

Role of the preoperative circulating tumor DNA *KRAS* mutation in patients with resectable pancreatic cancer

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Aim: The prognosis of resectable pancreatic cancer patients with the same stage of disease is highly variable. The purpose of this study is to establish a scoring system for preoperative screening of resectable patients. **Materials & methods:** The clinical information and laboratory tests of 105 resectable patients with pancreatic cancer were enrolled and analyzed. **Results:** The consistency of clinical stage and pathological stage was poor ($\kappa = 0.193$; $p < 0.003$). We performed a comprehensive scoring system with *KRAS* mutations in circulating tumor DNA (*mutKRAS* ctDNA) for the resectable patients. Patients with higher scores were more prone to early postoperative recurrence and poorer prognosis. **Conclusion:** The scoring system can help preoperatively screen out resectable patients who are prone to early postoperative recurrence.

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Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths worldwide, with a 5-year survival rate of approximately 9% [1]. Due to the lack of specific symptoms in the early stage, only 20% have the opportunity to undergo radical resection, which is currently the most effective way to prolong survival. However, even among patients who receive radical surgery, the 5-year survival rate is less than 25% [2]. Among them, some resectable patients suffer recurrence rapidly after surgery. It is very important to screen these patients before surgery, as these selected patients may benefit from neoadjuvant chemotherapy [3]. At present, the diagnosis and treatment strategy of pancreatic cancer (PC) mainly depends on the morphological stages of imaging and pathology. However, even at the same clinical or pathological stage, the prognosis of postoperative patients is variable. Currently, there is no suitable preoperative biomarker that can be used as a guide to the prognosis and progression of resectable patients [4].

Commonly used blood tumor markers such as carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) fail to be used to monitor the prognosis of PC due to their low sensitivity and specificity [5]. Circulating tumor DNA (ctDNA) is mainly derived from necrotic and apoptotic tumor cells, and often carries genetic changes such as tumor-related single-nucleotide variation, copy number alteration and structural variation [6,7]. These biological properties allow ctDNA to be used to monitor disease progression and guide targeted therapies. It has been reported that *KRAS* mutations in ctDNA (*mutKRAS* ctDNA) can be used as a tumor biomarker promising to predict the prognosis of patients in different cancers [8–11].

In this study, our goal was to investigate whether ^{mut}*KRAS* ctDNA can be used, independent of clinical and pathological stage, to establish a preoperative scoring system to evaluate early postoperative recurrence and long-term prognosis of patients with resectable PC.

Materials & methods

Patients & samples

In this prospective study, 105 patients with PC were recruited at the Tianjin Cancer Institute and Hospital between March 2016 and November 2017. The last follow-up date was January 2020. All patients had not received any treatment before enrollment, and the following conditions needed to be met: receiving R0 radical resection and standard adjuvant chemotherapy; histopathological confirmation of PC; all patients signed informed consent; no other history of malignant tumor. Preoperative CT or MRI examination is required for all patients to obtain the clinical stage. The clinical and pathological stage of patients was classified according to the 8th Edition of the AJCC system. Peripheral blood samples were collected from patients before surgery to detect tumor markers (CA199 and CEA) and to extract ctDNA in EDTA tubes. All samples were processed immediately after blood was drawn. This study was approved by the Ethics Committee of Tianjin Cancer Institute and Hospital.

Extraction of DNA & *KRAS* mutations analysis

A 10 ml peripheral blood was collected into EDTA tubes and DNA was purified from the blood using a QIAamp Circulating Nucleic Acid kit (Qiagen, Hilden, Germany), according to the instructions of manufacturer. The extracted DNA was detected by PCR with the *KRAS* mutation detection kit (GENETIC BIOTEK, Tianjin, China) (based on real-time bidirectional pyrophosphorolysis activated polymerization nucleic acid amplification) using Bio-Rad CFX96 real-time PCR detection system (BIO-RAD, CA, USA). This kit enables to detect the seven *KRAS* mutations (G12A, G12V, G12S, G12R, G12C, G12D and G13D), covering almost 98% of the mutation hotspots in *KRAS* oncogene [12].

