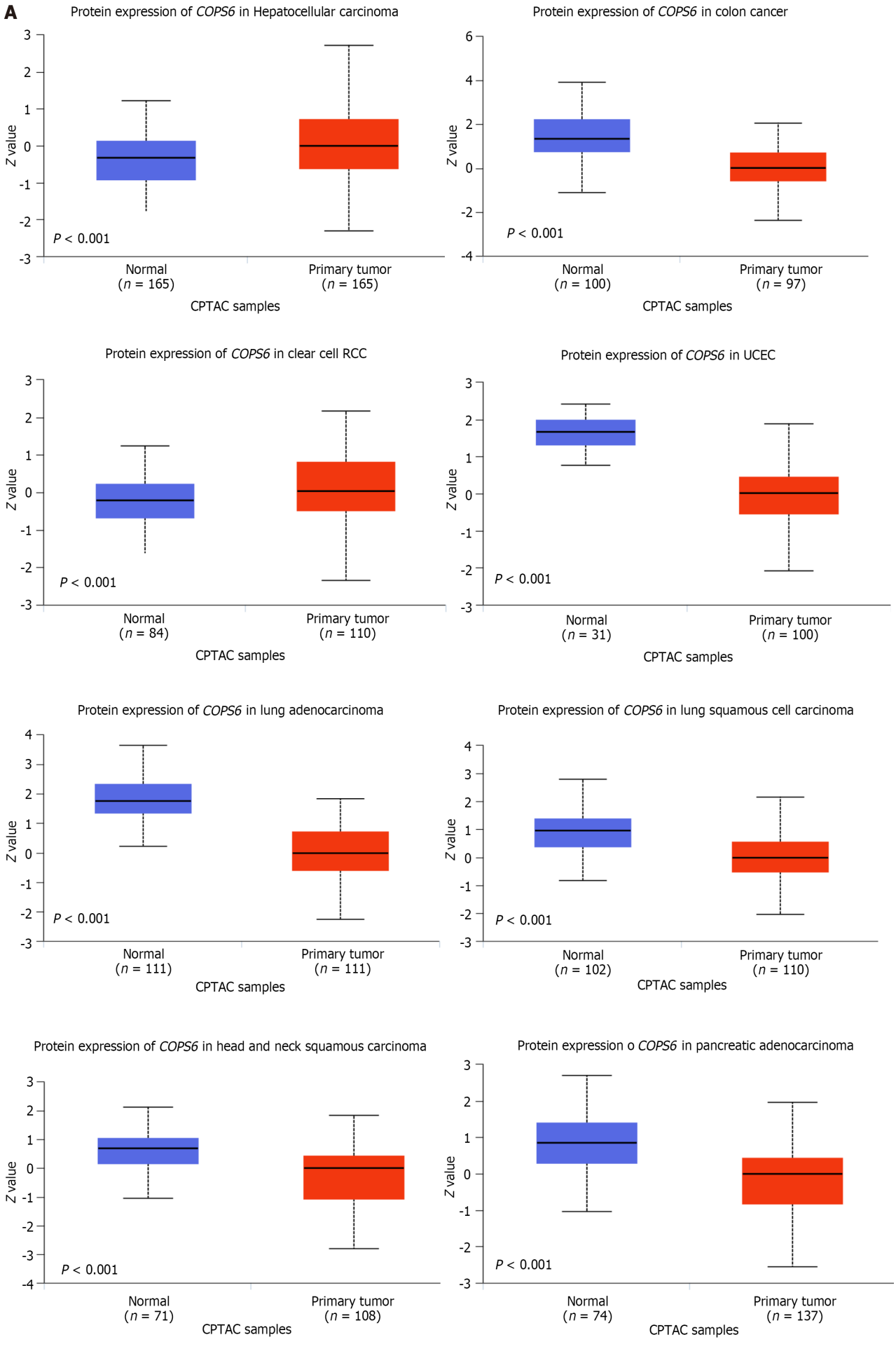
### *Correlation between COPS6 mutation and tumor progression*

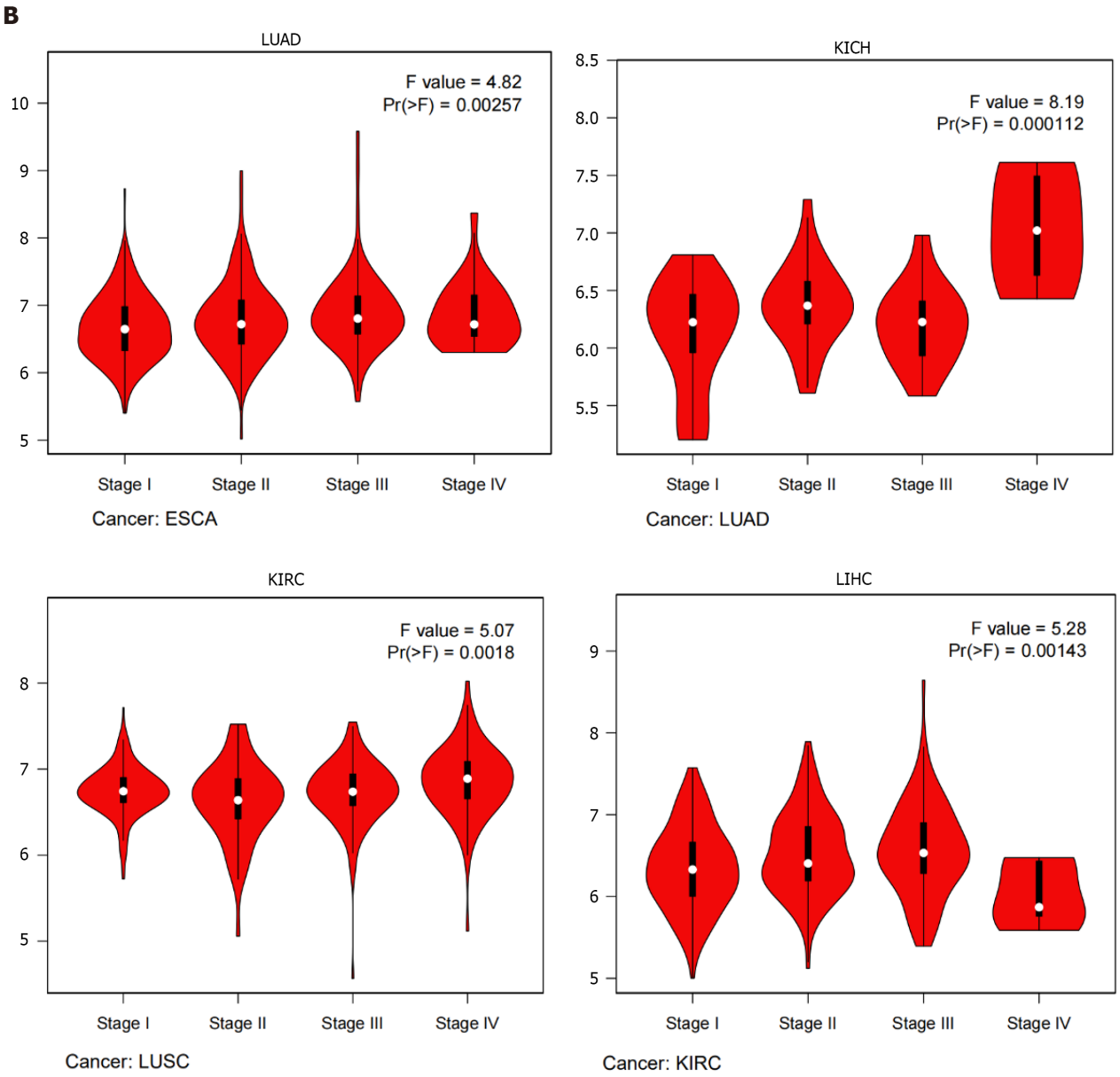
Using the cBioPortal website, comprehensive information was obtained regarding the mutation types, frequency, CNAs, and structural variants of *COPS6* across all TCGA tumors. Missense mutations were identified as the predominant mutation type. Among all TCGA tumors, the highest frequency of variations was found in esophageal adenocarcinoma (9.89%), with amplification being the most frequent alteration (9.34%) (Figure 5A). A 3D representation of the *COPS6* protein (Figure 5B) was constructed, revealing a notable mutation site, R197C/H, observed in one case each of adrenocortical carcinoma and endometrioid carcinoma (Figure 5C). Investigation of the relationship between *COPS6* mutations and prognosis in TCGA cases revealed no significant impact of mutation status on the prognosis of all types of cancer (Figure 5D).

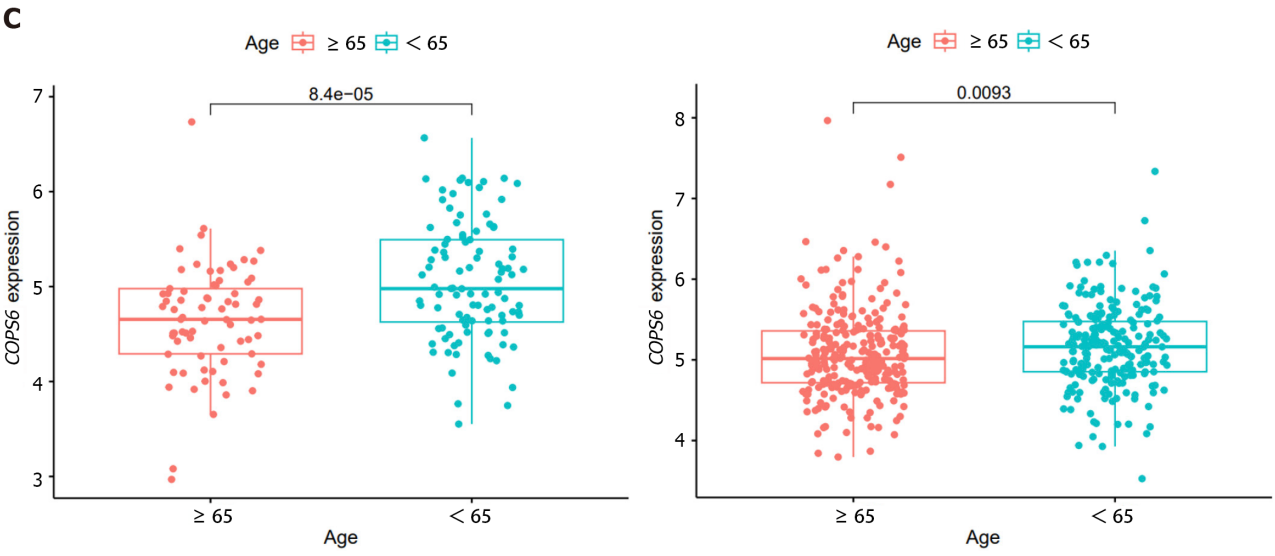
Furthermore, the correlations between *COPS6* expression level and tumor mutational burden (TMB) and microsatellite instability (MSI) were analyzed. Positive correlations were identified between *COPS6* expression level and TMB in LUAD, KIRP, LUSC, HNSC, PAAD, KICH, LIHC, KIRC, UCEC, LGG, BRCA, and PRAD ($P < 0.05$), while negative correlations were found in THYM, COAD, ESCA, and LAML ($P < 0.05$) (Figure 5E). Positive associations between *COPS6* expression level and MSI were observed in BRCA, USC, THCA, SKCM, SARC, PRAD, PAAD, KIRP, KIRC, HNSC, DLBC, and LIHC ($P < 0.05$), with a positive association observed in COAD ($P < 0.05$) (Figure 5F). Additionally, comparison of the *COPS6* promoter methylation level between normal and tumor samples revealed a higher methylation level in the tumor group in PRAD, LUSC, HNSC, BRCA, and KIRC, whereas a lower methylation level in BLCA (Figure 5G).

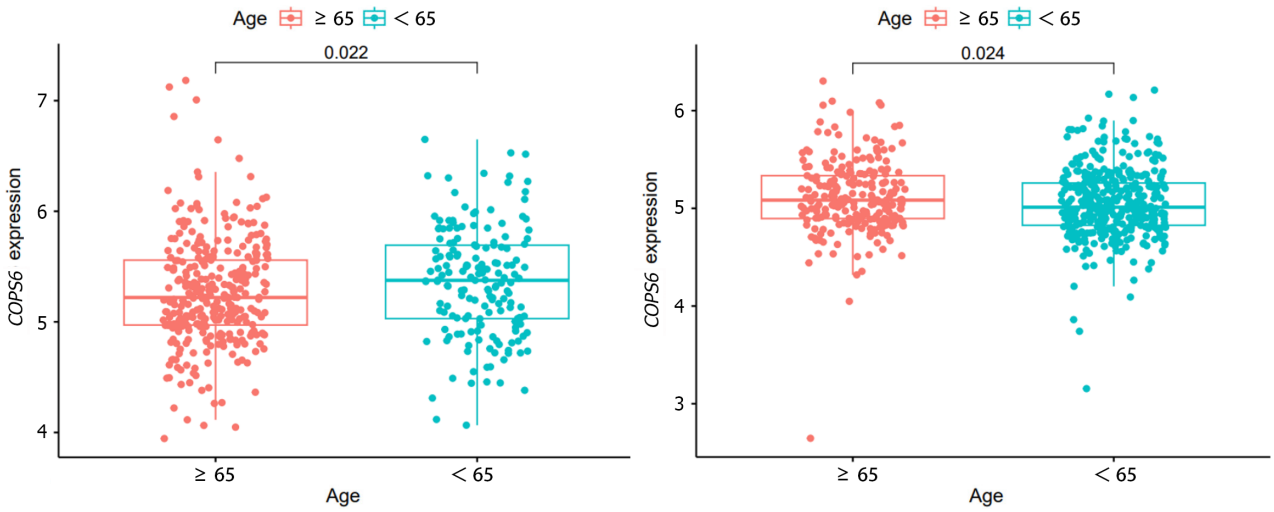
### *Correlation between COPS6 expression level and immune infiltration*

The relationship between *COPS6* expression level and immune-infiltrating cells in TCGA tumors was examined using the TIMER2 website. Across multiple algorithms, a negative correlation was found between *COPS6* expression level and CD8+ T cell infiltration in BRCA-LumA, HNSC, HNSC-HPV-, SKCM, SKCM-metastasis, and tenosynovial giant cell tumor (TGCT) (Figure 6A and B). Conversely, the correlation between cancer-associated fibroblast infiltration and *COPS6* expression level exhibited heterogeneity. Negative correlations were identified in DLBCL, OV, SARC, THYM, and THCA,


A
Protein expression of COPS6 in Hepatocellular carcinoma
Z value
P < 0.001
Normal (n = 165)
Primary tumor (n = 165)
CPTAC samples
Protein expression of COPS6 in colon cancer
Z value
P < 0.001
Normal (n = 100)
Primary tumor (n = 97)
CPTAC samples
Protein expression of COPS6 in clear cell RCC
Z value
P < 0.001
Normal (n = 84)
Primary tumor (n = 110)
CPTAC samples
Protein expression of COPS6 in UCEC
Z value
P < 0.001
Normal (n = 31)
Primary tumor (n = 100)
CPTAC samples
Protein expression of COPS6 in lung adenocarcinoma
Z value
P < 0.001
Normal (n = 111)
Primary tumor (n = 111)
CPTAC samples
Protein expression of COPS6 in lung squamous cell carcinoma
Z value
P < 0.001
Normal (n = 102)
Primary tumor (n = 110)
CPTAC samples
Protein expression of COPS6 in head and neck squamous carcinoma
Z value
P < 0.001
Normal (n = 71)
Primary tumor (n = 108)
CPTAC samples
Protein expression o COPS6 in pancreatic adenocarcinoma
Z value
P < 0.001
Normal (n = 74)
Primary tumor (n = 137)
CPTAC samples


B
LUAD
F value = 4.82
Pr(>F) = 0.00257
Stage I
Stage II
Stage III
Stage IV
Cancer: ESCA
KICH
F value = 8.19
Pr(>F) = 0.000112
Cancer: LUAD
KIRC
F value = 5.07
Pr(>F) = 0.0018
Cancer: LUSC
LIHC
F value = 5.28
Pr(>F) = 0.00143
Cancer: KIRC


C
Age ≥ 65 < 65
8.4e-05
COPS6 expression
≥ 65
< 65
Age
0.0093




**DOI**: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

**Figure 2 Association between *COP9 signalosome subunit 6* and clinical parameters in pan-cancer.** A: Differential analysis of *COP9 signalosome subunit 6 (COPS6)* protein expression in pan-cancers using the CPTAC database accessed through the UALCAN website; B: Relationship between *COPS6* expression and clinical stage analyzed with the GEPIA2 website; C: Correlation between *COPS6* expression and age using R software. KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma.

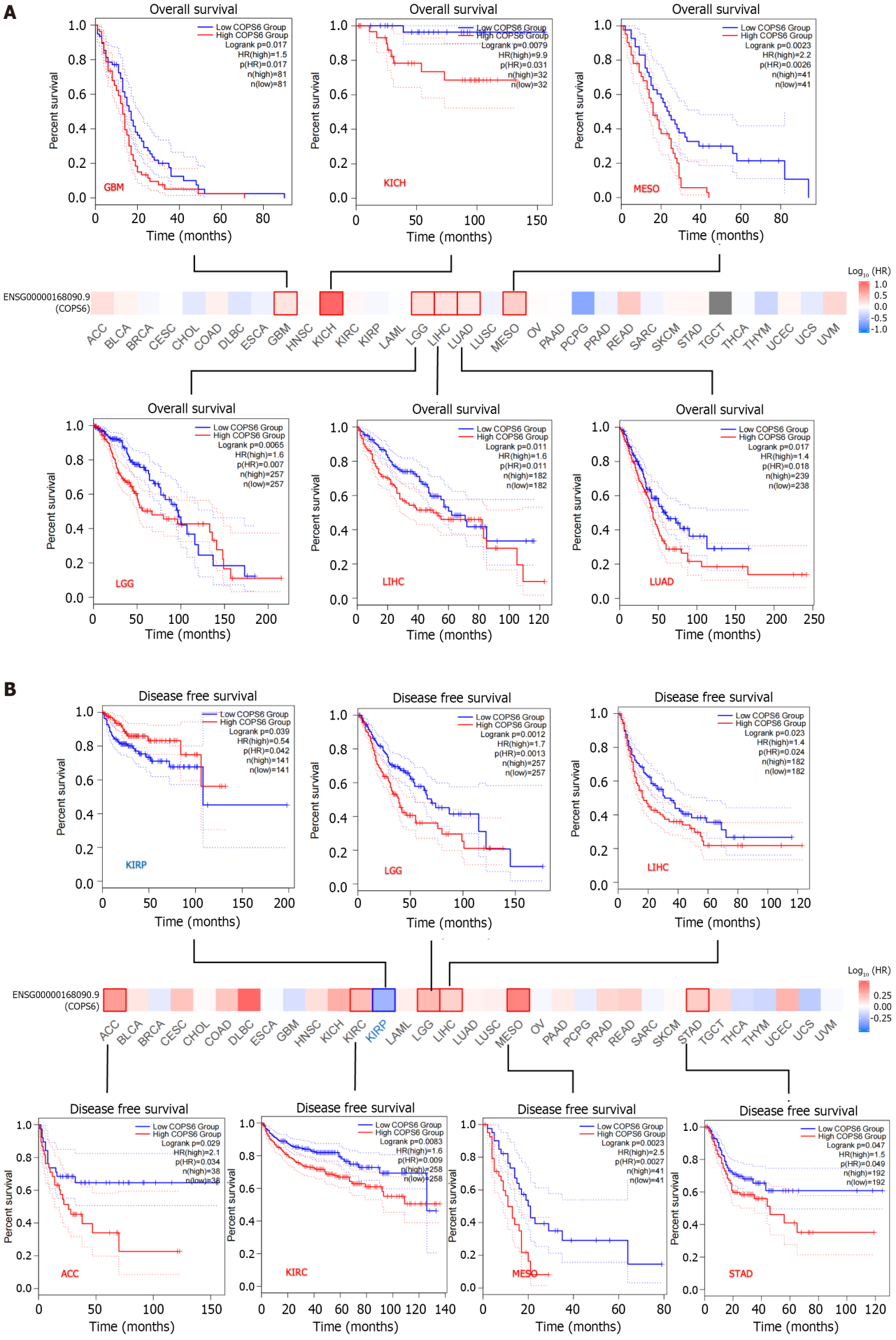
while positive correlations were found in HNSC, HNSC-HPV-, and TGCT (Figure 6C and D). The relationship between *COPS6* expression level and macrophage infiltration varied depending on macrophage subtype. An inverse association was detected between *COPS6* expression level and M1 macrophages, along with a positive association between *COPS6* expression level and M2 macrophages in certain tumors (Figure 6E and F). For instance, in DLBCL, four algorithms demonstrated a negative association between *COPS6* expression level and M1 macrophages, while TIDE algorithm revealed a positive association between *COPS6* expression level and M2 macrophages. Moreover, in BRCA, KIRC, THCA, and THYM, the TIDE algorithm indicated a positive correlation between *COPS6* expression level and M2 macrophages. In most of the tumors, NK cell infiltration exhibited a weak correlation with *COPS6* expression level, and clear associations were found only in a few tumors (Figure 6G and H). For instance, in THCA and THYM, *COPS6* expression level showed a negative correlation with NK cell infiltration. Further analysis of NK cell subtypes revealed a negative correlation between *COPS6* expression level and activated NK cell infiltration, as well as a positive correlation between *COPS6* expression level and resting NK cell infiltration.

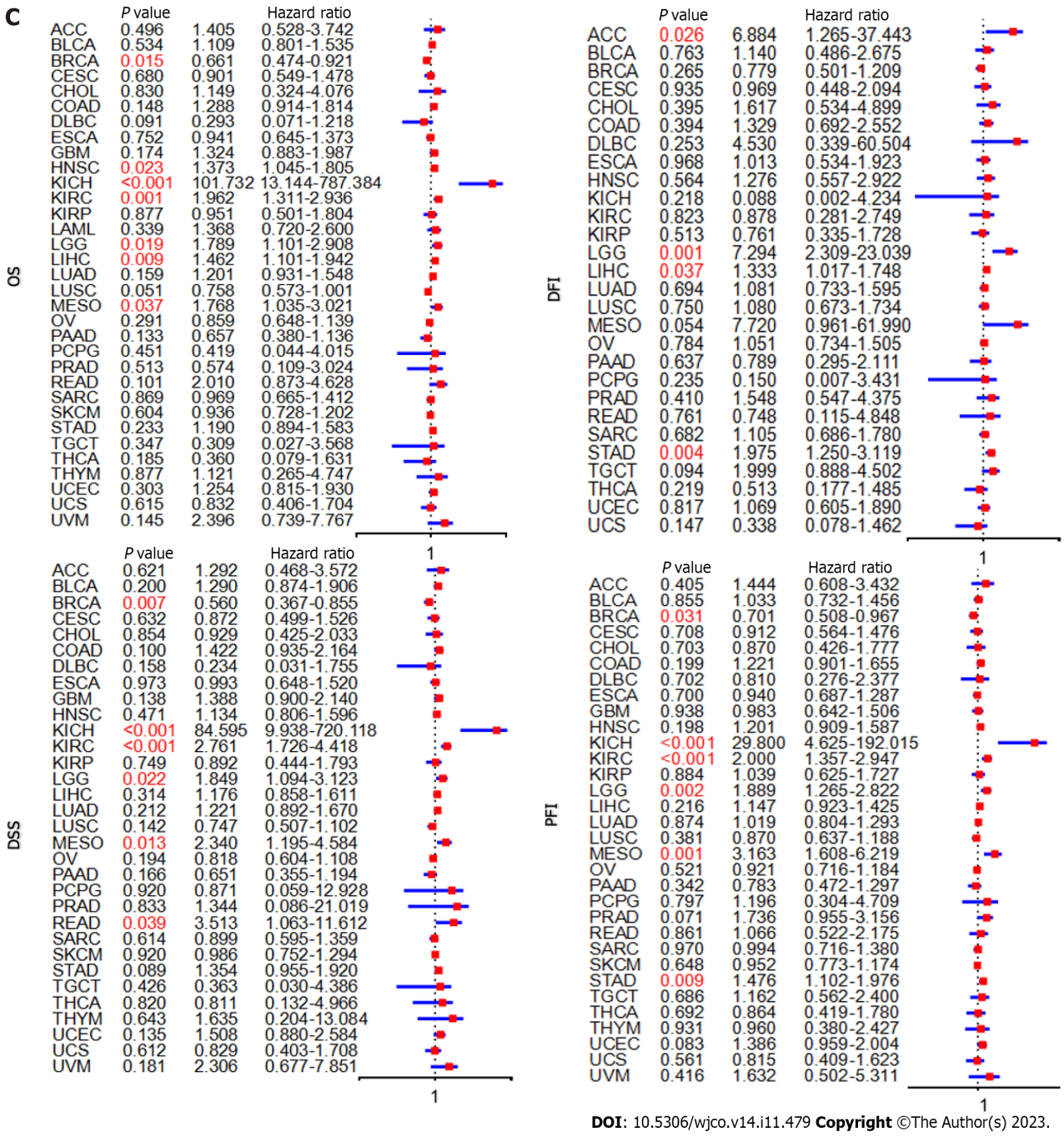
### *Enrichment analysis and correlation analysis of COPS6-associated genes*

To gain deeper insights into the molecular mechanisms involving *COPS6* in growth and progression of tumors, the GEPIA2 website was employed to screen the top 100 *COPS6*-associated genes. Subsequently, the top 5 genes with the highest correlation coefficients were identified and summarized as follows: POLR2J ($r = 0.69$, $P < 0.001$), BUD31 ($r = 0.65$, $P < 0.001$), TAF6 ($r = 0.66$, $P < 0.001$), ALKBH4 ($r = 0.62$, $P < 0.001$), and POP7 ($r = 0.61$, $P < 0.001$) (Figure 7A and B). The PPI network analysis was performed using the STRING website, resulting in the establishment of a network of 35 node genes (Figure 7C). The intersection of *COPS6*-associated genes obtained from the GEPIA2 and STRING led to the identification of *GPS1* and *TCEB2* (Figure 7D). Furthermore, the genes derived from both databases were merged, resulting in the detection of a total of 135 *COPS6*-related genes. Subsequently, GO and KEGG pathway enrichment analyses were conducted (Figure 7E and F). The KEGG pathway analysis revealed that *COPS6*-associated genes were enriched in pathways, such as ubiquitin-mediated proteolysis, nucleotide excision repair, human immunodeficiency virus 1 infection, Parkinson's disease, and circadian rhythm. The GO enrichment analysis indicated enrichment in the proteasomal protein catabolic process, proteasome-mediated ubiquitin-dependent protein catabolic process, protein modification by small protein removal, intrinsic apoptotic signaling pathway, protein deneddylation, COPS, Cullin-RING ubiquitin ligase (CRL) complex, SCF ubiquitin ligase complex, Cul4A-RING E3 ubiquitin ligase complex, Cullin family protein binding, ubiquitin-protein transferase activity, and ubiquitin-like protein transferase activity.

## DISCUSSION

The COP9 signalosome (CSN) is a complex protein composed of eight subunits (CSN1-CSN8), participating in various physiological processes. The CSN1, 2, 3, 4, 7, and 8 subunits contain a percutaneous coronary intervention domain, which acts as a scaffold in CSN assembly, while the *COPS6* and *COPS5* subunits possess an Mpr1-Pad 1-N-terminal (MPN) domain[17]. *COPS5* primarily exerts catalytic enzymatic activity, whereas *COPS6*, as an essential component of CSN, lacks the metal-binding site and isopeptidase activity associated with the *COPS5* MPN domain. The precise function of *COPS6* remains has still remained elusive[18]. *COPS6* is involved in various processes, including the ubiquitin proteasome system, signal transduction, DNA damage response, and tumor progression. It exhibits a high expression


A
Overall survival
Low COPS6 Group
High COPS6 Group
Logrank p=0.017
HR(high)=1.5
p(HR)=0.017
n(high)=81
n(low)=81
GBM
Overall survival
Logrank p=0.0079
HR(high)=9.9
p(HR)=0.031
n(high)=32
n(low)=32
KICH
Overall survival
Logrank p=0.0023
HR(high)=2.2
p(HR)=0.0026
n(high)=41
n(low)=41
MESO
Percent survival
Time (months)
ENSG00000168090.9
(COPS6)
Log10 (HR)
1.0
0.5
0.0
-0.5
-1.0
ACC BLCA BRCA CESC CHOL COAD DLBC ESCA GBM HNSC KICH KIRC KIRP LAML LGG LIHC LUAD LUSC MESO OV PAAD PCPG PRAD READ SARC SKCM STAD TGCT THCA THYM UCEC UCS UVM
Overall survival
Logrank p=0.0065
HR(high)=1.6
p(HR)=0.007
n(high)=257
n(low)=257
LGG
Overall survival
Logrank p=0.011
HR(high)=1.6
p(HR)=0.011
n(high)=182
n(low)=182
LIHC
Overall survival
Logrank p=0.017
HR(high)=1.4
p(HR)=0.018
n(high)=239
n(low)=238
LUAD
B
Disease free survival
Logrank p=0.039
HR(high)=0.54
p(HR)=0.042
n(high)=141
n(low)=141
KIRP
Disease free survival
Logrank p=0.0012
HR(high)=1.7
p(HR)=0.0013
n(high)=257
n(low)=257
LGG
Disease free survival
Logrank p=0.023
HR(high)=1.4
p(HR)=0.024
n(high)=182
n(low)=182
LIHC
0.25
0.00
-0.25
Disease free survival
Logrank p=0.029
HR(high)=2.1
p(HR)=0.034
n(high)=38
n(low)=38
ACC
Disease free survival
Logrank p=0.0083
HR(high)=1.6
p(HR)=0.009
n(high)=258
n(low)=258
KIRC
Disease free survival
Logrank p=0.0023
HR(high)=2.5
p(HR)=0.0027
n(high)=41
n(low)=41
MESO
Disease free survival
Logrank p=0.047
HR(high)=1.5
p(HR)=0.049
n(high)=192
n(low)=192
STAD

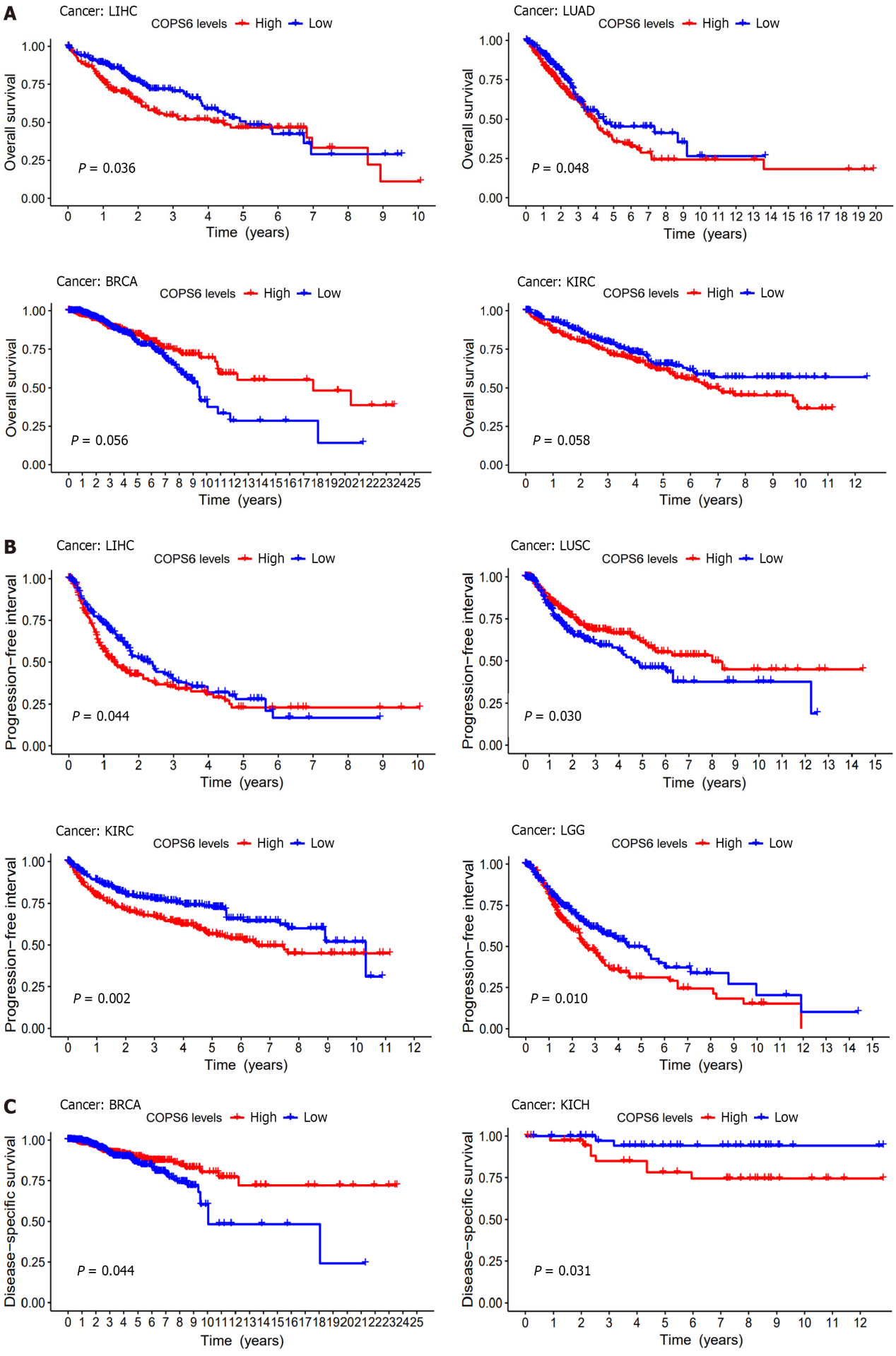


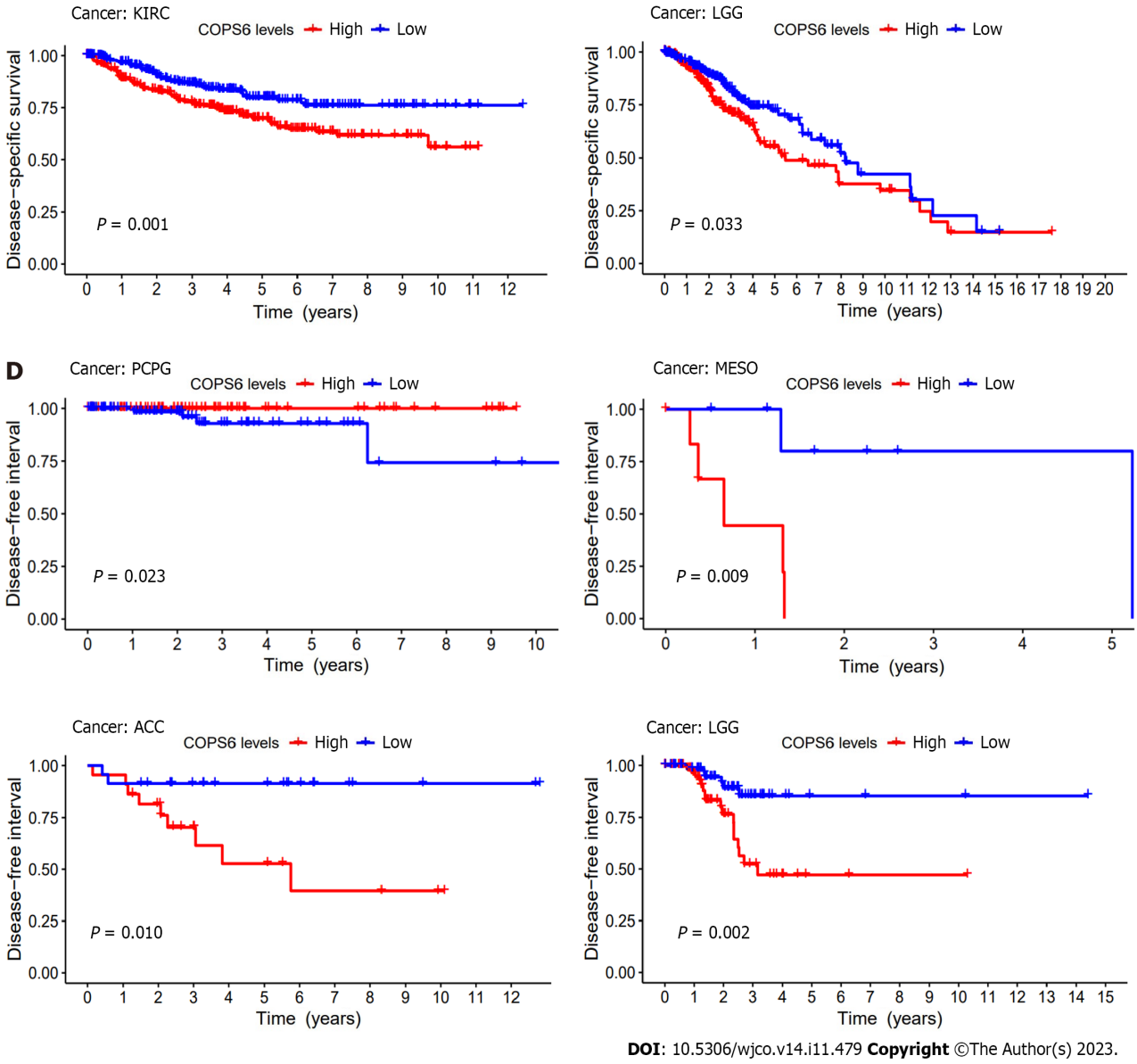

**DOI**: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

**Figure 3 Survival analysis of *COP9 signalosome subunit 6*.** A: Overall survival (OS) and disease-free survival analysis conducted with the GEPIA2 website using the log-rank test ($P < 0.05$); B: Forest plots illustrating disease-specific survival (DSS), OS, progression-free interval (PFI), and disease-free interval (DFI) analyzed with Cox analysis in R software. ACC: Adrenocortical carcinoma; GBM: Glioblastoma multiforme; KICH: Kidney chromophobe; KIRP: Kidney renal papillary cell carcinoma; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; MESO: Mesothelioma; STAD: Stomach adenocarcinoma.

level in diverse tumors, and studies have explored its role in cancer[19].

At present, there is a growing interest among researchers in investigating the role of *COPS6* in tumors, as it has been shown to predominantly promote cancer. The CRLs are involved in the ubiquitination of Myc, and Fbxw7, a CRL component, which participates in Myc ubiquitination. In mouse experiments, Chen *et al*[20] demonstrated that *COPS6* enhances Fbxw7 degradation through binding, thereby maintaining Myc stability and promoting tumor progression. Additionally, in a mouse model, Zhao *et al*[21] revealed that *COPS6* attenuates p53-mediated tumor suppression, promotes tumor growth by stabilizing MDM2 protein, and participates in DNA damage-associated apoptosis. In human tumors, *COPS6* also plays a significant role in tumor progression. Fang *et al*[22] demonstrated that *COPS6* overexpression in CRC is associated with a worse prognosis. Mechanistic studies suggested that ERK2 directly binds to CSN6 Leu163/Val165 and phosphorylates *COPS6* at Ser148, thereby regulating β-Trcp and stabilizing β-catenin expression, consequently blocking the ubiquitin-proteasome pathway and promoting CRC development.


A
Cancer: LIHC
COPS6 levels High Low
Overall survival
P = 0.036
Time (years)
Cancer: LUAD
COPS6 levels High Low
Overall survival
P = 0.048
Time (years)
Cancer: BRCA
COPS6 levels High Low
Overall survival
P = 0.056
Time (years)
Cancer: KIRC
COPS6 levels High Low
Overall survival
P = 0.058
Time (years)
B
Cancer: LIHC
COPS6 levels High Low
Progression−free interval
P = 0.044
Time (years)
Cancer: LUSC
COPS6 levels High Low
Progression−free interval
P = 0.030
Time (years)
Cancer: KIRC
COPS6 levels High Low
Progression−free interval
P = 0.002
Time (years)
Cancer: LGG
COPS6 levels High Low
Progression−free interval
P = 0.010
Time (years)
C
Cancer: BRCA
COPS6 levels High Low
Disease−specific survival
P = 0.044
Time (years)
Cancer: KICH
COPS6 levels High Low
Disease−specific survival
P = 0.031
Time (years)

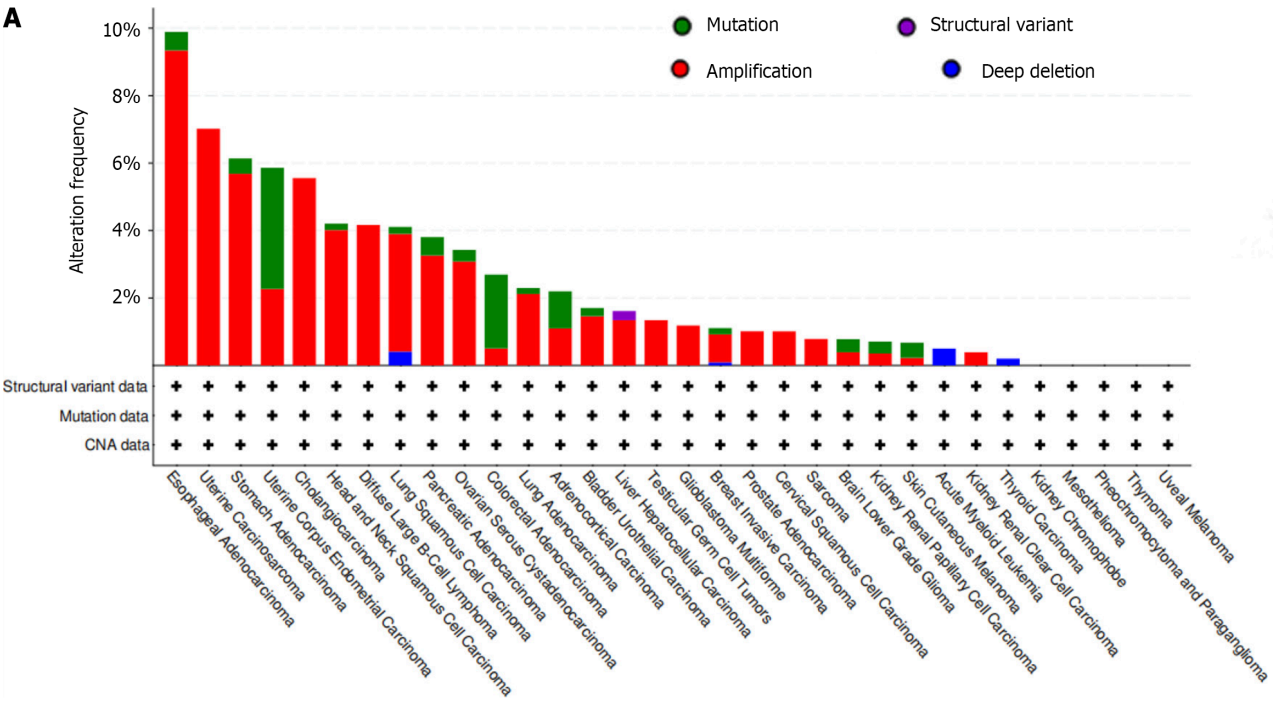


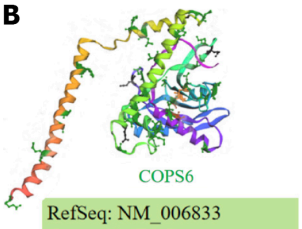

**DOI**: 10.5306/wjco.v14.i11.479 

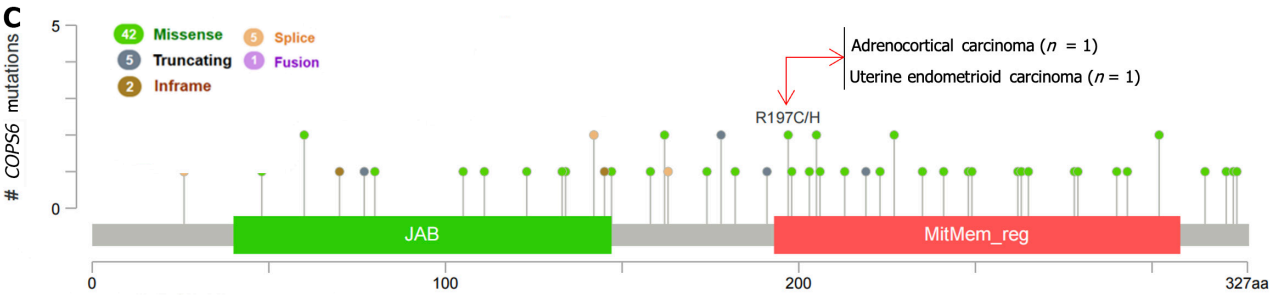
**Figure 4 Kaplan-Meier survival analysis of *COP9 signalosome subunit 6* in the The Cancer Genome Atlas database performed in R software.** A: Overall survival analysis. B: Progression-free interval analysis; C: Disease-specific survival analysis; D: Disease-free interval analysis. ACC: Adrenocortical carcinoma; BRCA: Breast invasive carcinoma; KIRC: Kidney renal clear cell carcinoma; LGG: Lower grade glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; MESO: Mesothelioma.