Pyrophosphorolysis activated polymerization PCR

The status of *KRAS* in plasma was analyzed using the Bio-Rad CFX96 real-time PCR system. We used a commercial *KRAS* gene mutation detection kit for PCR. The *KRAS* mutation in each blood sample was validated according to the corresponding mutation site (G12A, G12V, G12S, G12R, G12C, G12D and G13D) that had been determined. The reaction well was in freeze-dried powder form, containing DNA polymerase, reaction buffer, specific primers, dNTPs and tetrasodium pyrophosphate. The extracted DNA sample, negative control product, blank quality control product and positive quality control product was added to each group of test wells (8 wells/group) in order (17 µl/well). The cycling conditions were: 96°C for 2 min, 1 cycle; at 96°C for 12 s, at 64°C for 30 s and then at 68°C for 60 s for 40 cycles, 68–95°C increasing program, each increase of 0.5°C, for 5 s. Finally, the results were analyzed according to the threshold (Supplementary Tables 1 & 2). Each sample was repeated two or three times to detect the *KRAS* status in plasma.

Tumor biomarkers

CA19-9, CEA and CA242 levels were detected by chemi-luminescence immunoassay according to the manufacturer's instructions (Roche, Mannheim, Germany), and the cut-off values for them was 37 U/ml, 5 µg/l and 20 U/ml, respectively.

Statistical analysis

The SPSS 26.0 software was used for statistical analysis. The correlation between ^{mut}*KRAS* ctDNA and clinical characteristics was determined by the Chi-square test or Fisher's exact. To explore the consistency of clinical staging and pathological staging, Cohen's kappa coefficient analysis was used. Univariate and multivariate survival analysis for relapse-free survival and overall survival (OS) were estimated by the Cox regression. p-value and hazard ratio (HR) were calculated by Cox regression to determine the prognostic factors. Kaplan–Meier method was used to compare the survival between groups. The median survival in months and 95% CI were estimated as results. Relapse-free survival and OS were defined as the time between the date of surgery and the date of early recurrence and death for any reason or the last follow-up. p-values <0.05 were considered statistically significant.

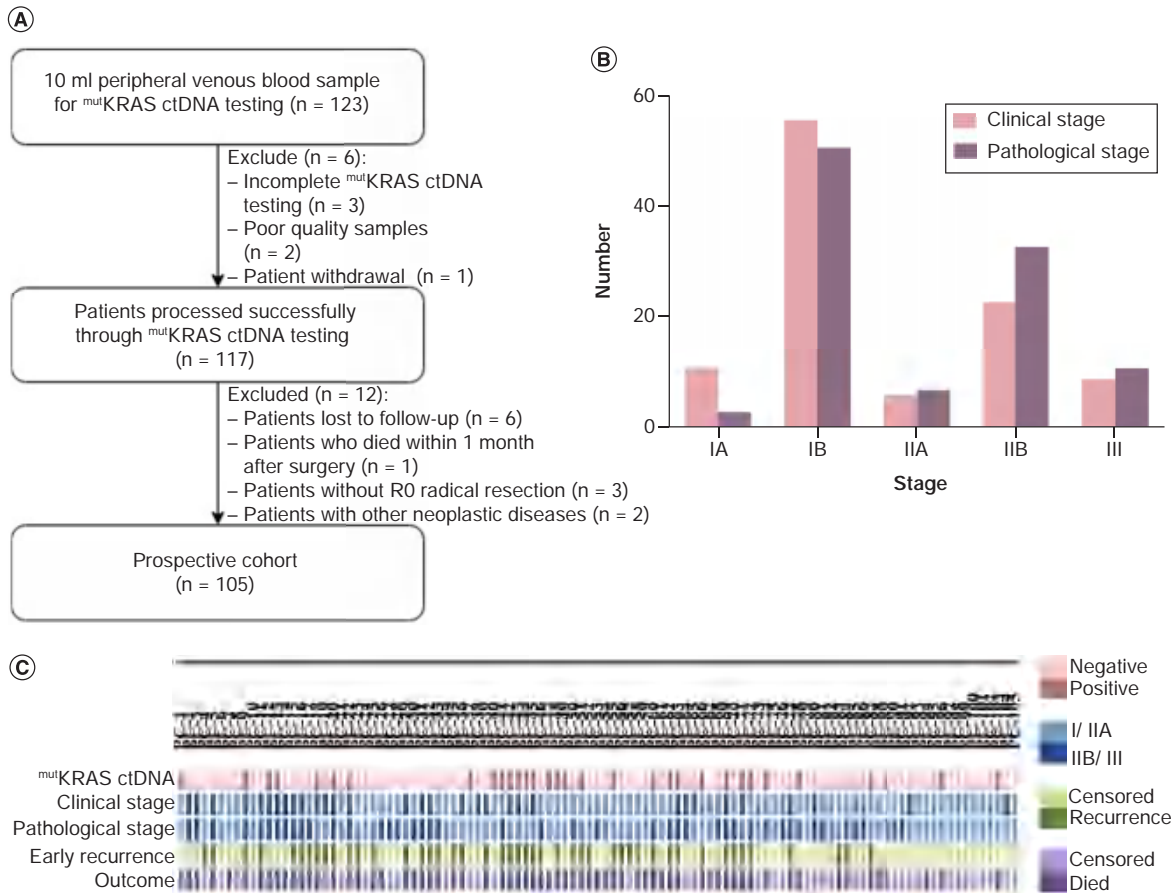


Figure 1. The clinicopathological characteristics of this study cohort. (A) Flow diagram of this study cohort inclusion. The study recruited 123 patients who agreed to venous blood sampling between March 2016 and November 2017. Among them, 18 patients were excluded. **(B)** The clinical and pathological stages of patients with radical resection. **(C)** The heat map showed the *mut*KRAS ctDNA status, clinical stage, pathological stage, early recurrence and long-term prognosis of 105 patients. ctDNA: Circulating tumor DNA.

Results

Patient characteristics & *mut*KRAS ctDNA analysis

A total of 105 patients were recruited in this study (Figure 1A), including 63 male (60%) and 42 female (40%) patients. The median age of these patients was 61 years ranging from 39 to 79 at diagnosis. All patients received radical resection, 30 (28.6%) of them received splenopancreatectomy because the tumor was located in the body or tail of pancreas, and 75 (71.4%) received pancreatoduodenectomy because the tumor was located in the pancreatic head or uncinate process. According to the pathological stage, there were 58 patients (55.2%) with early stage (I and IIA) PC, 47 patients (44.8%) with stage IIB and III. Based on the pathological characteristics, the tumor of seven cases (6.7%) were smaller than 2 cm, 76 cases (72.4%) with tumors between 2 and 4 cm and 22 cases (20.9%) with tumors larger than 4 cm. There were 47 patients with lymph node metastasis, and 11 of them had more than three lymph nodes metastases. Based on the clinical stage, 73 patients (69.5%) were in early stage, 32 patients (30.5%) were in stage IIB and III. Patients undergoing radical resection had a lower clinical stage than pathological stage, and the consistency of clinical stage and pathological stage was poor ($\kappa = 0.193$; $p < 0.003$, Figure 1B). When correlating clinical characteristics and *mut*KRAS ctDNA status, we found that *mut*KRAS ctDNA was not related to any clinical parameters including tumor size, lymph node status and stage (clinical stage and pathological stage) (Figure 1C, Table 1 & Supplementary Table 3). The clinicopathological information of patients was shown in Table 1.

Table 1. Clinical characteristics and ^{mut}KRAS ctDNA status.