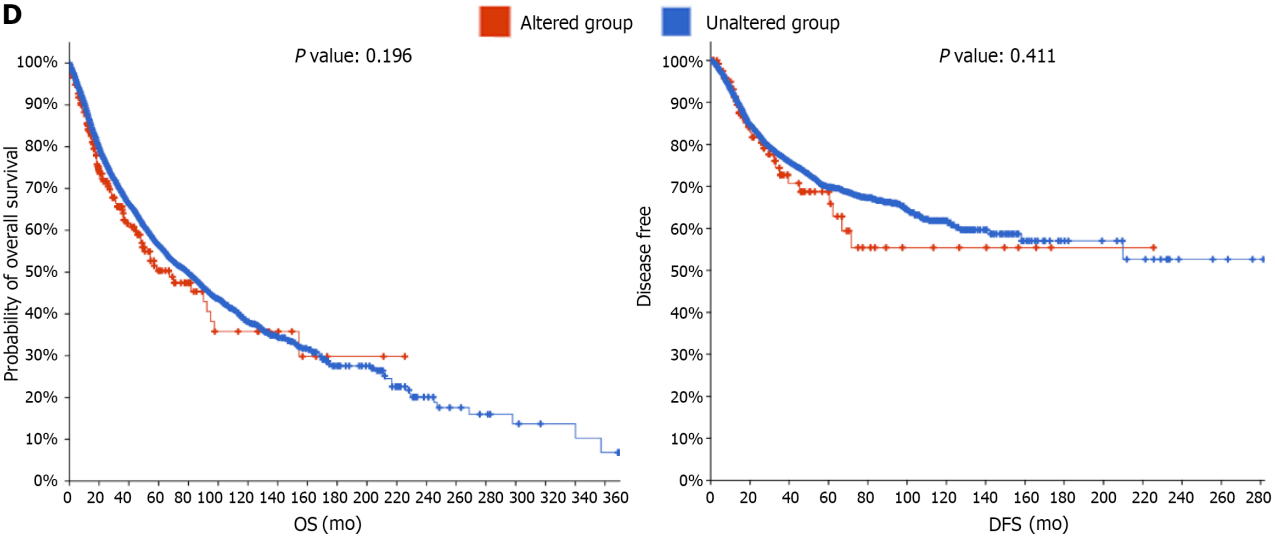
Programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) checkpoint blockade is an emerging immunotherapy modality in various tumors, while its regulatory mechanism remains uncertain. Su *et al*[23] demonstrated that *COPS6* expression level could be regulated by the EGFR-ERK pathway, inhibiting PD-L1 degradation and maintaining PD-L1 stability in GBM. Additionally, several studies have reported the involvement of *COPS6* in the epithelial-mesenchymal transition process in various tumors, promoting tumor invasion and metastasis. For instance, Zhang *et al*[24] revealed that the COPS6-UBR5-CDK9 axis could control melanoma proliferation and metastasis, while Mao *et al*[25] found that *COPS6* could promote migration and invasion of cervical cancer cells by regulating the expression level of cathepsin L through the autophagy-lysosomal system. Furthermore, *COPS6* was found to maintain the key transcription factor Snail1, promoting the invasion of breast cancer cells by inhibiting Snail1 ubiquitination[26].

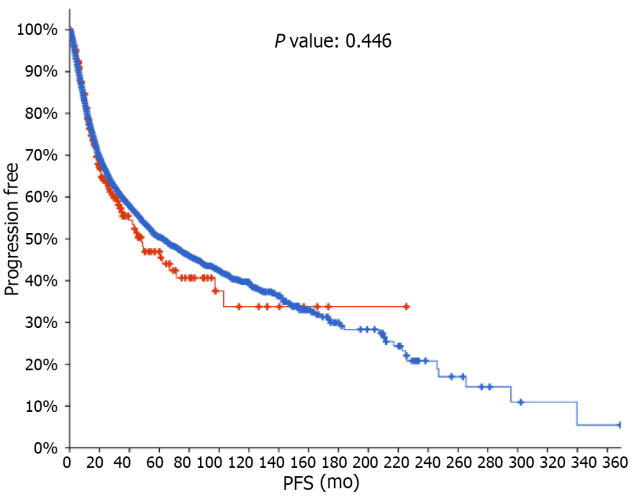
While previous studies have highlighted the significant role of *COPS6* in the progression of specific tumors, the heterogeneity of tumors suggests potential variations in its function across different cancer types. Therefore, a comprehensive analysis and screening are necessary to validate existing findings and provide direction for the future *COPS6*-related studies. In the present study, it was attempted to conduct comprehensive multilevel differential analysis and survival analysis of *COPS6* in pan-cancer data collected from various public databases and online analysis tools, including TCGA, GEO, CPTAC, GEPIA2, TIMER2, and UALCAN. The findings demonstrated that the expression level of the *COPS6* gene was significantly upregulated in the most types of cancer compared with normal tissues, except for KICH and LAML. Prognostic analysis revealed that the high expression level of *COPS6* was typically associated with worse prognosis in the majority of tumors, while showing a favorable prognosis in KIRP, BRCA, LUSC, and PCPG. Mutational analysis

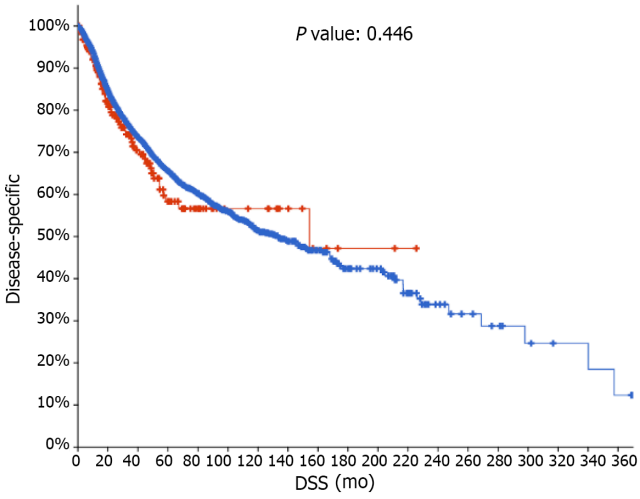

A
Mutation
Structural variant
Amplification
Deep deletion
Alteration frequency
10%
8%
6%
4%
2%
Structural variant data
Mutation data
CNA data
Esophageal Adenocarcinoma
Uterine Carcinosarcoma
Stomach Adenocarcinoma
Uterine Corpus Endometrial Carcinoma
Cholangiocarcinoma
Head and Neck Squamous Cell Carcinoma
Diffuse Large B-Cell Lymphoma
Lung Squamous Cell Carcinoma
Pancreatic Adenocarcinoma
Ovarian Serous Cystadenocarcinoma
Colorectal Adenocarcinoma
Lung Adenocarcinoma
Adrenocortical Carcinoma
Bladder Urothelial Carcinoma
Liver Hepatocellular Carcinoma
Testicular Germ Cell Tumors
Glioblastoma Multiforme
Breast Invasive Carcinoma
Prostate Adenocarcinoma
Cervical Squamous Cell Carcinoma
Sarcoma
Brain Lower Grade Glioma
Kidney Renal Papillary Cell Carcinoma
Skin Cutaneous Melanoma
Acute Myeloid Leukemia
Kidney Renal Clear Cell Carcinoma
Thyroid Carcinoma
Kidney Chromophobe
Mesothelioma
Pheochromocytoma and Paraganglioma
Thymoma
Uveal Melanoma

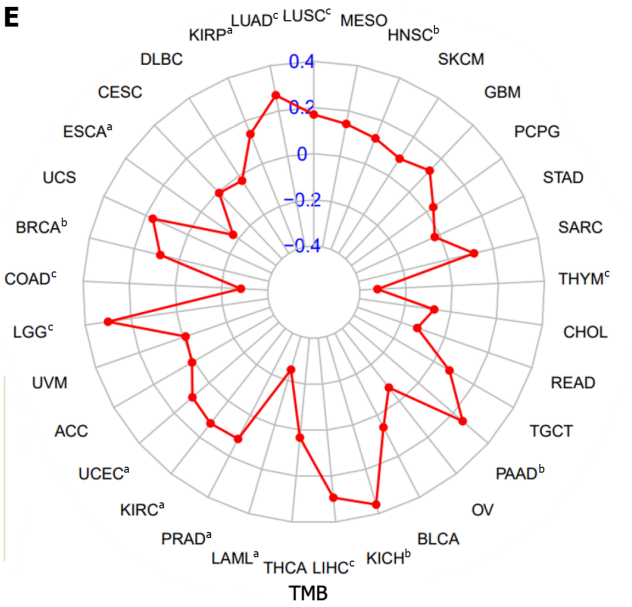

B
COPS6
RefSeq: NM_006833
Ensembl: ENST00000303904
CCDS: CCDS5682
UniProt: CNS6_HUMAN

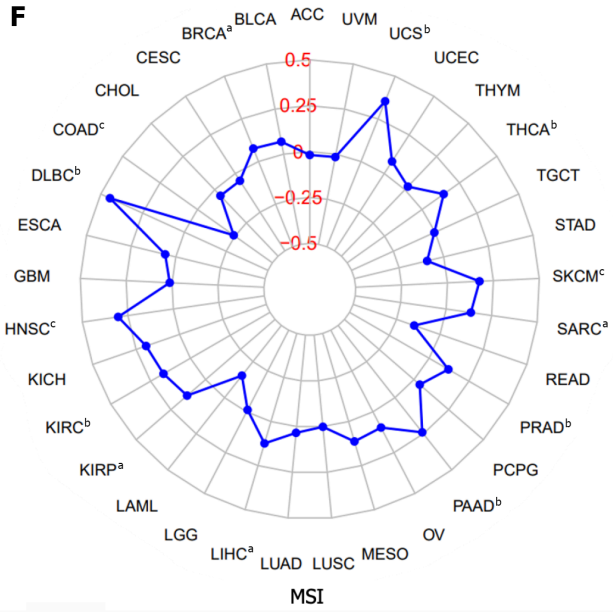

C
# COPS6 mutations
42 Missense
5 Truncating
2 Inframe
5 Splice
1 Fusion
Adrenocortical carcinoma (n = 1)
Uterine endometrioid carcinoma (n = 1)
R197C/H
JAB
MitMem_reg
0
100
200
327aa

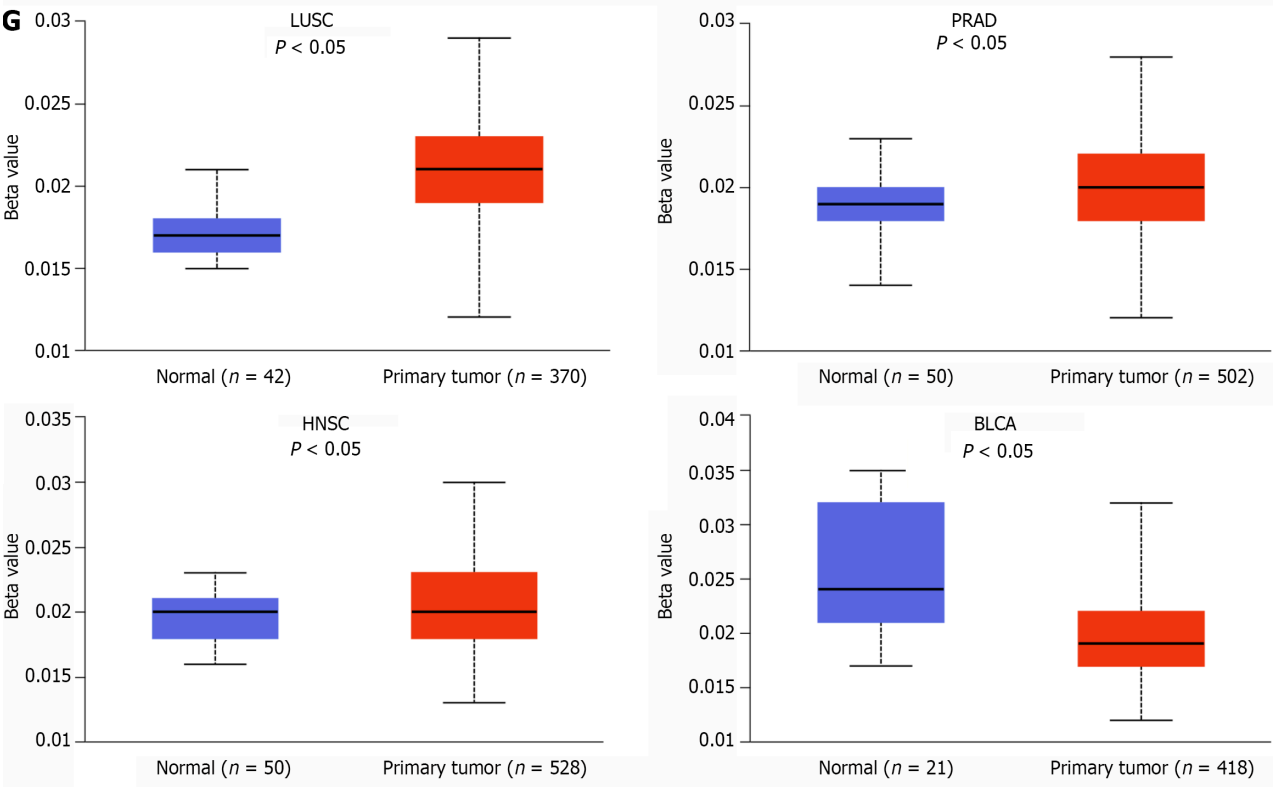

D
Altered group
Unaltered group
P value: 0.196
Probability of overall survival
OS (mo)
P value: 0.411
Disease free
DFS (mo)

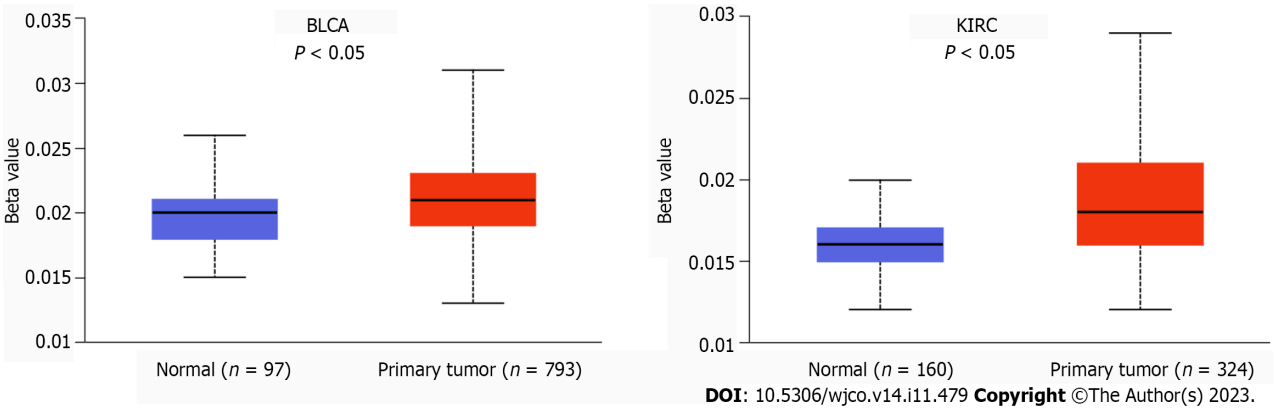

P value: 0.446
Progression free
PFS (mo)


P value: 0.446
Disease-specific
DSS (mo)


E
TMB


F
MSI


G
LUSC
P < 0.05
Normal (n = 42)
Primary tumor (n = 370)
PRAD
P < 0.05
Normal (n = 50)
Primary tumor (n = 502)
HNSC
P < 0.05
Normal (n = 50)
Primary tumor (n = 528)
BLCA
P < 0.05
Normal (n = 21)
Primary tumor (n = 418)
Beta value




**DOI**: 10.5306/wjco.v14.i11.479 

**Figure 5 Mutation analysis of *COP9 signalosome subunit 6*.** A: Mutation frequency and types visualized through the cBioPortal website; B: Three-dimensional structure highlighting the R197C/H mutation site in *COP9 signalosome subunit 6 (COPS6)*; C: Mutation sites depicted in the cBioPortal website; D: Survival analysis of *COPS6* mutations in pan-cancer; E: Correlation of *COPS6* with tumor mutational burden (tumor mutational burden) in pan-cancer using R software; F: Correlation of *COPS6* with microsatellite instability (microsatellite instability) in pan-cancer using R software; G: Promoter methylation levels of *COPS6* in prostate adenocarcinoma (PRAD), lung squamous cell carcinoma (LUSC), head and neck squamous cell carcinoma (HNSC), breast invasive carcinoma (BRCA), bladder cancer (BLCA), and kidney renal clear cell carcinoma (KIRC) accessed through the UALCAN website. OS: Overall survival; DFS: Disease-free survival; PFS: Progression-free survival; DSS: Disease-specific survival.

indicated that missense mutations were the predominant mutation type found in *COPS6*. Additionally, TMB and MSI exhibited a positive correlation with *COPS6* expression level in most of the tumors, and only few tumors showed a negative correlation. Further exploration of the impact of *COPS6* mutations on patient outcomes revealed that these mutations did not significantly contribute to a worse prognosis in any specific tumor types. However, a comprehensive analysis across all tumors indicated a trend towards a shorter OS associated with *COPS6* mutations. Therefore, it can be concluded that *COPS6* mutations have a limited effect on patient prognosis.

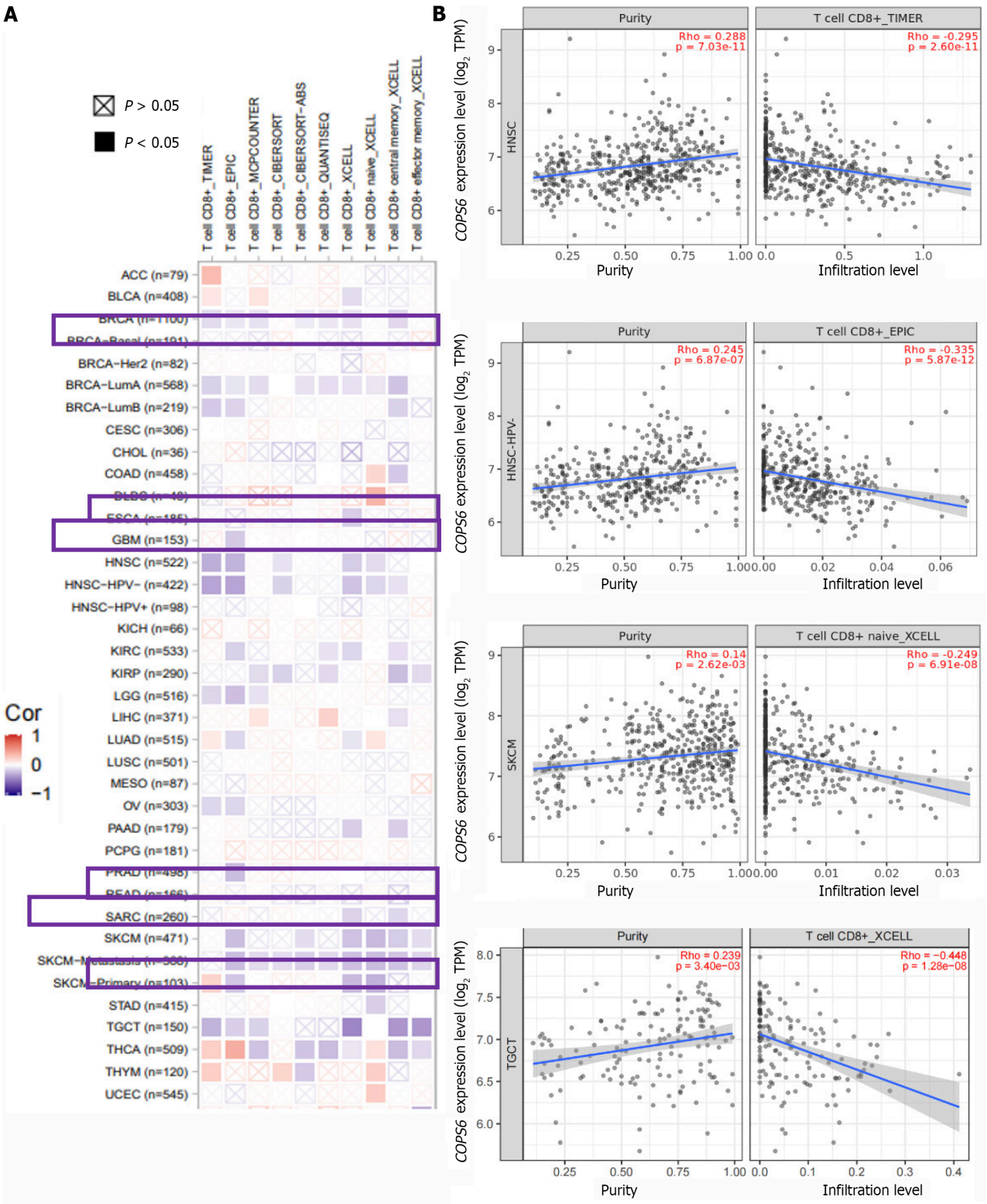
Subsequently, the association between *COPS6* expression level and the tumor immune microenvironment (TMIE) was investigated in various types of cancer. The TMIE plays a pivotal role in tumor progression, immune evasion, and therapeutic resistance, involving key components, such as CD8+ T cells, cancer-associated fibroblasts, macrophages, and NK cells[27]. The findings of the present study demonstrated a negative correlation between *COPS6* expression level and CD8+ T cell infiltration in several tumors, such as BRCA, HNSC, and TGCT. This aligns with Du *et al*[28]'s results, demonstrating that *COPS6* could inhibit CD8+ T cell infiltration within the tumor microenvironment (TME), thereby facilitating tumor immune evasion. Furthermore, a negative correlation was identified between *COPS6* expression level and the M1 phenotype of tumor-associated macrophages (TAMs), while a positive correlation was found with the M2 phenotype. TAMs, which are macrophages that infiltrate tumor tissue and differentiate from monocytes, predominantly adopt the immunosuppressive M2 phenotype in the TMIE[29]. The present study revealed a positive correlation between *COPS6* expression level and the M2 phenotype in the TIDE algorithm for DLBCL, BRCA, KIRC, THCA, THYM, and other tumors, while other algorithms exhibited a negative correlation with the M1 phenotype. However, it is noteworthy that in some tumors, only the TIDE algorithm yielded consistent results, while other algorithms suggested a negative or no correlation between *COPS6* expression level and the M2 phenotype. This discrepancy could be attributed to variations in the statistical methods employed by each algorithm, necessitating further experimental validation of these findings.

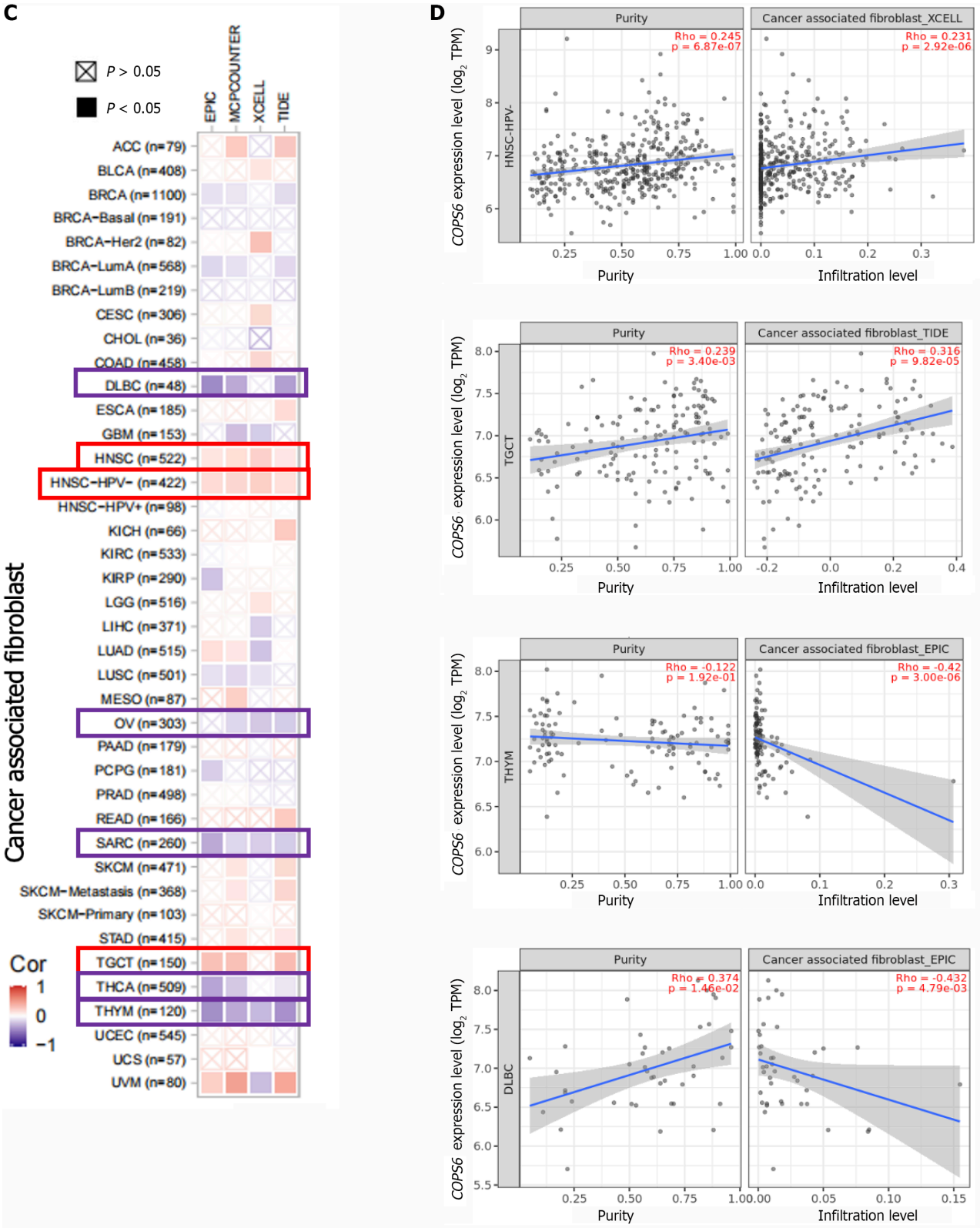
There is a scarcity of research regarding the interaction between *COPS6* Level and TME, highlighting the urgent need to explore the role of *COPS6* in the TME. Furthermore, in the present study, correlation and enrichment analyses of *COPS6* were conducted, and *GPS1* and *TCEB2* were identified as the two genes, exhibiting the strongest correlation. This investigation sheds light on the potential function and significance of *COPS6* as a novel biomarker in cancer, setting the stage for further research on its molecular mechanisms and the development of targeted therapies. Moreover, the findings emphasize the importance of studying the *COPS6*-related TIME. However, it should be noted that the current study of *COPS6* is preliminary, and the specific mechanisms of its action in different types of cancer remain elusive. Therefore, additional resources and efforts are warranted to delve deeper into the role of *COPS6* in cancer.

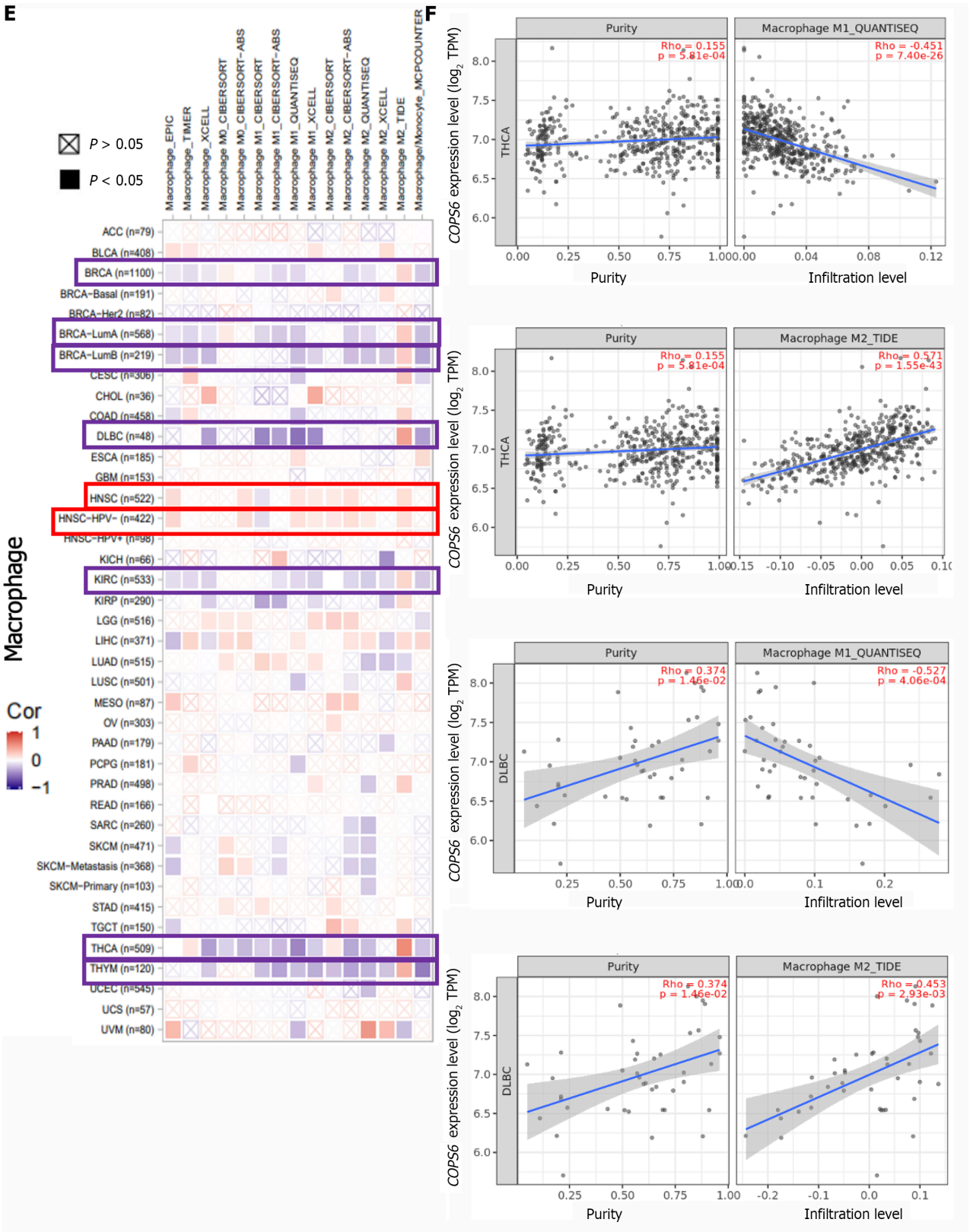
The present study revealed a potential association of *COPS6* with survival outcomes in various tumors. Notably, *GPS1* and *TCEB2* were identified as the two genes exhibiting the strongest correlation with *COPS6* at both the gene and protein levels, making them promising targets for future investigations. Additionally, a significant association was found between *COPS6* expression level and immune infiltration in diverse types of cancer, such as BRCA, HNSC, and TGCT, where research on the TIME remains limited.

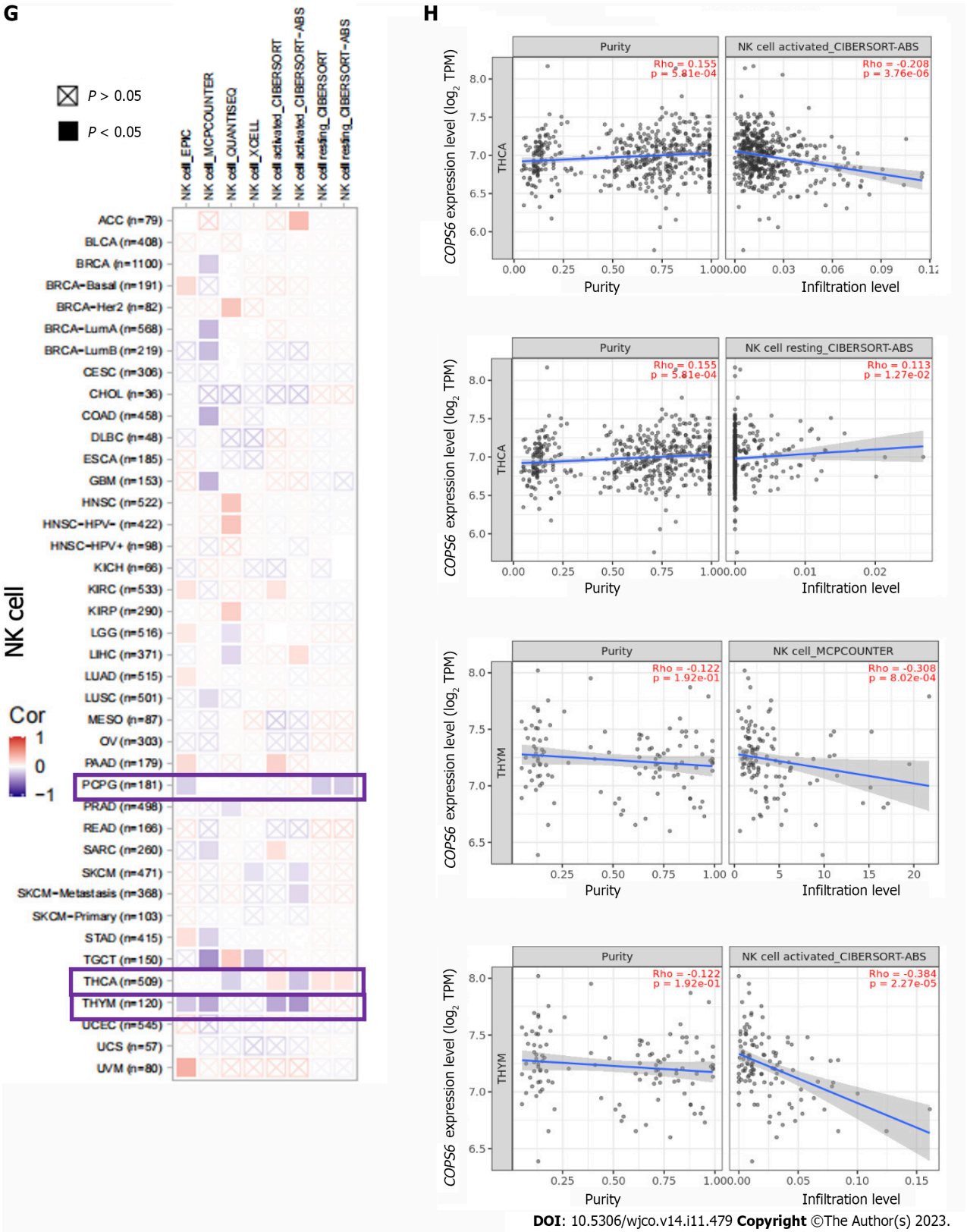
## CONCLUSION

This study is the first to explore the role of *COPS6* in pan-cancer, taking full use of the existing public database to investigate *COPS6* from the aspects of gene expression level, mutation, TIME, and prognosis. However, there are also some deficiencies in this study. For instance, only a multifaceted analysis of *COPS6* was conducted through


A
P > 0.05
P < 0.05
T cell CD8+_TIMER
T cell CD8+_EPIC
T cell CD8+_MCPCOUNTER
T cell CD8+_CIBERSORT
T cell CD8+_CIBERSORT-ABS
T cell CD8+_QUANTISEQ
T cell CD8+_XCELL
T cell CD8+ naive_XCELL
T cell CD8+ central memory_XCELL
T cell CD8+ effector memory_XCELL
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA-Basal (n=191)
BRCA-Her2 (n=82)
BRCA-LumA (n=568)
BRCA-LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC-HPV- (n=422)
HNSC-HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM-Metastasis (n=368)
SKCM-Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
Cor
1
0
−1
B
Purity
T cell CD8+_TIMER
Rho = 0.288
p = 7.03e-11
Rho = -0.295
p = 2.60e-11
HNSC
COPS6 expression level (log2 TPM)
Purity
Infiltration level
T cell CD8+_EPIC
Rho = 0.245
p = 6.87e-07
Rho = -0.335
p = 5.87e-12
HNSC-HPV-
T cell CD8+ naive_XCELL
Rho = 0.14
p = 2.62e-03
Rho = -0.249
p = 6.91e-08
SKCM
T cell CD8+_XCELL
Rho = 0.239
p = 3.40e-03
Rho = −0.448
p = 1.28e-08
TGCT

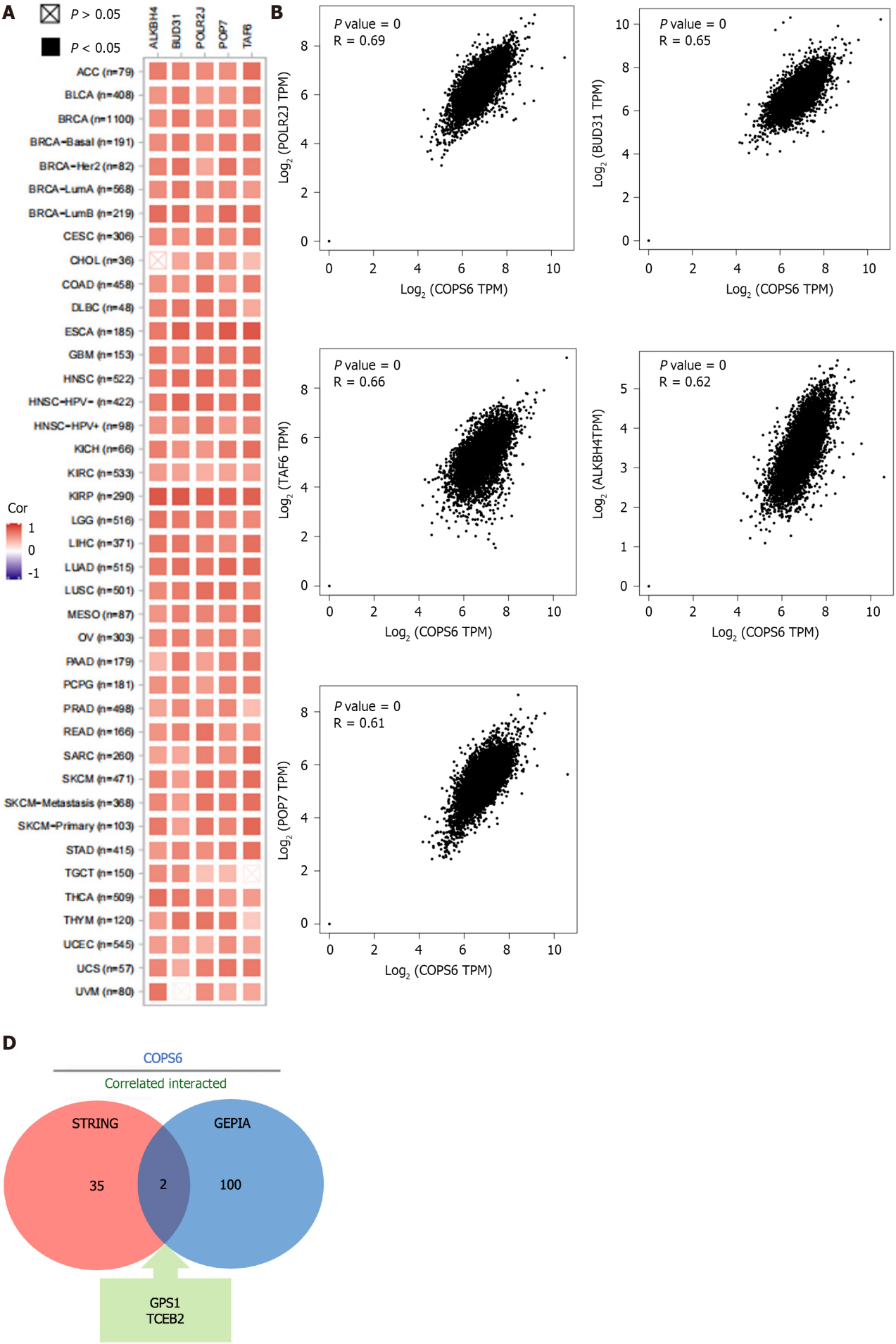

C
P > 0.05
P < 0.05
EPIC
MCPCOUNTER
XCELL
TIDE
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA−Basal (n=191)
BRCA−Her2 (n=82)
BRCA−LumA (n=568)
BRCA−LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC−HPV− (n=422)
HNSC−HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM−Metastasis (n=368)
SKCM−Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
UCS (n=57)
UVM (n=80)
Cancer associated fibroblast
Cor
1
0
−1
D
Purity
Cancer associated fibroblast_XCELL
Rho = 0.245
p = 6.87e-07
Rho = 0.231
p = 2.92e-06
HNSC−HPV−
COPS6 expression level (log2 TPM)
Purity
Infiltration level
Cancer associated fibroblast_TIDE
Rho = 0.239
p = 3.40e-03
Rho = 0.316
p = 9.82e-05
TGCT
Cancer associated fibroblast_EPIC
Rho = -0.122
p = 1.92e-01
Rho = -0.42
p = 3.00e-06
THYM
Rho = 0.374
p = 1.46e-02
Rho = -0.432
p = 4.79e-03
DLBC

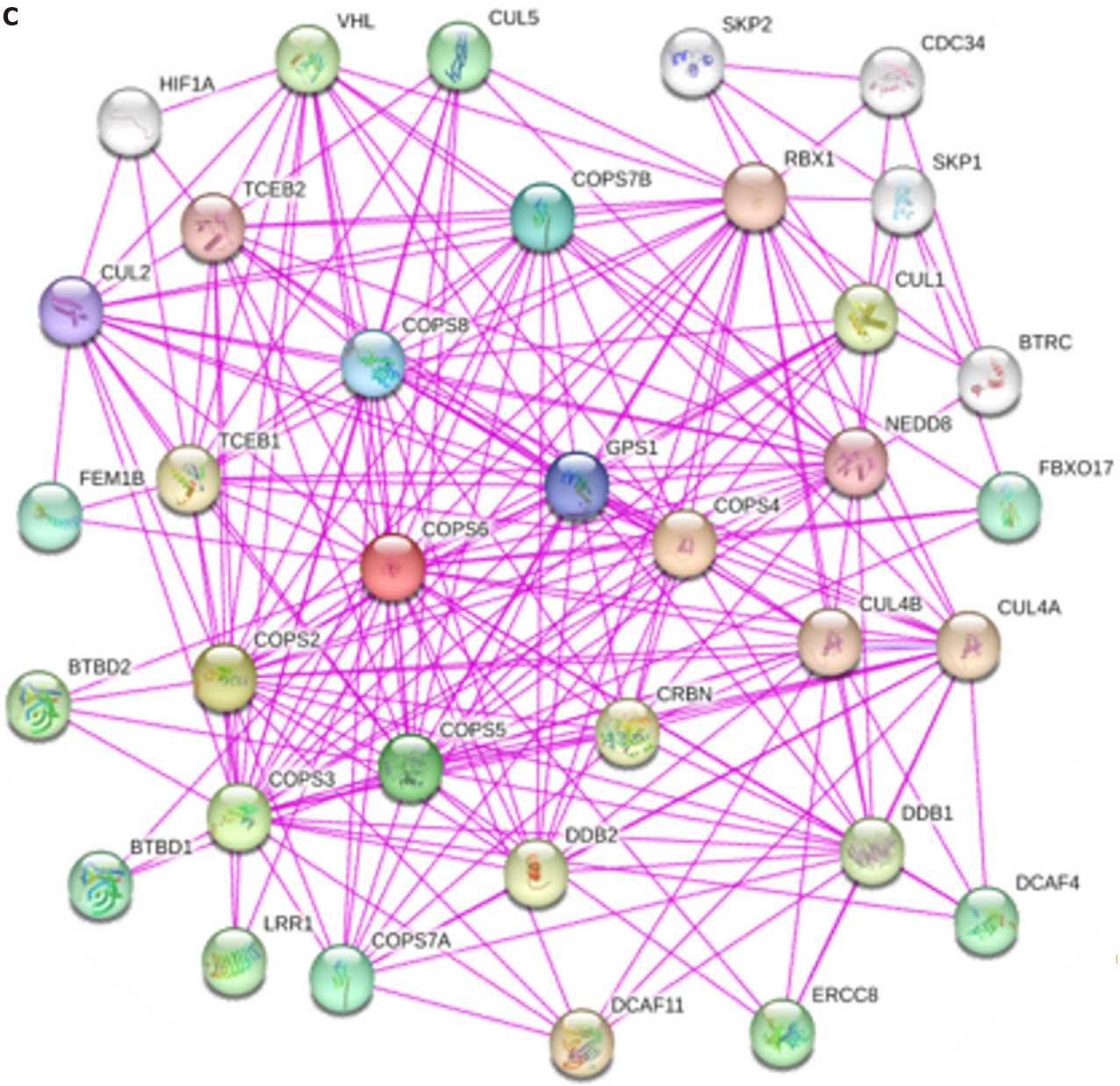

E
F
P > 0.05
P < 0.05
Macrophage_EPIC
Macrophage_TIMER
Macrophage_XCELL
Macrophage M0_CIBERSORT
Macrophage M0_CIBERSORT-ABS
Macrophage M1_CIBERSORT
Macrophage M1_CIBERSORT-ABS
Macrophage M1_QUANTISEQ
Macrophage M1_XCELL
Macrophage M2_CIBERSORT
Macrophage M2_CIBERSORT-ABS
Macrophage M2_QUANTISEQ
Macrophage M2_XCELL
Macrophage M2_TIDE
Macrophage/Monocyte_MCPCOUNTER
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA-Basal (n=191)
BRCA-Her2 (n=82)
BRCA-LumA (n=568)
BRCA-LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC-HPV- (n=422)
HNSC-HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM-Metastasis (n=368)
SKCM-Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
UCS (n=57)
UVM (n=80)
Macrophage
Cor
1
0
−1
Purity
Macrophage M1_QUANTISEQ
Rho = 0.155
p = 5.81e-04
Rho = -0.451
p = 7.40e-26
THCA
COPS6 expression level (log2 TPM)
Purity
Infiltration level
Purity
Macrophage M2_TIDE
Rho = 0.155
p = 5.81e-04
Rho = 0.571
p = 1.55e-43
THCA
COPS6 expression level (log2 TPM)
Purity
Infiltration level
Purity
Macrophage M1_QUANTISEQ
Rho = 0.374
p = 1.46e-02
Rho = -0.527
p = 4.06e-04
DLBC
COPS6 expression level (log2 TPM)
Purity
Infiltration level
Purity
Macrophage M2_TIDE
Rho = 0.374
p = 1.46e-02
Rho = 0.453
p = 2.93e-03
DLBC
COPS6 expression level (log2 TPM)
Purity
Infiltration level