| Characteristics | Value | ^{mut} KRAS ctDNA | | p-value |
|-----------------------------|---------------------------|---------------------------|----------|---------|
| | | Negative | Positive | |
| All | | 73 | 32 | – |
| Sex | Male | 44 | 19 | 0.931 |
| | Female | 29 | 13 | |
| Age (years) | <60 | 31 | 12 | 0.634 |
| | ≥60 | 42 | 20 | |
| Tumor location | Head and uncinate process | 52 | 23 | 0.947 |
| | Body and tail | 21 | 9 | |
| Tumor size [†] | T1 | 4 | 3 | 0.332 |
| | T2 | 56 | 20 | |
| | T3 | 13 | 9 | |
| Lymph node [†] | N0 | 40 | 18 | 0.127 |
| | N1 | 28 | 8 | |
| | N2 | 5 | 6 | |
| Pathological stage | I/IIA | 40 | 18 | 0.890 |
| | IIB/III | 33 | 14 | |
| Clinical stage | I/IIA | 53 | 20 | 0.301 |
| | IIB/III | 20 | 12 | |
| Grade | Moderate | 35 | 12 | 0.322 |
| | Poor | 38 | 20 | |
| Vascular cancer embolus | Present | 18 | 4 | 0.159 |
| | Absent | 55 | 28 | |
| Pancreatic capsule invasion | Present | 49 | 24 | 0.420 |
| | Absent | 24 | 8 | |
| Perineural invasion | Present | 52 | 21 | 0.566 |
| | Absent | 21 | 11 | |
| CA19-9 (U/ml) | <37 | 12 | 6 | 0.772 |
| | ≥37 | 61 | 26 | |
| CEA (μg/l) | <5 | 48 | 23 | 0.537 |
| | ≥5 | 25 | 9 | |
| CA242 (U/ml) [‡] | <20 | 34 | 20 | 0.150 |
| | ≥20 | 38 | 12 | |

[†]Tumor size and lymph node status depend on pathological diagnosis.
[‡]One patient lost the CA242 value.
 ctDNA: Circulating tumor DNA.

Analysis of postoperative early recurrence in patients with resectable PDAC

According to published research and clinical experience, several risk factors that may affect the prognosis of patients with PDAC were initially selected. Univariate analysis revealed that patients with large tumor size (HR = 1.820; CI = 1.089–3.040; *p* = 0.022), high clinical stage (HR = 2.616; CI = 1.589–4.306; *p* < 0.001), poor grade (HR = 1.934; CI = 1.165–3.213; *p* = 0.011) and ^{mut}KRAS ctDNA-positive (HR = 2.217; CI = 1.368–3.594; *p* = 0.001) were more likely to relapse after radical resection. In the univariate analysis, five candidate parameters were incorporated into the subsequent multivariate COX analysis because these *p*-values were less than 0.1. In multivariate analysis, clinical stage (HR = 2.423; CI = 1.467–4; *p* = 0.001), vascular cancer embolus (HR = 1.870; CI = 1.067–3.280; *p* = 0.029) and ^{mut}KRAS ctDNA (HR = 2.381; CI = 1.445–3.925; *p* = 0.001) were verified as the independent risk factors for early recurrence. Interestingly, pathological stage was not a risk factor for postoperative recurrence of PC patients undergoing radical resection. On the contrary, preoperative imaging were more meaningful for the assessment of patients' recurrence after surgery (Table 2 & Figure 2).

Table 2. Predictors of 1-year RFS and OS by Cox regression mode.

| Characteristic | 1-year RFS | | | | OS | | | |
|--|---------------------|---------------------|-----------------------|---------------------|---------------------|---------------------|-----------------------|---------------------|
| | Univariate analysis | | Multivariate analyses | | Univariate analysis | | Multivariate analyses | |
| | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) | p-value | HR (95% CI) |
| Sex (male vs female) | 0.312 | 0.769 (0.461–1.280) | | | 0.632 | 0.902 (0.593–1.374) | | |
| Age (<60 vs ≥60) | 0.970 | 1.009 (0.619–1.645) | | | 0.209 | 1.311 (0.859–2.001) | | |
| Tumor location (head vs body and tail) | 0.974 | 0.992 (0.592–1.662) | | | 0.952 | 1.014 (0.650–1.582) | | |
| Tumor size† (T1 and T2 vs T3) | 0.022 | 1.820 (1.089–3.040) | | | <0.001 | 2.433 (1.573–3.763) | 0.011 | 1.917 (1.163–3.158) |
| Lymph node† (N0 vs N1 and N2) | 0.173 | 1.396 (0.864–2.256) | | | <0.001 | 2.257 (1.477–3.448) | | |
| Clinical stage (I/IIA vs IIB/III) | <0.001 | 2.616 (1.589–4.306) | 0.001 | 2.423 (1.467–4.000) | 0.001 | 1.999 (1.316–3.037) | | |
| Pathological stage (I/IIA vs IIB/III) | 0.403 | 1.227 (0.760–1.982) | | | 0.003 | 1.877 (1.236–2.850) | | |
| Grade (moderate vs poor) | 0.011 | 1.934 (1.165–3.213) | | | 0.034 | 1.589 (1.036–2.437) | | |
| Vascular cancer embolus (present vs absent) | 0.052 | 1.713 (0.996–2.946) | 0.029 | 1.870 (1.067–3.280) | 0.941 | 1.019 (0.620–1.674) | | |
| Pancreatic capsule invasion (present vs absent) | 0.469 | 1.217 (0.715–2.071) | | | 0.893 | 1.031 (0.661–1.608) | | |
| Perineural invasion (present vs absent) | 0.860 | 0.953 (0.555–1.635) | | | 0.524 | 0.865 (0.554–1.351) | | |
| ^{mut} KRAS ctDNA (positive vs negative) | 0.001 | 2.217 (1.368–3.594) | 0.001 | 2.381 (1.445–3.925) | <0.001 | 2.858 (1.883–4.336) | <0.001 | 3.191 (2.057–4.950) |
| CA19-9 (≥37 U/ml vs <37 U/ml) | 0.613 | 0.855 (0.467–1.567) | | | 0.406 | 1.266 (0.726–2.207) | | |
| CEA (≥5 ng/ml vs <5 ng/ml) | 0.658 | 0.887 (0.521–1.509) | | | 0.020 | 1.649 (1.081–2.514) | | |
| CA242 (≥20 U/ml vs <20 U/ml) | 0.894 | 1.033 (0.640–1.668) | | | 0.564 | 1.129 (0.748–1.702) | | |

†Tumor size and lymph node status depend on pathological diagnosis.

HR: Hazard ratio; OS: Overall survival; RFS: Relapse-free survival.

Independent prognostic factors affecting the long-term survival of patients after radical resection

At a median follow-up time of 38 months, the median survival was 26 months ranging from 3 to 48 months for all enrolled patients. Univariate analysis results showed that large tumor size, positive lymph node, high stage, including pathological stage and clinical stage, poor grade, ^{mut}KRAS ctDNA-positive and CEA were all related to the worse long-term survival of patients. In order to eliminate the interaction between these parameters and reduce the collinearity of the parameters, the backward likelihood ratio method was adopted to incorporate these parameters into the multivariate analysis model. Multivariate analysis further confirmed that tumor size (HR = 1.917; CI = 1.163–3.158; p = 0.011) and ^{mut}KRAS ctDNA (HR = 3.191; CI = 2.057–4.950; p < 0.001) were independent risk factors for poor prognosis and survival of patients (Table 2 & Figure 3).

Preoperative combining ^{mut}KRAS ctDNA & imaging to predict early recurrence

At a median follow-up of 38 months, the median OS of the enrolled patients was 26 months. Among them, the median survival time of ^{mut}KRAS ctDNA positive group, ^{mut}KRAS ctDNA negative group, clinical stage I/IIA group and IIB/III group were 14, 37, 30 and 15 months, respectively. Within the positive group only three (3/32, 9.4%) patients were alive at 3 years, while within the negative group 27.4% (20/73) of patients were still alive at that time.

According to the preoperative status of ^{mut}KRAS ctDNA and clinical stage, we performed a comprehensive scoring system in resectable patients, which set the positive or high stage as 1 point, dividing into three grades of 0 (negative and low stage), 1 (positive or high stage) and 2 (positive and high stage) points. The combined scores of stage IIB/III and positive showed a strong correlation with early recurrence or OS which was shorter compared with it for the patients with stage IIB/III or positive alone (Figure 4).