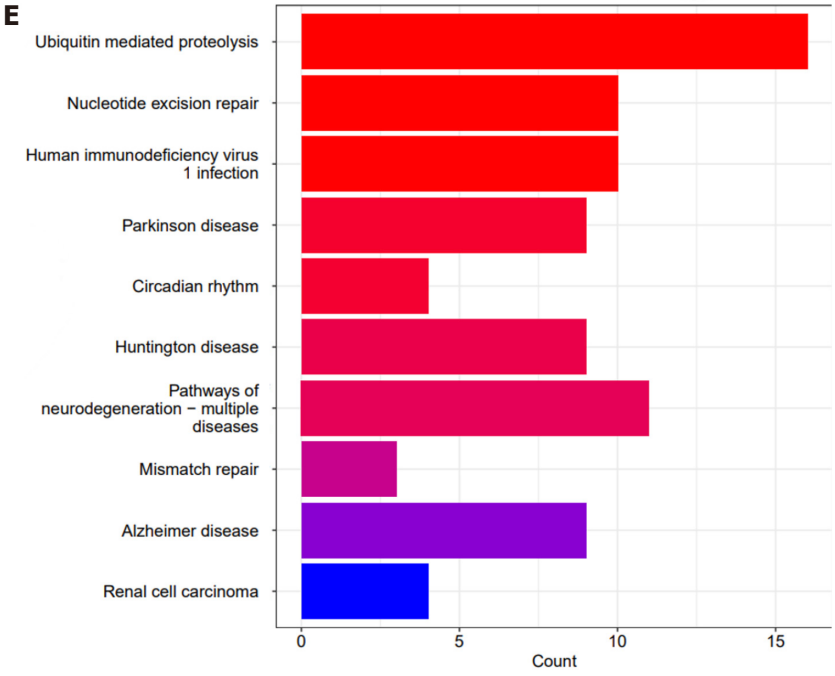


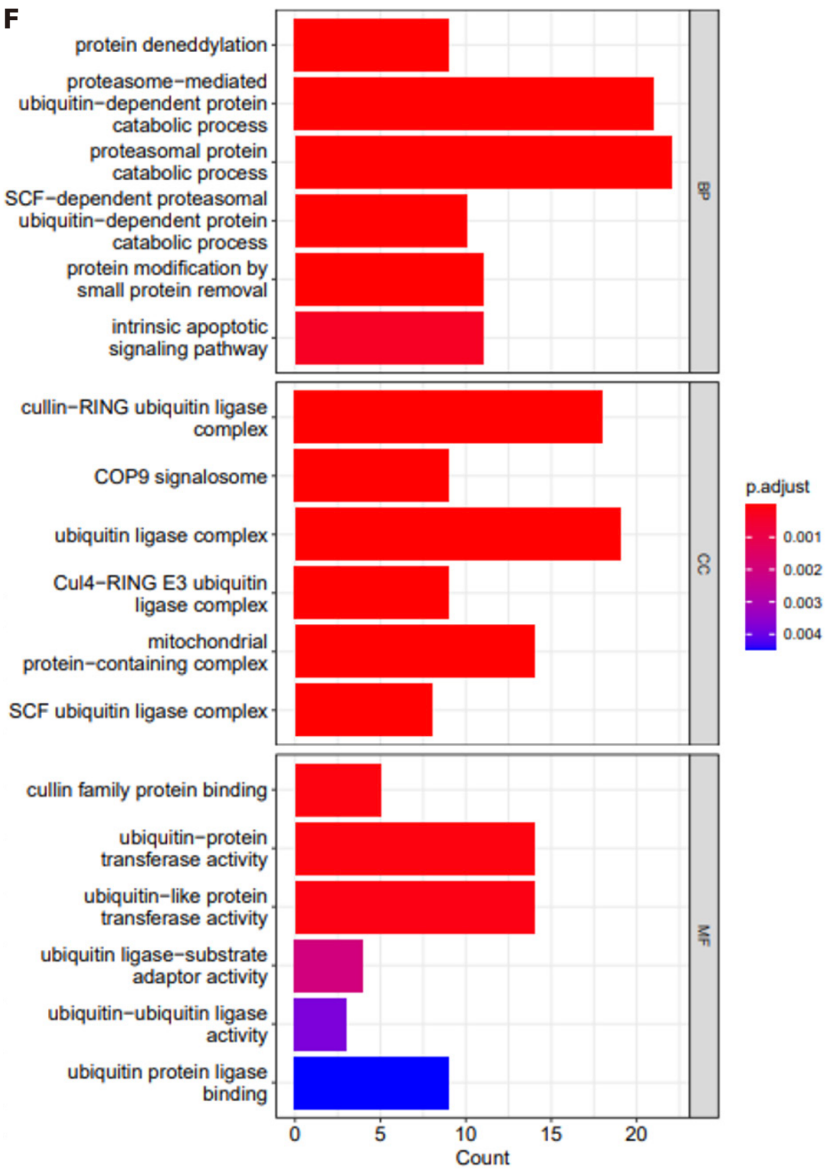

**DOI**: 10.5306/wjco.v14.i11.479 **Copyright** ©The Author(s) 2023.

**Figure 6 Immune infiltration analysis of *COP9 signalosome subunit 6* in the The Cancer Genome Atlas database using the TIMER2 website.** A: Heatmap depicting the correlation between *COP9 signalosome subunit 6 (COPS6)* and CD8+ T cells; B: Scatter plot illustrating the relationship between *COPS6* and CD8+ T cells; C: Heatmap displaying the correlation between *COPS6* and cancer-associated fibroblasts; D: Scatter plot demonstrating the relationship between *COPS6* and cancer-associated fibroblasts; E: Heatmap indicating the correlation between *COPS6* and macrophages; F: Scatter plot showing the relationship between *COPS6* and macrophages; G: Heatmap presenting the correlation between *COPS6* and natural killer (NK) cells; H: Scatter plot depicting the relationship between *COPS6* and NK cells. TPM: Transcripts per million.


A
P > 0.05
P < 0.05
ALKBH4
BUD31
POLR2J
POP7
TAF6
ACC (n=79)
BLCA (n=408)
BRCA (n=1100)
BRCA−Basal (n=191)
BRCA−Her2 (n=82)
BRCA−LumA (n=568)
BRCA−LumB (n=219)
CESC (n=306)
CHOL (n=36)
COAD (n=458)
DLBC (n=48)
ESCA (n=185)
GBM (n=153)
HNSC (n=522)
HNSC−HPV− (n=422)
HNSC−HPV+ (n=98)
KICH (n=66)
KIRC (n=533)
KIRP (n=290)
LGG (n=516)
LIHC (n=371)
LUAD (n=515)
LUSC (n=501)
MESO (n=87)
OV (n=303)
PAAD (n=179)
PCPG (n=181)
PRAD (n=498)
READ (n=166)
SARC (n=260)
SKCM (n=471)
SKCM−Metastasis (n=368)
SKCM−Primary (n=103)
STAD (n=415)
TGCT (n=150)
THCA (n=509)
THYM (n=120)
UCEC (n=545)
UCS (n=57)
UVM (n=80)
Cor
1
0
-1
B
P value = 0
R = 0.69
Log2 (POLR2J TPM)
Log2 (COPS6 TPM)
P value = 0
R = 0.65
Log2 (BUD31 TPM)
Log2 (COPS6 TPM)
P value = 0
R = 0.66
Log2 (TAF6 TPM)
Log2 (COPS6 TPM)
P value = 0
R = 0.62
Log2 (ALKBH4TPM)
Log2 (COPS6 TPM)
P value = 0
R = 0.61
Log2 (POP7 TPM)
Log2 (COPS6 TPM)
D
COPS6
Correlated interacted
STRING
GEPIA
35
2
100
GPS1
TCEB2


C
VHL
CUL5
SKP2
CDC34
HIF1A
RBX1
SKP1
TCEB2
COPS7B
CUL2
CUL1
COPS8
BTRC
NEDD8
TCEB1
GPS1
FEM1B
FBXO17
COPS4
COPS6
CUL4B
CUL4A
COPS2
BTBD2
CRBN
COPS5
COPS3
DDB1
BTBD1
DDB2
DCAF4
LRR1
COPS7A
DCAF11
ERCC8


E
Ubiquitin mediated proteolysis
Nucleotide excision repair
Human immunodeficiency virus 1 infection
Parkinson disease
Circadian rhythm
Huntington disease
Pathways of neurodegeneration − multiple diseases
Mismatch repair
Alzheimer disease
Renal cell carcinoma
0
5
10
15
Count




**DOI**: 10.5306/wjco.v14.i11.479 

**Figure 7 Correlation analysis and enrichment analysis of *COPS6*.** A: Heatmap displaying the top 5 genes correlated with *COPS6* in pan-cancer accessed through the TIMER2 website; B: Scatter plot illustrating the correlation between *COPS6* and the top 5 genes in pan-cancer using the GEPIA2 website; C: Protein-protein interaction network of *COPS6* obtained from the STRING database; D: Intersection of *COPS6*-related genes screened in GEPIA2 and STRING, resulting in GPS1 and TCEB2; E: Kyoto Encyclopedia of Genes and Genomes enrichment analysis of the combined *COPS6*-related genes from GEPIA2 and STRING; F: Gene ontology enrichment analysis of the combined *COPS6*-related genes from GEPIA2 and STRING. TPM: Transcripts per million.

bioinformatics, while no experiment was carried out to verify the results, hindering the generalization of the findings.

In conclusion, the present study provided early evidence that *COPS6* could be associated with clinicopathological characteristics in various tumors and could play a role in several cancer hallmarks. Additional research is needed to further elucidate the role of *COPS6* in cancer progression.

## ARTICLE HIGHLIGHTS

### *Research background*

The COP9 signaling body subunit 6 (*COPS6*) has been implicated in cancer progression, but its precise role in most types of cancer is unknown.

### *Research motivation*

This study aimed to investigate the functional and clinical relevance of *COPS6* in different tumor types, using publicly available databases.

### Research objectives
This study hopes to provide a basis for *COPS6* as a novel biomarker for cancer research by exploring the role of *COPS6* in different cancer types.

### Research methods
We used R software and online analysis databases to analyze the differential expression, prognosis, mutation and related functions of *COPS6* in pan-cancer.

### Research results
Differential expression analysis and survival analysis demonstrated that *COPS6* was highly expressed and associated with high-risk profiles in the majority of cancer types. Missense mutations are the main type of *COPS6* mutations, and in most types of cancer, the levels of *COPS6* expression are positively correlated with tumor mutation burden and microsatellite instability. Immune infiltration analysis found *COPS6* to play different roles in different cancers. Gene co-expression and enrichment analysis highlighted *COPS6*-related genes were predominantly involved in processes, such as ubiquitin-mediated proteolysis and human immunodeficiency virus 1 infection.

### Research conclusions
This study provides early evidence that *COPS6* may be associated with the clinicopathological features of various tumors and may play a role in several cancer features, providing a basis for subsequent studies related to *COPS6*.

### Research perspectives
Since this study mainly focused on data analysis, subsequent studies required experimental validation of relevant results.


## FOOTNOTES

**Co-first authors:** Shi-Lin Wang and Guang-Zheng Zhuo.

**Co-corresponding authors:** Yun-Bao Pan and Yi-Rong Li.

**Author contributions:** Pan YB designed the research; Wang SL and Zhuo GZ performed the research; Wang SL, Wang LP and Zhuo GZ contributed analytic tools; Wang SL and Zhuo GZ analyzed the data; Wang SL and Pan YB wrote the paper; Pan YB and Li YR were responsible for the supervision. Wang SL and Zhuo GZ contributed equally to this work as co-first authors. The reasons for designating Wang SL and Zhuo GZ as co-first authors are twofold. First, the research was performed as a collaborative effort, and the designation of co-corresponding authorship accurately reflects the distribution of responsibilities and burdens associated with the time and effort required to complete the study and the resultant paper. Second, Wang SL and Zhuo GZ contributed an equally substantial effort throughout the study. They are principal principals of paper writing and data analysis, selecting these researchers as co-first authors, recognizing and respecting this equal contribution. Pan YB and Li YR contributed equally to this work as co-corresponding authors. The reasons for designating Pan YB and Li YR as co-corresponding authors are twofold. First, the research was performed as a collaborative effort. This ensures effective communication and management of post-submission matters, ultimately enhancing the paper's quality and reliability. Second, the overall research team encompassed authors with a variety of expertise and skills from different fields, and the designation of co-corresponding authors best reflects this diversity. Pan YB and Li YR, as heads of both groups, contributed substantially to the experimental design, data analysis and revision, and were therefore listed as co-corresponding authors.

**Supported by** National Natural Science Foundation of China, No. 31900558; the Hubei Provincial Youth Talents Program for Public Health, No. WSJKRC2022013; Wuhan Young and Middle-Aged Medical Backbone Talents Training Project, No. WHQG201904.

**Conflict-of-interest statement:** The authors have no relevant financial or non-financial interests to disclose.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at [panyunbao@outlook.com]. Participants gave informed consent for data sharing.



**Country/Territory of origin:** China

**ORCID number:** Yun-Bao Pan 0000-0002-6311-2945; Yi-Rong Li 0000-0002-7493-045X.

**S-Editor:** Lin C
**L-Editor:** Filipodia
**P-Editor:** Zhang XD

## REFERENCES


1 **Siegel RL**, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin* 2023; **73**: 17-48 [PMID: 36633525 DOI: 10.3322/caac.21763]

2 **Feng R**, Su Q, Huang X, Basnet T, Xu X, Ye W. Cancer situation in China: what does the China cancer map indicate from the first national death survey to the latest cancer registration? *Cancer Commun (Lond)* 2023; **43**: 75-86 [PMID: 36397729 DOI: 10.1002/cac2.12393]

3 **Wang Y**, Yan Q, Fan C, Mo Y, Wang Y, Li X, Liao Q, Guo C, Li G, Zeng Z, Xiong W, Huang H. Overview and countermeasures of cancer burden in China. *Sci China Life Sci* 2023; 1-12 [PMID: 37071289 DOI: 10.1007/s11427-022-2240-6]

4 **Gupta S**, Harper A, Ruan Y, Barr R, Frazier AL, Ferlay J, Steliarova-Foucher E, Fidler-Benaoudia MM. International Trends in the Incidence of Cancer Among Adolescents and Young Adults. *J Natl Cancer Inst* 2020; **112**: 1105-1117 [PMID: 32016323 DOI: 10.1093/jnci/djaa007]

5 **Bhakta N**, Force LM, Allemani C, Atun R, Bray F, Coleman MP, Steliarova-Foucher E, Frazier AL, Robison LL, Rodriguez-Galindo C, Fitzmaurice C. Childhood cancer burden: a review of global estimates. *Lancet Oncol* 2019; **20**: e42-e53 [PMID: 30614477 DOI: 10.1016/S1470-2045(18)30761-7]

6 **Clough E**, Barrett T. The Gene Expression Omnibus Database. *Methods Mol Biol* 2016; **1418**: 93-110 [PMID: 27008011 DOI: 10.1007/978-1-4939-3578-9_5]

7 **Blum A**, Wang P, Zenklusen JC. SnapShot: TCGA-Analyzed Tumors. *Cell* 2018; **173**: 530 [PMID: 29625059 DOI: 10.1016/j.cell.2018.03.059]

8 **Ma XL**, Xu M, Jiang T. Crystal structure of the human CSN6 MPN domain. *Biochem Biophys Res Commun* 2014; **453**: 25-30 [PMID: 25242525 DOI: 10.1016/j.bbrc.2014.09.046]

9 **Lingaraju GM**, Bunker RD, Cavadini S, Hess D, Hassiepen U, Renatus M, Fischer ES, Thomä NH. Crystal structure of the human COP9 signalosome. *Nature* 2014; **512**: 161-165 [PMID: 25043011 DOI: 10.1038/nature13566]

10 **Faull SV**, Lau AMC, Martens C, Ahdash Z, Hansen K, Yebenes H, Schmidt C, Beuron F, Cronin NB, Morris EP, Politis A. Structural basis of Cullin 2 RING E3 ligase regulation by the COP9 signalosome. *Nat Commun* 2019; **10**: 3814 [PMID: 31444342 DOI: 10.1038/s41467-019-11772-y]

11 **Hou J**, Cui H. CSN6: a promising target for cancer prevention and therapy. *Histol Histopathol* 2020; **35**: 645-652 [PMID: 32016946 DOI: 10.14670/HH-18-206]

12 **Tang Z**, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res* 2019; **47**: W556-W560 [PMID: 31114875 DOI: 10.1093/nar/gkz430]

13 **Li T**, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res* 2020; **48**: W509-W514 [PMID: 32442275 DOI: 10.1093/nar/gkaa407]

14 **Chandrashekar DS**, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK, Varambally S. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* 2017; **19**: 649-658 [PMID: 28732212 DOI: 10.1016/j.neo.2017.05.002]

15 **Wu P**, Heins ZJ, Muller JT, Katsnelson L, de Bruijn I, Abeshouse AA, Schultz N, Fenyö D, Gao J. Integration and Analysis of CPTAC Proteomics Data in the Context of Cancer Genomics in the cBioPortal. *Mol Cell Proteomics* 2019; **18**: 1893-1898 [PMID: 31308250 DOI: 10.1074/mcp.TIR119.001673]

16 **Szklarczyk D**, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 2021; **49**: D605-D612 [PMID: 33237311 DOI: 10.1093/nar/gkaa1074]

17 **Birol M**, Enchev RI, Padilla A, Stengel F, Aebersold R, Betzi S, Yang Y, Hoh F, Peter M, Dumas C, Echalier A. Structural and biochemical characterization of the Cop9 signalosome CSN5/CSN6 heterodimer. *PLoS One* 2014; **9**: e105688 [PMID: 25144743 DOI: 10.1371/journal.pone.0105688]

18 **Xu M**, Zhen L, Lin L, Wu K, Wang Y, Cai X. Overexpression of CSN6 promotes the epithelial-mesenchymal transition and predicts poor prognosis in hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol* 2020; **44**: 340-348 [PMID: 31422034 DOI: 10.1016/j.clinre.2019.07.012]

19 **Choi HH**, Lee MH. CSN6-COP1 axis in cancer. *Aging (Albany NY)* 2015; **7**: 461-462 [PMID: 26186957 DOI: 10.18632/aging.100778]

20 **Chen J**, Shin JH, Zhao R, Phan L, Wang H, Xue Y, Post SM, Ho Choi H, Chen JS, Wang E, Zhou Z, Tseng C, Gully C, Velazquez-Torres G, Fuentes-Mattei E, Yeung G, Qiao Y, Chou PC, Su CH, Hsieh YC, Hsu SL, Ohshiro K, Shaikenov T, Yeung SC, Lee MH. CSN6 drives carcinogenesis by positively regulating Myc stability. *Nat Commun* 2014; **5**: 5384 [PMID: 25395170 DOI: 10.1038/ncomms6384]

21 **Zhao R**, Yeung SC, Chen J, Iwakuma T, Su CH, Chen B, Qu C, Zhang F, Chen YT, Lin YL, Lee DF, Jin F, Zhu R, Shaikenov T, Sarbassov D, Sahin A, Wang H, Lai CC, Tsai FJ, Lozano G, Lee MH. Subunit 6 of the COP9 signalosome promotes tumorigenesis in mice through stabilization of MDM2 and is upregulated in human cancers. *J Clin Invest* 2011; **121**: 851-865 [PMID: 21317535 DOI: 10.1172/JCI44111]

22 **Fang L**, Lu W, Choi HH, Yeung SC, Tung JY, Hsiao CD, Fuentes-Mattei E, Menter D, Chen C, Wang L, Wang J, Lee MH. ERK2-Dependent Phosphorylation of CSN6 Is Critical in Colorectal Cancer Development. *Cancer Cell* 2015; **28**: 183-197 [PMID: 26267535 DOI: 10.1016/j.ccell.2015.07.004]

23 **Su L**, Guo W, Lou L, Nie S, Zhang Q, Liu Y, Chang Y, Zhang X, Li Y, Shen H. EGFR-ERK pathway regulates CSN6 to contribute to PD-L1 expression in glioblastoma. *Mol Carcinog* 2020; **59**: 520-532 [PMID: 32134157 DOI: 10.1002/mc.23176]

24 **Zhang Y**, Hou J, Shi S, Du J, Liu Y, Huang P, Li Q, Liu L, Hu H, Ji Y, Guo L, Shi Y, Cui H. CSN6 promotes melanoma proliferation and metastasis by controlling the UBR5-mediated ubiquitination and degradation of CDK9. *Cell Death Dis* 2021; **12**: 118 [PMID: 33483464 DOI: 10.1038/s41419-021-03398-0]

25 **Mao Z**, Sang MM, Chen C, Zhu WT, Gong YS, Pei DS. CSN6 Promotes the Migration and Invasion of Cervical Cancer Cells by Inhibiting Autophagic Degradation of Cathepsin L. *Int J Biol Sci* 2019; **15**: 1310-1324 [PMID: 31223289 DOI: 10.7150/ijbs.32987]

26 **Mou J**, Wei L, Liang J, Du W, Pei D. CSN6 promotes the cell migration of breast cancer cells by positively regulating Snail1 stability. *Int J Med Sci* 2020; **17**: 2809-2818 [PMID: 33162808 DOI: 10.7150/ijms.50206]

27 **Ruffin AT**, Li H, Vujanovic L, Zandberg DP, Ferris RL, Bruno TC. Improving head and neck cancer therapies by immunomodulation of the tumour microenvironment. *Nat Rev Cancer* 2023; **23**: 173-188 [PMID: 36456755 DOI: 10.1038/s41568-022-00531-9]

28 **Du WQ**, Zhu ZM, Jiang X, Kang MJ, Pei DS. COPS6 promotes tumor progression and reduces CD8(+) T cell infiltration by repressing IL-6 production to facilitate tumor immune evasion in breast cancer. *Acta Pharmacol Sin* 2023; **44**: 1890-1905 [PMID: 37095198 DOI: 10.1038/s41401-023-01085-8]

29 **Wang S**, Liu G, Li Y, Pan Y. Metabolic Reprogramming Induces Macrophage Polarization in the Tumor Microenvironment. *Front Immunol* 2022; **13**: 840029 [PMID: 35874739 DOI: 10.3389/fimmu.2022.840029]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

*World J Clin Oncol* 2023 November 24; 14(11): 504-517

DOI: 10.5306/wjco.v14.i11.504

ISSN 2218-4333 (online)

META-ANALYSIS

# Circulating tumor cells as potential prognostic biomarkers for early-stage pancreatic cancer: A systematic review and meta-analysis

Zi-Han Zhang, Yi-Wen Bao, Ya-Jun Zhao, Jian-Quan Wang, Jin-Tao Guo, Si-Yu Sun

**Specialty type:** Oncology

**Provenance and peer review:** Invited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

**P-Reviewer:** Kitamura K, Japan; Mizuno S, Japan

**Received:** June 28, 2023
**Peer-review started:** June 28, 2023
**First decision:** August 25, 2023
**Revised:** September 14, 2023
**Accepted:** October 26, 2023
**Article in press:** October 26, 2023
**Published online:** November 24, 2023

**Zi-Han Zhang, Yi-Wen Bao, Ya-Jun Zhao, Jian-Quan Wang, Jin-Tao Guo, Si-Yu Sun,** Department of Gastroenterology, Shengjing Hospital of China Medical University, Shenyang 110004, Liaoning Province, China

**Corresponding author:** Jin-Tao Guo, MD, PhD, Professor, Department of Gastroenterology, Shengjing Hospital of China Medical University, No. 36 Sanhao Street, Shenyang 110004, Liaoning Province, China. guojt@sj-hospital.org

## Abstract

### *BACKGROUND*

Pancreatic cancer is difficult to be diagnosed early clinically, while often leads to poor prognosis. If optimal personalized treatment plan can be provided to pancreatic cancer patient at an earlier stage, this can greatly improve overall survival (OS). Circulating tumor cells (CTCs) are a collective term for various types of tumor cells present in the peripheral blood (PB), which are formed by detachment during the development of solid tumor lesions. Most CTCs undergo apoptosis or are phagocytosed after entering the PB, whereas a few can escape and anchor at distal sites to develop metastasis, increasing the risk of death for patients with malignant tumors.

### *AIM*

To investigate the significance of CTCs in predicting the prognosis of early pancreatic cancer patients.

### *METHODS*

The PubMed, EMBASE, Web of Science, Cochrane Library, China National Knowledge Infrastructure, China Biology Medicine, and ChinaInfo databases were searched for articles published through December 2022. Studies were considered qualified if they included patients with early pancreatic cancer, analyzed the prognostic value of CTCs, and were full papers reported in English or Chinese. Researches were selected and assessed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocol and the Newcastle-Ottawa Scale criteria. We used a funnel plot to assess publication bias.

### *RESULTS*

From 1595 publications, we identified eight eligible studies that collectively enrolled 355 patients with pancreatic cancer. Among these original studies, two were carried out in China; three in the United States; and one each in Italy, Spain, and Norway. All eight studies analyzed the relevance between CTCs and the

prognosis of patients with early-stage pancreatic cancer after surgery. A meta-analysis showed that the patients that were positive pre-treatment or post-treatment for CTCs were associated with decreased OS [hazard ratio (HR) = 1.93, 95% confidence interval (CI): 1.197-3.126, $P$ = 0.007] and decreased relapse-free/disease-free/progression-free survival (HR = 1.27, 95%CI: 1.137-1.419, $P$ < 0.001) in early-stage pancreatic cancer. Additionally, the results suggest no statistically noticeable publication bias for overall, disease-free, progression-free, and recurrence-free survival.

*CONCLUSION*

This pooled meta-analysis shows that CTCs, as biomarkers, can afford reliable prognostic information for patients with early-stage pancreatic cancer and help develop individualized treatment plans.

**Key Words:** Pancreatic cancer; Surgery; Prognosis; Systematic review; Meta-analysis; Biomarkers



**Core Tip:** There is no consensus regarding the prognostic value of circulating tumor cells (CTCs) in early-stage pancreatic cancer after surgery. This is the first systematic review and meta-analysis to investigate the potential of CTCs in predicting survival time in early pancreatic cancer. We pooled the analyses of the relationship between CTCs and overall/disease-free/progression-free/relapse-free survival in related studies. Patients testing positive for CTCs pre- or intra-surgery may have worse prognoses, requiring more intense chemotherapy and closer follow-up.

**Citation:** Zhang ZH, Bao YW, Zhao YJ, Wang JQ, Guo JT, Sun SY. Circulating tumor cells as potential prognostic biomarkers for early-stage pancreatic cancer: A systematic review and meta-analysis. *World J Clin Oncol* 2023; 14(11): 504-517
**URL:** https://www.wjgnet.com/2218-4333/full/v14/i11/504.htm
**DOI:** https://dx.doi.org/10.5306/wjco.v14.i11.504

## INTRODUCTION

Pancreatic cancer is a fatal disease with poor prognosis. In 2018, pancreatic cancer was the seventh leading cause of cancer-related mortality worldwide[1]. The number of annual diagnoses of and deaths related to pancreatic cancer in China have exceeded those in the United States[2]. The incidence of pancreatic cancer continues to increase at a rate of 0.5%-1.0% each year, and it is projected to be the second deadliest cancer by 2030 in Western countries[3]. Despite continuous advances in chemotherapy, radiation, and surgical techniques; the prognosis of patients with pancreatic cancer remains significantly poor. Approximately half of the patients experience reoccurrence within the first year, mainly due to metastatic disease occurrence after surgical resection. Consequently, one of the most important challenges is to identify a factor that can assess the survival outcome of patients with early pancreatic cancer before surgery, optimize treatment, and assist in the development of monitoring strategies.

Currently, technological innovations in imaging and endoscopy are being used to improve the diagnostic accuracy of pancreatic cancer. Computed tomography (CT) and magnetic resonance imaging (MRI) remain first-line diagnostic modalities for clinical suspicion. Endoscopic ultrasound (EUS) and EUS-guided fine-needle aspiration (EUS-FNA), also play important roles in its diagnosis. However, imaging features of early pancreatic cancer are subtle, and the general consensus is that it is difficult to detect early lesions[4]. Liquid biopsy is a new technology that detects biomarkers, such as carcinoembryonic antigen, carbohydrate antigen 19-9 (CA19-9), or CA125, from nonsolid biological tissues, such as blood[5], and has received increased attention because of its convenience and noninvasiveness[6]. However, these biomarkers all lack sufficient sensitivity and specificity for diagnostic purposes.

With the development of medical testing technologies, circulating tumor cells (CTCs) have emerged as a popular research topic over the last few decades. CTCs are a small number of tumor cells that detach from primary tumors, circulate through the bloodstream, and are the main source of its dissemination and metastasis[7]. Unlike traditional histopathological examinations, which are usually complex and risky for difficult-to-biopsy tumors such as pancreatic cancer, CTCs are noninvasive. CTCs can provide real-time and comprehensive information about the tumor because they are enriched in the bloodstream and originate from different regions of the original tumor or metastasis. Moreover, CTCs can present large-scale health information, such as the expression of genes and proteins and alteration of cellular contents and cell membranes, which are essential for improving diagnostic accuracy and developing individualized treatments[8]. Recently, an increasing number of studies have revealed that CTCs show promise for prognostic evaluation in several tumors, including lung[9], renal[10], breast[11], gastric[12], and colorectal cancers[13].

Nonetheless, the prognostic effect of CTCs in early pancreatic cancer remains ambiguous, mainly because of the increasing number of CTC isolation methods and different study designs. Therefore, this study aimed to perform a structural meta-analysis of currently available evidence on the prognostic value of CTCs in early-stage pancreatic cancer.

## MATERIALS AND METHODS

### Search strategy

Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we conducted a comprehensive literature search of all studies published in database repositories before December 2022 that were related to the use of CTCs for the diagnosis of pancreatic cancer. The search was performed only in English and Chinese databases including PubMed, EMBASE, Web of Science, Cochrane Library, China National Knowledge Infrastructure (CNKI), China Biology Medicine (CBM), and ChinaInfo. Only studies published in Chinese and English were included in the analysis. Search terms included these keywords, MeSH (medical subject headings) terms, and their entry terms: "Pancreatic Neoplasms"(MeSH), "Neoplasm, Pancreatic," "Pancreatic Neoplasm," "Pancreas Neoplasms," "Neoplasm, Pancreas," "Neoplasms, Pancreas," "Pancreas Neoplasm," "Neoplasms, Pancreatic," "Cancer of Pancreas," "Pancreas Cancers," "Pancreas Cancer," "Cancer, Pancreas," "Cancers, Pancreas," "Pancreatic Cancer," "Cancer, Pancreatic," "Cancers, Pancreatic," "Pancreatic Cancers," "Cancer of the Pancreas," "Neoplastic Cells, Circulating"(MeSH), "Neoplasm Circulating Cells," "Circulating Neoplastic Cells," "Cell, Circulating Neoplastic," "Cells, Circulating Neoplastic," "Circulating Neoplastic Cell," "Neoplastic Cell, Circulating," "Circulating Tumor Cells," "Cell, Circulating Tumor," "Cells, Circulating Tumor," "Circulating Tumor Cell," "Tumor Cell, Circulating," "Tumor Cells, Circulating," "Cells, Neoplasm Circulating," "Cell, Neoplasm Circulating," "Neoplasm Circulating Cell," "Circulating Cells, Neoplasm," "Tumor Cells, Embolic," "Cell, Embolic Tumor," "Cells, Embolic Tumor," "Embolic Tumor Cell," "Tumor Cell, Embolic," "Embolic Tumor Cells," "Embolism, Tumor," "Embolisms, Tumor," "Tumor Embolism," "Tumor Embolisms," "Prognostic," "Prognosis," "Prognos*". These terms were supplemented by the logical operators "and" and "or." To expand the literature, we reviewed and evaluated the references of the included studies.

### Selection criteria

Two independent investigators reviewed the article titles and abstracts according to inclusion and exclusion criteria to exclude irrelevant studies. Subsequently, the full texts of the included studies were analyzed to determine their suitability for meta-analysis. In case of any contradictions, a third reviewer was consulted for adjudication. The inclusion and exclusion criteria were formulated based on the PRISMA of Diagnostic Test Accuracy Studies guidelines. Inclusion criteria: (1) Studies included participants of any age with histologically or cytologically confirmed early pancreatic cancer (early pancreatic cancer was defined as pancreatic adenocarcinoma with a maximum tumor diameter of 4 cm, regional lymph node metastasis of no more than three nodes, and no distant metastasis); (2) studies that investigated pre- or intra-operative CTCs as a prognostic biomarker in blood for early pancreatic cancer patients' survival results after surgery; (3) sufficient published data available for calculating hazard ratio (HR) and 95% confidence interval (CI) of overall survival (OS) and progression-free survival (PFS); (4) studies that were published in English or Chinese; and (5) studies that were reported as full paper publications. Exclusion criteria: (1) Patients were diagnosed with pancreatic cancer at advanced stages or studies that didn't analyze the results of early pancreatic cancer patients independently; (2) studies that did not provide adequate data on the prognostic performance of CTCs for early pancreatic cancer patients; (3) patients didn't receive surgical therapy; and (4) articles were published in languages other than English and Chinese. Overall, five non-human studies, case reports, comments, meta-analyses, reviews, and published clinical and treatment guidelines were excluded.

### Data extraction

To decrease systematic errors during data collection, two reviewers independently selected the studies. Any conflicting cases were carefully reviewed, and a third reviewer was consulted to reach a consensus. Data extraction from the eligible papers included the following items: (1) General information about the article: first author, publication date, and country; (2) study information: number of patients, evidence of confirmed pancreatic cancer, CTCs separation solution, CTCs determination criteria, and follow-up time; and (3) data for the meta-analysis: HR with 95%CI for OS, PFS or disease-free survival (DFS), recurrence-free survival (RFS). For articles without HR and 95%CI, we used Engauge Digitizer 11.3 to calculate them based on the survival rate extracted from Kaplan-Meier curves.

### Risk of bias

The quality of the included studies was assessed based on the Newcastle-Ottawa Scale (NOS) criteria for non-randomized studies, which included three key domains covering "selection," "comparability," and "outcome." A star rating system was used to semi-quantitatively evaluate study quality, and those who met the standards for each item were awarded one or two stars. Scores range from zero to nine. A score equal to or greater than seven indicates high quality. This tool objectively evaluates the risk of bias and assesses concerns regarding its applicability. The quality assessment was performed by two independent reviewers. Any disagreements were discussed until an agreement was reached. Publication bias was investigated using a funnel plot, with $P < 0.05$ indicating a significant publication bias.

### Statistical analysis

Review Manager 5.3 software (The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark) was used to assess the pooled HR effect size. Heterogeneity between studies was assessed using Cochran's Q test and $I^2$ statistics. According to Higgins and Thompson, $I^2 > 50\%$ or $P < 0.1$ was viewed as consistent significant heterogeneity. A fixed-effect model was used when minor heterogeneity was observed. Otherwise, a random-effects model was used to calculate the pooled HR. Subgroup analyses based on ethnicity, separation solution, treatment, and follow-up time were performed to explore potential sources of heterogeneity. Finally, we conducted a sensitivity analysis to evaluate the effect

of a single study on overall outcomes.

## RESULTS

### Characteristics and quality of the included studies

A total of 1963 articles were collected, including 91 from CNKI, 273 from ChianInfo, 90 from CBM, 258 from PubMed, 55 from EMBASE, 31 from Cochrane, and 1165 from Web of Science. After deleting 368 duplicate references, we screened the titles and abstracts of 1595 residual articles. In total, 1551 articles were excluded (520 meta-analyses or reviews; 32 non-human studies; 15 articles published in languages other than English and Chinese; 161 meeting abstracts, case reports, guidelines, or letters; and 823 irrelevant studies). According to the inclusion and exclusion criteria, we read the full text of the 44 remaining studies, of which 36 articles were ultimately excluded (5 irrelevant studies and 31 articles with data deficiency). Consequently, eight studies involving 355 patients with early-stage pancreatic cancer were included in our meta-analysis[14-21]. A flow diagram of the literature search and filtering process is shown in Figure 1.

The characteristics of these studies are summarized in Table 1. The overall research quality was rated as moderate by the NOS, with an average score of 6.75 (Table 2). Among these original studies, two were conducted in China; three in the United States; and one each in Italy, Spain, and Norway. The publication years were 2014 ($n$ = 1), 2018 ($n$ = 1), 2021 ($n$ = 5), and 2022 ($n$ = 1). All eight studies analyzed the correlation between CTCs and prognosis in patients with early-stage pancreatic cancer after surgery. While four studies included early pancreatic cancer patients only[14,19-21], the remaining four studies contained early and advanced pancreatic cancer patients with early pancreatic cancer patients accounting for at least 30% of the total patients[15-18]. In total, five studies detected CTCs in the peripheral blood (PB), two studies detected CTCs in the PB and portal venous blood (PVB), and one study detected CTCs in the central venous catheter and portal blood. Blood samples were collected solely before surgery in four studies[16,17,21], before and after surgery in three studies[14,15,18], and during surgery in only one study[19]. Only pre- and intra-operative CTC data were included in this meta-analysis. Although eight studies applied different CTC enrichment and separation methods, including commercially available CTC detection kits[14,17], dielectrophoresis-field flow fractionation (DEP-FFF)[15], and CTC isolation systems[18-21], most methods essentially rely on the density characteristics of CTCs as well as the epithelial and mesenchymal markers expressed by CTCs. Most studies applied DFS, OS, PFS, and RFS as survival outcomes, while only Hugenschmidt *et al*[20] adopted cancer-specific survival as an outcome indicator. Seven of the eight studies reported the adjusted HR and 95%CI for the association between DFS/PFS/RFS and positive CTCs. For the one that did not, we calculated these values according to the Kaplan-Meier curves provided in the articles. In addition, only three of the eight included studies reported the adjusted HR and 95%CI for the association between OS and positive CTCs while another two studies provided Kaplan-Meier curves for OS.

### The relationship between CTCs and prognosis of early-stage pancreatic cancer patients after surgery

We conducted a meta-analysis to assess the association between CTCs detected in the blood samples of patients with early-stage pancreatic cancer and their prognosis after surgery. The degree of heterogeneity among the five studies that provided the adjusted HR and 95%CI for OS was low ($I^2$ = 41%, $P$ = 0.15); therefore, we chose a fixed-effect model for analysis. The pooled analyses of the five studies, containing 169 patients, showed that positive detection of pre-treatment or post-treatment CTCs was associated with decreased OS (HR = 1.93, 95%CI: 1.20-3.13) that was statistically significant ($Z$ = 2.69, $P$ = 0.007) (Figure 2A). There was a moderate degree of heterogeneity among the seven studies available for the adjusted HR and 95%CI of RFS/DFS/PFS ($I^2$ = 65%, $P$ = 0.01); therefore, we chose a random-effects model for analysis. The results showed that positive pre-treatment or post-treatment CTCs were associated with decreased DFS/PFS/RFS (HR = 1.97, 95%CI: 1.20-3.25) that were statistically significant ($Z$ = 2.67, $P$ = 0.008) (Figure 2B).

### Subgroup analysis

Because of the high heterogeneity in the DFS/PFS/RFS results, meta-regression was conducted to explore the sources of heterogeneity. No apparent deviation was found in the ethnicity, treatment, and follow-up time subgroups. Later analysis demonstrated that CTC-positive patients were associated with decreased DFS/PFS/RFS in subgroups that detected CTCs by the CellSearch system (HR = 2.62, 95%CI: 1.65-4.16, $Z$ = 4.09, $P$ < 0.001), and the heterogeneity between subgroups was low ($P$ = 0.018) (Figure 3).

### Sensitivity analysis

To explore the potential sources of this difference, we conducted sensitivity analysis by sequentially excluding each study. When the study by Xing *et al*[14] was excluded, the heterogeneity of the remaining studies was significantly reduced (OS: $I^2$ = 0%, $P$ = 0.44; DFS/PFS/RFS: $I^2$ = 0%, $P$ = 0.76), and the pooled results for both OS and DFS/PFS/RFS were increased (OS: HR = 3.06 95%CI: 1.59-5.86, $Z$ = 3.36, $P$ < 0.001; DFS/PFS/RFS: HR = 2.60 95%CI: 1.78-3.812, $Z$ = 4.001, $P$ < 0.001) (Figure 4). Nevertheless, the direction of the pooled results for the OS and DFS/PFS/RFS subgroups were not affected, indicating a negative association between CTC positivity and lower OS or DFS/PFS/RFS.

### Publication bias

We used a funnel plot to assess publication bias in this meta-analysis. The results showed no publication bias for OS ($P$ = 0.653) and DFS/PFS/RFS ($P$ = 0.117) (Figure 5).

**Table 1 Characteristics of the included studies**

| Ref. | Year | Country | Sample | Separation Solution | Markers and expression level on PC CTC | Cases | No-positive | Treatment | Outcome | Follow-up time |
|---|---|---|---|---|---|---|---|---|---|---|
| Xing *et al*[14] | 2021 | China | Peripheral blood | SE-iFISH | CD44+ CTEC: DAPI +/CD45-/CD31 +/CD44 +/Vimentin (+ or -) with aneuploid CEP8 | 73 | Not reported | Surgery | OS/DFS | Median 10.8 mo (1.2-31.8 mo) |
| Semaan *et al* [15] | 2021 | United States | Peripheral blood | DEP-FFF | pEMT-CTC: CD45 -, EpCAM + and/or Pan-CK +, Vimentin +, DAPI + | 31 early stages (74 total) | 28 | Surgery/neoadjuvant treatment | OS/PFS | Median 15.4 mo (0-43.1 mo) |
| Padillo-Ruiz *et al*[21] | 2021 | Spain | Central venous catheter and portalblood | ICC | DAPI +/CK +/CD45 - | 35 | 35 | Surgery/chemotherapy | OS/DFS | 24 mo |
| Court *et al*[16] | 2018 | United States | Peripheral blood | NanoVelcro chip | DAPI +/CD45 -/CK +; CD45 positivity greater than 2 × background | 40 early stages (126 total) | 27 | Surgery/chemotherapy | OS/RFS | ≥ 24 mo |
| Cheng *et al*[17] | 2022 | China | Peripheral blood | LT-PCR | FR + CTC | 25 early stages (44 total) | 13 | Surgery/chemotherapy | OS/DFS | Median 20 mo (6-28 mo) |
| White *et al*[18] | 2021 | United States | Peripheral blood and portal venous blood | CellSearch | DAPI +/CK +/CD45 - | 33 early stages (34 total) | 21 | Surgery/neoadjuvant treatment | OS/RFS | Median 14.1 mo (0.86-1.97 mo) |
| Bissolati *et al* [19] | 2014 | Italy | Peripheral blood and portal venous blood | CellSearch | DAPI +/CK +/CD45 - | 20 | 9 | Surgery/chemotherapy | OS/PFS | Median 39.2 mo (36-45 mo) |
| Hugenschmidt *et al*[20] | 2021 | Norway | Peripheral blood | CellSearch | EpCAM +/DAPI +/CK +/CD45 - | 98 | 7 | Surgery | CSS/DFS | Median 96 mo (63-126 mo) |

DEP-FFF: Dielectrophoresis-field flow fractionation; ICC: Immunocytochemistry; SE-iFISH: Subtraction enrichment and immunostaining-fluorescence in situ hybridization; PC: Pancreatic cacner ; CTCs: Circulating tumor cells; LT-PCR: Ligand-targeted polymerase chain reaction; CK: Cytokeratin; DAPI: 4',6-diamidino-2-phenylindole; EpCAM: Epithelial cell adhesion molecule; CSS: Cancer specific survival; DFS: Disease-free survival; OS: Overall survival; PFS: Progression-free survival; pEMT: Partial epithelial-mesenchymal transition.

### *The relationship between CA19-9 and the prognosis of early-stage pancreatic cancer patients after surgery*

In order to compare the potential of CTC and CA19-9 in predicting patient prognosis, we extracted the adjusted HR and 95%CI of DFS related to CA19-9 in the included articles. Only four studies had the required data[14,16-18]. The degree of heterogeneity of the adjusted HR and 95%CI for DFS was high ($I^2$ =73%, $P$ = 0.01), so we used a random-effects model for the analysis. The pooled analyses of the four studies, containing 171 patients, showed that the CA19-9 level in the PVB of early-stage pancreatic cancer patients was not an independent predictor of a shorter time to recurrence (HR = 1, 95%CI: 1.00-1.00; $Z$ = 0.36, $P$ = 0.72) (Figure 6). Considering the impact of sampling time on the results, we excluded the study of White *et al*[18], which collected venous blood after resection. Though the heterogeneity of the remaining studies was significantly reduced ($I^2$ = 48%, $P$ = 0.15), the combined HR was still 1 (95%CI: 1.00-1.00; $Z$ =2.20, $P$ = 0.03) (Figure 7).

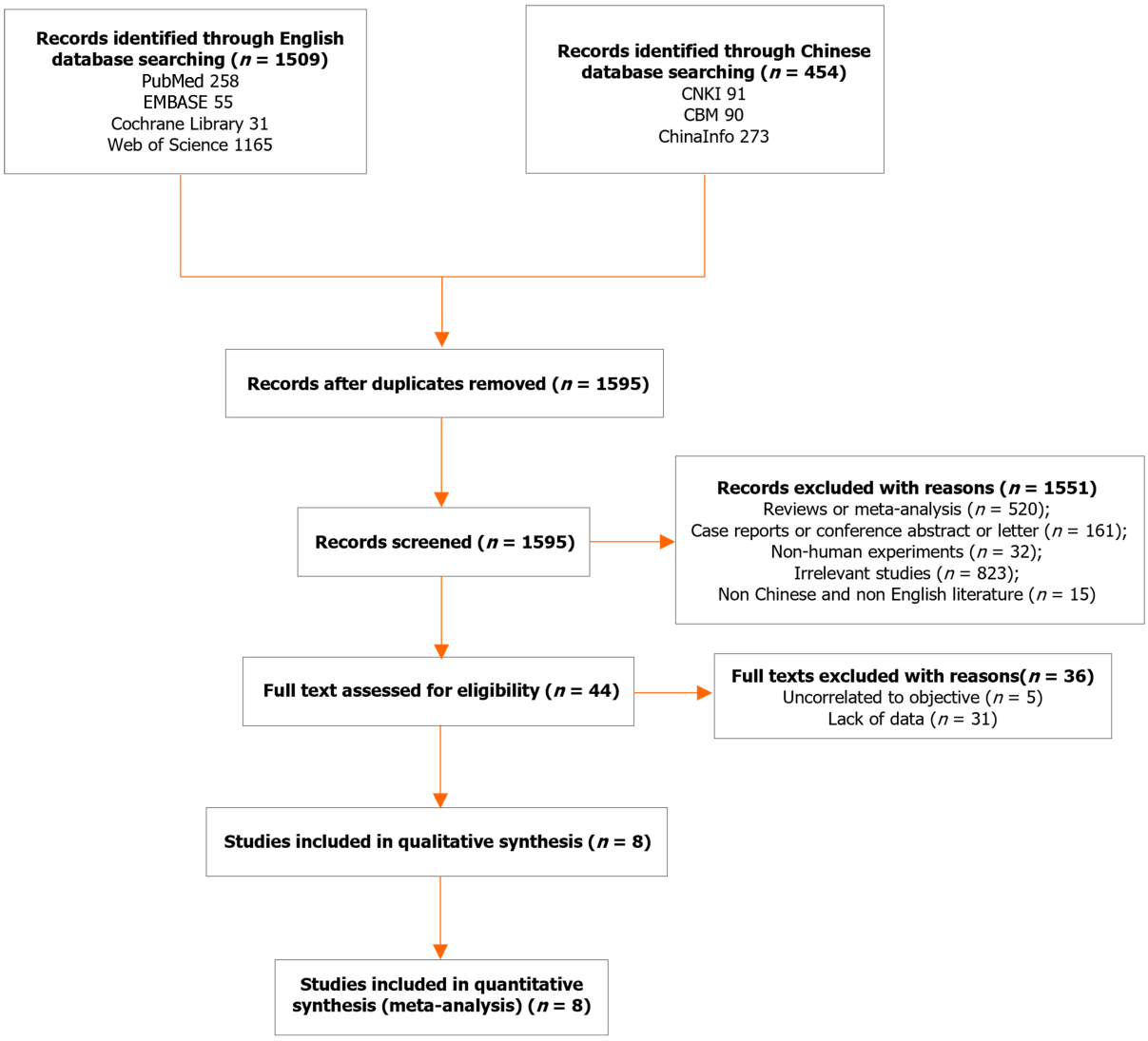
**Table 2 Quality assessment of the included studies according to the Newcastle-Ottawa Scale criteria for non-randomized studies**

| Ref. | Selection | | | | Comparability | | | Outcome | | Total stars |
|---|---|---|---|---|---|---|---|---|---|---|
| | Representativeness | Selection of non-exposed | Ascertainment of exposure | Outcome not present at start | Comparability on most important factors | Comparability on other risk factors | Assessment of outcome | Long enough follow-up (median  24 mo) | Completeness of follow-up | |
| Xing *et al*[14] | - | [1] | [1] | [1] | [1] | - | [1] | - | [1] | 6 |
| Semaan *et al*[15] | - | [1] | [1] | [1] | [1] | - | [1] | - | [1] | 6 |
| Padillo-Ruiz *et al*[21] | - | [1] | [1] | [1] | [1] | - | [1] | [1] | [1] | 7 |
| Court *et al*[16] | - | [1] | [1] | [1] | [1] | - | [1] | [1] | [1] | 7 |
| Cheng *et al*[17] | [1] | [1] | [1] | [1] | [1] | - | [1] | - | [1] | 7 |
| White *et al*[18] | - | [1] | [1] | [1] | [1] | - | [1] | - | [1] | 6 |
| Bissolati *et al*[19] | [1] | [1] | [1] | [1] | [1] | - | [1] | [1] | [1] | 8 |
| Hugenschmidt *et al*[20] | - | [1] | [1] | [1] | [1] | - | [1] | [1] | [1] | 7 |

[1]Meets the criteria of the scale. A maximum of one 'star' for each item within the 'Selection' and 'Outcome' categories; maximum of two 'stars' for 'Comparability'; -Does not meet the criteria of the scale.

## DISCUSSION

In this meta-analysis, we found that CTC detected in patients with early pancreatic cancer is negatively correlated with the prognosis time after surgery. After data collection and filtering, eight studies from 2014 to 2022, including 355 patients with early-stage pancreatic cancer, were included in our analysis. Through data sorting and meta-analysis, we demonstrated that testing positive pre- or intra-treatment for CTCs was associated with decreased OS and RFS/DFS/PFS in early-stage pancreatic cancer. Nevertheless, our meta-analysis showed a high degree of heterogeneity in DFS/PFS/RFS. To determine the potential sources of the heterogeneity, we conducted a subgroup analysis. The pooled results were not affected by ethnicity, treatment, or follow-up time, whereas subgroup analysis by CTCs separation solution decreased the heterogeneity between the groups, indicating that this might have caused the heterogeneity. Studies that enumerated CTCs by the CellSearch system demonstrated a more obvious correlation between CTC-positive patients and decreased DFS/PFS/RFS. However, except for this system, all other separation solutions contained only one study; therefore, we were unable to obtain pooled results. Sensitivity analysis was conducted by using the "leave-one-out method." Notably, when a study by Xing *et al*[14] was removed, the heterogeneity of the remaining studies was significantly reduced, and the pooled results for both OS and DFS/PFS/RFS were relatively elevated. We infer that the differences present in this study may be derived from the separation solutions employed as well as the stemness markers used to identify the stem cell-like phenotype of CTCs. Importantly, the results demonstrating the survival-jeopardizing effects of CTCs were maintained. We suggest that patients testing positive for CTCs pre- or intra-treatment may have a worse prognosis and



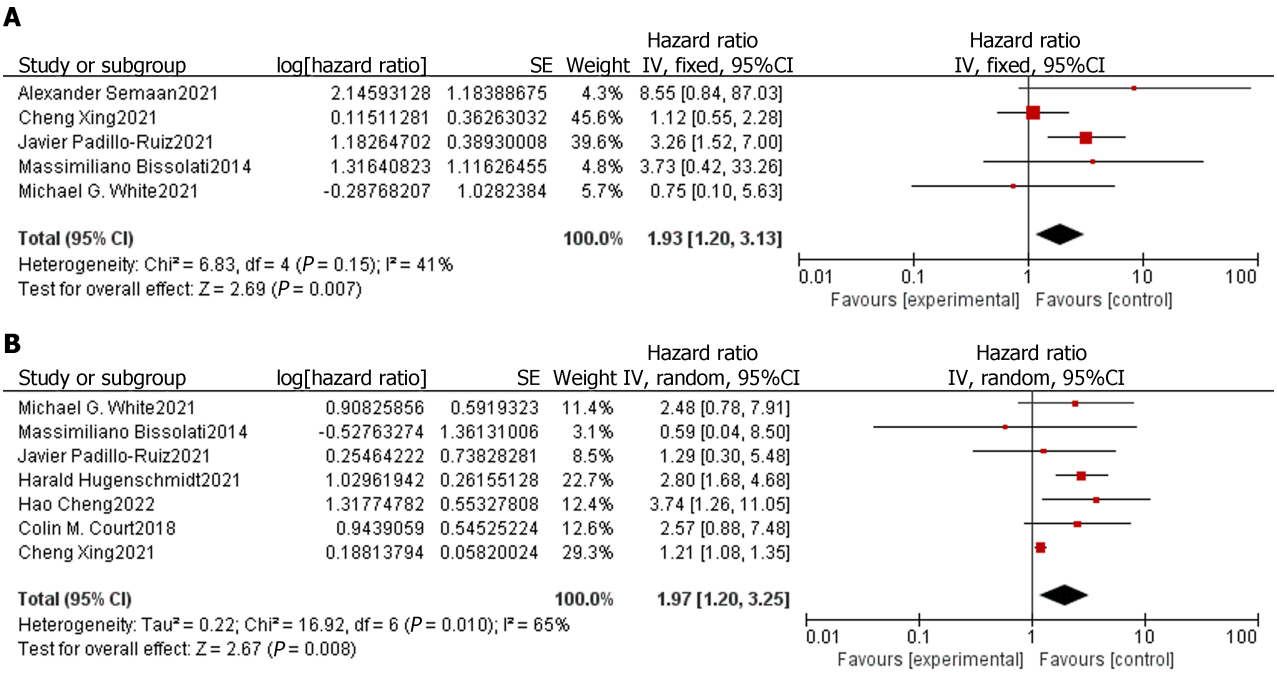

**DOI**: 10.5306/wjco.v14.i11.504 

**Figure 1 Flow diagram for searching and filtering eligible studies included in the meta-analysis.**

require more intense chemotherapy and closer follow-up. Furthermore, we collected data regarding CA19-9 in the included studies and four studies containing 171 patients, that met our requirements. Our meta-analysis showed that the preoperative CA19-9 level in the PVB of early-stage pancreatic cancer patients was not an independent predictor for shorter time of recurrence after resection.

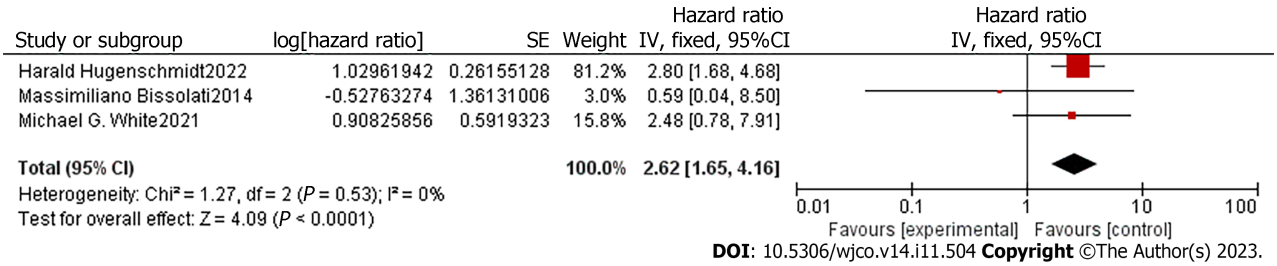
The prevalence of pancreatic cancer has increased dramatically globally, and is expected to become a leading cause of cancer-related mortality[22]. Only 10%-15% of patients have localized pancreatic cancer suitable for surgery[3], which is the only potentially curative therapy known to date. Even for patients with a localized disease, a high proportion experience postoperative recurrence within 5 years, caused by local tumor recurrence or distant metastases. The 1- and 5-year survival rates are 63% and 17%, respectively[23]. Therefore, identifying high-risk populations for screening and prevention, early diagnosis, and establishing personalized treatment plans are currently the primary challenges[24].

Imaging, including EUS, CT and MRI, which can provide a convenient and noninvasive diagnosis, remains the first-line diagnostic modality for pancreatic cancer and is used to evaluate therapeutic efficacy in many organs[25-28]. However, cross-sectional imaging is limited in the visualization of small and metastatic tumors, which can frequently result in underestimation of the pancreatic cancer stage[29]. EUS-FNA or EUS-fine needle biopsy (EUS-FNB) can localize pancreatic lesions measuring < 3 cm, providing a minimally invasive tissue biopsy[30]. EUS-FNB is increasingly becoming a practical tool for diagnosing malignancy in various pancreatic solid lesions[31,32]. EUS-FNA combined with needle-based confocal laser endomicroscopy (nCLE) can achieve real-time imaging for in vivo tissue analysis[33]. However, these examinations requires anesthesia and may be accompanied by complications such as acute pancreatitis, tumor dissemination, and postoperative hemorrhage. Besides, nCLE is limited by the duration of the surgical time and the operability of the 19G FNA needle[33]. More recently, liquid biopsy has received a great deal of attention for its ability to assess a comprehensive cancer profile in a noninvasive and real-time manner[26]. Serum CA19-9 level is the only diagnostic biomarker approved by the United States Food and Drug Administration for pancreatic cancer. Moreover, CA19-9 can be an independent predictor of prognosis for pancreatic cancer patients[34]. An elevated CA19-9 level after




**DOI**: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

**Figure 2 Forest plots of the five studies show a shorter overall survival and disease-free survival/progression-free survival/recurrence-free survival in early pancreatic patients with positive pre-treatment or post-treatment circulating tumor cells detection.** A: Overall survival; B: Disease-free survival/progression-free survival/recurrence-free survival. CI: Confidence interval.



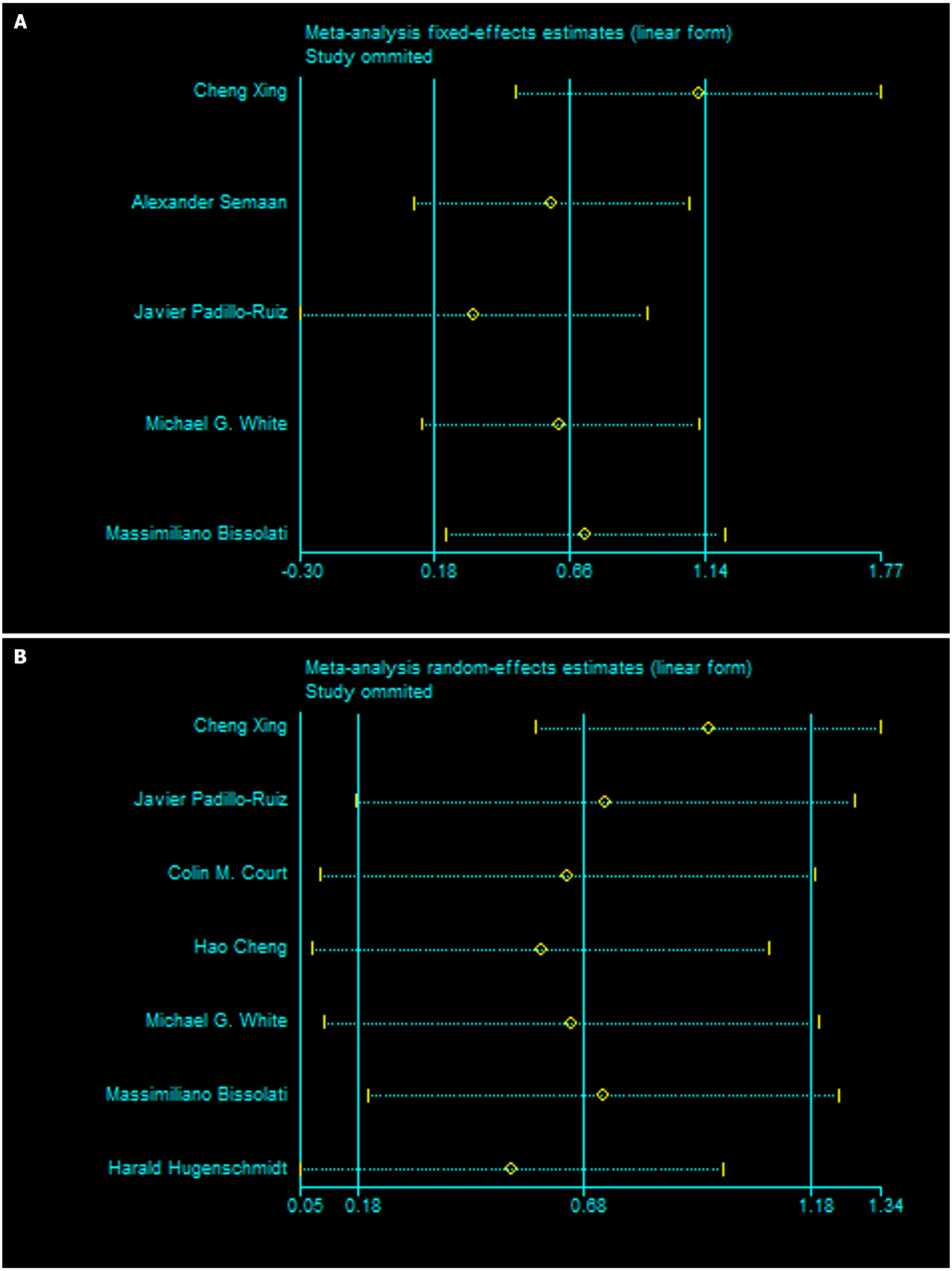

**DOI**: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

**Figure 3 Forest plot of the three studies that used the CellSearch system shows a decreased disease-free survival/progression-free survival/recurrence-free survival in early pancreatic patients that were positive pre-treatment or post-treatment for circulating tumor cells.** CI: Confidence interval.

resection or during chemotherapy predicts a high probability of tumor recurrence or progression[35]. However, the prognostic ability of preoperative CA19-9 is still disputed, since it is mostly detected in advanced stages, it is neither sensitive nor specific enough to identify early-stage patients or for the differential diagnosis of patients at different stages; additionally, it is also positive in many other benign and malignant pancreatic diseases such as pancreatitis, cholestasis, and gastric cancer[7]. In this meta-analysis, the pooled results of the four studies, that we examined, demonstrated that the prognostic effect of CA19-9 in peripheral venous blood on early postoperative recurrence of early-stage pancreatic cancer is not as obvious as that of CTCs.

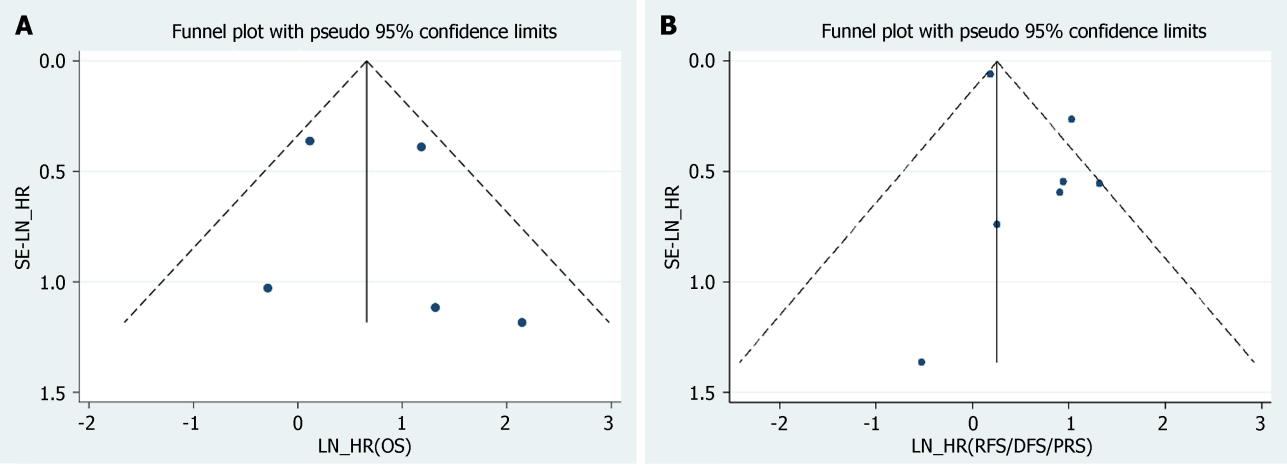
In the past decade, an increasing number of studies have examined the prognostic value of CTCs in cancers of various organs, as the formation of tumor metastases relies heavily on the survival of CTCs and their ability to mediate angiogenesis in target organs[36]. CTCs are tumor cells shed from both primary and secondary foci and are found circulating in the bloodstream; therefore, they can provide valuable information about primary tumors and secondary deposits. In addition, isolation and in vivo cultures of animal xenografts provide deeper information on individual tumor characteristics[37]. Prospective observational studies have also revealed that CTC numbers rarely drop to zero even after complete resection in both chemo-naïve and post-neoadjuvant patients and can be observed longitudinally before disease recurrence[38]. However, the deficiency of relevant studies and different research designs make the clinical significance of CTCs in early pancreatic cancer prognosis a controversial topic. A thorough analysis of the prognostic performance of CTCs is critical for monitoring and developing treatment strategies for patients with pancreatic cancer.

CTCs in the bloodstream are difficult to capture and identify because their concentration in the circulatory system is extremely low (1-10 cells/10 mL) in most cases[39]. Furthermore, CTCs are scattered among an enormous number of erythrocytes and leukocytes, posing tremendous challenges for their complete collection and accurate detection. To



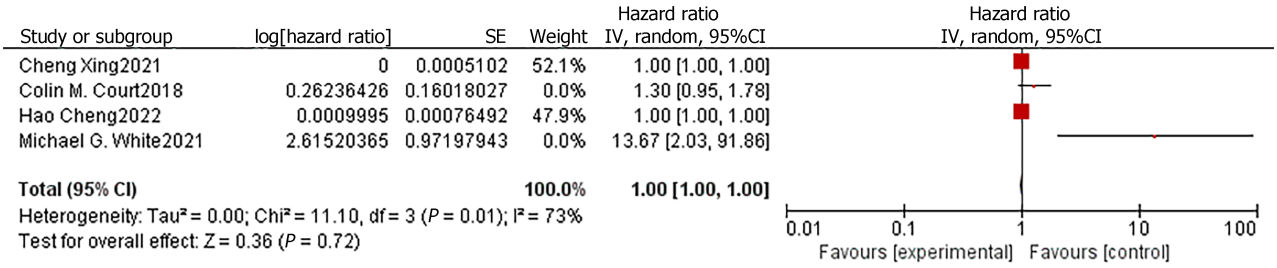

**DOI**: 10.5306/wjco.v14.i11.504 

**Figure 4 Sensitivity analysis of the influence of each study on the pooled results for the overall survival and disease-free survival/progression-free survival/recurrence-free survival subgroups.** A: Overall survival; B: Disease-free survival/progression-free survival/recurrence-free survival.



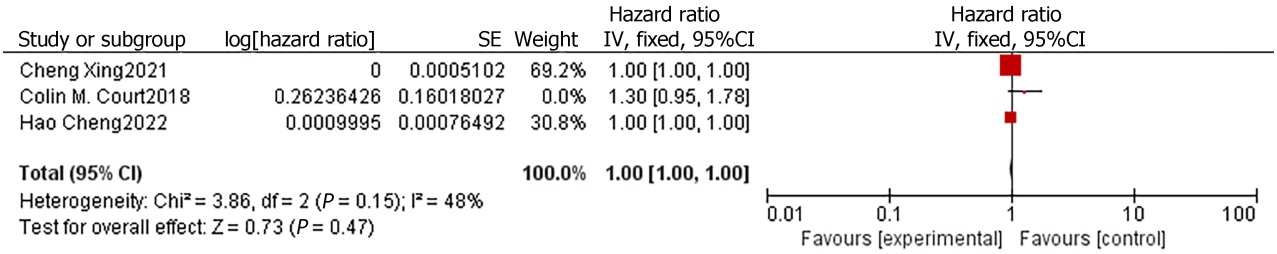

**DOI**: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

**Figure 5 Funnel plots were generated for the included studies to determine whether or not publication bias was found for both the overall survival and disease-free survival/progression-free survival/recurrence-free survival subgroups.** A: Overall survival; B: Disease-free survival/progression-free survival/recurrence-free survival.




**DOI**: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

**Figure 6 Forest plots of the four studies show that the carbohydrate antigen 19-9 level in the venous blood of early-stage pancreatic cancer patients was not an independent predictor of a shorter time to recurrence.** CI: Confidence interval.




**DOI**: 10.5306/wjco.v14.i11.504 **Copyright** ©The Author(s) 2023.

**Figure 7 Forest plots of the three studies, that detected the carbohydrate antigen 19-9 level in the venous blood before surgery, show that it was not an independent predictor of a shorter time to recurrence for early-stage pancreatic cancer patients.** CI: Confidence interval.

improve this situation, several methods have been used for CTC enrichment, such as density centrifugation, immunomagnetic enrichment with anti-CD45 monoclonal antibodies, epithelial cell adhesion molecule (EpCAM), and cell filtration technology. Additionally, epithelial cell-specific markers, such as the cytokeratin (CK) family, and mesenchymal markers, such as N-cadherin and vimentin, have been used to identify epithelial and mesenchymal CTCs, respectively[40, 41]. Numerous platforms based on these technologies have been developed. The CellSearch system is the only platform approved by the Food and Drug Administration for confirming the presence of CTCs in patients with pancreatic cancer. This system first enriches CTCs by taking advantage of their characteristic expression of the EpCAM on their membrane surface and then distinguishing different cells by immunostaining markers such as CD45, 4',6-diamidino-2-phenylindole (DAPI), and CK 8, 18, 19[42]. However, the detection rates are only 20% and 5%-42% for resectable and advanced pancreatic cancers, respectively [19,43]. This is because most CTCs undergo epithelial-mesenchymal transition (EMT) and thus lack or express low levels of epithelial markers that are generally used as the basis for the detection of these cells [44]. In addition, separation solutions based on these epithelial markers may exclude cells that play important roles in

metastasis and chemoresistance[45]. In our included studies, Court *et al*[16] used the microfluidic NanoVelcro CTC chip to evaluate the presence and number of CTCs. This platform greatly improves CTC capture and identification by utilizing anti-EpCAM-coated 3D-nanosubstrates in conjunction with microfluidic chaotic mixers and by adding tumor identification markers, which enhance the synergistic effects of cell–substrate contact frequency as well as its affinity[46]. This platform also allows seamless integration with laser capture microdissection for single CTC isolation[47]; moreover, the separated cells can be subjected to downstream molecular analyses[48]. However, an important limitation of this assay is that it is similar to the CellSearch system. In addition, using membrane filtration may reduce specificity, as some CTCs are found to be equal or smaller in size than nucleated blood cells[49]. The combination of specific mesenchymal markers may be a future direction for these assays. Considering their failure to detect EpCAM- or CK-negative CTCs, Lin *et al*[50] developed an integrated tumor cell surface molecule-independent SE-iFISH platform in 2015. Using fluorescence in situ hybridization with a specific chromosome centromere probe, this system can detect aneuploidy in the PB, which is a common manifestation of chromosome instability and malignant solid tumors. Moreover, Semaan *et al*[15] used an antigen-independent approach called DEP-FFF, which utilizes the physical properties of cells and allows the isolation of phenotypically distinct CTCs. In doing so, they obtained not only epithelial and mesenchymal CTCs, but also intermediate-state CTCs, which may show greater invasiveness and therapeutic resistance. Another valid method to detect the molecular characterization of CTCs is PCR. This is most likely due to the detection of multiple tumor markers, which could downgrade the effect of the heterogeneity that exists in CTCs. Among the included studies, Cheng *et al*[17] used immunomagnetic depletion and ligand-targeted polymerase chain reaction to detect the expression rate of folate receptor + CTCs in patients with different stages of pancreatic cancer. Traditional genetic studies of CTCs are limited by the low specificity of the enrichment methods, which contributes to the presence of nuclear blood cells, necrotic cells, tumor-derived exosomes, and cellular fragments. Therefore, the obtained nucleic acids may not accurately reflect the hereditary properties of CTCs[42]. In recent years, single-cell separation and whole-genome amplification of CTCs have been developed to overcome this challenge. However, studies on pancreatic cancer are still lacking.

Although the pooled results of our meta-analysis indicated that CTCs were associated with shorter OS and DFS/PFS/RFS in patients with early pancreatic cancer after surgery, three of the eight included studies showed no significant correlation between the existence of CTCs and both OS and DFS/PFS/RFS. Colin *et al*[48] found that CTCs were independent predictors of RFS following surgery and could correctly identify patients with occult metastatic disease preoperatively. However, when the analysis was limited to the early-stage subset, CTC count was no longer associated with shorter RFS. One possibility is that they used only 4 mL of PB, which may be too small to detect CTCs in patients with early-stage pancreatic cancer. For example, White *et al*[18], the CTC number in the PB did not contribute to predicting the OS or RFS of patients after resection, and the CTC number in the PVB was not associated with RFS. However, their results showed complete collinearity between the number of CTCs detected in the PVB and the OS. They hypothesized that this was caused by pancreatic venous drainage, as well as the capture and dilution effects of the liver for CTCs in the portal circulation. Similarly, the research of Bissolati *et al*[19] showed no significant correlation between the number of CTCs and survival time. However, they found that patients with a positive intraoperative detection of CTCs in the PVB had a higher liver metastasis rate, indicating that CTCs are of great significance for guiding adjuvant chemotherapy and postoperative follow-up monitoring. Overall, these studies revealed that CTCs in the blood of patients with pancreatic cancer are difficult to detect, particularly in early-stage patients.

In contrast, despite the different separation technologies and biomarkers used, the prognostic value of CTCs as a survival indicator for patients with early-stage pancreatic cancer was demonstrated in the remaining five studies. Moreover, they indicated that CTCs could provide tumor characteristics. Xing *et al*[14] used CD44+ as a marker to detect CTCs, which represent a more stem-like phenotype and can cause tumor metastasis, promoting tumor growth, angiogenesis, and drug resistance. Semaan *et al*[15] detected the longitudinal characterization of CTC subtypes and described EMT-CTCs as a more aggressive phenotype that is more prone to therapeutic resistance. In addition, the preoperative CTCs level in the PVB of early-stage pancreatic cancer patients may be a better prognostic marker then the preoperative CA19-9 level. This may broaden the selectivity of biomarkers for pancreatic cancer.

Our study has several limitations. First, the small number of included studies as well as the numerous types of separation strategies and research designs limited the power of our analysis. Second, several studies did not provide HR and 95%CI directly; therefore, we estimated them based on Kaplan-Meier curves, which may cause deviations. Finally, four of the eight studies recruited patients with pancreatic cancer at all stages, and we only extracted data about patients with early-stage pancreatic cancer; hence, the information was not detailed.

## CONCLUSION

This meta-analysis reveals the potential of CTCs as a prognostic biomarker for early-stage pancreatic cancer. Although we rigorously gathered and analyzed the data, the essential limitations of the included studies caused a high degree of heterogeneity and hindered deeper exploration, which reduced the confidence level of our study. To overcome these difficulties, large-scale multicenter cohort studies are urgently needed to explore the full potential of CTCs.

## ARTICLE HIGHLIGHTS

### *Research background*

Pancreatic cancer is a terribly invasive and poorly prognosis disease with a five-year survival less than 10%. Recently, more and more studies demonstrated that circulating tumor cells (CTCs) can be a significant prognostic marker of pancreatic cancer.

### *Research motivation*

In this study, we conducted a meta-analysis to analyse the prognostic role of CTCs in patients with pancreatic cancer and investigated whether CTCs can provide prognostic information and assist develop personalized treatment plans.

### *Research objectives*

Our research aims at exploring the predictive effect of CTCs on survival indicators of pancreatic cancer patients in different studies.

### *Research methods*

A standardized literature search of databases was conducted for articles about CTCs published through December 2022. After screening based on inclusion and exclusion criteria, data relevant to prognosis were extracted for analysis. We used a fixed- or random-effect model to calculate the pooled hazard ratio (HR) and 95% confidence interval (CI) of overall survival (OS) and progression-free survival (PFS) according to the degree of heterogeneity.

### *Research results*

Eight eligible studies with a total number of 355 patients with early-stage pancreatic cancer were included. This meta-analysis showed that positive pre-treatment or post-treatment CTCs was associated with shorter OS (HR = 1.93, 95%CI: 1.197-3.126, $P$ = 0.007) and decreased relapse-free/disease-free/PFS (HR = 1.27, 95%CI: 1.137-1.419, $P$ < 0.001) in patients with early-stage pancreatic cancer. While the CA19-9 level in the portal venous blood of early-stage pancreatic cancer patients showed no significant correlation with postoperative recurrence time of patients (HR = 1, 95%CI: 1.00-1.00, $P$ = 0.03).

### *Research conclusions*

Our meta-analysis indicates that CTCs are closely related to the prognosis of early pancreatic cancer patients and can serve as a guiding indicator for developing patient important treatment plans.

### *Research perspectives*

Researchers should extend follow-up time to observe the relationship between CTC and OS. Besides, large-scale multicenter cohort studies are urgently needed to explore the full potential of CTCs.

## ACKNOWLEDGEMENTS

We thank Professor Si-Yu Sun, for his support and guidance, as well as all other doctors who participated in this study.

## FOOTNOTES

**Author contributions:** Zhang ZH contributed to the data acquisition and analysis, and article drafting; Bao YW, Zhao YJ, and Wang JQ and Sun SY oversaw acquiring and analyzing data; Guo JT was the study supervisor and was responsible for the revision of the manuscript; all authors read and gave their final approval for this version of the manuscript to be submitted.

**Supported by** Liaoning Province Applied Basic Research Program Joint Program Project, No. 2022JH2/101500076; Shenyang Young and Middle-aged Science and Technology Innovation Talent Support Program, No. RC200438; Tree planting program of Shengjing Hospital, No. M1595.

**Conflict-of-interest statement:** The authors deny any conflict of interest.

**PRISMA 2009 Checklist statement:** The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.



**Country/Territory of origin:** China

**ORCID number:** Zi-Han Zhang 0000-0001-7636-9400; Yi-Wen Bao 0000-0002-2224-1118; Ya-Jun Zhao 0009-0008-6326-6590; Jian-Quan Wang 0009-0008-8907-5311; Jin-Tao Guo 0000-0001-5722-6359; Si-Yu Sun 0000-0002-7308-0473.

**S-Editor:** Qu XL
**L-Editor:** A
**P-Editor:** Chen YX

## REFERENCES

1 **Park W**, Chawla A, O'Reilly EM. Pancreatic Cancer: A Review. *JAMA* 2021; **326**: 851-862 [PMID: 34547082 DOI: 10.1001/jama.2021.13027]
2 **Lin QJ**, Yang F, Jin C, Fu DL. Current status and progress of pancreatic cancer in China. *World J Gastroenterol* 2015; **21**: 7988-8003 [PMID: 26185370 DOI: 10.3748/wjg.v21.i26.7988]
3 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020; **70**: 7-30 [PMID: 31912902 DOI: 10.3322/caac.21590]
4 **Kamyabi N**, Bernard V, Maitra A. Liquid biopsies in pancreatic cancer. *Expert Rev Anticancer Ther* 2019; **19**: 869-878 [PMID: 31533487 DOI: 10.1080/14737140.2019.1670063]
5 **Crowley E**, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; **10**: 472-484 [PMID: 23836314 DOI: 10.1038/nrclinonc.2013.110]
6 **Marrugo-Ramírez J**, Mir M, Samitier J. Blood-Based Cancer Biomarkers in Liquid Biopsy: A Promising Non-Invasive Alternative to Tissue Biopsy. *Int J Mol Sci* 2018; **19** [PMID: 30248975 DOI: 10.3390/ijms19102877]
7 **Hou J**, Li X, Xie KP. Coupled liquid biopsy and bioinformatics for pancreatic cancer early detection and precision prognostication. *Mol Cancer* 2021; **20**: 34 [PMID: 33593396 DOI: 10.1186/s12943-021-01309-7]
8 **Gall TMH**, Belete S, Khanderia E, Frampton AE, Jiao LR. Circulating Tumor Cells and Cell-Free DNA in Pancreatic Ductal Adenocarcinoma. *Am J Pathol* 2019; **189**: 71-81 [PMID: 30558725 DOI: 10.1016/j.ajpath.2018.03.020]
9 **Kanayama M**, Kuwata T, Mori M, Nemoto Y, Nishizawa N, Oyama R, Matsumiya H, Taira A, Shinohara S, Takenaka M, Yoneda K, Kuroda K, Ohnaga T, Tanaka F. Prognostic impact of circulating tumor cells detected with the microfluidic "universal CTC-chip" for primary lung cancer. *Cancer Sci* 2022; **113**: 1028-1037 [PMID: 34964211 DOI: 10.1111/cas.15255]
10 **Guan Y**, Xu F, Tian J, Gao K, Wan Z, Wang Y, Gao M, Wang Z, Chong T. The prognostic value of circulating tumour cells (CTCs) and CTC white blood cell clusters in patients with renal cell carcinoma. *BMC Cancer* 2021; **21**: 826 [PMID: 34271857 DOI: 10.1186/s12885-021-08463-7]
11 **Strati A**, Nikolaou M, Georgoulias V, Lianidou ES. RNA-Based CTC Analysis Provides Prognostic Information in Metastatic Breast Cancer. *Diagnostics (Basel)* 2021; **11** [PMID: 33799422 DOI: 10.3390/diagnostics11030513]
12 **Zong W**, Feng W, Jiang Y, Ju S, Cui M, Jing R. Evaluating the diagnostic and prognostic value of serum long non-coding RNA CTC-497E21.4 in gastric cancer. *Clin Chem Lab Med* 2019; **57**: 1063-1072 [PMID: 30763257 DOI: 10.1515/cclm-2018-0929]
13 **Cai D**, Li N, Jin L, Qi X, Hua D, Wang T. High CTC-TRPC5 Expression Significantly Associated With Poor Prognosis in Radical Resected Colorectal Cancer Patients. *Front Mol Biosci* 2021; **8**: 727864 [PMID: 34422911 DOI: 10.3389/fmolb.2021.727864]
14 **Xing C**, Li Y, Ding C, Wang S, Zhang H, Chen L, Li P, Dai M. CD44+ Circulating Tumor Endothelial Cells Indicate Poor Prognosis in Pancreatic Ductal Adenocarcinoma After Radical Surgery: A Pilot Study. *Cancer Manag Res* 2021; **13**: 4417-4431 [PMID: 34103996 DOI: 10.2147/CMAR.S309115]
15 **Semaan A**, Bernard V, Kim DU, Lee JJ, Huang J, Kamyabi N, Stephens BM, Qiao W, Varadhachary GR, Katz MH, Shen Y, San Lucas FA, Gascoyne P, Alvarez HA, Maitra A, Guerrero PA. Characterisation of circulating tumour cell phenotypes identifies a partial-EMT sub-population for clinical stratification of pancreatic cancer. *Br J Cancer* 2021; **124**: 1970-1977 [PMID: 33785875 DOI: 10.1038/s41416-021-01350-9]
16 **Court CM**, Ankeny JS, Sho S, Winograd P, Hou S, Song M, Wainberg ZA, Girgis MD, Graeber TG, Agopian VG, Tseng HR, Tomlinson JS. Circulating Tumor Cells Predict Occult Metastatic Disease and Prognosis in Pancreatic Cancer. *Ann Surg Oncol* 2018; **25**: 1000-1008 [PMID: 29442211 DOI: 10.1245/s10434-017-6290-8]
17 **Cheng H**, Yang J, Fu X, Mao L, Chu X, Lu C, Li G, Qiu Y, He W. Folate receptor-positive circulating tumor cells predict survival and recurrence patterns in patients undergoing resection for pancreatic cancer. *Front Oncol* 2022; **12**: 1012609 [PMID: 36313690 DOI: 10.3389/fonc.2022.1012609]
18 **White MG**, Lee A, Vicente D, Hall C, Kim MP, Katz MHG, Lee JE, Ikoma N, Lucci A, Tzeng CD. Measurement of Portal Vein Blood Circulating Tumor Cells is Safe and May Correlate With Outcomes in Resected Pancreatic Ductal Adenocarcinoma. *Ann Surg Oncol* 2021; **28**: 4615-4622 [PMID: 33415562 DOI: 10.1245/s10434-020-09518-y]
19 **Bissolati M**, Sandri MT, Burtulo G, Zorzino L, Balzano G, Braga M. Portal vein-circulating tumor cells predict liver metastases in patients with resectable pancreatic cancer. *Tumour Biol* 2015; **36**: 991-996 [PMID: 25318603 DOI: 10.1007/s13277-014-2716-0]
20 **Hugenschmidt H**, Labori KJ, Borgen E, Brunborg C, Schirmer CB, Seeberg LT, Naume B, Wiedswang G. Preoperative CTC-Detection by CellSearch((R)) Is Associated with Early Distant Metastasis and Impaired Survival in Resected Pancreatic Cancer. *Cancers (Basel)* 2021; **13** [DOI: 10.20944/preprints202012.0556.v1]
21 **Padillo-Ruiz J**, Suarez G, Pereira S, Calero-Castro FJ, Tinoco J, Marin L, Bernal C, Cepeda-Franco C, Alamo JM, Almoguera F, Macher HC, Villanueva P, García-Fernandez FJ, Gallego I, Romero M, Gomez-Bravo MA, Denninghoff V, Serrano MJ. Circulating Tumor Cells Enumeration from the Portal Vein for Risk Stratification in Early Pancreatic Cancer Patients. *Cancers (Basel)* 2021; **13** [PMID: 34944773 DOI: 10.3390/cancers13246153]
22 **Klein AP**. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 493-502 [PMID: 34002083 DOI: 10.1038/s41575-021-00457-x]
23 **Forgensen J**. Resected adenocarcinoma of the pancreas--616 patients: results, outcomes, and prognostic indicators. *J Gastrointest Surg* 2001; **5**: 681; author reply 681 [PMID: 12086909 DOI: 10.1016/s1091-255x(01)80113-x]
24 **Neoptolemos JP**, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer DH. Therapeutic developments in pancreatic cancer: current and future

perspectives. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 333-348 [PMID: 29717230 DOI: 10.1038/s41575-018-0005-x]