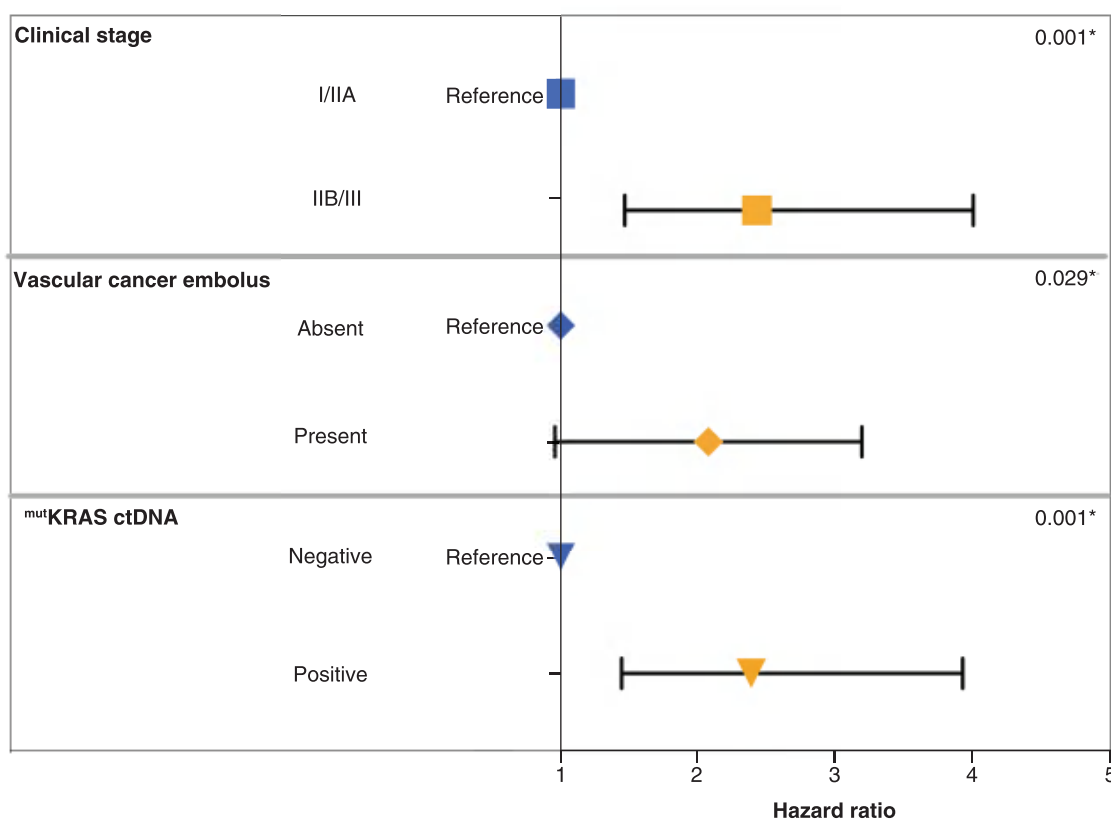


Figure 2. Clinical stage, vascular cancer embolus and ^{mut}KRAS ctDNA were independent predictors of 1-year relapse-free survival. The forest map of multivariate regression analysis. The x-axis represents hazard ratio.

*p-value < 0.05.

ctDNA: Circulating tumor DNA.

Discussion

In clinical practice, we found significant differences in the prognosis of patients with PDAC who underwent radical resection. Even if patients at the same stage undergo radical resection, some patients experience rapid relapse and progression. According to previous reports, we set the time threshold for early recurrence at 1 year [3]. About 43.8% (46/105) of patients experienced early recurrence after radical resection. The treatment strategy of PDAC is mainly based on imaging and intraoperative anatomy. Malignant tumors are highly heterogeneous, and even the morphology and cell composition displayed between different regions of the same tumor tissue are significantly different [13,14]. The heterogeneity may not be fully reflected in morphologically based diagnosis [15]. This may explain different outcomes for different patients in the same stage. Currently, there is a lack of biomarkers that predict early recurrence in patients with resectable PC. ctDNA is a noninvasive 'liquid biopsy' that can detect genetic mutations in patients and enable real-time monitoring of disease progression and treatment effects [16]. This study evaluated factors that may affect early postoperative recurrence in 105 patients, including basic patient information, laboratory tests and liquid biopsy (^{mut}KRASctDNA), imaging and postoperative pathological results. Among them, age, gender and tumor node metastasis stage (TNM stage) are common risk factors for tumor prognosis. Studies have shown that the clinical stage and pathological stage of resectable PC are poorly correlated [17]. Therefore, we include both the preoperative clinical stage and postoperative pathological stage of the patient into the risk factors that may affect the prognosis. At the same time, tumor markers CA19-9, CEA and CA24-2 that reflect tumorigenesis and progression were included [18]. In this study, all parameters were incorporated in univariate and multivariate COX risk regression models, and the results showed that the preoperative clinical stage and ^{mut}KRAS ctDNA of resectable patients were independent risk factors for early postoperative recurrence. In tumor markers, we did not find that preoperative CA19-9, CEA and CA24-2 were independent risk factors after PDAC radical surgery. As we all know, CA19-9 is the first choice biomarker for the diagnosis and prognosis of PC in clinical practice