25 **Liu P**, Zhu H, Zhang X, Feng A, Zhu X, Sun Y. Predicting Survival for Hepatic Arterial Infusion Chemotherapy of Unresectable Colorectal Liver Metastases: Radiomics Analysis of Pretreatment Computed Tomography. *J Transl Int Med* 2022; **10**: 56-64 [PMID: 35702189 DOI: 10.2478/jtim-2022-0004]

26 **Yang J**, Xu R, Wang C, Qiu J, Ren B, You L. Early screening and diagnosis strategies of pancreatic cancer: a comprehensive review. *Cancer Commun (Lond)* 2021; **41**: 1257-1274 [PMID: 34331845 DOI: 10.1002/cac2.12204]

27 **Yin H**, Yang X, Sun L, Pan P, Peng L, Li K, Zhang D, Cui F, Xia C, Huang H, Li Z. The value of artificial intelligence techniques in predicting pancreatic ductal adenocarcinoma with EUS images: A meta-analysis and systematic review. *Endosc Ultrasound* 2023; **12**: 50-58 [PMID: 35313419 DOI: 10.4103/EUS-D-21-00131]

28 **Sagami R**, Hayasaka K, Ujihara T, Iwaki T, Katsuyama Y, Harada H, Ome Y, Honda G, Horiguchi SI, Murakami K, Amano Y. Role of EUS combined with a newly modified scoring system to detect pancreatic high-grade precancerous lesions. *Endosc Ultrasound* 2023; **12**: 111-119 [PMID: 36861510 DOI: 10.4103/EUS-D-21-00187]

29 **Bian Y**, Jiang H, Zheng J, Shao C, Lu J. Basic Pancreatic Lesions: Radiologic-pathologic Correlation. *J Transl Int Med* 2022; **10**: 18-27 [PMID: 35702187 DOI: 10.2478/jtim-2022-0003]

30 **Hewitt MJ**, McPhail MJ, Possamai L, Dhar A, Vlavianos P, Monahan KJ. EUS-guided FNA for diagnosis of solid pancreatic neoplasms: a meta-analysis. *Gastrointest Endosc* 2012; **75**: 319-331 [PMID: 22248600 DOI: 10.1016/j.gie.2011.08.049]

31 **Facciorusso A**, Gkolfakis P, Tziatzios G, Ramai D, Papanikolaou IS, Triantafyllou K, Lisotti A, Fusaroli P, Mangiavillano B, Chandan S, Mohan BP, Crinò SF. Comparison between EUS-guided fine-needle biopsy with or without rapid on-site evaluation for tissue sampling of solid pancreatic lesions: A systematic review and meta-analysis. *Endosc Ultrasound* 2022; **11**: 458-465 [PMID: 36537383 DOI: 10.4103/EUS-D-22-00026]

32 **Thomsen MM**, Larsen MH, Di Caterino T, Hedegaard Jensen G, Mortensen MB, Detlefsen S. Accuracy and clinical outcomes of pancreatic EUS-guided fine-needle biopsy in a consecutive series of 852 specimens. *Endosc Ultrasound* 2022; **11**: 306-318 [PMID: 35708361 DOI: 10.4103/EUS-D-21-00180]

33 **Saghir SM**, Dhindsa BS, Daid SGS, Mashiana HS, Dhaliwal A, Cross C, Singh S, Bhat I, Ohning GV, Adler DG. Efficacy of EUS-guided needle-based confocal laser endomicroscopy in the diagnosis of pancreatic lesions: A systematic review and meta-analysis. *Endosc Ultrasound* 2022; **11**: 275-282 [PMID: 33666181 DOI: 10.4103/EUS-D-20-00122]

34 **Hartwig W**, Strobel O, Hinz U, Fritz S, Hackert T, Roth C, Büchler MW, Werner J. CA19-9 in potentially resectable pancreatic cancer: perspective to adjust surgical and perioperative therapy. *Ann Surg Oncol* 2013; **20**: 2188-2196 [PMID: 23247983 DOI: 10.1245/s10434-012-2809-1]

35 **Ferrone CR**, Finkelstein DM, Thayer SP, Muzikansky A, Fernandez-delCastillo C, Warshaw AL. Perioperative CA19-9 levels can predict stage and survival in patients with resectable pancreatic adenocarcinoma. *J Clin Oncol* 2006; **24**: 2897-2902 [PMID: 16782929 DOI: 10.1200/JCO.2005.05.3934]

36 **Wang Y**, Yu X, Hartmann D, Zhou J. Circulating tumor cells in peripheral blood of pancreatic cancer patients and their prognostic role: a systematic review and meta-analysis. *HPB (Oxford)* 2020; **22**: 660-669 [PMID: 31786054 DOI: 10.1016/j.hpb.2019.11.003]

37 **Khoo BL**, Grenci G, Lim YB, Lee SC, Han J, Lim CT. Expansion of patient-derived circulating tumor cells from liquid biopsies using a CTC microfluidic culture device. *Nat Protoc* 2018; **13**: 34-58 [PMID: 29215634 DOI: 10.1038/nprot.2017.125]

38 **Gemenetzis G**, Groot VP, Yu J, Ding D, Teinor JA, Javed AA, Wood LD, Burkhart RA, Cameron JL, Makary MA, Weiss MJ, He J, Wolfgang CL. Circulating Tumor Cells Dynamics in Pancreatic Adenocarcinoma Correlate With Disease Status: Results of the Prospective CLUSTER Study. *Ann Surg* 2018; **268**: 408-420 [PMID: 30080739 DOI: 10.1097/SLA.0000000000002925]

39 **Li Y**, Wu S, Bai F. Molecular characterization of circulating tumor cells-from bench to bedside. *Semin Cell Dev Biol* 2018; **75**: 88-97 [PMID: 28899718 DOI: 10.1016/j.semcdb.2017.09.013]

40 **Pantel K**, Alix-Panabières C. The clinical significance of circulating tumor cells. *Nat Clin Pract Oncol* 2007; **4**: 62-63 [PMID: 17259923 DOI: 10.1038/ncponc0737]

41 **Alix-Panabières C**, Pantel K. Challenges in circulating tumour cell research. *Nat Rev Cancer* 2014; **14**: 623-631 [PMID: 25154812 DOI: 10.1038/nrc3820]

42 **Cen P**, Ni X, Yang J, Graham DY, Li M. Circulating tumor cells in the diagnosis and management of pancreatic cancer. *Biochim Biophys Acta* 2012; **1826**: 350-356 [PMID: 22683404 DOI: 10.1016/j.bbcan.2012.05.007]

43 **Bidard FC**, Huguet F, Louvet C, Mineur L, Bouché O, Chibaudel B, Artru P, Desseigne F, Bachet JB, Mathiot C, Pierga JY, Hammel P. Circulating tumor cells in locally advanced pancreatic adenocarcinoma: the ancillary CirCe 07 study to the LAP 07 trial. *Ann Oncol* 2013; **24**: 2057-2061 [PMID: 23676420 DOI: 10.1093/annonc/mdt176]

44 **Pecot CV**, Bischoff FZ, Mayer JA, Wong KL, Pham T, Bottsford-Miller J, Stone RL, Lin YG, Jaladurgam P, Roh JW, Goodman BW, Merritt WM, Pircher TJ, Mikolajczyk SD, Nick AM, Celestino J, Eng C, Ellis LM, Deavers MT, Sood AK. A novel platform for detection of CK+ and CK- CTCs. *Cancer Discov* 2011; **1**: 580-586 [PMID: 22180853 DOI: 10.1158/2159-8290.CD-11-0215]

45 **Liu H**, Sun B, Wang S, Liu C, Lu Y, Li D, Liu X. Circulating Tumor Cells as a Biomarker in Pancreatic Ductal Adenocarcinoma. *Cell Physiol Biochem* 2017; **42**: 373-382 [PMID: 28558380 DOI: 10.1159/000477481]

46 **Wang S**, Liu K, Liu J, Yu ZT, Xu X, Zhao L, Lee T, Lee EK, Reiss J, Lee YK, Chung LW, Huang J, Rettig M, Seligson D, Duraiswamy KN, Shen CK, Tseng HR. Highly efficient capture of circulating tumor cells by using nanostructured silicon substrates with integrated chaotic micromixers. *Angew Chem Int Ed Engl* 2011; **50**: 3084-3088 [PMID: 21374764 DOI: 10.1002/anie.201005853]

47 **Hou S**, Zhao L, Shen Q, Yu J, Ng C, Kong X, Wu D, Song M, Shi X, Xu X, OuYang WH, He R, Zhao XZ, Lee T, Brunicardi FC, Garcia MA, Ribas A, Lo RS, Tseng HR. Polymer nanofiber-embedded microchips for detection, isolation, and molecular analysis of single circulating melanoma cells. *Angew Chem Int Ed Engl* 2013; **52**: 3379-3383 [PMID: 23436302 DOI: 10.1002/anie.201208452]

48 **Court CM**, Ankeny JS, Sho S, Hou S, Li Q, Hsieh C, Song M, Liao X, Rochefort MM, Wainberg ZA, Graeber TG, Tseng HR, Tomlinson JS. Reality of Single Circulating Tumor Cell Sequencing for Molecular Diagnostics in Pancreatic Cancer. *J Mol Diagn* 2016; **18**: 688-696 [PMID: 27375074 DOI: 10.1016/j.jmoldx.2016.03.006]

49 **Zou D**, Cui D. Advances in isolation and detection of circulating tumor cells based on microfluidics. *Cancer Biol Med* 2018; **15**: 335-353 [PMID: 30766747 DOI: 10.20892/j.issn.2095-3941.2018.0256]

50 **Lin PP**. Integrated EpCAM-independent subtraction enrichment and iFISH strategies to detect and classify disseminated and circulating tumors cells. *Clin Transl Med* 2015; **4**: 38 [PMID: 26718583 DOI: 10.1186/s40169-015-0081-2]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

*World J Clin Oncol* 2023 November 24; 14(11): 518-534

DOI: 10.5306/wjco.v14.i11.518

ISSN 2218-4333 (online)

SCIENTOMETRICS

# Bibliometric analysis of the global research status and trends of mechanotransduction in cancer

Yi-Zhan Zhang, Meng-Zhu Li, Guang-Xin Wang, Da-Wei Wang

**Specialty type:** Oncology

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): C
Grade D (Fair): D, D
Grade E (Poor): 0

**P-Reviewer:** Fu L, China; Jeong KY, South Korea; Safarzadeh Kozani P, Iran; Gupta MK, Germany

**Received:** August 2, 2023
**Peer-review started:** August 2, 2023
**First decision:** August 16, 2023
**Revised:** September 14, 2023
**Accepted:** October 16, 2023
**Article in press:** October 16, 2023
**Published online:** November 24, 2023

**Yi-Zhan Zhang, Meng-Zhu Li, Da-Wei Wang,** Department of Endocrinology, Shandong Provincial Hospital, Shandong University, Jinan 250021, Shandong Province, China

**Yi-Zhan Zhang, Meng-Zhu Li, Da-Wei Wang,** Key Laboratory of Endocrine Glucose & Lipids Metabolism and Brain Aging, Ministry of Education, Jinan 250021, Shandong Province, China

**Guang-Xin Wang,** Shandong Innovation Center of Intelligent Diagnosis, Central Hospital Affiliated to Shandong First Medical University, Jinan 250013, Shandong Province, China

**Corresponding author:** Da-Wei Wang, DPhil, Associate Professor, Postdoc, Postdoctoral Fellow, Department of Endocrinology, Shandong Provincial Hospital, Shandong University, No. 544 Jingsi Road, Huaiyin District, Jinan 250021, Shandong Province, China.
wangdawei@sdfmu.edu.cn

## Abstract

### *BACKGROUND*

The development of cancer is thought to involve the dynamic crosstalk between the tumor cells and the microenvironment they inhabit. Such crosstalk is thought to involve mechanotransduction, a process whereby the cells sense mechanical cues such as stiffness, and translate these into biochemical signals, which have an impact on the subsequent cellular activities. Bibliometric analysis is a statistical method that involves investigating different aspects (including authors' names and affiliations, article keywords, journals and citations) of large volumes of literature. Despite an increase in mechanotransduction-related research in recent years, there are currently no bibliometric studies that describe the global status and trends of mechanotransduction-related research in the cancer field.

### *AIM*

To investigate the global research status and trends of mechanotransduction in cancer from a bibliometric viewpoint.

### *METHODS*

Literature on mechanotransduction in cancer published from January 1, 1900 to December 31, 2022 was retrieved from the Web of Science Core Collection. Excel and GraphPad software carried out the statistical analysis of the relevant author, journal, organization, and country information. The co-authorship, keyword co-occurrence, and keyword burst analysis were visualized with VOSviewer and CiteSpace.

***RESULTS***

Of 597 publications from 745 institutions in 45 countries were published in 268 journals with 35510 citation times. With 270 articles, the United States is a well-established global leader in this field, and the University of California system, the most productive ($n$ = 36) and influential institution ($n$ = 4705 citations), is the most highly active in collaborating with other organizations. *Cancers* was the most frequent publisher with the highest H-index. The most productive researcher was Valerie M. Weaver, with 10 publications. The combined analysis of concurrent and burst keywords revealed that the future research hotspots of mechanotransduction in cancer were related to the plasma membrane, autophagy, piezo1/2, heterogeneity, cancer diagnosis, and post-transcriptional modifications.

***CONCLUSION***

Mechanotransduction-related cancer research remains a hot topic. The United States is in the leading position of global research on mechano-oncology after almost 30 years of investigations. Research group cooperations exist but remain largely domestic, lacking cross-national communications. The next big topic in this field is to explore how the plasma membrane and its localized mechanosensor can transduce mechanical force through post-transcriptional modifications and thereby participate in cellular activity regulations and cancer development.

**Key Words:** Bibliometric analysis; Mechanotransduction; Cancer; Visualization; Signal transduction



**Core Tip:** Through bibliometric analysis, we found that mechanotransduction-related cancer research remains a hot topic, with approximately 100 papers and 5000 citations generated per year in the past three years. Additionally, the United States is a well-established global leader of this field, and the University of California system is the most influential organization in this field. We predict that investigating how the plasma membrane and its localized mechanosensors transduce mechanical forces *via* post-transcriptional modifications and thereby participate in the regulation of cellular activity will be the next big research topic in the cancer field.

**Citation:** Zhang YZ, Li MZ, Wang GX, Wang DW. Bibliometric analysis of the global research status and trends of mechanotransduction in cancer. *World J Clin Oncol* 2023; 14(11): 518-534
**URL:** https://www.wjgnet.com/2218-4333/full/v14/i11/518.htm
**DOI:** https://dx.doi.org/10.5306/wjco.v14.i11.518

## INTRODUCTION

Cancer is a devastating disease characterized by the transduction of abnormal cell signals, which leads to oncogenic cellular behaviors such as uncontrolled proliferation and resistance to death[1]. According to the latest statistics, cancer is the second most common cause of death worldwide after cardiovascular disease[2]. Therefore, it is crucial to determine the pathogenesis of this disease so that effective therapeutic approaches can be identified. Although cancer is primarily regarded as being a genetic disease caused by the stepwise accumulation of gene mutations, an increasing number of studies suggest that environmental factors might also have a significant influence on the development of this disease[3]. The tumor microenvironment (TME) consists of cancer-associated fibroblasts (CAFs), endothelial cells, immune cells, and the extracellular matrix (ECM), and together these provide the biochemical and mechanical signals required to stimulate the occurrence, survival, and development of cancer[4]. While the importance of biochemical stimuli (such as small molecules, growth factors, and cytokines) in cancer progression is well-established, the role of mechanical force is still relatively underexplored although recent investigations suggest that it is on par with the chemical factors[5-7].

In cancerous tissues, mechanical forces such as shear stress, tension, and compression, are suggested to be generated during cell-TME and cell-cell contact events[8]. These forces are then translated into biochemical signaling cascades with the help of various mechanoreceptors, including G protein-coupled receptors, ion channels, and cell junction proteins[9, 10]. This process of transducing specific mechanosignals into distinct intracellular biochemical signals is called mechanotransduction[5]. Abnormal mechanical signals (generated by the environment or cells), can alter the expression of genes and transduction of signaling pathways. This leads to the dysregulated cell behaviors that are associated with many diseases, including cancer[11]. For example, when compared with normal tissues, cancerous tissues have a more rigid ECM, which contributes to the aberrant mechanotransduction observed[12]. This leads to an increase in the expression of many oncogenic transcription factors (such as YAP/TAZ, Twist1, and β-catenin), which enhances cell proliferation, the epithelial-mesenchymal transition (EMT), and/or cell migration in cancer cells[13]. Interestingly, YAP/TAZ has also been reported to contribute to tissue stiffness by increasing the expression of the crucial ECM modifiers, CTGF and CYR61[14-17]. Therefore, a positive mechanotransductive feedback loop between cancer cells and the ECM is regarded as one of the major culprits for cancer malignancy[17]. In addition, when cancer cells are surrounded by a rigid, crosslinked ECM, then this impedes immune cell invasion and drug distribution, which leads to immune surveillance escape and drug resistance

[6,18].

The concept of mechanotransduction also helps to explain the mechanisms of cancer initiation, invasion, and metastasis from a new perspective. Considering the fundamental role of mechanotransduction in cancer development, researchers are now developing new cancer therapeutics based on the specific mechanical properties of and mechano-signal transducers generated by tumors. For example, Simtuzumab and Simvastatin, which reduce ECM stiffness and inhibit YAP/TAZ hyperactivation, respectively, have been evaluated for their anti-cancer efficacy in preclinical studies [18-20]. Mechanotransduction is therefore already showing great promise for clinical applications, and so further comprehensive investigations in this field will likely reveal more therapeutic strategies for cancer treatment. Thus, gaining a better understanding of the current research status of mechanotransduction in cancer is likely to shed light on important research questions and directions for future study.

Bibliometric analysis is a useful quantitative method to comprehensively analyze publications from many different aspects (including the authors and their affiliations, journal title and article keywords), to reveal collaborative networks and emerging trends in specific research areas[21-23]. Although increasing numbers of research papers now focus on the role of mechanotransduction in cancer, to date, no bibliometric analysis has been conducted to quantify the situation. To this end, here we present the updated bibliometric analysis of research conducted on mechanotransduction in cancer and reveal the current research being conducted in this field. In this way, we aim to provide a better understanding of the present status of this study area and predict key topics of future investigation in this promising research field.

## MATERIALS AND METHODS

### *Searching strategies*

We retrieved literature on 'mechanotransduction in cancer' research from the Web of Science Core Collection (WoSCC). The publication period was set from January 1, 1900 to December 31, 2022, and only English publications were included. The search strategy is illustrated in Figure 1. All recorded data, including the authors' names, institutions, and countries, as well as keywords, were downloaded from the WoSCC and normalized to a standard format. To avoid ambiguity, we cross-checked duplicate authors among the documents. Meeting abstracts, editorial materials, corrections, and retractions were excluded from our research.

### *Keyword analysis*

The keywords were extracted from the keyword section of articles. To avoid potential deviations, similar or same keywords with different expressions were manually standardized to correct and/or group similarities as previously suggested[23-25], before VOSviewer or CiteSpace analysis. Burst keywords were assessed using CiteSpace (V6.2R4 SE) with the following parameters: time slicing (from January 1994 to December 2022), years per slice (1), node type (keyword), the minimum burst duration (1 year), γ (0.39) and others (default). A keyword co-occurrence analysis was conducted with VOSviewer (version 1.6.18) with the following parameters: Type of analysis (co-occurrence), unit of analysis (keywords), counting method (full counting), minimum number of occurrences of a keyword (3).

### *Data visualization*

The number of publications and citations in the indicated time was presented using GraphPad Prism 8. Visualization of bibliometric information, including co-authorship analysis, keyword co-occurrence, and burst analysis, was conducted with VOSviewer (version 1.6.18) and CiteSpace (V6.2R4 SE).

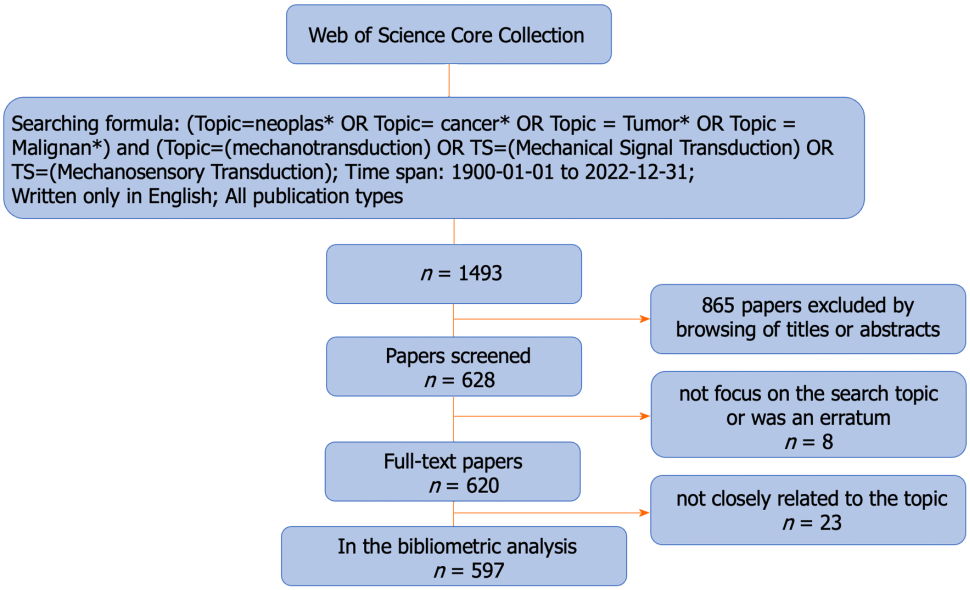
## RESULTS

### *General information*

A total of 597 publications comprising 388 research and 209 review articles, were extracted for deep analysis. Although the concept of mechanotransduction was established progressively from the 1950s to the 1980s[26-28], the first paper describing mechanotransduction in cancer was only published in 1994[29]. Since then, this topic has gradually gained more attention from researchers in the cancer research field. Based on the number of publications and citations analysed in our bibliometric study on mechanotransduction-related cancer research, we prepared a growth curve comprising three clear stages. The first stage described the period from 1994 to 2010, during which time < 10 papers were published each year. In the second stage, which ran from 2012 to 2017, a slow growth rate was observed, and in the third stage (2018-2022), the growth rate started to accelerate, with almost 100 articles being published in the final year (Figure 2A).

In the 597 articles that were published, there was an average of 59.48 citations per paper. The top 10 most cited papers included six reviews and four research articles; these are listed in Supplementary Table 1 and they are ranked by the number of times they were cited. The most highly cited review article ($n$ = 2882 citations) was published by Chambers *et al*[30], and the most highly cited research article ($n$ = 1021 citations) was contributed by Aragona *et al*[31].

During our exploration of mechanotransduction in cancer, we sub-divided the publications into 56 research categories to indicate the multidisciplinary crossovers that occurred. After the top 10 categories were generated (Figure 2B), we found that cell biology ($n$ = 191), oncology ($n$ = 130), and biochemistry molecular biology ($n$ = 117), accounted for 73.37% of all the publications. This indicates that molecular cytology is a key contributor to research on the role of mechanical force in cancer development.




**DOI**: 10.5306/wjco.v14.i11.518 

**Figure 1 The strategy we used to search for publications about mechanotransduction in cancer.** 597 publications closely correlated with mechanotransduction in cancer were extracted based on this strategy.

### *Analysis of countries and institutions*

Although a total of 45 countries was found to contribute publications on the topic of mechanotransduction in cancer, the top 10 most productive countries generated 87.6% of all the papers. Of these, the United States was the most productive as it was responsible for 270 (*i.e.*, 45.23%) of the publications. China ($n$ = 119, 19.93%) and Italy ($n$ = 41, 6.87%) held the second and third positions, respectively, but both lagged behind the United States (Table 1). However, when we ranked the countries in terms of the average citation times, Canada occupied the first place with an average of 197.33 citations per paper. This was followed by Italy and Spain with values of 109.17 and 93.09, respectively. In addition, according to this ranking approach, the United States (73.74 times) held a middle position, whereas China (20.34 times) was at the bottom of the ranking list. To evaluate the cooperation between different countries, a co-authorship network was established with a criterion of at least three publications in each country. As shown in Figure 3, the United States (with the most extended research history in this field), collaborated most with other countries, followed by the United Kingdom. In addition, it was interesting to find that although 37.78% (*i.e.*, 17/45) of the countries only published one or two papers, most of this work was conducted in the recent five years. This indicates that more countries are stepping into this research field.

Our data analysis also showed that 745 organizations were involved in research related to mechanotransduction in cancer. After ranking these according to the number of publications, we found that the top 10 organizations accounted for 35.85% (214/597) of all papers. The University of California system ranked first with 36 articles, and this was followed sequentially by UDICE-French Research Universities ($n$ = 27), Centre National de la Recherche Scientifique ($n$ = 25), the University of Illinois system ($n$ = 21), and Institut National de la Sante et de la Recherche Medicale (Inserm). It was striking to find that three institutions from France occupied three of the top five positions with a total of 31 publications (Table 2). Another analysis of the global inter-institutional network showed that the University of California system collaborated most with other organizations, followed sequentially by the University of Padua, Harvard University, and University College London (Figure 4).

### *Analysis of journals and authors*

Articles related to the field of mechanotransductive cancer research were distributed among 268 different academic journals. The top 10 journals to publish papers in this field accounted for 22.61% (135/597) of all the articles (Supplementary Table 2), but 162 journals (60.45%) only published one paper in this research area. *Cancers* ranked first with 29 (4.86%) publications, followed by the *International Journal of Molecular Sciences* ($n$ = 20, 3.35%) and the *Journal of Cell Science* ($n$ = 17, 2.85%).

We also found that a total of 3001 authors contributed to these 597 publications. After analyzing the top 10 authors in terms of the number of papers (Supplementary Table 3), we found that Valerie M. Weaver from the University of California system was the most productive researcher with $n$ = 10 publications. She was followed by Stefano Piccolo from the University of Padua ($n$ = 9), Antonios Gargalionis from the National & Kapodistrian University of Athens ($n$ = 9), and Marc D. Basson from John D. Dingell Veterans Affairs Medical Center ($n$ = 8). Notably, according to the total (T) and average (A) citation times of the papers generated from each author, Stefano Piccolo (T = 3167; A = 351.89), Tito Panciera (T = 2076, A = 296.57), Valerie M. Weaver (T = 2495, A = 249.5), Michelangelo Cordenonsi (T = 1983, A = 330.5), and Patricia J. Keely (T = 1478, A = 246.33) ranked the top 5, indicating that they were in positions of authority. To investigate if there were any collaborations between the various authors in this field, an authorship network analysis was performed by identifying those with at least three publications. As shown in Figure 5, the authors were distributed into 43 clusters,

**Table 1 The top 10 productive countries that published papers about mechanotransduction in cancer**

| Rank | Country | Publications | % | Total citations | Average citations | Connections | H-index |
|---|---|---|---|---|---|---|---|
| 1 | United States | 270 | 45.23 | 19911 | 73.74 | 22 | 69 |
| 2 | China | 119 | 19.93 | 2421 | 20.34 | 10 | 25 |
| 3 | Italy | 41 | 6.87 | 4476 | 109.17 | 12 | 22 |
| 4 | Germany | 37 | 6.20 | 2072 | 56.00 | 10 | 18 |
| 5 | United Kingdom | 34 | 7.54 | 2098 | 61.71 | 15 | 20 |
| 6 | France | 31 | 5.19 | 2466 | 79.55 | 12 | 19 |
| 7 | Spain | 22 | 3.69 | 2048 | 93.09 | 11 | 14 |
| 8 | Canada | 21 | 3.52 | 4144 | 197.33 | 8 | 13 |
| 9 | Japan | 20 | 3.35 | 761 | 38.05 | 4 | 14 |
| 10 | South Korea | 20 | 3.35 | 355 | 17.75 | 2 | 9 |

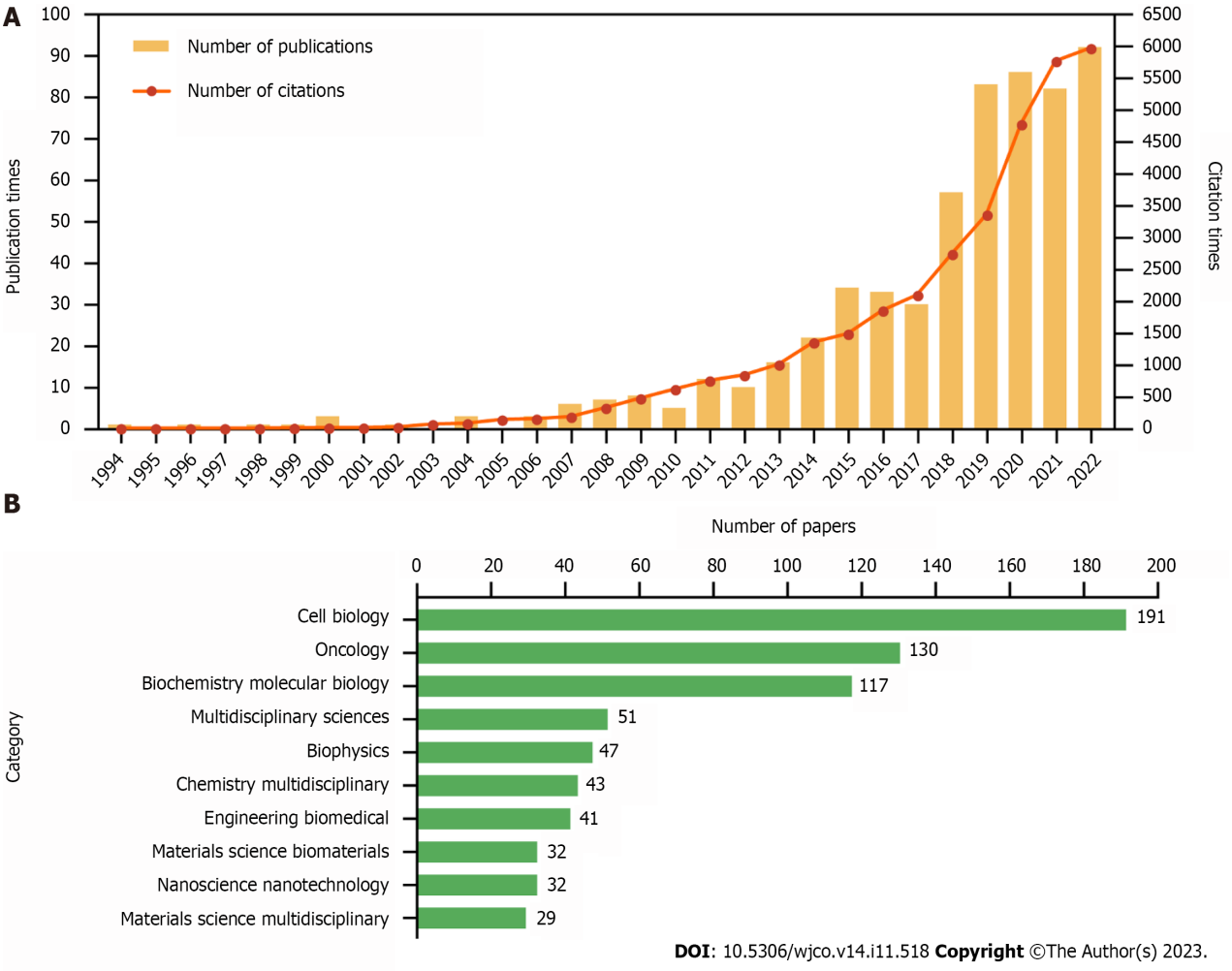
**Table 2 The top 10 productive organizations publishing papers about mechanotransduction in cancer**

| Rank | Organization | Publications | Citations | Average citation | Country | % | H-index |
|---|---|---|---|---|---|---|---|
| 1 | University of California System | 36 | 4705 | 130.69 | United States | 6.03 | 21 |
| 2 | UDICE-French Research Universities | 27 | 1875 | 69.44 | France | 4.52 | 17 |
| 3 | Centre National de la Recherche Scientifique | 25 | 1843 | 73.72 | France | 4.19 | 16 |
| 4 | University of Illinois System | 21 | 1013 | 48.24 | United States | 3.52 | 14 |
| 5 | Institut National de la Sante et de la Recherche Medicale | 20 | 1845 | 92.25 | France | 3.35 | 13 |
| 6 | University of Texas System | 20 | 1619 | 80.95 | United States | 3.35 | 15 |
| 7 | University of London | 18 | 1388 | 77.11 | United Kingdom | 3.02 | 13 |
| 8 | University of Padua | 16 | 3399 | 212.44 | Italy | 2.68 | 12 |
| 9 | Harvard University | 16 | 2463 | 153.94 | United States | 2.68 | 13 |
| 10 | Vanderbilt University | 15 | 691 | 46.07 | United States | 2.51 | 10 |

with 4 clusters containing at least ten authors. This indicates that there were partial connections between the different groups in this research area.

### *Keyword analysis*

To explore the current research themes and discover hot topics in mechanotransduction-related cancer research, we acquired 666 keywords by collating those with the same meaning or category from all 597 papers. Among them, the top 4 co-occurrent keywords that were consistent with our research topic, were: Mechanotransduction, cancer, mechanical force, and mechanical property. However, when these keywords were restricted to at least 3 co-occurrences, only 93 items could satisfy this criterion. We next established a network based on these 93 keywords and found that they could be further subdivided into eight clusters (Figure 6A and Supplementary Table 4). We found that 19 keywords were included in cluster 1 (red) as follows: Angiogenesis, cadherin, caveolin, cell death, endothelial cell, epithelial cell, growth factor/receptor, hypoxia, integrin, invadopodia, mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK), mechanical force, matrix metalloproteinases (MMPs), mechanistic target of rapamycin (mTOR), NF-κB, phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), plasma membrane, signal transduction, and traction force microscopy. Cluster 2 (green) also contained 19 items. These were: AMP-activated protein kinase, cell cycle, cell morphology, cytoskeleton or cytoskeleton remodeling, epigenetic regulation, gene regulation, heterogeneity, lamin, linc complex, mechanotransduction, metabolism, microfluidics, migration, nuclear envelope, nuclear mechanics, nucleoskeleton, nucleus, pancreatic stellate cell, and SRC. Cluster 3 (blue) contained the following 16 items: Calcium, cancer, DNA damage, dormancy, immunology, invasion, ion channel, mechanobiology, metastasis, motility, oxidative stress, piezo1/2, post-translational modification, SRF, stem cell, and TRP. Cluster 4 (yellow) contained 11 items: 3D culture, autophagy, cancer diagnosis, cancer stem cell (CSC), drug resistance, hydrogel, immunotherapy, organoid, programmed cell death protein 1/programmed cell death ligand 1, pharmacology, and polycystin. Cluster 5 (purple) contained 8 items: Atomic force microscopy, CAF, ECM or ECM remodeling, mechanical property, oncophysics, therapy, TME, and tumor model. Cluster 6 (cyan) contained 8 items: Contact inhibition, G protein-coupled receptor (GPCR), growth, Hippo, polarity, TAZ, transcription factor, and YAP. Cluster 7 (orange) contained 7 items: Catenin, EMT, fibrosis, noncoding




**DOI**: 10.5306/wjco.v14.i11.518 

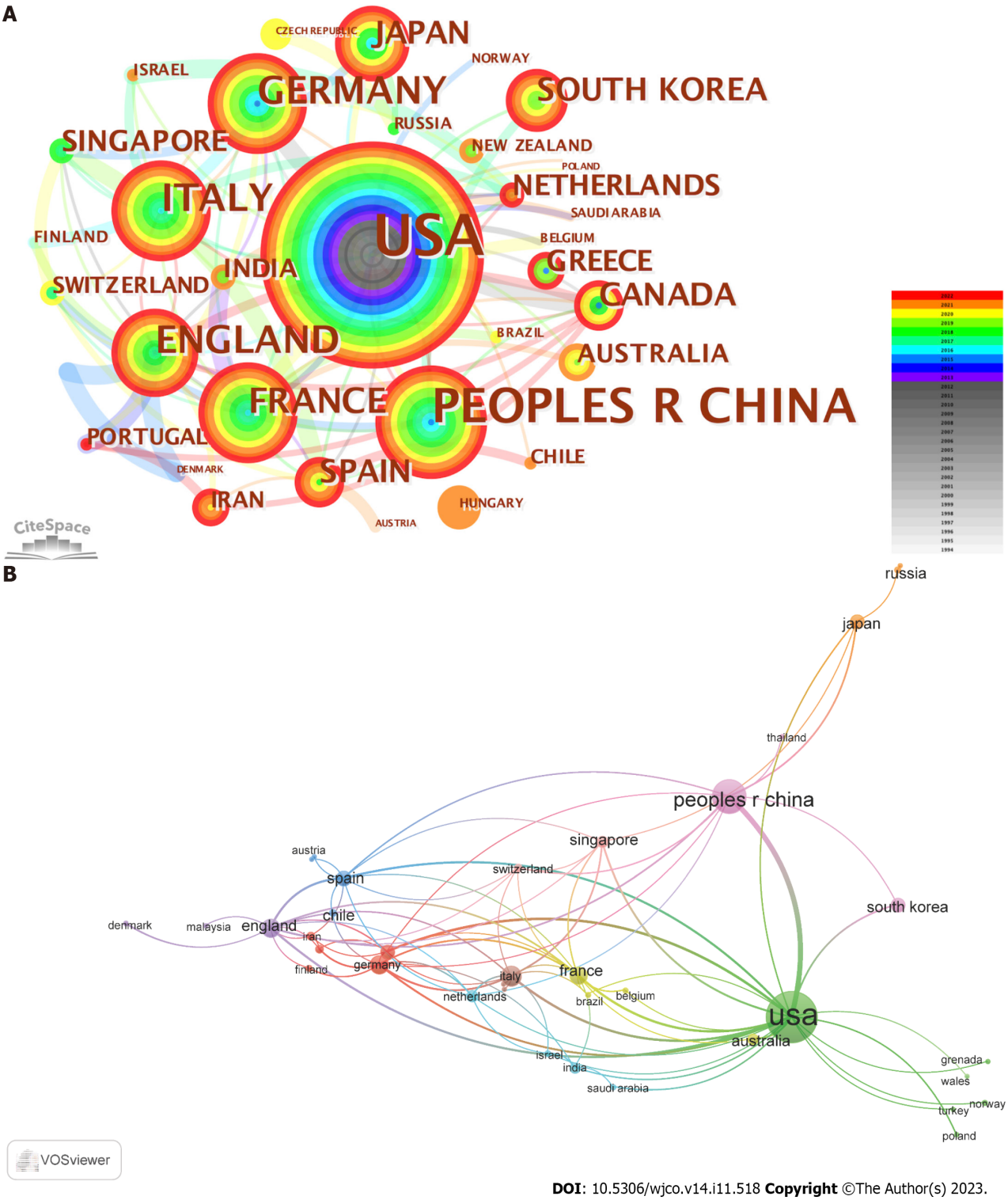
**Figure 2 Trends and categories of publications about mechanotransduction in cancer.** A: The annual number of publications and citations from 1994 to 2022; B: The research categories of publications related to mechanotransduction in cancer.

RNA, transforming growth factor-beta (TGF-β), transcription, and Wnt, and cluster 8 (brown) had 5 items: Adhesion, focal adhesion kinase (FAK), mechanical microenvironment, morphogenesis, and RhoA/Rho/ROCK. These grouped keywords were further marked according to the average publication year to reflect their yearly development. Figure 6B shows that the keywords in clusters 1 and 8 were mainly labeled in purple, suggesting that they appeared relatively early on in this field. In contrast, there were more keywords labeled in yellow in clusters 4 and 5, indicating that the topics in these two clusters gained more attention in recent years. Of note, some keywords exhibited a relatively late mean publication year and low mean frequency of occurrence, and so we suggest that these might be the next big topics to be investigated in this area. These include "plasma membrane [average appearing year (AAY) = 2021.00]", "autophagy (AAY = 2021.00)", "piezo1/2 (AAY = 2021.17)", and "heterogeneity (AAY = 2021.25)".

In addition to our analysis of co-occurring keywords, a burst keyword analysis (which identifies keywords that were frequently used over a certain period), was carried out with the CiteSpace software. We found that "mechanical force" was the most potent keyword; this appeared in 2006 with a burst strength of 3.99. In addition, the longest burst duration occurred for "signal transduction" (with a burst strength of 2.19); this started in 2004 and lasted for 9 years. Apart from these two keywords, "cancer diagnosis", "post-transcriptional modification" and "plasma membrane" were shown to have the highest more recent burst time (Figure 6C), indicating possible new research topics in mechanotransduction-associated cancer studies.

## DISCUSSION

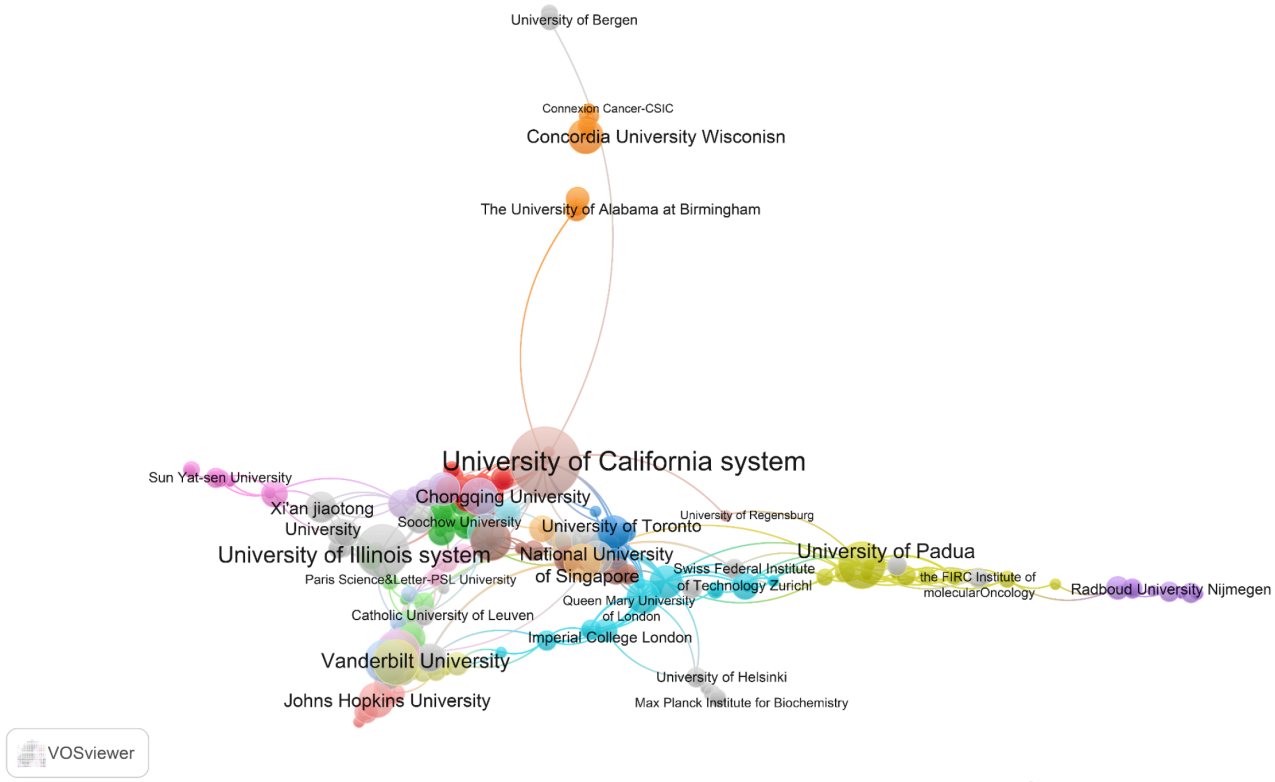
The concept of mechanotransduction developed gradually between the 1950s and 1980s as researchers studied how stretching forces influenced membrane depolarization in excitable cells such as nerves[26-28]. The fact that mechanotransduction might play a role in cancer was first suggested in 1994, and since then it has helped to explain many puzzles in the cancer research field[29]. However, compared to biochemical signal transduction, the role of mechanotransduction in cancer development was largely overlooked from 1994 to 2006. The field then gradually received more attention, and more than 40 papers were published between 2006 and 2016, probably due to the boom in research on the Hippo




**DOI**: 10.5306/wjco.v14.i11.518 

**Figure 3 Analysis of countries working on mechanotransduction in cancer.** A: CiteSpace was used to conduct the cooperation network between countries. The number of publications is represented by the node size and the different publication years are indicated by the different colors; B: VOSviewer was used to visualize the cooperation between country network. The number of publications is represented by the size of the node and the connection strength is indicated by the line thickness.

pathway[32]. Notably, there was then a slight reduction in the number of publications in 2017, followed by a substantial increase in 2018. Since then, this field has become far more popular among cancer researchers, such that in the last three years, > 80 papers were published per year. As the number of publications increased, so did the number of times that the publications were cited. Indeed, the average citation time reached 59.48 citations per paper, suggesting the high quality of research conducted in this field. Furthermore, Drs David Julius and Ardem Patapoutian were awarded the 2021 Nobel Prize in Physiology or medicine for their work on mechanosensitive ion channels[33]. Therefore, this might encourage more groups to conduct mechanotransduction-related cancer research.

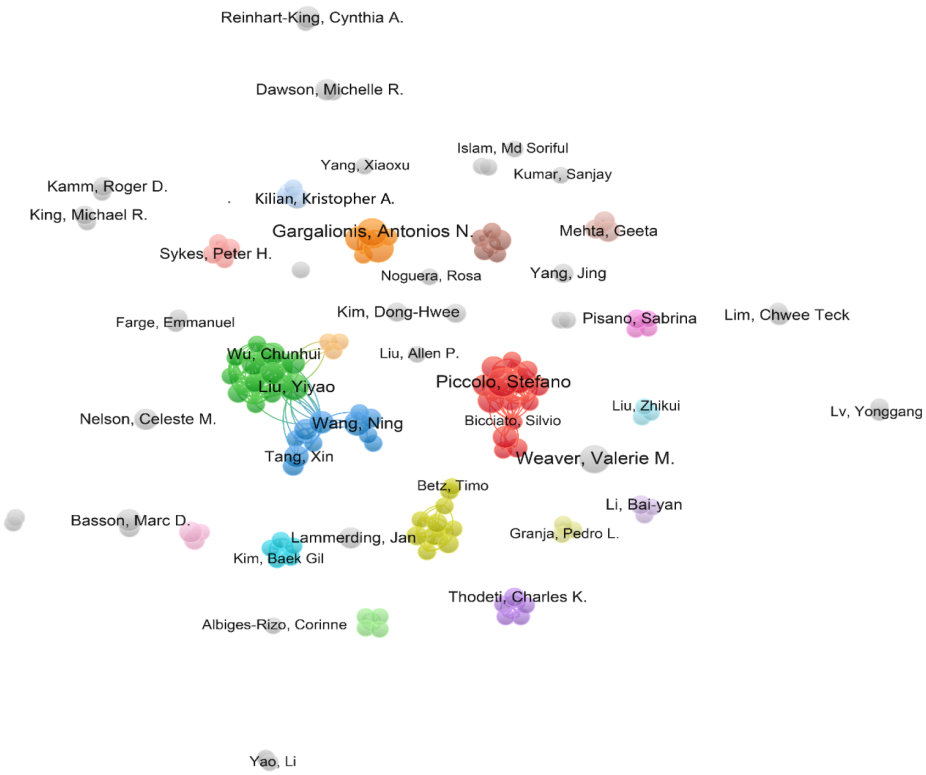



**DOI**: 10.5306/wjco.v14.i11.518 

**Figure 4 Network analysis of organizations.** The collaboration between organizations was analysed with VOSviewer.

By ranking the top 10 productive countries in this area, we found that all except China are advanced countries. The United States has established the lead position of global research on mechano-oncology after nearly 30 years of investigations. However, the number of publications from China indicates that they are in a catch-up position, although the average citation time for their papers is still relatively low. This might be due to the relatively low quality of the publications so far, even though the quantity is high. In addition, the low number of publications in the past from China and the more recent fast growth rate might inherently overly inflate the contribution of highly cited papers to the average citation time. To address this phenomenon, the Chinese government should provide more financial and political support for this research field and encourage original research. For Chinese scholars, discovering new research frontiers as early as possible and carrying out in-depth research is indispensable for improving their international influence and academic standing. It is also important to note that although Canada published 21 articles and is ranked only eighth according to the number of publications, it has the highest average citation rate. One reason for this might be that a review paper published by a Canadian oncology group (Chambers *et al*[30]), systematically describes the role of mechanical factors on the various physiological stages of cancer metastasis, and this attracts the highest citation frequency in this field.

We also found that cooperations existed between each country, with China and the United States having the most exchanges and collaborations. At the institutional level, we found that cooperations existed in developed countries such as the United States, France, and the United Kingdom. However, in other countries mutual partnerships tended to be insufficient, especially in undeveloped countries, including China. In addition, although several connections between different research groups were found on our authorship network map, these group cooperations largely remained domestic, lacking cross-national communications. Furthermore, three of the top productive and influential scientists in this field (*i.e.*, Valerie M. Weaver, Stefano Piccolo, and Antonios Gargalionis), have relatively few collaborations with other researchers. The study of mechanotransduction in cancer not only requires an in-depth exploration of molecular mechanisms but also necessitates a large number of clinical samples or populations for clinical validation or translation. At this point, inter-country or inter-institutional collaboration should be advocated, either by sharing clinical databases or by dividing the project into concrete tasks based on the respective expertise. With this approach, significant breakthroughs in this field might be achieved at the earliest time. In addition to collaboration between research groups, interdisciplinary collaborations are also essential for a research field to flourish. Mechanotransduction-related cancer research involves various different disciplines, including biology, physics, and medicine, and so interdisciplinary exchanges are beneficial for the diversity of research and to create new perspectives and questions. For example, *in vivo* mechanosensing is based on force-dependent protein deformation and reorganisation[34]. However, due to a lack of molecular resolution in cellular imaging techniques, the intracellular mechanisms are unknown. Recently, with the development of super-resolution microscopy and molecular force sensors, it is now possible to gain molecular insights into mechanosensing in living cells[35]. Moreover, the development of novel imaging techniques has helped to advance our knowledge of the molecular mechanisms involved in mechanotransduction[36].



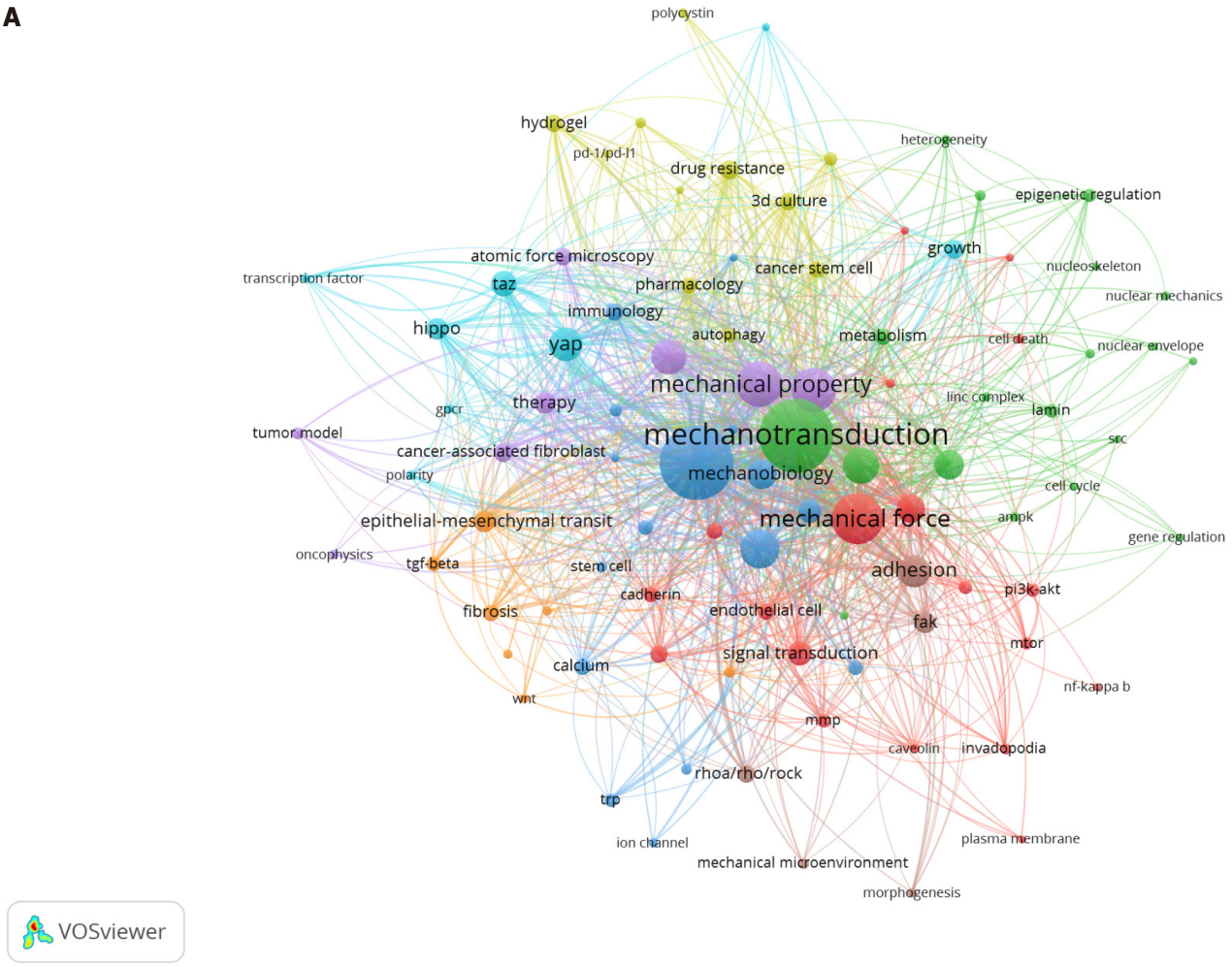

**DOI**: 10.5306/wjco.v14.i11.518 **Copyright** ©The Author(s) 2023.

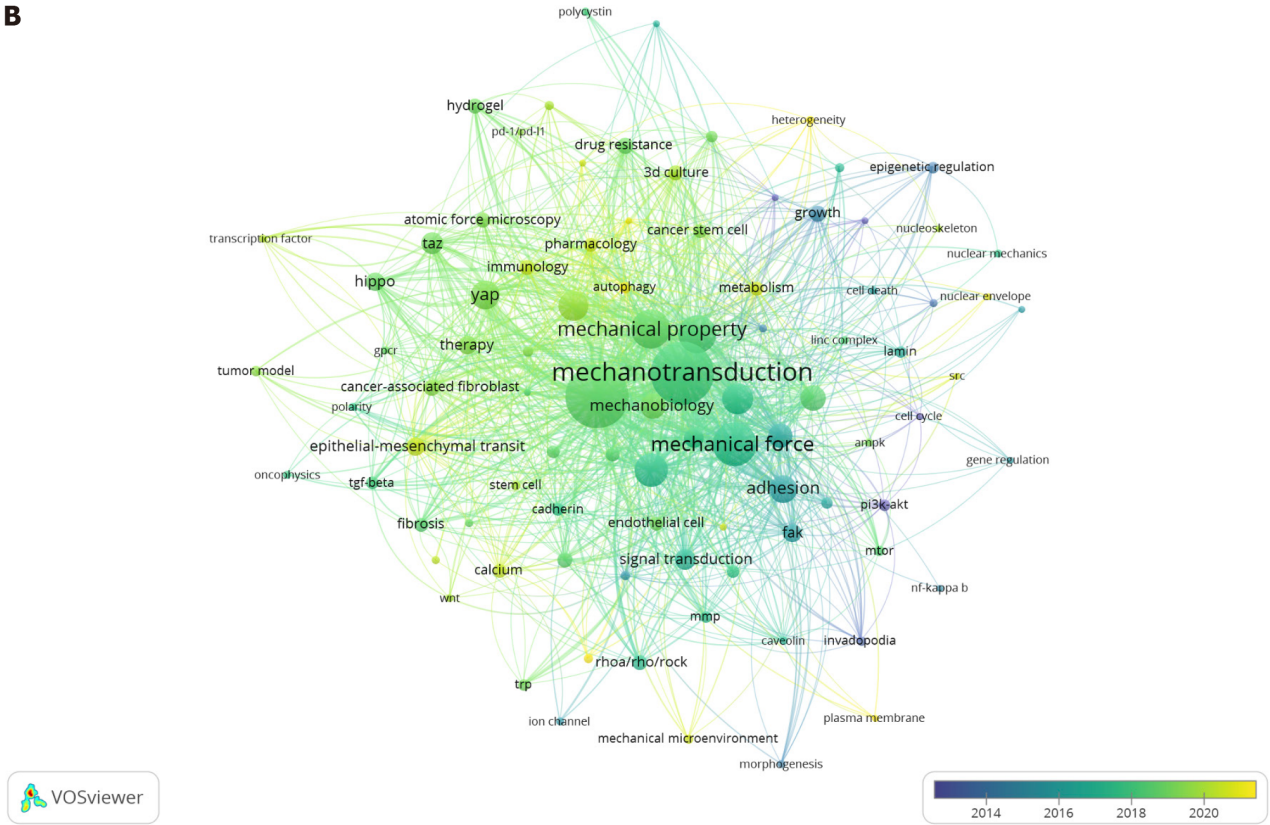
**Figure 5 Visualization of author cooperation.** VOSviewer was used to visualize the co-authorship network. The clusters are indicated by the different colors and the lines represent the various collaborations between authors.

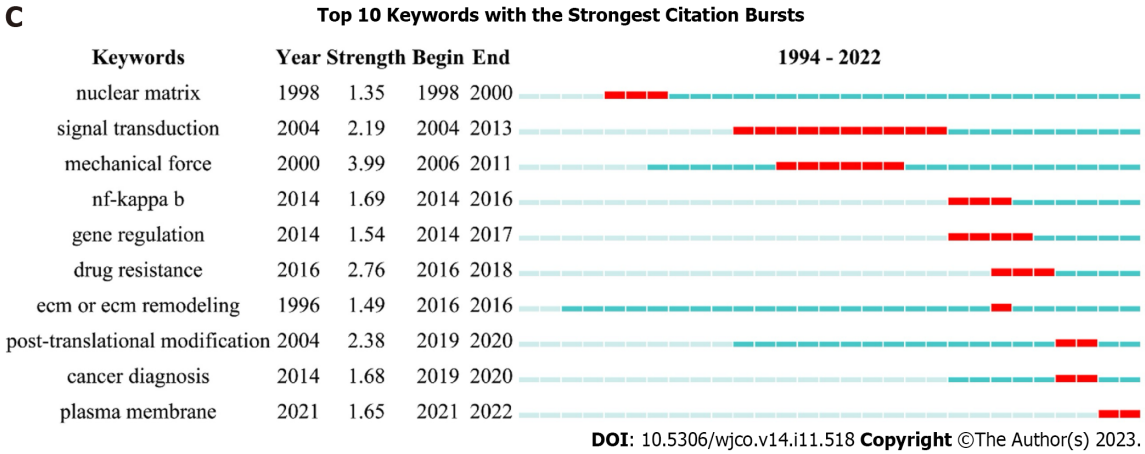
The 597 papers we found were published in almost 300 journals. This suggests that this field is widely recognized by publishers. Notably, some highly prestigious and influential journals, such as *Nature*, *Cell*, and *Cancer Cell*, have accepted relevant articles in recent years, which indicates that this research direction is significant and holds great potential for future investigations. We also found that there was a positive correlation between the average number of times that papers published in the top 10 most productive journals were cited, and the impact factor of the journal. This reflects the vibrancy and attractiveness of this research field. However, except for the *Proceedings of the National Academy of Science of the United States of America* and *Cancer Research*, we found that most of these journals such as *Cancers* and *International Journal of Molecular Sciences* are less highly qualified; therefore, more in-depth explorations are required in the future.

Eight clusters were enriched through a keywords co-occurrence analysis. Cluster 1, with 19 keywords, appeared to elucidate the mechanisms of tumor angiogenesis from the viewpoint of mechanotransduction. Indeed, by providing oxygen and nutrition, tumor angiogenesis, which is initiated by vascular endothelial cell activation, is one of the most fundamental factors for tumor growth and metastasis[37,38]. Hypoxia and growth factors plus their receptors (including vascular endothelial-derived growth factor/vascular endothelial-derived growth factor receptor and fibroblast growth factor/fibroblast growth factor receptor) are known to induce endothelial cell proliferation *via* various signal transduction pathways such as *via* hypoxia-inducible factor-1, PI3K/Akt/mTOR, MAPK/ERK, integrin, and NF-κB signaling[39,40]. In addition, MMPs facilitate endothelial cell invasion by degrading the surrounding ECM components[41,42]. As well as these biochemical pathways, the shear force of the blood flow and stiffness of the tumor tissues are also considered to be essential parameters that influence angiogenesis by regulating endothelial (tip and stalk) cell migration and vessel stabilization[43,44]. For example, it has been demonstrated that caveolin-1 and caveolae act as mechanosensors to respond to altered shear forces for endothelial cell stimulation[45,46]. The mechanical forces exerted on cells can be measured using traction force microscopy, and so this technique might help researchers discover more plasma membrane-localized mechanosensors during angiogenesis[47,48].

Clusters 2 and 3 emphasize the molecular mechanisms of mechanotransduction in cancer metastasis from the perspective of cytoskeletal and nucleoskeletal remodeling, and ion channels, respectively. During metastasis, cancer cells detach from the site of the primary tumor. They then migrate and invade the surrounding microenvironments, and after intravasation into vessels and escaping immune surveillance, they seed and colonize distant sites[49]. As essential initial steps during metastasis, the migration and invasion processes subject cancer cells to different mechanical forces. These are generated due to contact with the TME, and are characterized by increased solid stress, fluid pressure, and tissue stiffness when compared with their counterparts in normal tissue[17]. Although the rigid TME imposes compressive stress on migrating cancer cells, which can cause DNA damage, these mechanical forces can in turn activate the DNA repair system, which limits genotoxicity and maintains regular cellular activity[50]. Various ion channels, such as TRP, polycystin, and piezo1/2, are involved in this process as mechanosensors. For example, in the presence of membrane


A
polycystin
hydrogel
pd-1/pd-l1
heterogeneity
drug resistance
3d culture
epigenetic regulation
growth
atomic force microscopy
cancer stem cell
nucleoskeleton
transcription factor
taz
pharmacology
nuclear mechanics
immunology
hippo
autophagy
metabolism
cell death
nuclear envelope
yap
mechanical property
therapy
linc complex
gpcr
lamin
tumor model
mechanotransduction
src
cancer-associated fibroblast
polarity
mechanobiology
cell cycle
epithelial-mesenchymal transit
mechanical force
ampk
gene regulation
oncophysics
tgf-beta
stem cell
adhesion
pi3k-akt
cadherin
fibrosis
endothelial cell
fak
mtor
calcium
signal transduction
nf-kappa b
wnt
mmp
caveolin
invadopodia
rhoa/rho/rock
trp
ion channel
plasma membrane
mechanical microenvironment
morphogenesis
VOSviewer


B
polycystin
hydrogel
pd-1/pd-l1
heterogeneity
drug resistance
3d culture
epigenetic regulation
growth
atomic force microscopy
cancer stem cell
nucleoskeleton
transcription factor
taz
pharmacology
nuclear mechanics
immunology
hippo
autophagy
metabolism
cell death
nuclear envelope
yap
mechanical property
therapy
linc complex
gpcr
lamin
tumor model
mechanotransduction
src
cancer-associated fibroblast
polarity
mechanobiology
cell cycle
epithelial-mesenchymal transit
mechanical force
ampk
gene regulation
oncophysics
tgf-beta
stem cell
adhesion
pi3k-akt
cadherin
fibrosis
endothelial cell
fak
mtor
calcium
signal transduction
nf-kappa b
wnt
mmp
caveolin
invadopodia
rhoa/rho/rock
trp
ion channel
plasma membrane
mechanical microenvironment
morphogenesis
VOSviewer
2014
2016
2018
2020




**DOI**: 10.5306/wjco.v14.i11.518 

**Figure 6 Keyword co-occurrence and burst analysis.** A: VOSviewer was applied in our keyword co-occurrence analysis. The colors indicate different clusters and the node size indicates the number of publications; B: A keyword overlay was visualized with VOSviewer. The circle size reflects the number of publications and the average published year is shown by different colored circles; C: CiteSpace was used to conduct the keyword burst analysis. The red rectangles represent the duration of a keyword.

tension, TRP or piezo1/2 undergo conformational changes, which leads to their activation and an influx of extracellular $Ca^{2+}$[51-53]. Such alterations in the $Ca^{2+}$ influx often result in cytoskeleton rearrangements, which directly affect downstream signaling by changing the affinity of cytoskeletal protein binding and regulatory proteins[54]. Then, with the assistance of the linker of the nucleoskeleton and cytoskeleton complex, the mechanical forces are transferred to the nucleoskeleton, which distorts the nuclear envelope, impacts the epigenetic state and regulates gene expression[55].

Clusters 4 and 5 highlight the contributing factors that determine the mechanical properties during cancer progression and so these might be useful for the development of relevant pharmaceutical interventions and therapeutical applications. CSCs are the main culprits for the heterogeneity of cancer as they are responsible for cancer initiation, invasive front formation, and drug resistance. It is suggested that the mechanical forces induced by the dense ECM might activate the autophagy and Hippo pathways to promote CSC proliferation and stemness[56,57]. Thus, an alternative explanation for the generation of heterogenous CSC populations (except for their intrinsic differences) might be that the heterogenous TME exerts differential mechanical forces on these cells[58]. CAFs are a key component of the TME in most solid tumor tissues, and the secretomes that originate from these cells contribute to the formation of the ECM[59]. During cancer development, CAFs and cancer cells secrete proteolytic enzymes, cytokines, growth factors, and/or other ECM components, which result in high ECM deposition and increased stiffness[60]. The upregulation of programmed cell death ligand 1 under these stiffer ECM conditions enables cancer cells to evade the immune system; a characteristic that is positively correlated with increased malignancy[61]. As expected, these unique cancer ECM characteristics have already been applied to cancer diagnosis[62]. The three-way biochemical and mechanical crosstalk between CAFs, cancer cells, and the ECM facilitate the remodeling of the latter. The resulting altered mechanical properties of the ECM are favorable for cancer survival, proliferation, drug resistance, and metastasis[63]. Therefore, mechanistic investigations of mechanotransduction during oncogenesis are crucial for the development of relevant pharmaceutical interventions and therapeutical applications[64]. Due to the development of 3D cell culture systems and atomic force microscopy, the mechanical properties can now be mimicked and evaluated to reflect the TME and physical cell-matrix interactions *in vivo*. These methods could therefore be used to screen anti-cancer drugs and in mechanotransduction-related cancer research[65,66].

Cluster 6 shows how cancer cells can override contact inhibition from the viewpoint of mechanotransduction. In cell culture conditions, when the density of normal cells reaches a high enough level, the close physical contact between the cells leads to cell cycle arrest and the suppression of proliferation[67]. Several GPCRs can sense and transduce the higher pressure resulting from the increased cell contacts, to downstream signals. This leads to the cytoplasmic translocation of YAP and TAZ, which nullifies their transcriptional activation[68]. In addition to an inhibition of proliferation in cell dense regions, cell migration is also affected when two normal cells collide. Indeed, cell repolarisation is necessary to separate cells after a collision, and RACK1-dependent cytoskeletal reorganization at the migratory front is also crucial for this process[69,70]. However, these events do not occur during carcinogenesis, and so cancer outgrowth and metastasis is the result[71]. The hyperactivation of YAP and TAZ (caused by a loss of E-cadherin or spectrin), helps to explain the reduced contact inhibition that occurs in cancer from the proliferation perspective[72,73]. However, the reasons for the loss of contact inhibition from the cell migration viewpoint, remain elusive and require further investigation.

Cluster 7 describes the role of Wnt signaling in the mechanical force-driven EMT. The EMT is a process by which epithelial cells are transformed into mesenchymal cells, which then migrate and secrete more ECM components, and so it is crucial for cancer metastasis and drug resistance[74,75]. The activation of the WNT/β-catenin signaling pathway contributes to the transcription of EMT regulators such as *Snail* and *Slug*, both of which inhibit the expression of E-cadherin, and therefore reduce intercellular adhesion and increase cell motility[76]. In addition, TGF-β and some long noncoding RNAs respond to the increase in ECM stiffness, and in this way, they regulate WNT/β-catenin activity and participate in the EMT[18,77].

Finally, cluster 8 demonstrates the impact of the mechanical microenvironment on cell adhesion. The three main characteristics of tumor mechanical microenvironments are an increase in matrix stiffness, solid stress, and interstitial fluid pressure, and these are proposed to activate FAK, which drives focal adhesion formation and primes the RhoA/ROCK signaling cascade[78,79]. This signaling pathway is involved in regulating the organization of the actin cytoskeleton and, therefore, it enables cells to alter their shape and migrate from their primary sites[80].

After performing co-occurring and burst keyword analyses, we found that the keywords "plasma membrane", "autophagy", "piezo1/2", "heterogeneity", "cancer diagnosis", and "post-transcriptional modification" are likely to be the next topics of interest in this field. Interestingly, both keyword analysis methods indicated that the plasma membrane, a mechanosensing structure that transduces the mechanical stimulus to downstream biochemical signal transduction pathways, is a popular topic for future research. Contrary to the increase in substrate stiffness that occurs in cancerous tissues, the plasma membrane of cancer cells is softer than in normal cells, which means that they can migrate more easily [81]. Moreover, the softer plasma membrane along with the underlying cytoskeleton, are more likely to undergo alterations in configuration in response to external mechanical stimuli, and this affects the subsequent transmission of biochemical signals[82]. Since membrane tension is closely associated with cancer cell behavior, this characteristic has recently been used for the diagnosis and prognosis of a low-grade glioma *via* the establishment of a membrane tension-related gene signature[83]. In addition, many cell membrane-localized proteins such as ion channels and other mechano-sensitive proteins are reported to be highly expressed in cancers and act as mechanosensors, which respond to the rigid TME by changing their conformation[84,85]. Of note, the 2021 Nobel Prize was awarded for the discovery of piezo1/2 as mechanosensitive ion channel proteins, and this has initiated a burst of related studies, especially in the cancer research field[33]. Indeed, piezo proteins are closely associated with several cancers and so their potential as diagnostic and prognostic cancer biomarkers is indisputable. In addition, due to the contribution of mechanical force from the TME in the regulation of tumor heterogeneity, identifying the mechanical properties of areas surrounding a tumor and developing therapeutics to counter the mechanical forces with carcinogenic impact would be favorable for precise cancer diagnosis and treatment[86].

The main biochemical signals generated from the mechanical forces, are transduced in the form of phosphorylations. Therefore, identifying more post-transcriptional modification types under different mechanical stimuli might provide some novel perspectives for determining how extracellular cues influence intracellular activities[87,88]. For example, it is now accepted that autophagy is activated by mechanical stress and plays a role in tumorigenesis[89-91]. Therefore, further investigations to explore how the plasma membrane and its localized mechanosensors transduce mechanical forces through post-transcriptional modifications (and thereby participate in the regulation of cellular activity), will not only help to reveal the reasons behind tumor heterogeneity but will also benefit the diagnosis, treatment, and prognosis of cancer. For example, the increase in stiffness is a well-recognized feature of cancer mechanics that has been used previously for cancer diagnosis and prognosis[12]. The continued development and validation of mechanobiological biomarkers that reflect the mechanical properties of tissue microenvironments are likely to facilitate the clinical application of mechano-oncology. Moreover, the mechanosensitivity of cancer cells is suggested to promote malignant cell behaviors[92,93], and mechanical abnormalities are the main culprit that drives cancer chemoresistance *via* the activation of cellular drug efflux or DNA repair systems[94]. Therefore, deciphering the detailed signaling pathways such as autophagy and post-transcriptional modifications involved in mechanotransduction might allow the development of new drugs that can be used in combination with current cancer therapies. This would increase the likelihood of therapeutic success and minimize the chance of developing drug resistance, which is advantageous for the prognosis of cancer patients.

### *Limitations*

Here, we used a bibliometric approach to analyze the trends and important issues regarding mechanotransduction studies in cancer research. While this analysis provides a relatively complete and understandable picture of the state of research today, there are several inevitable limitations. First, while the WoSCC used in this study, is regarded as being a reliable database for bibliometric analysis, the use of additional database sources would provide a more comprehensive view of the situation. Second, some papers that are already included in the associated databases might be delayed being included in the WoSCC, leading to statistical bias and a loss of precision. Finally, analyzing and summarizing research trends based on keywords alone, might be subjective and therefore lack a depth of exploration when compared with traditional literature reviews.

## CONCLUSION

Our results show that mechanotransduction-related cancer research is an increasingly popular topic in the world today. The United States is in the leading position of global research on mechano-oncology after almost 30 years of investigations, and the University of California system (with the largest number of collaborators), is the most influential organization based on its publication and citation times. Research group cooperations exist but remain largely domestic, lacking cross-national communications. Our findings suggest that investigations exploring how the plasma membrane and its localized mechanosensors might transduce mechanical force through post-transcriptional modifications and thereby participate in the cellular activity regulations and cancer development, will be the next big topic in this field.

## ARTICLE HIGHLIGHTS

### *Research background*

Mechanical stimuli, generated by the contact between cells (both tumor and non-tumur) or with the non-cellular microenvironment, have been demonstrated to play a significant role in the development of cancer. Unlike biochemical transduction, which depends on small molecules, growth factors, and cytokines, mechanotransduction is a process whereby cells sense mechanical cues in their external environment and translate them into biochemical signals to impact their intracellular activities. Indeed, in recent investigations, the importance of mechanical stimulation in cancer development is described as being on par with biochemical factors. Drs David Julius and Ardem Patapoutian were awarded the 2021 Nobel Prize in Physiology or Medicine for their work on mechanosensitive ion channels, which has already acted as a new catalyst for the increasing numbers of researchers to conduct mechanotransduction-related cancer research. Bibliometrics is a useful quantitative method to comprehensively analyze publications in multiple aspects, including the authors, organizations, countries, journals, and keywords, to uncover collaboration conditions and emerging trends in specific research areas. Although increasing numbers of research papers are now starting to focus on the role of mechanotransduction in cancer, to date no bibliometric analysis has been conducted to quantify the situation.

### *Research motivation*

The deep understanding of mechanotransduction in cancer will not only help determine the reasons behind the tumor heterogeneity, but also facilitate the development of more versatile approaches to cancer diagnosis and therapy.

### *Research objectives*

To provides an objective evaluation of the dynamics and emerging trends of mechanotransduction-related cancer research.

### *Research methods*

We present the first bibliometric analysis of research conducted on mechanotransduction in cancer and reveal the current trends and hot topics in this field.

### *Research results*

This study showed that mechanotransduction-related cancer research remains a hot topic, with approximately 100 papers and 5000 citations generated per year in the past three years. Additionally, the United States is a well-established global leader of this field, and the University of California system is the most influential organization in this field. The keywords "plasma membrane", "autophagy", "piezo1/2", "heterogeneity", "cancer diagnosis", and "post-transcriptional modification" are likely to be the next topics of interest in this field.

### *Research conclusions*

Our results found that mechanotransduction-related cancer research is an increasingly popular topic in the world today. The United States is in the leading position of global research on mechano-oncology after almost 30 years of investigations, and the University of California system (with the largest number of collaborators), is the most influential organization based on its publication and citation times. Research group cooperations exist but remain largely domestic, lacking cross-national communications.

### *Research perspectives*

We predict that the next 'hot' topic in cancer research will be investigating how localized mechanosensors in the plasma membrane transduce mechanical forces *via* post-transcriptional modifications to participate in the regulation of cellular activity.

## FOOTNOTES

**Author contributions:** Wang DW conceptualized and designed this study; Wang DW, Zhang YZ, Li MZ, and Wang GX collected and analyzed the database; Wang DW, Zhang YZ, and Li MZ wrote the manuscript; and all authors have read and approve the final manuscript.

**Supported by** the National Natural Science Foundation of China, No. 32200557; Natural Science Foundation of Shandong Province, No. ZR2022QH271; and the Postdoctoral Innovative Projects of Shandong Province, No. SDCX-ZG-202203047.

**Conflict-of-interest statement:** All the authors report no relevant conflicts of interest for this article.

**PRISMA 2009 Checklist statement:** The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

**Country/Territory of origin:** China

**ORCID number:** Da-Wei Wang 0000-0001-7902-001X.

**S-Editor:** Wang JJ
**L-Editor:** A
**P-Editor:** Zhang XD

## REFERENCES

1 **Vaghari-Tabari M**, Ferns GA, Qujeq D, Andevari AN, Sabahi Z, Moein S. Signaling, metabolism, and cancer: An important relationship for therapeutic intervention. *J Cell Physiol* 2021; **236**: 5512-5532 [PMID: 33580511 DOI: 10.1002/jcp.30276]

2 **Global Burden of Disease 2019 Cancer Collaboration**, Kocarnik JM, Compton K, Dean FE, Fu W, Gaw BL, Harvey JD, Henrikson HJ, Lu D, Pennini A, Xu R, Ababneh E, Abbasi-Kangevari M, Abbastabar H, Abd-Elsalam SM, Abdoli A, Abedi A, Abidi H, Abolhassani H, Adedeji IA, Adnani QES, Advani SM, Afzal MS, Aghaali M, Ahinkorah BO, Ahmad S, Ahmad T, Ahmadi A, Ahmadi S, Ahmed Rashid T, Ahmed Salih Y, Akalu GT, Aklilu A, Akram T, Akunna CJ, Al Hamad H, Alahdab F, Al-Aly Z, Ali S, Alimohamadi Y, Alipour V, Aljunid SM, Alkhayyat M, Almasi-Hashiani A, Almasri NA, Al-Maweri SAA, Almustanyir S, Alonso N, Alvis-Guzman N, Amu H, Anbesu EW, Ancuceanu R, Ansari F, Ansari-Moghaddam A, Antwi MH, Anvari D, Anyasodor AE, Aqeel M, Arabloo J, Arab-Zozani M, Aremu O, Ariffin H, Aripov T, Arshad M, Artaman A, Arulappan J, Asemi Z, Asghari Jafarabadi M, Ashraf T, Atorkey P, Aujayeb A, Ausloos M, Awedew AF, Ayala Quintanilla BP, Ayenew T, Azab MA, Azadnajafabad S, Azari Jafari A, Azarian G, Azzam AY, Badiye AD, Bahadory S, Baig AA, Baker JL, Balakrishnan S, Banach M, Bärnighausen TW, Barone-Adesi F, Barra F, Barrow A, Behzadifar M, Belgaumi UI, Bezabhe WMM, Bezabih YM, Bhagat DS, Bhagavathula AS, Bhardwaj N, Bhardwaj P, Bhaskar S, Bhattacharyya K, Bhojaraja VS, Bibi S, Bijani A, Biondi A, Bisignano C, Bjørge T, Bleyer A, Blyuss O, Bolarinwa OA, Bolla SR, Braithwaite D, Brar A, Brenner H, Bustamante-Teixeira MT, Butt NS, Butt ZA, Caetano Dos Santos FL, Cao Y, Carreras G, Catalá-López F, Cembranel F, Cerin E, Cernigliaro A, Chakinala RC, Chattu SK, Chattu VK, Chaturvedi P, Chimed-Ochir O, Cho DY, Christopher DJ, Chu DT, Chung MT, Conde J, Cortés S, Cortesi PA, Costa VM, Cunha AR, Dadras O, Dagnew AB, Dahlawi SMA, Dai X, Dandona L, Dandona R, Darwesh AM, das Neves J, De la Hoz FP, Demis AB, Denova-Gutiérrez E, Dhamnetiya D, Dhimal ML, Dhimal M, Dianatinasab M, Diaz D, Djalalinia S, Do HP, Doaei S, Dorostkar F, Dos Santos Figueiredo FW, Driscoll TR, Ebrahimi H, Eftekharzadeh S, El Tantawi M, El-Abid H, Elbarazi I, Elhabashy HR, Elhadi M, El-Jaafary SI, Eshrati B, Eskandarieh S, Esmaeilzadeh F, Etemadi A, Ezzikouri S, Faisaluddin M, Faraon EJA, Fares J, Farzadfar F, Feroze AH, Ferrero S, Ferro Desideri L, Filip I, Fischer F, Fisher JL, Foroutan M, Fukumoto T, Gaal PA, Gad MM, Gadanya MA, Gallus S, Gaspar Fonseca M, Getachew Obsa A, Ghafourifard M, Ghashghaee A, Ghith N, Gholamalizadeh M, Gilani SA, Ginindza TG, Gizaw ATT, Glasbey JC, Golechha M, Goleij P, Gomez RS, Gopalani SV, Gorini G, Goudarzi H, Grosso G, Gubari MIM, Guerra MR, Guha A, Gunasekera DS, Gupta B, Gupta VB, Gupta VK, Gutiérrez RA, Hafezi-Nejad N, Haider MR, Haj-Mirzaian A, Halwani R, Hamadeh RR, Hameed S, Hamidi S, Hanif A, Haque S, Harlianto NI, Haro JM, Hasaballah AI, Hassanipour S, Hay RJ, Hay SI, Hayat K, Heidari G, Heidari M, Herrera-Serna BY, Herteliu C, Hezam K, Holla R, Hossain MM, Hossain MBH, Hosseini MS, Hosseini M, Hosseinzadeh M, Hostiuc M, Hostiuc S, Househ M, Hsairi M, Huang J, Hugo FN, Hussain R, Hussein NR, Hwang BF, Iavicoli I, Ibitoye SE, Ida F, Ikuta KS, Ilesanmi OS, Ilic IM, Ilic MD, Irham LM, Islam JY, Islam RM, Islam SMS, Ismail NE, Isola G, Iwagami M, Jacob L, Jain V, Jakovljevic MB, Javaheri T, Jayaram S, Jazayeri SB, Jha RP, Jonas JB, Joo T, Joseph N, Joukar F, Jürisson M, Kabir A, Kahrizi D, Kalankesh LR, Kalhor R, Kaliyadan F, Kalkonde Y, Kamath A, Kameran Al-Salihi N, Kandel H, Kapoor N, Karch A, Kasa AS, Katikireddi SV, Kauppila JH, Kavetskyy T, Kebede SA, Keshavarz P, Keykhaei M, Khader YS, Khalilov R, Khan G, Khan M, Khan MN, Khan MAB, Khang YH, Khater AM, Khayamzadeh M, Kim GR, Kim YJ, Kisa A, Kisa S, Kissimova-Skarbek K, Kopec JA, Koteeswaran R, Koul PA, Koulmane Laxminarayana SL, Koyanagi A, Kucuk Bicer B, Kugbey N, Kumar GA, Kumar N, Kurmi OP, Kutluk T, La Vecchia C, Lami FH, Landires I, Lauriola P, Lee SW, Lee SWH, Lee WC, Lee YH, Leigh J, Leong E, Li J, Li MC, Liu X, Loureiro JA, Lunevicius R, Magdy Abd El Razek M, Majeed A, Makki A, Male S, Malik AA, Mansournia MA, Martini S, Masoumi SZ, Mathur P, McKee M, Mehrotra R, Mendoza W, Menezes RG, Mengesha EW, Mesregah MK, Mestrovic T, Miao Jonasson J, Miazgowski B, Miazgowski T, Michalek IM, Miller TR, Mirzaei H, Mirzaei HR, Misra S, Mithra P, Moghadaszadeh M, Mohammad KA, Mohammad Y, Mohammadi M, Mohammadi SM, Mohammadian-Hafshejani A, Mohammed S, Moka N, Mokdad AH, Molokhia M, Monasta L, Moni MA, Moosavi MA, Moradi Y, Moraga P, Morgado-da-Costa J, Morrison SD, Mosapour A, Mubarik S, Mwanri L, Nagarajan AJ, Nagaraju SP, Nagata C, Naimzada MD, Nangia V, Naqvi AA, Narasimha Swamy S, Ndejjo R, Nduaguba SO, Negoi I, Negru SM, Neupane Kandel S, Nguyen CT, Nguyen HLT, Niazi RK, Nnaji CA, Noor NM, Nuñez-Samudio V, Nzoputam CI, Oancea B, Ochir C, Odukoya OO, Ogbo FA, Olagunju AT, Olakunde BO, Omar E, Omar Bali A, Omonisi AEE, Ong S, Onwujekwe OE, Orru H, Ortega-Altamirano DV, Otstavnov N, Otstavnov SS, Owolabi MO, P A M, Padubidri JR, Pakshir K, Pana A, Panagiotakos D, Panda-Jonas S, Pardhan S, Park EC, Park EK, Pashazadeh Kan F, Patel HK, Patel JR, Pati S, Pattanshetty SM, Paudel U, Pereira DM, Pereira RB, Perianayagam A, Pillay JD, Pirouzpanah S, Pishgar F, Podder I, Postma MJ, Pourjafar H, Prashant A, Preotescu L, Rabiee M, Rabiee N, Radfar A, Radhakrishnan RA, Radhakrishnan V, Rafiee A, Rahim F, Rahimzadeh S, Rahman M, Rahman MA, Rahmani AM, Rajai N, Rajesh A, Rakovac I, Ram P, Ramezanzadeh K, Ranabhat K, Ranasinghe P, Rao CR, Rao SJ, Rawassizadeh R, Razeghinia MS, Renzaho AMN, Rezaei N, Rezapour A, Roberts TJ, Rodriguez JAB, Rohloff P, Romoli M, Ronfani L, Roshandel G, Rwegerera GM, S M, Sabour S, Saddik B, Saeed U, Sahebkar A, Sahoo H, Salehi S, Salem MR, Salimzadeh H, Samaei M, Samy AM, Sanabria J, Sankararaman S, Santric-Milicevic MM, Sardiwalla Y, Sarveazad A, Sathian B, Sawhney M, Saylan M, Schneider IJC, Sekerija M, Seylani A, Shafaat O, Shaghaghi Z, Shaikh MA, Shamsoddin E, Shannawaz M, Sharma R, Sheikh A, Sheikhbahaei S, Shetty A, Shetty JK, Shetty PH, Shibuya K, Shirkoohi R, Shivakumar KM, Shivarov V, Siabani S, Siddappa Malleshappa SK, Silva DAS, Singh JA, Sintayehu Y, Skryabin VY, Skryabina AA, Soeberg MJ, Sofi-Mahmudi A, Sotoudeh H, Steiropoulos P, Straif K, Subedi R, Sufiyan MB, Sultan I, Sultana S, Sur D, Szerencsés V, Szócska M, Tabarés-Seisdedos R, Tabuchi T, Tadbiri H, Taherkhani A, Takahashi K, Talaat IM, Tan KK, Tat VY, Tedla BAA, Tefera YG, Tehrani-Banihashemi A, Temsah MH, Tesfay FH, Tessema GA, Thapar R, Thavamani A, Thoguluva Chandrasekar V, Thomas N, Tohidinik HR, Touvier M,

Tovani-Palone MR, Traini E, Tran BX, Tran KB, Tran MTN, Tripathy JP, Tusa BS, Ullah I, Ullah S, Umapathi KK, Unnikrishnan B, Upadhyay E, Vacante M, Vaezi M, Valadan Tahbaz S, Velazquez DZ, Veroux M, Violante FS, Vlassov V, Vo B, Volovici V, Vu GT, Waheed Y, Wamai RG, Ward P, Wen YF, Westerman R, Winkler AS, Yadav L, Yahyazadeh Jabbari SH, Yang L, Yaya S, Yazie TSY, Yeshaw Y, Yonemoto N, Younis MZ, Yousefi Z, Yu C, Yuce D, Yunusa I, Zadnik V, Zare F, Zastrozhin MS, Zastrozhina A, Zhang J, Zhong C, Zhou L, Zhu C, Ziapour A, Zimmermann IR, Fitzmaurice C, Murray CJL, Force LM. Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life Years for 29 Cancer Groups From 2010 to 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *JAMA Oncol* 2022; **8**: 420-444 [PMID: 34967848 DOI: 10.1001/jamaoncol.2021.6987]

3 **Mbemi A**, Khanna S, Njiki S, Yedjou CG, Tchounwou PB. Impact of Gene-Environment Interactions on Cancer Development. *Int J Environ Res Public Health* 2020; **17** [PMID: 33153024 DOI: 10.3390/ijerph17218089]

4 **de Visser KE**, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. *Cancer Cell* 2023; **41**: 374-403 [PMID: 36917948 DOI: 10.1016/j.ccell.2023.02.016]

5 **Broders-Bondon F**, Nguyen Ho-Bouldoires TH, Fernandez-Sanchez ME, Farge E. Mechanotransduction in tumor progression: The dark side of the force. *J Cell Biol* 2018; **217**: 1571-1587 [PMID: 29467174 DOI: 10.1083/jcb.201701039]

6 **Mierke CT**. The fundamental role of mechanical properties in the progression of cancer disease and inflammation. *Rep Prog Phys* 2014; **77**: 076602 [PMID: 25006689 DOI: 10.1088/0034-4885/77/7/076602]

7 **Butcher DT**, Alliston T, Weaver VM. A tense situation: forcing tumour progression. *Nat Rev Cancer* 2009; **9**: 108-122 [PMID: 19165226 DOI: 10.1038/nrc2544]

8 **Liu Q**, Luo Q, Ju Y, Song G. Role of the mechanical microenvironment in cancer development and progression. *Cancer Biol Med* 2020; **17**: 282-292 [PMID: 32587769 DOI: 10.20892/j.issn.2095-3941.2019.0437]

9 **Fang Y**, Wu D, Birukov KG. Mechanosensing and Mechanoregulation of Endothelial Cell Functions. *Compr Physiol* 2019; **9**: 873-904 [PMID: 30873580 DOI: 10.1002/cphy.c180020]

10 **Lin HH**, Ng KF, Chen TC, Tseng WY. Ligands and Beyond: Mechanosensitive Adhesion GPCRs. *Pharmaceuticals (Basel)* 2022; **15** [PMID: 35215331 DOI: 10.3390/ph15020219]

11 **Panciera T**, Azzolin L, Cordenonsi M, Piccolo S. Mechanobiology of YAP and TAZ in physiology and disease. *Nat Rev Mol Cell Biol* 2017; **18**: 758-770 [PMID: 28951564 DOI: 10.1038/nrm.2017.87]

12 **Winkler J**, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat Commun* 2020; **11**: 5120 [PMID: 33037194 DOI: 10.1038/s41467-020-18794-x]

13 **Abdollahiyan P**, Oroojalian F, Baradaran B, de la Guardia M, Mokhtarzadeh A. Advanced mechanotherapy: Biotensegrity for governing metastatic tumor cell fate *via* modulating the extracellular matrix. *J Control Release* 2021; **335**: 596-618 [PMID: 34097925 DOI: 10.1016/j.jconrel.2021.06.002]

14 **Chaqour B**, Goppelt-Struebe M. Mechanical regulation of the Cyr61/CCN1 and CTGF/CCN2 proteins. *FEBS J* 2006; **273**: 3639-3649 [PMID: 16856934 DOI: 10.1111/j.1742-4658.2006.05360.x]

15 **Hong W**, Guan KL. The YAP and TAZ transcription co-activators: key downstream effectors of the mammalian Hippo pathway. *Semin Cell Dev Biol* 2012; **23**: 785-793 [PMID: 22659496 DOI: 10.1016/j.semcdb.2012.05.004]

16 **Nasrollahi S**, Walter C, Loza AJ, Schimizzi GV, Longmore GD, Pathak A. Past matrix stiffness primes epithelial cells and regulates their future collective migration through a mechanical memory. *Biomaterials* 2017; **146**: 146-155 [PMID: 28918264 DOI: 10.1016/j.biomaterials.2017.09.012]

17 **Mohammadi H**, Sahai E. Mechanisms and impact of altered tumour mechanics. *Nat Cell Biol* 2018; **20**: 766-774 [PMID: 29950570 DOI: 10.1038/s41556-018-0131-2]

18 **Jiang Y**, Zhang H, Wang J, Liu Y, Luo T, Hua H. Targeting extracellular matrix stiffness and mechanotransducers to improve cancer therapy. *J Hematol Oncol* 2022; **15**: 34 [PMID: 35331296 DOI: 10.1186/s13045-022-01252-0]

19 **Er EE**, Tello-Lafoz M, Huse M. Mechanoregulation of Metastasis beyond the Matrix. *Cancer Res* 2022; **82**: 3409-3419 [PMID: 35877197 DOI: 10.1158/0008-5472.CAN-22-0419]

20 **Ni W**, Mo H, Liu Y, Xu Y, Qin C, Zhou Y, Li Y, Zhou A, Yao S, Zhou R, Huo J, Che L, Li J. Targeting cholesterol biosynthesis promotes anti-tumor immunity by inhibiting long noncoding RNA SNHG29-mediated YAP activation. *Mol Ther* 2021; **29**: 2995-3010 [PMID: 33992804 DOI: 10.1016/j.ymthe.2021.05.012]

21 **Donthu N**, Kumar S, Mukherjee D, Pandey N, Lim WM. How to conduct a bibliometric analysis: An overview and guidelines. *J Bus Res* 2021; **133**: 285-296 [DOI: 10.1016/j.jbusres.2021.04.070]

22 **Ugolini D**, Puntoni R, Perera FP, Schulte PA, Bonassi S. A bibliometric analysis of scientific production in cancer molecular epidemiology. *Carcinogenesis* 2007; **28**: 1774-1779 [PMID: 17548902 DOI: 10.1093/carcin/bgm129]

23 **Song M**, Lu Q, Xu M, Li Y, Zhao Y, Gong C, Ou X. The global research and emerging trends in autophagy of pancreatic cancer: A bibliometric and visualized study. *Front Oncol* 2022; **12**: 987026 [PMID: 36263211 DOI: 10.3389/fonc.2022.987026]

24 **Li M**, Zhang Y, Zhao J, Wang D. The global landscape and research trend of phase separation in cancer: a bibliometric analysis and visualization. *Front Oncol* 2023; **13**: 1170157 [PMID: 37333812 DOI: 10.3389/fonc.2023.1170157]

25 **Kong H**, Li M, Deng CM, Wu YJ, He ST, Mu DL. A comprehensive overview of clinical research on dexmedetomidine in the past 2 decades: A bibliometric analysis. *Front Pharmacol* 2023; **14**: 1043956 [PMID: 36865921 DOI: 10.3389/fphar.2023.1043956]

26 **KATZ B**. Depolarization of sensory terminals and the initiation of impulses in the muscle spindle. *J Physiol* 1950; **111**: 261-282 [PMID: 14795439 DOI: 10.1113/jphysiol.1950.sp004479]

27 **Guharay F**, Sachs F. Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J Physiol* 1984; **352**: 685-701 [PMID: 6086918 DOI: 10.1113/jphysiol.1984.sp015317]

28 **Erxleben C**. Stretch-activated current through single ion channels in the abdominal stretch receptor organ of the crayfish. *J Gen Physiol* 1989; **94**: 1071-1083 [PMID: 2482327 DOI: 10.1085/jgp.94.6.1071]

29 **Weiss L**. Cell adhesion molecules: a critical examination of their role in metastasis. *Invasion Metastasis***14**: 192-197 [PMID: 7657512]

30 **Chambers AF**, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* 2002; **2**: 563-572 [PMID: 12154349 DOI: 10.1038/nrc865]

31 **Aragona M**, Panciera T, Manfrin A, Giulitti S, Michielin F, Elvassore N, Dupont S, Piccolo S. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell* 2013; **154**: 1047-1059 [PMID: 23954413 DOI: 10.1016/j.cell.2013.07.042]

32 **Ma S**, Meng Z, Chen R, Guan KL. The Hippo Pathway: Biology and Pathophysiology. *Annu Rev Biochem* 2019; **88**: 577-604 [PMID: 30566373 DOI: 10.1146/annurev-biochem-013118-111829]

33 **Martinac B**. 2021 Nobel Prize for mechanosensory transduction. *Biophys Rev* 2022; **14**: 15-20 [PMID: 35340591 DOI: 10.1007/s12551-022-00935-9]

34 **Massou S**, Nunes Vicente F, Wetzel F, Mehidi A, Strehle D, Leduc C, Voituriez R, Rossier O, Nassoy P, Giannone G. Cell stretching is amplified by active actin remodelling to deform and recruit proteins in mechanosensitive structures. *Nat Cell Biol* 2020; **22**: 1011-1023 [PMID: 32719553 DOI: 10.1038/s41556-020-0548-2]

35 **Nunes Vicente F**, Chen T, Rossier O, Giannone G. Novel imaging methods and force probes for molecular mechanobiology of cytoskeleton and adhesion. *Trends Cell Biol* 2023; **33**: 204-220 [PMID: 36055943 DOI: 10.1016/j.tcb.2022.07.008]

36 **Lavrenyuk K**, Conway D, Dahl KN. Imaging methods in mechanosensing: a historical perspective and visions for the future. *Mol Biol Cell* 2021; **32**: 842-854 [PMID: 33788578 DOI: 10.1091/mbc.E20-10-0671]

37 **Folkman J**. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**: 1182-1186 [PMID: 4938153 DOI: 10.1056/nejm197111182852108]

38 **Folkman J**. Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 2002; **29**: 15-18 [PMID: 12516034 DOI: 10.1053/sonc.2002.37263]

39 **Liu G**, Chen T, Ding Z, Wang Y, Wei Y, Wei X. Inhibition of FGF-FGFR and VEGF-VEGFR signalling in cancer treatment. *Cell Prolif* 2021; **54**: e13009 [PMID: 33655556 DOI: 10.1111/cpr.13009]

40 **Pugh CW**, Ratcliffe PJ. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med* 2003; **9**: 677-684 [PMID: 12778166 DOI: 10.1038/nm0603-677]

41 **Alaseem A**, Alhazzani K, Dondapati P, Alobid S, Bishayee A, Rathinavelu A. Matrix Metalloproteinases: A challenging paradigm of cancer management. *Semin Cancer Biol* 2019; **56**: 100-115 [PMID: 29155240 DOI: 10.1016/j.semcancer.2017.11.008]

42 **Kessenbrock K**, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010; **141**: 52-67 [PMID: 20371345 DOI: 10.1016/j.cell.2010.03.015]

43 **Kai F**, Laklai H, Weaver VM. Force Matters: Biomechanical Regulation of Cell Invasion and Migration in Disease. *Trends Cell Biol* 2016; **26**: 486-497 [PMID: 27056543 DOI: 10.1016/j.tcb.2016.03.007]

44 **Kretschmer M**, Rüdiger D, Zahler S. Mechanical Aspects of Angiogenesis. *Cancers (Basel)* 2021; **13** [PMID: 34638470 DOI: 10.3390/cancers13194987]

45 **Shihata WA**, Michell DL, Andrews KL, Chin-Dusting JP. Caveolae: A Role in Endothelial Inflammation and Mechanotransduction? *Front Physiol* 2016; **7**: 628 [PMID: 28066261 DOI: 10.3389/fphys.2016.00628]

46 **Frank PG**, Lisanti MP. Role of caveolin-1 in the regulation of the vascular shear stress response. *J Clin Invest* 2006; **116**: 1222-1225 [PMID: 16670766 DOI: 10.1172/jci28509]

47 **Boldock L**, Wittkowske C, Perrault CM. Microfluidic traction force microscopy to study mechanotransduction in angiogenesis. *Microcirculation* 2017; **24** [PMID: 28164414 DOI: 10.1111/micc.12361]

48 **Hur SS**, Jeong JH, Ban MJ, Park JH, Yoon JK, Hwang Y. Traction force microscopy for understanding cellular mechanotransduction. *BMB Rep* 2020; **53**: 74-81 [PMID: 31964473 DOI: 10.5483/BMBRep.2020.53.2.308]

49 **Fares J**, Fares MY, Khachfe HH, Salhab HA, Fares Y. Molecular principles of metastasis: a hallmark of cancer revisited. *Signal Transduct Target Ther* 2020; **5**: 28 [PMID: 32296047 DOI: 10.1038/s41392-020-0134-x]

50 **Montagner M**, Dupont S. Mechanical Forces as Determinants of Disseminated Metastatic Cell Fate. *Cells* 2020; **9** [PMID: 31963820 DOI: 10.3390/cells9010250]

51 **Pethő Z**, Najder K, Bulk E, Schwab A. Mechanosensitive ion channels push cancer progression. *Cell Calcium* 2019; **80**: 79-90 [PMID: 30991298 DOI: 10.1016/j.ceca.2019.03.007]

52 **Pedersen SF**, Nilius B. Transient receptor potential channels in mechanosensing and cell volume regulation. *Methods Enzymol* 2007; **428**: 183-207 [PMID: 17875418 DOI: 10.1016/s0076-6879(07)28010-3]

53 **Jiang Y**, Yang X, Jiang J, Xiao B. Structural Designs and Mechanogating Mechanisms of the Mechanosensitive Piezo Channels. *Trends Biochem Sci* 2021; **46**: 472-488 [PMID: 33610426 DOI: 10.1016/j.tibs.2021.01.008]

54 **Li X**, Wang J. Mechanical tumor microenvironment and transduction: cytoskeleton mediates cancer cell invasion and metastasis. *Int J Biol Sci* 2020; **16**: 2014-2028 [PMID: 32549750 DOI: 10.7150/ijbs.44943]

55 **Janota CS**, Calero-Cuenca FJ, Gomes ER. The role of the cell nucleus in mechanotransduction. *Curr Opin Cell Biol* 2020; **63**: 204-211 [PMID: 32361559 DOI: 10.1016/j.ceb.2020.03.001]

56 **Li Y**, Randriantsilefisoa R, Chen J, Cuellar-Camacho JL, Liang W, Li W. Matrix Stiffness Regulates Chemosensitivity, Stemness Characteristics, and Autophagy in Breast Cancer Cells. *ACS Appl Bio Mater* 2020; **3**: 4474-4485 [PMID: 35025446 DOI: 10.1021/acsabm.0c00448]

57 **Li YR**, Fang Y, Lyu Z, Zhu Y, Yang L. Exploring the dynamic interplay between cancer stem cells and the tumor microenvironment: implications for novel therapeutic strategies. *J Transl Med* 2023; **21**: 686 [PMID: 37784157 DOI: 10.1186/s12967-023-04575-9]

58 **Prasetyanti PR**, Medema JP. Intra-tumor heterogeneity from a cancer stem cell perspective. *Mol Cancer* 2017; **16**: 41 [PMID: 28209166 DOI: 10.1186/s12943-017-0600-4]

59 **Wu F**, Yang J, Liu J, Wang Y, Mu J, Zeng Q, Deng S, Zhou H. Signaling pathways in cancer-associated fibroblasts and targeted therapy for cancer. *Signal Transduct Target Ther* 2021; **6**: 218 [PMID: 34108441 DOI: 10.1038/s41392-021-00641-0]

60 **Najafi M**, Farhood B, Mortezaee K. Extracellular matrix (ECM) stiffness and degradation as cancer drivers. *J Cell Biochem* 2019; **120**: 2782-2790 [PMID: 30321449 DOI: 10.1002/jcb.27681]

61 **Miyazawa A**, Ito S, Asano S, Tanaka I, Sato M, Kondo M, Hasegawa Y. Regulation of PD-L1 expression by matrix stiffness in lung cancer cells. *Biochem Biophys Res Commun* 2018; **495**: 2344-2349 [PMID: 29274784 DOI: 10.1016/j.bbrc.2017.12.115]

62 **Petersen EV**, Chudakova DA, Skorova EY, Anikin V, Reshetov IV, Mynbaev OA. The Extracellular Matrix-Derived Biomarkers for Diagnosis, Prognosis, and Personalized Therapy of Malignant Tumors. *Front Oncol* 2020; **10**: 575569 [PMID: 33425730 DOI: 10.3389/fonc.2020.575569]

63 **Jang I**, Beningo KA. Integrins, CAFs and Mechanical Forces in the Progression of Cancer. *Cancers (Basel)* 2019; **11** [PMID: 31137693 DOI: 10.3390/cancers11050721]

64 **Gargalionis AN**, Basdra EK, Papavassiliou AG. Tumor mechanosensing and its therapeutic potential. *J Cell Biochem* 2018; **119**: 4304-4308 [PMID: 29479734 DOI: 10.1002/jcb.26786]

65 **Trache A**, Xie L, Huang H, Glinsky VV, Meininger GA. Applications of Atomic Force Microscopy for Adhesion Force Measurements in Mechanotransduction. *Methods Mol Biol* 2018; **1814**: 515-528 [PMID: 29956252 DOI: 10.1007/978-1-4939-8591-3_30]

66 **Zonderland J**, Moroni L. Steering cell behavior through mechanobiology in 3D: A regenerative medicine perspective. *Biomaterials* 2021; **268**: 120572 [PMID: 33285439 DOI: 10.1016/j.biomaterials.2020.120572]

67 **McClatchey AI**, Yap AS. Contact inhibition (of proliferation) redux. *Curr Opin Cell Biol* 2012; **24**: 685-694 [PMID: 22835462 DOI: 10.1016/j.ceb.2012.06.009]

68 **Luo J**, Yu FX. GPCR-Hippo Signaling in Cancer. *Cells* 2019; **8** [PMID: 31072060 DOI: 10.3390/cells8050426]

69 **Duff D**, Long A. Roles for RACK1 in cancer cell migration and invasion. *Cell Signal* 2017; **35**: 250-255 [PMID: 28336233 DOI: 10.1016/j.cellsig.2017.03.005]

70 **Roycroft A**, Mayor R. Molecular basis of contact inhibition of locomotion. *Cell Mol Life Sci* 2016; **73**: 1119-1130 [PMID: 26585026 DOI: 10.1007/s00018-015-2090-0]

71 **Mayor R**, Carmona-Fontaine C. Keeping in touch with contact inhibition of locomotion. *Trends Cell Biol* 2010; **20**: 319-328 [PMID: 20399659 DOI: 10.1016/j.tcb.2010.03.005]

72 **Kim NG**, Koh E, Chen X, Gumbiner BM. E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components. *Proc Natl Acad Sci U S A* 2011; **108**: 11930-11935 [PMID: 21730131 DOI: 10.1073/pnas.1103345108]

73 **Fletcher GC**, Elbediwy A, Khanal I, Ribeiro PS, Tapon N, Thompson BJ. The Spectrin cytoskeleton regulates the Hippo signalling pathway. *EMBO J* 2015; **34**: 940-954 [PMID: 25712476 DOI: 10.15252/embj.201489642]

74 **Pastushenko I**, Blanpain C. EMT Transition States during Tumor Progression and Metastasis. *Trends Cell Biol* 2019; **29**: 212-226 [PMID: 30594349 DOI: 10.1016/j.tcb.2018.12.001]

75 **Bakir B**, Chiarella AM, Pitarresi JR, Rustgi AK. EMT, MET, Plasticity, and Tumor Metastasis. *Trends Cell Biol* 2020; **30**: 764-776 [PMID: 32800658 DOI: 10.1016/j.tcb.2020.07.003]

76 **Kalluri R**, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009; **119**: 1420-1428 [PMID: 19487818 DOI: 10.1172/JCI39104]

77 **Xu X**, Zhang Y, Wang X, Li S, Tang L. Substrate Stiffness Drives Epithelial to Mesenchymal Transition and Proliferation through the NEAT1-Wnt/β-Catenin Pathway in Liver Cancer. *Int J Mol Sci* 2021; **22** [PMID: 34769497 DOI: 10.3390/ijms222112066]

78 **Zebda N**, Dubrovskyi O, Birukov KG. Focal adhesion kinase regulation of mechanotransduction and its impact on endothelial cell functions. *Microvasc Res* 2012; **83**: 71-81 [PMID: 21741394 DOI: 10.1016/j.mvr.2011.06.007]

79 **Chuang HH**, Zhen YY, Tsai YC, Chuang CH, Hsiao M, Huang MS, Yang CJ. FAK in Cancer: From Mechanisms to Therapeutic Strategies. *Int J Mol Sci* 2022; **23** [PMID: 35163650 DOI: 10.3390/ijms23031726]

80 **Crosas-Molist E**, Samain R, Kohlhammer L, Orgaz JL, George SL, Maiques O, Barcelo J, Sanz-Moreno V. Rho GTPase signaling in cancer progression and dissemination. *Physiol Rev* 2022; **102**: 455-510 [PMID: 34541899 DOI: 10.1152/physrev.00045.2020]

81 **Nguyen HL**, Man VH, Li MS, Derreumaux P, Wang J, Nguyen PH. Elastic moduli of normal and cancer cell membranes revealed by molecular dynamics simulations. *Phys Chem Chem Phys* 2022; **24**: 6225-6237 [PMID: 35229839 DOI: 10.1039/d1cp04836h]

82 **Uray IP**, Uray K. Mechanotransduction at the Plasma Membrane-Cytoskeleton Interface. *Int J Mol Sci* 2021; **22** [PMID: 34768998 DOI: 10.3390/ijms222111566]

83 **Li J**, Lv F, Jin T. Structuring and validating a prognostic model for low-grade gliomas based on the genes for plasma membrane tension. *Front Neurol* 2022; **13**: 1024869 [PMID: 36408514 DOI: 10.3389/fneur.2022.1024869]

84 **Haining AW**, Lieberthal TJ, Del Río Hernández A. Talin: a mechanosensitive molecule in health and disease. *FASEB J* 2016; **30**: 2073-2085 [PMID: 27252130 DOI: 10.1096/fj.201500080R]

85 **Yu JL**, Liao HY. Piezo-type mechanosensitive ion channel component 1 (Piezo1) in human cancer. *Biomed Pharmacother* 2021; **140**: 111692 [PMID: 34004511 DOI: 10.1016/j.biopha.2021.111692]

86 **Bejarano L**, Jordão MJC, Joyce JA. Therapeutic Targeting of the Tumor Microenvironment. *Cancer Discov* 2021; **11**: 933-959 [PMID: 33811125 DOI: 10.1158/2159-8290.CD-20-1808]

87 **Buwa N**, Mazumdar D, Balasubramanian N. Caveolin1 Tyrosine-14 Phosphorylation: Role in Cellular Responsiveness to Mechanical Cues. *J Membr Biol* 2020; **253**: 509-534 [PMID: 33089394 DOI: 10.1007/s00232-020-00143-0]

88 **Iqbal J**, Zaidi M. Molecular regulation of mechanotransduction. *Biochem Biophys Res Commun* 2005; **328**: 751-755 [PMID: 15694410 DOI: 10.1016/j.bbrc.2004.12.087]

89 **Hernández-Cáceres MP**, Munoz L, Pradenas JM, Pena F, Lagos P, Aceiton P, Owen GI, Morselli E, Criollo A, Ravasio A, Bertocchi C. Mechanobiology of Autophagy: The Unexplored Side of Cancer. *Front Oncol* 2021; **11**: 632956 [PMID: 33718218 DOI: 10.3389/fonc.2021.632956]

90 **Ravasio A**, Morselli E, Bertocchi C. Mechanoautophagy: Synergies Between Autophagy and Cell Mechanotransduction at Adhesive Complexes. *Front Cell Dev Biol* 2022; **10**: 917662 [PMID: 35721483 DOI: 10.3389/fcell.2022.917662]

91 **Levy JMM**, Towers CG, Thorburn A. Targeting autophagy in cancer. *Nat Rev Cancer* 2017; **17**: 528-542 [PMID: 28751651 DOI: 10.1038/nrc.2017.53]

92 **Abidine Y**, Constantinescu A, Laurent VM, Sundar Rajan V, Michel R, Laplaud V, Duperray A, Verdier C. Mechanosensitivity of Cancer Cells in Contact with Soft Substrates Using AFM. *Biophys J* 2018; **114**: 1165-1175 [PMID: 29539402 DOI: 10.1016/j.bpj.2018.01.005]

93 **Chen X**, Momin A, Wanggou S, Wang X, Min HK, Dou W, Gong Z, Chan J, Dong W, Fan JJ, Xiong Y, Talipova K, Zhao H, Chen YX, Veerasammy K, Fekete A, Kumar SA, Liu H, Yang Q, Son JE, Dou Z, Hu M, Pardis P, Juraschka K, Donovan LK, Zhang J, Ramaswamy V, Selvadurai HJ, Dirks PB, Taylor MD, Wang LY, Hui CC, Abzalimov R, He Y, Sun Y, Li X, Huang X. Mechanosensitive brain tumor cells construct blood-tumor barrier to mask chemosensitivity. *Neuron* 2023; **111**: 30-48.e14 [PMID: 36323321 DOI: 10.1016/j.neuron.2022.10.007]

94 **Kalli M**, Poskus MD, Stylianopoulos T, Zervantonakis IK. Beyond matrix stiffness: targeting force-induced cancer drug resistance. *Trends Cancer* 2023; **9**: 937-954 [PMID: 37558577 DOI: 10.1016/j.trecan.2023.07.006]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

*World J Clin Oncol* 2023 November 24; 14(11): 535-543

DOI: 10.5306/wjco.v14.i11.535

ISSN 2218-4333 (online)

CASE REPORT

# Autoimmune diabetes from pembrolizumab: A case report and review of literature

Hardikkumar Bhanderi, Farhan Khalid, Zubair Hassan Bodla, Tayyeb Muhammad, Doantrang Du, Trishala Meghal

**Specialty type:** Oncology

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

**P-Reviewer:** Wu QN, China; Zhang H, China

**Received:** July 13, 2023
**Peer-review started:** July 13, 2023
**First decision:** September 26, 2023
**Revised:** October 13, 2023
**Accepted:** October 30, 2023
**Article in press:** October 30, 2023
**Published online:** November 24, 2023

**Hardikkumar Bhanderi, Farhan Khalid, Tayyeb Muhammad, Doantrang Du,** Department of Internal Medicine, Monmouth Medical Center, Long branch, NJ 07740, United States

**Zubair Hassan Bodla,** Department of Internal Medicine, University of Central Florida College of Medicine, Gainesville, FL 32303, United States

**Trishala Meghal,** Department of Hematology-Oncology, Monmouth Medical Center, Long Branch, NJ 07740, United States

**Corresponding author:** Hardikkumar Bhanderi, MD, Staff Physician, Department of Internal Medicine, Monmouth Medical Center, 300 2nd Avenue, Long Branch, NJ 07740, United States. dr.hardikkumar.bhanderi@gmail.com

## Abstract

*BACKGROUND*

Immunotherapy, specifically the use of checkpoint inhibitors such as pembrolizumab, has become an important tool in personalized cancer therapy. These inhibitors target proteins on T-cells that regulate the immune response against tumor cells. Pembrolizumab, which targets the programmed cell death 1 receptor on T-cells, has been approved for the treatment of metastatic melanoma and non-small cell lung cancer. However, it can also lead to immune-related side effects, including pneumonitis, colitis, thyroid abnormalities, and rare cases of type 1 diabetes.

*CASE SUMMARY*

The case presented involves an adult patient in 30s with breast cancer who developed hyperglycemia after receiving pembrolizumab treatment. The patient was diagnosed with diabetic ketoacidosis and further investigations were performed to evaluate for new-onset type 1 diabetes. The patient had a history of hypothyroidism and a family history of breast cancer. Treatment for diabetic ketoacidosis was initiated, and the patient was discharged for close follow-up with an endocrinologist.

*CONCLUSION*

This literature review highlights the occurrence of diabetic ketoacidosis and new-onset type 1 diabetes in patients receiving pembrolizumab treatment for different types of cancer. Overall, the article emphasizes the therapeutic benefits of immunotherapy in cancer treatment, particularly pembrolizumab, while also

highlighting the potential side effect of immune-related diabetes that can occur in a small percentage of patients. Here we present a case where pembrolizumab lead to development of diabetes after a few cycles highlighting one of the rare yet a serious toxicity of the drug.

**Key Words:** Pembrolizumab; Breast cancer; Autoimmune diabetes; Keytruda; Immunotherapy; Case report



**Core Tip:** Our review highlights an important and rare adverse effect of Pembrolizumab. We have also reviewed the number of cycles patients were treated with Keytruda before the onset of diabetes. Clinicians should be watchful for the signs and symptoms. Early discontinuation of immunotherapy is needed to prevent significant morbidity and mortality.

**Citation:** Bhanderi H, Khalid F, Bodla ZH, Muhammad T, Du D, Meghal T. Autoimmune diabetes from pembrolizumab: A case report and review of literature. *World J Clin Oncol* 2023; 14(11): 535-543
**URL:** https://www.wjgnet.com/2218-4333/full/v14/i11/535.htm
**DOI:** https://dx.doi.org/10.5306/wjco.v14.i11.535

## INTRODUCTION

Immunotherapy has become an essential tool in the treatment of cancers and represents therapeutic advancement in the individualized cancer therapy[1]. The role of immunotherapy is based on the ability to recognize abnormal tissue and enhance body's immune system against tumor cells. Immune system has both stimulators and inhibitors for the immune response generation in order to maintain balance and avoid auto-immune response to self antigens by means of positive selection of T cells. But sometimes this positive selection leads to lack of required immune response against tumor cells, which leads to tumor growth[2]. There are multiple check-points in cell production have been identified like T cell immunoglobulin and mucin-domain containing-3, T cell immunoglobulin and ITIM domain, lymphocyte activation gene-3, indoleamine 2, 3-dioxygenase 1, and V-domain immunoglobulin suppressor of T cell activation, but to date only United States Food and Drug Administration (FDA) approved check-point inhibitors are those which targets cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), or programmed cell death protein-ligand 1 (PD-L1)[3]. The mechanism behind is the inhibition of check point inhibitors namely CTLA-4, PD-1, or PD-L1 which results in the increased anti-tumor immune response. These check point inhibitors are expressed on T-cells and their activation leads to the decreased T-cell proliferation from inhibition of T-cell receptor mediated signaling, reduced cytokines secretion limiting inflammatory response and autoimmunity[4]. The immune check point inhibitors are the monoclonal antibodies directed against the above mentioned ligands which results in the immune activation against the tumor cells[5]. Pembrolizumab is a monoclonal antibody designed against check point inhibitor PD-1 receptor on surface of T-cells resulting in the proliferation of T-cells and enhanced intrinsic immune mediated anticancer activity[6]. PD-1 receptor is a cell surface protein expressed on activated T cells which on binding with the ligands PD-L1 and PD-L2 leads to the inhibition of kinase signaling pathways causing suppression of T-cell[7]. Pembrolizumab was originally approved by FDA for metastatic melanoma in 2014 and for non-small cell lung cancer in 2014[1]. Since then it has been widely used in the treatment of different cancers especially those with resistance to first line therapies. Excessive immune activation has been a frequent and serious side effect of the immune therapies. Most common adverse effects reported from the clinical trials are pneumonitis, colitis, thyroid abnormalities, liver and kidney issues[8]. Type 1 diabetes was only reported in 0.1% of the patients in the clinical trials making this rare but significant side effect of the treatment[1]. Here we present a case of a young female who presented with hyperglycemia after getting treatment with the pembrolizumab for the breast cancer.

## CASE PRESENTATION

### *Chief complaints*

Nausea, Vomiting and Hyperglycemia at outpatient chemotherapy infusion center.

### *History of present illness*

The patient presented to the emergency department for the evaluation of hyperglycemia, which was found at the infusion center during 4$^{th}$ cycle. The patient complained of nausea, vomiting which was non-bilious and non-bloody associated with dizziness. The patient denied any fever, shortness of breath, chest pain, abdominal pain or loss of consciousness, recent weight loss, travel history, constipation or diarrhea.

### *History of past illness*

Hypothyroidism and triple negative left invasive mammary breast carcinoma with Ki 67%-90% diagnosed an year ago which was at anatomical stage 2A/Clinical prognostic stage 2B status post chemotherapy with carboplatin and Paclitaxel along with 3 cycles of Pembrolizumab.

### *Personal and family history*

The patient social history was significant for 2-3 cigarettes a day before getting diagnosed with breast cancer and occasional alcohol consumption and marijuana consumption. The family history was significant for breast cancer in mother and sister.

### *Physical examination*

Physical examination was unremarkable.

### *Laboratory examinations*

The initial blood work up revealed Hemoglobin level of 12.8 g/dL, white cell count of 4.7 K/CMM, Hematocrits of 37.2% and platelet count of 332 K/CMM. Complete metabolic panel was significant for sodium level of 133 mEq/L, Bicarbonate level of < 10 mEq/L with anion gap of > 19 mEq/L, blood glucose level of 343 mg/dL. Liver and Kidney functions were benign. Beta hydroxy butyrate level was found to be elevated > 46.8 mg/dL. Urinalysis was positive for glucose and ketones. Amylase and lipase level were within normal limits. Arterial blood gas analysis showed pH of 7.13 with pCO2 of 23 mmHg, pO2 of 65 mmHg, and bicarbonate level of 8 mmol/L. HbA1c level was found to be 6.8. During hospitalization, work up for the new onset type 1 diabetes mellitus (T1DM) was done. IA-2 antibody, Insulin antibody, glutamic acid decarboxylase antibody was negative. C-peptide level was found to be low at 0.24. Cortisol and Thyroid stimulating hormone level was within normal limits. So, involvements of other endocrine abnormalities were ruled out.

### *Imaging examinations*

No imaging studies were done.

## MULTIDISCIPLINARY EXPERT CONSULTATION

Endocrinologist was consulted because of labile glucose level and to optimize insulin regimen on discharge.

## FINAL DIAGNOSIS

Diabetic ketoacidosis.

## TREATMENT

Intensive care unit was consulted and the patient was managed as per protocol for diabetic ketoacidosis.

## OUTCOME AND FOLLOW-UP

Patient was discharged for the close follow up with endocrinologist. Pembrolizumab was stopped and the chemotherapy was continued.

Our literature review mentions the prior studies highlighting the effects of pembrolizumab leading to autoimmune diabetes. The mean number of cycles was 4 and the mean number of weeks leading to presentation after the start of treatment was 15.4. The mean HbA1c of the patients was 7.97. Below mentioned are the baseline characteristics of the patients along with the disease presentation (Tables 1 and 2)[9-56].

## DISCUSSION

This study presents a comprehensive literature review of similar cases that were reported on various databases. These patients were started on various chemotherapy regimens for different cancer, but after no or little improvement from those modalities, were eventually started on immunotherapy particularly pembrolizumab. These patients presented to the emergency department with various chief complaints including from asymptomatic hyperglycemia to diabetic ketoacidosis (DKA) and were eventually diagnosed with insulin dependent diabetes mellitus. The time of presentation for all these patients varied a lot in terms of range from after just one cycle to as long as 19 cycles with average beingcycles. This average number of cycles is skewed most probably, because the most number of patients developed this

**Table 1 Baseline characteristics**

| Ref. | No. of patients | Age/Sex | Type of cancer | Time from first administration (wk) | No. of cycle |
|---|---|---|---|---|---|
| de Filette *et al*[9], 2019 | | 61/M | NSCLC | 8 | 2 |
| de Filette *et al*[9], 2019 | 91 | 65 | Melanoma/NSCLC | | 4.5 |
| Clotman *et al*[10], 2018 | | 73/F | Melanoma | 8 | 2 |
| Clotman *et al*[10], 2018 | 14 | 63 | | 6 | 3 |
| Farina *et al*[11], 2019 | 10 | 62 | Melanoma/Lung cancer | | 5 |
| Kyriacou *et al*[12], 2020 | | 68/F | Lung cancer | 7 | 2 |
| Banatwalla *et al*[13], 2021 | | 83/F | Melanoma | 23 | 7 |
| Hernandez *et al*[14], 2021 | | 67/M | SCC tongue | 3 | 1 |
| Bansal *et al*[15], 2022 | | 85/F | Lung adeno | 9 | 3 |
| Kedzior *et al*[16], 2021 | | 51/F | Lung adeno | 8 | 2 |
| Cunha *et al*[17], 2022 | | 59/F | Lung adeno | 3 | 1 |
| Kähler *et al*[18], 2020 | 5 | 74-85, 3F and 2M | Melanoma | | 4 |
| Tohi *et al*[19], 2019 | | 75/M | Urothelial CA | 10 | 3 |
| Edahiro *et al*[20], 2019 | | 61/F | Lung adeno | 25 | 8 |
| Magis *et al*[21], 2018 | | 41/F | Melanoma | 57 | 19 |
| Samoa *et al*[22], 2020 | | 12/M | Hodgkin's lymphoma | 15 | 5 |
| Li *et al*[23], 2018 | | 67/M | NSCLC | 10 | 3 |
| Boyle *et al*[24], 2019 | | 56/M | Melanoma | 22 months | |
| Boyle *et al*[24], 2019 | | 74/F | Merkel cell cancer | 23 | 7 |
| Sankar *et al*[25], 2021 | | 85/F | Bladder CA | 9 months | |
| Hakami *et al*[26], 2019 | | 52/M | Melanoma | 21 | 7 |
| Chaudry *et al*[27], 2020 | | 75/M | NSCLC | 12 | 4 |
| Kotwal *et al*[28], 2019 | 11 | 61 | | | 4 |
| Zand *et al*[29], 2022 | | 81/F | Melanoma | 26 | 8 |
| Maamari *et al*[30], 2019 | | 47/F | Cardiac angiosarcoma | 6 | 1 |
| Alrifai *et al*[31], 2019 | | 69/M | NSCLC | 15 | 4 |
| Hong *et al*[32], 2020 | | 76/M | Lung | 11 | 3 |
| Hong *et al*[32], 2020 | | 78/F | Melanoma | 4 | 1 |
| Hong *et al*[32], 2020 | | 65/F | Biliary CA | 21 | 7 |
| Skorpen *et al*[33], 2019 | | 60s/M | Lung adeno | 8 | 2 |
| Martin-Liberal *et al*[34], 2015 | | 54/F | Melanoma | 9 | 3 |
| Gaudy *et al*[35], 2015 | | 44/F | Melanoma | 8 | 2 |
| Aleksova *et al*[36], 2016 | | 61/M | Melanoma | 6 | 1 |
| M A *et al*[37], 2016 | | 55/M | Melanoma | 27 | 9 |
| Hansen *et al*[38], 2016 | | 58/M | Melanoma | | 17 |
| Alhusseini *et al*[39], 2017 | | 65/M | Lung adenocarcinoma | 3 | 1 |
| F A *et al*[40], 2017 | | 48/F | Melanoma | 2 | 1 |
| Tay *et al*[41], 2017 | | 74/F | Melanoma | 3 | 1 |
| Chae *et al*[42], 2017 | | 76/M | Lung adenocarcinoma | 1 | 1 |
| Smith-Cohn *et al*[43], 2017 | | 61/F | Cholangiocarcinoma | 18 | 6 |
| C M *et al*[44], 2017 | | 58/M | Melanoma | | 4 |

| | | | | |
|---|---|---|---|---|
| Abayev *et al*[45], 2018 | 71/M | Melanoma | 26 | |
| Ioana *et al*[46], 2018 | 52/M | Melanoma | 13 | |
| Kalkan *et al*[47], 2018 | 73/F | NSCLC | 9 | 3 |
| Reslan *et al*[48], 2018 | 79/M | Melanoma | 24 | 5 |
| Fernandez *et al*[49], 2019 | 15/M | Soft tissue sarcoma | 2 | 1 |
| Sfeir *et al*[50], 2019 | 90/M | Melanoma | | |
| Talib *et al*[51], 2019 | 67/F | Esophageal squamous cell CA | 8 | 2 |
| Gunjur *et al*[52], 2019 | 77/F | Melanoma | 3 Days | 1 |
| Singh *et al*[53], 2019 | 70/M | Melanoma | 10 | 3 |
| Akopyan *et al*[54], 2020 | 66/F | Urothelial CA | 6 months | |
| Zagouras *et al*[55], 2020 | 52/M | Lung adenocarcinoma | 9 | 3 |
| Kethireddy *et al*[56], 2021 | 85/M | | 9 | 3 |

NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma.

diabetic complication earlier rather than later in the course of starting immunotherapy. This observation is also supported by a relatively lower value of glycated hemoglobin value as compared to classic type 1 diabetic patients who develop diabetic ketoacidosis[57]. At the same time, a diagnosis of T1DM was established by presence of one or the other classic antibodies in most of the patients. Among patients who were tested for these antibodies, many of them were positive for anti-glutamic acid decarboxylase antibodiesand some of them were positive for others like islet cell antibodies or insulin antigen 2 antibodies. This conclusion is based on the data from the patients who were tested for these antibodies. To some extent this data suggest that presence of these antibodies is lower in these patients as compared to patients with classic T1DM, where a presence of at least one antibody in 97.8%[58]. On reviewing the literature it was found that incidence of newly diagnosed diabetic ketoacidosis is more in patients receiving pembrolizumab dose of 400 mg every 6 wk as compared to conventional 200 mg every 3 wk[18]. These patients are more prone to develop other endocrinopathies as well particularly thyroid related issues along with diabetes[59]. The pathophysiology of these immune checkpoint inhibitors induced diabetes mellitus is still not clear. Human leukocyte antigen is the key structure involved in the presentation of different peptides, one of which might be containing "diabetogenic peptide" in genetically susceptible individuals[60]. Recognition of this complex by T cell receptor stimulates cytotoxic T-cells that lead to destruction of B-cells in pancreas. Alternatively, these auto-antigenic peptides gets presented to the regulatory T cells, stimulation of which leads to secretion of different kind of cytokines like Interleukin 1, Interleukin 2, Interferon gamma, Tumor necrosis factor alpha and beta. These cytokines in turn stimulate cytotoxic T cells and eventual destruction of B cells ensues. To avoid this phenomenon, interaction between PD-1 and its PD-L1 is really important to maintain self tolerance against pancreatic islets[9]. Different Immune checkpoint inhibitors affect different pathways. Pembrolizumab in particular inhibits the PD-1/PD-L1 pathway, which leads to destruction of pancreatic islet cells and development of T1DM.

The predisposing factors in an individual for development of immune checkpoint inhibitors induced diabetes is not well defined as opposed to individuals with classic T1DM. Individuals with certain genotypes like DR3-DQ2 and DR4-DQ8 have shown to have higher risk of developing classic T1DM as compared to the general population[61]. In our study, we have not included genotypes of patients as there was not much data available regarding that in most cases, but studies particularly focusing on these aspects have shown that individuals with high risk genotypes have developed diabetes more while being on immune checkpoint inhibitors as compared individuals with other genotypes[9]. So, these individuals were at a high risk, but rapid onset of diabetes with presentation of ketoacidosis and relatively a low glycated hemoglobin value as compared to classic T1DM makes it different. In our study, some of the patients also had history of autoimmune disease, which makes them more susceptible to develop other autoimmune disease. Patients with already diagnosed and well controlled type 2 diabetes are also shown to be at high risk of worsening diabetes and presenting with diabetic ketoacidosis along with blood work showing presence of autoantibodies.

Although this is one of the rare side effects of the immunotherapies but with development of new immunotherapy agents, these cases should be kept in mind particularly while giving therapies to patients with high risk factors. Initial check for glycated haemoglobin before starting therapy for both diagnosed and undiagnosed diabetic patients can be useful for the risk stratification. A regular and timely checkup for glucose along with education for signs and symptoms of hyperglycemia should be introduced in patients receiving these agents. This could lead to detection of early development or worsening diabetes. Another significant finding in most of the patients was continuation of immuno-therapy after initial management of diabetic ketoacidosis was possible with introduction of as needed long and short acting insulin regimen. This is not the best option, but stopping immunotherapy in advanced malignancies, where very few treatment options are available is not desirable. The prognosis particularly because of the development of these endocrinopathies did not seem to change in most of the patients.

**Table 2 Diabetes characteristics**

| Ref. | Presentation | HbA1C | C-peptide | Auto Ab | Outcome |
|---|---|---|---|---|---|
| de Filette *et al*[9] | DKA | | 0.02 nmol/L | GADA | Not known |
| de Filette *et al*[9] | 71% DKA | 7.6 | Low in 84% | 51% GADA 18% IA213% ICA26% Anti-Insulin | Not known |
| Clotman *et al*[10] | DKA | 7.1 | Low | GADA, ICA | Stayed on insulin |
| Clotman *et al*[10] | 70% DKA | 7.5 | Low in 93% | 56% GADA | |
| Farina *et al*[11] | 69% DKA | 7.76 | 0.1 | 50% GADA+ | 97% remained on Insulin therapy |
| Kyriacou *et al*[12] | DKA | 7 | Low | GADA+ | Stayed on insulin |
| Banatwalla *et al*[13] | DKA | 8.2 | 0.09 | All neg | Stayed on insulin |
| Hernandez *et al*[14] | DKA | 6.9 | | | Stayed on insulin |
| Bansal *et al*[15] | HHS | 8.3 | Normal | GADA + | Stayed on insulin |
| Kedzior *et al*[16] | DKA | 8.3 | Undetected | GADA+ | |
| Cunha *et al*[17] | DKA | 5.6 | Undetected | GADA+ | Stayed on insulin |
| Kähler *et al*[18] | DKA | 9.7, 6.5, 7.5, No data for other 2 | Low in 1 | GADA+ in 2 | |
| Tohi *et al*[19] | DKA | 6.7 | Undetected | GADA negative | Stayed on insulin |
| Edahiro *et al*[20] | DKA | 8.4 | Low | GADA negative | Stayed on insuline |
| Magis *et al*[21] | DKA | 6.8 | < 0.003 | GADA negative,IA-2 Positive | Stayed on insulin |
| Samoa *et al*[22] | DKA | 8.9, Intial was 6.0 | Low | GADA negative,IA-2 PositiveIA Positive | Stayed on insulin |
| Li *et al*[23] | DKA | 8 | Low | All ab negative | Stayed on insuline |
| Boyle *et al*[24] | DKA | 7.4 | Low | All ab negative | Stayed on insulin |
| Boyle *et al*[24] | DKA | | Low | All ab negative | |
| Sankar *et al*[25] | DKA | 6.8 | | All ab negative | Stayed on insulin |
| Hakami *et al*[26] | DKA | 8.3 | < 0.001 | All ab negative | Stayed on insulin |
| Chaudry *et al*[27] | DKA | | | GADA + | Stayed on insulin |
| Kotwal *et al*[28] | 8 DKA,1 HHS,1 Ketosis,1 Hyperglycemia | 9.7 | 5/6 Low | 4/7 GADA+, 1/7 IAA+, 1/7 IA2A+ | Stayed on insulin |
| Zand *et al*[29] | DKA | 8.9 | | All ab negative | Stayed on insulin |
| Maamari *et al*[30] | DKA | 6.4 | Low | GADA+ | Stayed on insulin |
| Alrifai *et al*[31] | DKA | 9.2 | Low | GADA+ | Stayed on insulin |
| Hong *et al*[32] | DKA | 10.4 | Low | All ab negative | Stayed on insulin |
| Hong *et al*[32] | DKA | 11.4 | Low | All ab negative | Stayed on insulin |
| Hong *et al*[32] | DKA | 5.8 | Low | All ab negative | Stayed on insulin |
| Skorpen *et al*[33] | DKA | 8.4 | Undetected | All ab negative | Stayed on insulin |
| Martin-Liberal *et al* [34] | DKA | | | GADA+ | Stayed on insulin |
| Gaudy *et al*[35] | DKA | 6.85 | Undetected | All ab negative | Stayed on insulin |
| Aleksova *et al*[36] | DKA | | Low | All ab negative | Stayed on insulin |
| M A *et al*[37] | DKA | 10.7 | | All ab negative | Stayed on insulin |
| Hansen *et al*[38] | Simple T1DM | 7.1 | Low | GADA+ | Dced insulin |
| Alhusseini *et al*[39] | DKA | 8.5 | Undectable | GADA+, ICA+ | Stayed on insulin |
| F A *et al*[40] | DKA | 8 | Undectable | GADA+, IA+ | Stayed on insulin |

| | | | | | |
|---|---|---|---|---|---|
| Tay *et al*[41] | DKA | 9.3 | Undectable | All ab Negative | Stayed on insulin |
| Chae *et al*[42] | DKA | 5.8 | Low | GADA+, ICA+ | Stayed on insulin |
| Smith-Cohn *et al*[43] | DKA | 8.7 | | GADA+ | Stayed on insulin |
| C M *et al*[44] | DKA | 7.4 | Undetectable | All ab Negative | |
| Abayev *et al*[45] | DKA | 11.8 | Normal | All ab Negative | Stayed on insulin |
| Ioana *et al*[46] | DKA | 8.3 | Undetectable | All ab Negative | |
| Kalkan *et al*[47] | DKA | | Low | All ab Negative | |
| Reslan *et al*[48] | DKA | 7.5 | | | Stayed on insulin |
| Fernandez *et al*[49] | DKA | 5.5 | Low | GADA+ | |
| Sfeir *et al*[50] | DKA | 10.2 | Low | All ab negative | Stayed on insulin |
| Talib *et al*[51] | DKA | 7.9 | Low | GADA+ | |
| Gunjur *et al*[52] | DKA | 6.9 | Low | GADA+, ICA+ | Stayed on insulin |
| Singh *et al*[53] | DKA | | | GADA+ | Stayed on insulin |
| Akopyan *et al*[54] | DKA | | | All ab negative | Stayed on insulin |
| Zagouras *et al*[55] | Hyperglycemia | 5.7 | Low | GADA+ | Stayed on insulin |
| Kethireddy *et al*[56] | T1DM | 9 | | GADA+ | Stayed on insulin |

NSCLC: Non-small cell lung cancer; SCC: Squamous cell carcinoma; DKA: Diabetic ketoacidosis; GADA: Glutamic acid decarboxylase antibody; IA-2: Islet antibody; ICA: Islet cell antibodies; IAA: Insulin autoantibodies; ab: Antibodies.

## CONCLUSION

In the end, there is a need for a lot of research in this particular aspect regarding recognition of high risk individuals for developing these rare side effects, which might eventually help patients to avoid these side effects. Identifying different biomarkers apart from classic autoantibodies can also help in early detection of diabetes. More studies are needed to find out exact pathophysiology behind this side effect which is also the need of the hour.

## FOOTNOTES

**Author contributions:** Bhanderi H did conceptualization and did literature review; Khalid F wrote case presentation; Bodla ZH and Muhammad T helped in discussion; Du D and Meghal T helped in final editing.

**Informed consent statement:** Informed written consent was obtained from the patient for publication of this report and any accompanying images.

**Conflict-of-interest statement:** Authors does not have any conflict of interest.

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).



**Country/Territory of origin:** United States

**ORCID number:** Hardikkumar Bhanderi 0000-0002-7939-0958.

**S-Editor:** Liu JH
**L-Editor:** A
**P-Editor:** Chen YX

## REFERENCES


1 **Abdel-Wahab N**, Shah M, Suarez-Almazor ME. Adverse Events Associated with Immune Checkpoint Blockade in Patients with Cancer: A Systematic Review of Case Reports. *PLoS One* 2016; **11**: e0160221 [PMID: 27472273 DOI: 10.1371/journal.pone.0160221]

2 **Wang Y**, Zhang H, Liu C, Wang Z, Wu W, Zhang N, Zhang L, Hu J, Luo P, Zhang J, Liu Z, Peng Y, Tang L, Cheng Q. Immune checkpoint modulators in cancer immunotherapy: recent advances and emerging concepts. *J Hematol Oncol* 2022; **15**: 111 [PMID: 35978433 DOI: 10.1186/s13045-022-01325-0]

3 **Zhang H**, Dai Z, Wu W, Wang Z, Zhang N, Zhang L, Zeng WJ, Liu Z, Cheng Q. Regulatory mechanisms of immune checkpoints PD-L1 and CTLA-4 in cancer. *J Exp Clin Cancer Res* 2021; **40**: 184 [PMID: 34088360 DOI: 10.1186/s13046-021-01987-7]

4 **Pardoll DM**. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12**: 252-264 [PMID: 22437870 DOI: 10.1038/nrc3239]

5 **Guleria I**, Gubbels Bupp M, Dada S, Fife B, Tang Q, Ansari MJ, Trikudanathan S, Vadivel N, Fiorina P, Yagita H, Azuma M, Atkinson M, Bluestone JA, Sayegh MH. Mechanisms of PDL1-mediated regulation of autoimmune diabetes. *Clin Immunol* 2007; **125**: 16-25 [PMID: 17627890 DOI: 10.1016/j.clim.2007.05.013]

6 **Faghfuri E**, Faramarzi MA, Nikfar S, Abdollahi M. Nivolumab and pembrolizumab as immune-modulating monoclonal antibodies targeting the PD-1 receptor to treat melanoma. *Expert Rev Anticancer Ther* 2015; **15**: 981-993 [PMID: 26313415 DOI: 10.1586/14737140.2015.1074862]

7 **Godwin JL**, Jaggi S, Sirisena I, Sharda P, Rao AD, Mehra R, Veloski C. Nivolumab-induced autoimmune diabetes mellitus presenting as diabetic ketoacidosis in a patient with metastatic lung cancer. *J Immunother Cancer* 2017; **5**: 40 [PMID: 28515940 DOI: 10.1186/s40425-017-0245-2]

8 **Chan MM**, Kefford RF, Carlino M, Clements A, Manolios N. Arthritis and tenosynovitis associated with the anti-PD1 antibody pembrolizumab in metastatic melanoma. *J Immunother* 2015; **38**: 37-39 [PMID: 25415286 DOI: 10.1097/CJI.0000000000000060]

9 **de Filette JMK**, Pen JJ, Decoster L, Vissers T, Bravenboer B, Van der Auwera BJ, Gorus FK, Roep BO, Aspeslagh S, Neyns B, Velkeniers B, Kharagjitsingh AV. Immune checkpoint inhibitors and type 1 diabetes mellitus: a case report and systematic review. *Eur J Endocrinol* 2019; **181**: 363-374 [PMID: 31330498 DOI: 10.1530/EJE-19-0291]

10 **Clotman K**, Janssens K, Specenier P, Weets I, De Block CEM. Programmed Cell Death-1 Inhibitor-Induced Type 1 Diabetes Mellitus. *J Clin Endocrinol Metab* 2018; **103**: 3144-3154 [PMID: 29955867 DOI: 10.1210/jc.2018-00728]

11 **Farina KA**, Kane MP. Programmed Cell Death-1 Monoclonal Antibody Therapy and Type 1 Diabetes Mellitus: A Review of the Literature. *J Pharm Pract* 2021; **34**: 133-140 [PMID: 31269868 DOI: 10.1177/0897190019850929]

12 **Kyriacou A**, Melson E, Chen W, Kempegowda P. Is immune checkpoint inhibitor-associated diabetes the same as fulminant type 1 diabetes mellitus? *Clin Med (Lond)* 2020; **20**: 417-423 [PMID: 32675150 DOI: 10.7861/clinmed.2020-0054]

13 **Banatwalla R**, Kirresh OZ, Ahmed FW. Pembrolizumab-induced diabetes. *Endokrynol Pol* 2021; **72**: 414-415 [PMID: 34057191 DOI: 10.5603/EP.a2021.0053]

14 **Hernandez A**, Zeidan B, Desai P, Frunzi J. Diabetic Ketoacidosis Secondary to New Onset Type 1 Diabetes Mellitus Related to Pembrolizumab Therapy. *Cureus* 2021; **13**: e13302 [DOI: 10.7759/cureus.13302]

15 **Bansal S**, Saca D, Getz D, Rab A, Singhania N. New-Onset Type 1 Diabetes in an Elderly Female on Pembrolizumab. *Am J Med Sci* 2022; **363**: 371-372 [PMID: 34995577 DOI: 10.1016/j.amjms.2021.10.024]

16 **Kedzior SK**, Jacknin G, Hudler A, Mueller SW, Kiser TH. A Severe Case of Diabetic Ketoacidosis and New-Onset Type 1 Diabetes Mellitus Associated with Anti-Glutamic Acid Decarboxylase Antibodies Following Immunotherapy with Pembrolizumab. *Am J Case Rep* 2021; **22**: e931702 [PMID: 34185763 DOI: 10.12659/AJCR.931702]

17 **Cunha C**, Silva E, Vieira AC, Saraiva C, Duarte S. New onset autoimmune diabetes mellitus and hypothyroidism secondary to pembrolizumab in a patient with metastatic lung cancer. *Endocrinol Diabetes Metab Case Rep* 2022; **2022** [PMID: 35140188 DOI: 10.1530/EDM-21-0123]

18 **Kähler KC**, Kosova K, Bohne AS, Schreiber S, Hauschild A. Increased risk of immune checkpoint inhibitor-induced type 1 diabetes mellitus with the new approved 6-week scheme of pembrolizumab in patients with melanoma? *Eur J Cancer* 2020; **138**: 169-171 [PMID: 32890812 DOI: 10.1016/j.ejca.2020.07.028]

19 **Tohi Y**, Fujimoto K, Suzuki R, Suzuki I, Kubota M, Kawakita M. Fulminant type 1 diabetes mellitus induced by pembrolizumab in a patient with urothelial carcinoma: A case report. *Urol Case Rep* 2019; **24**: 100849 [PMID: 31211060 DOI: 10.1016/j.eucr.2019.100849]

20 **Edahiro R**, Ishijima M, Kurebe H, Nishida K, Uenami T, Kanazu M, Akazawa Y, Yano Y, Mori M. Continued administration of pembrolizumab for adenocarcinoma of the lung after the onset of fulminant type 1 diabetes mellitus as an immune-related adverse effect: A case report. *Thorac Cancer* 2019; **10**: 1276-1279 [PMID: 30964601 DOI: 10.1111/1759-7714.13065]

21 **Magis Q**, Gaudy-Marqueste C, Basire A, Loundou A, Malissen N, Troin L, Monestier S, Mallet S, Hesse S, Richard MA, Valéro R, Beliard S, Grob JJ. Diabetes and Blood Glucose Disorders Under Anti-PD1. *J Immunother* 2018; **41**: 232-240 [PMID: 29481423 DOI: 10.1097/CJI.0000000000000218]

22 **Samoa RA**, Lee HS, Kil SH, Roep BO. Anti-PD-1 Therapy-Associated Type 1 Diabetes in a Pediatric Patient With Relapsed Classical Hodgkin Lymphoma. *Diabetes Care* 2020; **43**: 2293-2295 [PMID: 32616607 DOI: 10.2337/dc20-0740]

23 **Li S**, Zhang Y, Sun Z, Hu J, Fang C. Anti-PD-1 pembrolizumab induced autoimmune diabetes in Chinese patient: A case report. *Medicine (Baltimore)* 2018; **97**: e12907 [PMID: 30407284 DOI: 10.1097/MD.0000000000012907]

24 **Boyle V**, Cundy T, Cutfield R. Rapid onset type 1 diabetes associated with the programmed cell death-1 inhibitor pembrolizumab. *Intern Med J* 2019; **49**: 930-931 [PMID: 31295781 DOI: 10.1111/imj.14340]

25 **Sankar K**, Macfarlane M, Cooper O, Falk J. Pembrolizumab-Induced Diabetic Ketoacidosis: A Review of Critical Care Case. *Cureus* 2021; **13**: e18983 [DOI: 10.7759/cureus.18983]

26 **Hakami OA**, Ioana J, Ahmad S, Tun TK, Sreenan S, McDermott JH. A case of pembrolizumab-induced severe DKA and hypothyroidism in a patient with metastatic melanoma. *Endocrinol Diabetes Metab Case Rep* 2019; **2019** [PMID: 30836329 DOI: 10.1530/EDM-18-0153]

27 **Chaudry A**, Chaudry M, Aslam J. Pembrolizumab: An Immunotherapeutic Agent Causing Endocrinopathies. *Cureus* 2020; **12**: e8836 [DOI: 10.7759/cureus.8836]

28 **Kotwal A**, Haddox C, Block M, Kudva YC. Immune checkpoint inhibitors: an emerging cause of insulin-dependent diabetes. *BMJ Open Diabetes Res Care* 2019; **7**: e000591 [PMID: 30899528 DOI: 10.1136/bmjdrc-2018-000591]

29 **Zand Irani A**, Almuwais A, Gibbons H. Immune checkpoint inhibitor-induced diabetes mellitus with pembrolizumab. *BMJ Case Rep* 2022; **15** [PMID: 35039353 DOI: 10.1136/bcr-2021-245846]

30 **Maamari J**, Yeung SJ, Chaftari PS. Diabetic ketoacidosis induced by a single dose of pembrolizumab. *Am J Emerg Med* 2019; **37**: 376.e1-376.e2 [PMID: 30361152 DOI: 10.1016/j.ajem.2018.10.040]

31 **Alrifai T**, Ali FS, Saleem S, Ruiz DCM, Rifai D, Younas S, Qureshi F. Immune Checkpoint Inhibitor Induced Diabetes Mellitus Treated with Insulin and Metformin: Evolution of Diabetes Management in the Era of Immunotherapy. *Case Rep Oncol Med* 2019; **2019**: 8781347 [PMID: 31781446 DOI: 10.1155/2019/8781347]

32 **Hong AR**, Yoon JH, Kim HK, Kang HC. Immune Checkpoint Inhibitor-Induced Diabetic Ketoacidosis: A Report of Four Cases and Literature Review. *Front Endocrinol (Lausanne)* 2020; **11**: 14 [PMID: 32047478 DOI: 10.3389/fendo.2020.00014]

33 **Skorpen PK**, Margull J. Diabetic ketoacidosis following immunotherapy for lung cancer. *Tidsskr Nor Laegeforen* 2019; **139** [PMID: 30808100 DOI: 10.4045/tidsskr.18.0597]

34 **Martin-Liberal J**, Furness AJ, Joshi K, Peggs KS, Quezada SA, Larkin J. Anti-programmed cell death-1 therapy and insulin-dependent diabetes: a case report. *Cancer Immunol Immunother* 2015; **64**: 765-767 [PMID: 25828465 DOI: 10.1007/s00262-015-1689-1]

35 **Gaudy C**, Clévy C, Monestier S, Dubois N, Préau Y, Mallet S, Richard MA, Grob JJ, Valéro R, Béliard S. Anti-PD1 Pembrolizumab Can Induce Exceptional Fulminant Type 1 Diabetes. *Diabetes Care* 2015; **38**: e182-e183 [PMID: 26310693 DOI: 10.2337/dc15-1331]

36 Abstracts of the Endocrine Society of Australia Annual Scientific Meeting 2015, 23-26 August 2015. *Clin Endocrinol (Oxf)* 2016; **84** Suppl 1: 1-49 [PMID: 26935124 DOI: 10.1111/cen.13010]

37 **Humayun MA**, Poole R. A case of multiple immune toxicities from Ipilimumab and pembrolizumab treatment. *Hormones (Athens)* 2016; **15**: 303-306 [PMID: 27376434 DOI: 10.14310/horm.2002.1656]

38 **Hansen E**, Sahasrabudhe D, Sievert L. A case report of insulin-dependent diabetes as immune-related toxicity of pembrolizumab: presentation, management and outcome. *Cancer Immunol Immunother* 2016; **65**: 765-767 [PMID: 27067877 DOI: 10.1007/s00262-016-1835-4]

39 **Alhusseini M**, Samantray J. Autoimmune diabetes superimposed on type 2 diabetes in a patient initiated on immunotherapy for lung cancer. *Diabetes Metab* 2017; **43**: 86-88 [PMID: 27291328 DOI: 10.1016/j.diabet.2016.05.007]

40 **Abdul Aziz MHariz F**, Fernando IP, Lenkanpally A, Fernando DJS. Diabetic ketoacidosis after treatment with pembrolizumab. *J Clin Transl Endocrinol Case Rep* 2017; **5**: 4-5 [DOI: 10.1016/j.jecr.2017.05.002]

41 **Tay WL**, Loh WJ, Kek PC. A case of pembrolizumab-induced type 1 diabetes mellitus presenting with severe diabetic ketoacidosis. *J Clin Transl Endocrinol Case Rep* 2017; **6**: 9-10 [DOI: 10.1016/j.jecr.2017.07.002]

42 **Chae YK**, Chiec L, Mohindra N, Gentzler R, Patel J, Giles F. A case of pembrolizumab-induced type-1 diabetes mellitus and discussion of immune checkpoint inhibitor-induced type 1 diabetes. *Cancer Immunol Immunother* 2017; **66**: 25-32 [PMID: 27761609 DOI: 10.1007/s00262-016-1913-7]

43 **Smith-Cohn MA**, Gill D, Voorhies BN, Agarwal N, Garrido-Laguna I. Case report: pembrolizumab-induced Type 1 diabetes in a patient with metastatic cholangiocarcinoma. *Immunotherapy* 2017; **9**: 797-804 [PMID: 28877632 DOI: 10.2217/imt-2017-0042]

44 **Mizab Mellah C**, Sánchez Pérez M, Santos Rey MD, Hernández García M. Fulminant type 1 diabetes mellitus associated with pembrolizumab. *Endocrinol Diabetes Nutr* 2017; **64**: 272-273 [PMID: 29056250 DOI: 10.1016/j.endinu.2017.01.005]

45 **Abayev J**, Dhawan N, Jetty V, Khan A, Goldenberg N. Abstract #100 Atypical Presentation of Congenitial Adrenal Hyperplasia. *Endocr Pract* 2018; **24**: 1 [DOI: 10.4158/1530-891X-24.s1.1]

46 Irish Endocrine Society 42nd Annual Meeting, 19th and 20th October 2018. *Ir J Med Sci* 2018; **187**: 173-226 [PMID: 30120710 DOI: 10.1007/s11845-018-1877-z]

47 **Ryder M**, Randolph G. 88(th) Annual Meeting of the American Thyroid Association October 3-7, 2018 Marriott Marquis, Washington, DC. *Thyroid* 2018; **28**: 1227-1228 [PMID: 30141375 DOI: 10.1089/thy.2018.0524]

48 **Reslan Z**, Bennett M, Wong E, Jacques S, Lee A. Pembrolizumab-induced auto-immune type-1 diabetes in a patient with metastatic melanoma. *J Pharm Pract Res* 2018; **48**: 262-264 [DOI: 10.1002/jppr.1329]

49 9 2019 ASPHO ABSTRACTS. Pediatr Blood Cancer. 2019; 66: e27713 [DOI: 10.1002/pbc.27713]

50 Paper Abstract. *J Am GeriatrSoc* 2019; **67**: S1-384 [DOI: 10.1111/jgs.15898]

51 **Talib U**, ALpert A, Mena J, Matos A, Nagus I, Sycheva T, Blaski C. Pembrolizumab-induced type 1 diabetes presenting as diabetic ketoacidosis. *Chest* 2019; **156**: A2146-A2147

52 **Gunjur A**, Klein O, Kee D, Cebon J. Anti-programmed cell death protein 1 (anti-PD1) immunotherapy induced autoimmune polyendocrine syndrome type II (APS-2): a case report and review of the literature. *J Immunother Cancer* 2019; **7**: 241 [PMID: 31488221 DOI: 10.1186/s40425-019-0713-y]

53 **Singh NR**. Pembrolizumab: a case of drug-induced autoimmune diabetes mellitus and colitis. *J Pharm Pract Res* 2019; **49**: 50-54 [DOI: 10.1002/jppr.1436]

54 **Akopyan K**, Siddiqui A, Otekeiwebia A, Kroker-Bode C. Finding the culprit: a case of new onset type i diabetes mellitus (DM) and severe diabetic ketoacidosis (DKA) after the initiation of pembrolizumab. *Chest* 2020; **158**: A869 [DOI: 10.1016/j.chest.2020.08.807]

55 **Zagouras A**, Patil PD, Yogi-Morren D, Pennell NA. Cases from the Immune-Related Adverse Event Tumor Board: Diagnosis and Management of Immune Checkpoint Blockade-Induced Diabetes. *Oncologist* 2020; **25**: 921-924 [PMID: 32564463 DOI: 10.1634/theoncologist.2019-0806]

56 **Kethireddy N**, Thomas S, Bindal P, Shukla P, Hegde U. Multiple autoimmune side effects of immune checkpoint inhibitors in a patient with metastatic melanoma receiving pembrolizumab. *J Oncol Pharm Pract* 2021; **27**: 207-211 [PMID: 32390537 DOI: 10.1177/1078155220921543]

57 **Lamsal R**, Klyachman L, Adeyinka A, Kondamudi N, Pierre L. Is HbA1c an Indicator of Diabetic Ketoacidosis Severity in the Pediatric Population? *Pediatr Emerg Care* 2023; **39**: 216-218 [PMID: 36727771 DOI: 10.1097/PEC.0000000000002859]

58 **Basu M**, Pandit K, Banerjee M, Mondal SA, Mukhopadhyay P, Ghosh S. Profile of Auto-antibodies (Disease Related and Other) in Children with Type 1 Diabetes. *Indian J Endocrinol Metab* 2020; **24**: 256-259 [PMID: 33083265 DOI: 10.4103/ijem.IJEM_63_20]

59 **Paepegaey AC**, Lheure C, Ratour C, Lethielleux G, Clerc J, Bertherat J, Kramkimel N, Groussin L. Polyendocrinopathy Resulting From Pembrolizumab in a Patient With a Malignant Melanoma. *J Endocr Soc* 2017; **1**: 646-649 [PMID: 29264517 DOI: 10.1210/js.2017-00170]

60 **Abel M**, Krokowski M. Pathophysiology of immune-mediated (type 1) diabetes mellitus: potential for immunotherapy. *BioDrugs* 2001; **15**: 291-301 [PMID: 11437693 DOI: 10.2165/00063030-200115050-00002]

61 **Steck AK**, Rewers MJ. Genetics of type 1 diabetes. *Clin Chem* 2011; **57**: 176-185 [PMID: 21205883 DOI: 10.1373/clinchem.2010.148221]

World Journal of Clinical Oncology

Submit a Manuscript: https://www.f6publishing.com

*World J Clin Oncol* 2023 November 24; 14(11): 544-548

DOI: 10.5306/wjco.v14.i11.544

ISSN 2218-4333 (online)

*CASE REPORT*

# Calcitriol induced hypercalcemia - a rare phenomenon in lung cancer: A case report

Amulya Prakash, Farhan Khalid, Ahmad Alalwan, Husam Bader, Doantrang Du, Trishala Meghal

**Specialty type:** Medicine, research and experimental

**Provenance and peer review:** Unsolicited article; Externally peer reviewed.

**Peer-review model:** Single blind

**Peer-review report's scientific quality classification**

Grade A (Excellent): A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

**P-Reviewer:** Haddadi S, Algeria; Musetti C, Italy

**Received:** July 26, 2023
**Peer-review started:** July 26, 2023
**First decision:** September 4, 2023
**Revised:** September 20, 2023
**Accepted:** October 23, 2023
**Article in press:** October 23, 2023
**Published online:** November 24, 2023

**Amulya Prakash,** Department of Internal Medicine, Haywood Regional Medical Center, Clyde, NC 28721, United States

**Amulya Prakash, Farhan Khalid, Ahmad Alalwan, Husam Bader, Doantrang Du,** Department of Internal Medicine, Monmouth Medical Center, Long Branch, NJ 07740, United States

**Trishala Meghal,** Department of Hematology-Oncology, Monmouth Medical Center, Long Branch, NJ 07740, United States

**Corresponding author:** Farhan Khalid, MD, Chief Physician, Department of Internal Medicine, Monmouth Medical Center, 200 Second Avenue, Long Branch, NJ 07740, United States. farhan.khalid.md@gmail.com

## Abstract

### *BACKGROUND*

Calcitriol-induced hypercalcemia has been rarely reported in cases of lung cancer; however, it is frequently reported in cases of lymphoid malignancy and granulomatous disease. We present a rare case of hypercalcemia associated with squamous cell cancer of the lung with elevated calcitriol level.

### *CASE SUMMARY*

A 61-year-old Caucasian female with severe hypercalcemia of 15 mg/dL, which led to a new diagnosis of metastatic lung cancer. Since the parathyroid hormone-related peptide (PTHrP) level was minimally elevated at 2.1 pmol/L, we believe excessive calcitriol production by tumor cells was the underlying mechanism for hypercalcemia. Calcitriol was significantly elevated at 130 pg/mL with a low 25-hydroxyvitamin D level of 25.9 ng/mL and suppressed PTH level of 8 pg/mL. Corticosteroids are generally used to treat calcitriol-induced hypercalcemia, but we successfully treated our patient with bisphosphonate, highlighting the further utility of bisphosphonates in hypercalcemia treatment.

### *CONCLUSION*

We believe that the underlying cause of hypercalcemia, in this case of metastatic squamous cell lung carcinoma, was elevated calcitriol, which was likely produced by the tumor cells. In addition to PTHrP, calcitriol levels should be included in the workup for hypercalcemia in cases of lung cancer. However, the pathophysiology and prognostic significance of dysregulated calcitriol production in solid tumors remain unclear and warrant further research. Bisphosphonate may be used as a steroid-sparing therapy even in cases of calcitriol-induced hypercalcemia and

warrants further investigation.

**Key Words:** Hypercalcemia associated malignancy; Lung cancer; Denosumab; Calcitriol; Vitamin D; Case report



**Core Tip:** Our case report illuminates a rare mechanism of hypercalcemia in lung malignancies, characterized by elevated calcitriol. Despite its rarity, it sheds light on the pathophysiology of hypercalcemia in solid malignancies, notably lung cancers. To the best of our knowledge, this is the first documented case in medical literature to present this mechanism. Moreover, the successful management of this condition with bisphosphonates highlights the potential efficacy of this treatment approach for future cases involving similar symptoms. By emphasizing this novel observation, our report contributes to the expanding body of knowledge regarding hypercalcemia in lung cancers and paves the way for the development of novel therapeutic strategies for the treatment of such cases.

**Citation:** Prakash A, Khalid F, Alalwan A, Bader H, Du D, Meghal T. Calcitriol induced hypercalcemia - a rare phenomenon in lung cancer: A case report. *World J Clin Oncol* 2023; 14(11): 544-548
**URL:** https://www.wjgnet.com/2218-4333/full/v14/i11/544.htm
**DOI:** https://dx.doi.org/10.5306/wjco.v14.i11.544

## INTRODUCTION

Hypercalcemia associated with malignancy (HAM) is a common clinical finding and may even present as an oncologic emergency. It has been found in up to 30% of cases of malignancy[1]. The estimated yearly prevalence of hypercalcemia for all cancers is 1.46% to 2.74%[2]. Calcitriol overproduction is a rare etiology of HAM and accounts for merely 1% of cases of HAM[3]. It has been frequently reported in cases of Hodgkin and non-Hodgkin lymphoma and also in some cases of ovarian dysgerminoma, pancreatic neuroendocrine tumors, seminomas, and renal cell carcinoma[4-6]. In our extensive literature search, we came across just one case report of squamous cell lung cancer by Akai *et al*[7], where calcitriol overproduction was exclusively responsible for hypercalcemia and treated with tumor resection. We present a rare case of squamous cell lung carcinoma with hypercalcemia and elevated calcitriol levels, which was treated successfully with bisphosphonate. To the best of our knowledge, bisphosphonates have never been reported for the treatment of calcitriol-induced hypercalcemia in the case of squamous cell lung carcinoma.

## CASE PRESENTATION

### *Chief complaints*

Hypercalcemia on routine blood work investigation.

### *History of present illness*

The patient didn't complain of any significant symptoms at the time of presentation. However, during detailed history taking, she reported vague complaints of nausea, fatigue, and generalized weakness but denied any other symptoms like constipation, palpitation, confusion, *etc.*

### *History of past illness*

She has a past medical history of diabetes, hyperlipidemia, and depression.

### *Personal and family history*

The patient has a remote history of tobacco smoking more than 15 years ago. She denies any supplementation with vitamin A, vitamin D, or calcium, frequent use of antacids, or excessive consumption of dairy products. Her current medications include atorvastatin and sertraline. Patient denies any significant family history.

### *Physical examination*

Her vital signs and physical exam were within normal limits.

### *Laboratory examinations*

Three months prior to the presentation, her calcium level was noted to be within the normal range of 10.1 (range: 8.6-10.3 mg/dL). In a routine lab work performed one week before the presentation, her corrected serum calcium was noted to be elevated first time at 13 mg/dL. She was asymptomatic at that point so decision was made to monitor with serial labs.

Our patient presented for repeat lab draw a week later and now her corrected serum calcium was 14.3 mg/dL, so she was instructed to visit the emergency department for further evaluation. She was admitted 12 h later on the same day and her corrected serum calcium further worsened to 15 mg/dL with an ionized calcium value of 7.5 mg/dL (range: 4.5-5.6). Her albumin was 3.4 mg/dL, creatinine 0.5 mg/dL, estimated glomerular filtration rate > 60 mL/min/1.73 $m^2$, magnesium 1.9 mg/dL (range: 1.8-2.4), phosphorus 2.4 mg/dL (range: 2.5-4.9). 25-hydroxyvitamin D was noted to be low: 25.9 ng/mL (30-100 ng/mL), with elevated calcitriol level: 130 pg/mL (24.8-81.5 pg/mL) and suppressed intact parathyroid hormone (PTH) level: 8 pg/mL (15-65 pg/mL) and minimally elevated PTH-related peptide (PTHrP) 2.1 pmol/L (normal < 2.0 pmol/L). Complete blood count and urinalysis were within normal limits.

### *Imaging examinations*

Chest X-ray reveals a left hilar opacity, which was concerning for lung neoplasm. Upon further investigation with contrasted pan-computed tomography, the patient was noted to have a left lower lobe mass with an epicenter in the left lower lobe bronchus with invasion into the mediastinum and multiple hepatic metastases. No metastasis to bones, brain or spleen was noted. A core biopsy was performed on one of the liver metastases. Histopathology findings were consistent with metastatic squamous cell carcinoma, moderately differentiated. Immunohistochemistry of biopsied tissue sample was positive for markers p63, cytokeratin 5/6 heterogeneously positive; negative for thyroid transcription factor, cytokeratins 7, caudal-related homeobox transcription factor 2 and GATA binding protein 3. Thus, immunohistochemistry findings were also consistent with squamous cell carcinoma of lung. The tissue sample shows high programmed cell death ligand 1 expression (Figure 1).

## FINAL DIAGNOSIS

Calcitriol induced hypercalcemia.

## TREATMENT

Our patient was paucisymptomatic but had rapid rise in serum calcium level so we decided to treat her hypercalcemia. The patient was managed with intravenous normal saline, subcutaneous calcitonin administered twice, and a single infusion of zoledronic acid 4 mg during the course of the hospital stay. Her calcium level improved rapidly to 9.8 mg/dL within 48 h and she was discharged from the hospital. Her calcium level 2-wk later was noted to be elevated again at 13 mg/dL and was given another dose of zolendronic acid. Four weeks after discharge, her calcium level was noted to be within the normal range at 9.9 mg/dL.

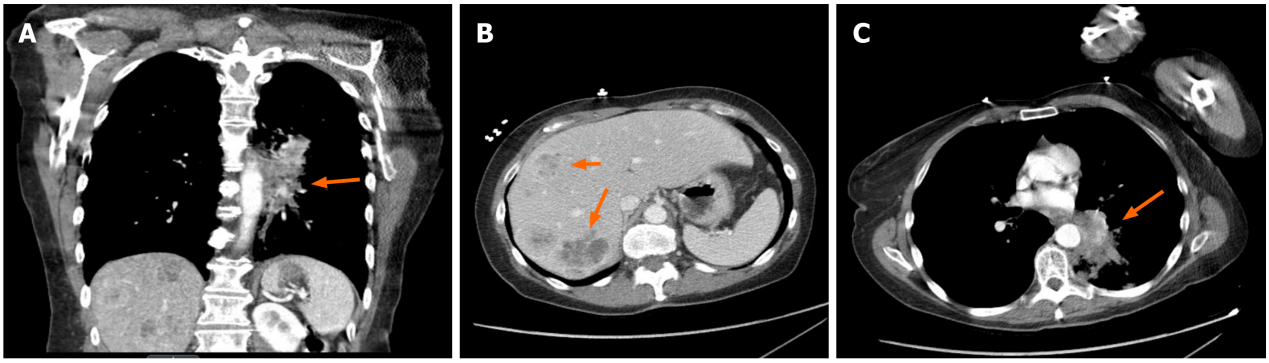
## OUTCOME AND FOLLOW-UP

The patient is scheduled to receive infusion of zolendronic acid once every month to manage hypercalcemia. She is planned to undergo chemotherapy with carboplatin, paclitaxel and pembrolizumab.

## DISCUSSION

Hypercalcemia results from dysregulation between normal bone formation and the degradation cycle. The pathophysiology of HAM can be broadly classified into: (1) Local osteolytic hypercalcemia; (2) Humoral hypercalcemia mediated through PTHrP; and (3) Excess production of 1,25-dihydroxy vitamin D (calcitriol).

In the current case, calcitriol level was noted to be significantly elevated with suppressed PTH level and minimal elevation of PTHrP. The question is about what's causing the calcitriol elevation in this case. Squamous cell cancer of the lung is usually associated with hypercalcemia driven by PTHrP elevation. In granulomatous disease such as sacrcoidosis, there is extrarenal production of calcitriol *via* autonomous 1-α-hydroxylase activity in tissue macrophages. PTHrP can also upregulate 1-alpha hydroxylase activity and calcitriol production in mice models, but it does not increase calcitriol production in humans[8,9]. In this patient, we tend to believe that hypercalcemia was due to a PTHrP-independent mechanism since PTHrP was minimally elevated. Additionally, extrarenal synthesis of calcitriol is dependent on its substrate 25-hydroxyvitamin D, which was low in this case, thus excluding extrarenal calcitriol production as an underlying mechanism. It's very possible that it was being ectopically produced by tumor cells in an autonomous fashion [10]. Although staining of the biopsy sample for 1,25-dihydroxy vitamin D and 1-alpha-hydroxylase was not done to confirm calcitriol's ectopic production, the treatment response further solidifies our hypothesis. Increased calcitriol level in cases of granulomatous disease is believed to be due to the upregulation of 1-alpha hydroxylase activity, and its autocrine regulation is sensitive to corticosteroid therapy. Hence, corticosteroid is indicated in the treatment of hypercalcemia in such cases[11,12]. However, in our case, hypercalcemia responded remarkably to treatment with bispho -sphonate and didn't require any steroid treatment, making us doubtful of any increased 1-alpha-hydroxylase activity.




**DOI**: 10.5306/wjco.v14.i11.544 **Copyright** ©The Author(s) 2023.

**Figure 1 Computed tomography images.** A: Computed tomography (CT) showing left lower lobe mass involving left bronchus; B: CT abdomen with contrast showing liver metastases; C: CT chest showing left lower lobe mass invasion into mediastinum.

The standard treatment approach for HAM is aimed at: (1) Promoting renal calcium excretion through intravenous normal saline administration and even loop diuretics, sometimes; and (2) Reducing bone absorption through the use of bisphosphonates and denosumab in refractory cases. Calcitonin can also be used as an adjunctive therapy with bisphosphonates, but tachyphylaxis develops within 48 h. Corticosteroids are the first-line agents for the treatment of calcitriol-mediated hypercalcemia by inhibiting the transcription of 25-hydroxyvitamin D-1-hydroxylase; however, it was not required in our case. That being said, bisphosphonates may have more of a role in the treatment of HAM. Bisphosphonate may inhibit the adhesion of osteoclast precursors to stromal osteoblasts through the increased expression of intercellular adhesion molecule-1, which is promoted by calcitriol[13,14]. In a case series reported by Rizzoli *et al*[15], bisphosphonate was more effective than steroids in the treatment of hypercalcemia, probably due to its bone anti-resorptive effect. Bisphosphonates may have additional effects, including induction of apoptosis, inhibition of invasion, and antiangiogenic properties, as seen in some preclinical studies[16]. It would be worthwhile to conduct further research to investigate the cellular effects of calcitriol and bisphosphonate in patients with lung cancer.

## CONCLUSION

We believe that the underlying cause of hypercalcemia, in this case of metastatic squamous cell lung carcinoma, was elevated calcitriol, which was likely produced by the tumor cells. In addition to PTHrP, calcitriol levels should be included in the workup for hypercalcemia in cases of lung cancer. However, the pathophysiology and prognostic significance of dysregulated calcitriol production in solid tumors remain unclear and warrant further research. Bisphosphonate may be used as a steroid-sparing therapy even in cases of calcitriol-induced hypercalcemia and warrants further investigation.

## FOOTNOTES

**Author contributions:** Alalwan A conceived the idea, did the literature search, managed the case on medical floor, contributed to case presenrtation and the introduction; Khalid F and Bader H contributed the discussion; Du D and Meghal T edited and revised the manuscript.

**Informed consent statement:** Informed consent was obtained by the patient.

**Conflict-of-interest statement:** All the authors report no relevant conflicts of interest for this article.

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).



**Country/Territory of origin:** United States

**ORCID number:** Farhan Khalid 0000-0002-8456-5118.

**S-Editor:** Wang JJ

**L-Editor:** A
**P-Editor:** Wang JJ

## REFERENCES

1 **Stewart AF**. Clinical practice. Hypercalcemia associated with cancer. *N Engl J Med* 2005; **352**: 373-379 [PMID: 15673803 DOI: 10.1056/NEJMcp042806]
2 **Jick S**, Li L, Gastanaga VM, Liede A. Prevalence of hypercalcemia of malignancy among cancer patients in the UK: analysis of the Clinical Practice Research Datalink database. *Cancer Epidemiol* 2015; **39**: 901-907 [PMID: 26520619 DOI: 10.1016/j.canep.2015.10.012]
3 **Goldner W**. Cancer-Related Hypercalcemia. *J Oncol Pract* 2016; **12**: 426-432 [PMID: 27170690 DOI: 10.1200/JOP.2016.011155]
4 **Rodríguez-Gutiérrez R**, Zapata-Rivera MA, Quintanilla-Flores DL, Camara-Lemarroy CR, Lavalle-Gonzalez FJ, González-González JG, Villarreal-Pérez JZ. 1,25-dihydroxyvitamin D and PTHrP mediated malignant hypercalcemia in a seminoma. *BMC Endocr Disord* 2014; **14**: 32 [PMID: 24721620 DOI: 10.1186/1472-6823-14-32]
5 **Shivnani SB**, Shelton JM, Richardson JA, Maalouf NM. Hypercalcemia of malignancy with simultaneous elevation in serum parathyroid hormone--related peptide and 1,25-dihydroxyvitamin D in a patient with metastatic renal cell carcinoma. *Endocr Pract* 2009; **15**: 234-239 [PMID: 19364692 DOI: 10.4158/EP.15.3.234]
6 **Hibi M**, Hara F, Tomishige H, Nishida Y, Kato T, Okumura N, Hashimoto T, Kato R. 1,25-dihydroxyvitamin D-mediated hypercalcemia in ovarian dysgerminoma. *Pediatr Hematol Oncol* 2008; **25**: 73-78 [PMID: 18231957 DOI: 10.1080/08880010701774033]
7 **Akai PS**, Wong T, Chang-Poon V, Green F, Whitelaw WA, Hanley DA. Resectable bronchogenic carcinoma presenting with hypercalcemia: tumor-associated granulomatous reaction and probable production of 1,25-dihydroxyvitamin D. *Clin Invest Med* 1989; **12**: 212-216 [PMID: 2743640]
8 **Horwitz MJ**, Tedesco MB, Sereika SM, Syed MA, Garcia-Ocaña A, Bisello A, Hollis BW, Rosen CJ, Wysolmerski JJ, Dann P, Gundberg C, Stewart AF. Continuous PTH and PTHrP infusion causes suppression of bone formation and discordant effects on 1,25(OH)2 vitamin D. *J Bone Miner Res* 2005; **20**: 1792-1803 [PMID: 16160737 DOI: 10.1359/JBMR.050602]
9 **Schilling T**, Pecherstorfer M, Blind E, Leidig G, Ziegler R, Raue F. Parathyroid hormone-related protein (PTHrP) does not regulate 1,25-dihydroxyvitamin D serum levels in hypercalcemia of malignancy. *J Clin Endocrinol Metab* 1993; **76**: 801-803 [PMID: 8445039 DOI: 10.1210/jcem.76.3.8445039]
10 **Chukir T**, Liu Y, Hoffman K, Bilezikian JP, Farooki A. Calcitriol Elevation Is Associated with a Higher Risk of Refractory Hypercalcemia of Malignancy in Solid Tumors. *J Clin Endocrinol Metab* 2020; **105**: e1115-e1123 [PMID: 31841590 DOI: 10.1210/clinem/dgz278]
11 **Fuss M**, Pepersack T, Gillet C, Karmali R, Corvilain J. Calcium and vitamin D metabolism in granulomatous diseases. *Clin Rheumatol* 1992; **11**: 28-36 [PMID: 1582115 DOI: 10.1007/BF02207080]
12 **Kallas M**, Green F, Hewison M, White C, Kline G. Rare causes of calcitriol-mediated hypercalcemia: a case report and literature review. *J Clin Endocrinol Metab* 2010; **95**: 3111-3117 [PMID: 20427501 DOI: 10.1210/jc.2009-2673]
13 **Mateo RCI**, Ortiz R, Rosen HN. BISPHOSPHONATES FOR THE TREATMENT OF CALCITRIOL-INDUCED HYPERCALCEMIA. *AACE Clin Case Rep* 2019; **5**: e316-e320 [PMID: 31967061 DOI: 10.4158/ACCR-2019-0101]
14 **Okada Y**, Morimoto I, Ura K, Watanabe K, Eto S, Kumegawa M, Raisz L, Pilbeam C, Tanaka Y. Cell-to-Cell adhesion *via* intercellular adhesion molecule-1 and leukocyte function-associated antigen-1 pathway is involved in 1alpha,25(OH)2D3, PTH and IL-1alpha-induced osteoclast differentiation and bone resorption. *Endocr J* 2002; **49**: 483-495 [PMID: 12402981 DOI: 10.1507/endocrj.49.483]
15 **Rizzoli R**, Stoermann C, Ammann P, Bonjour JP. Hypercalcemia and hyperosteolysis in vitamin D intoxication: effects of clodronate therapy. *Bone* 1994; **15**: 193-198 [PMID: 8086237 DOI: 10.1016/8756-3282(94)90707-2]
16 **Clezardin P**. Potential anticancer properties of bisphosphonates: insights from preclinical studies. *Anticancer Agents Med Chem* 2012; **12**: 102-113 [PMID: 21864232 DOI: 10.2174/1871520127990149777]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
**Telephone:** +1-925-3991568
**E-mail:** bpgoffice@wjgnet.com
**Help Desk:** https://www.f6publishing.com/helpdesk
https://www.wjgnet.com