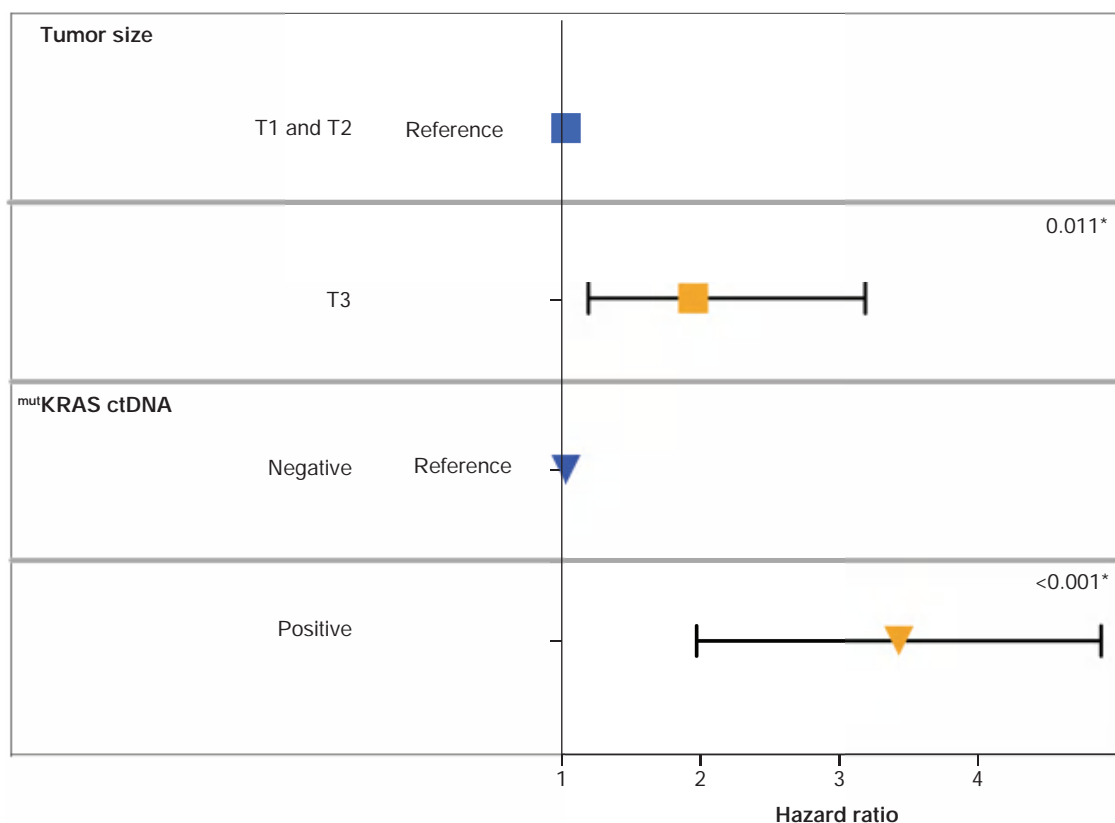


Figure 3. The independent predictors of overall survival. The forest map of multivariate regression analysis. The x-axis represents hazard ratio.
*p-value less than 0.05.

due to its high specificity (90%) and sensitivity (70–90%) [19]. Published research confirms that 6.9% of patients lack the Lewis antigen and staging has different effects on CA199 [20]. The population for this study was mainly relatively early PDAC patients, whose CA199 was less sensitive than late PDAC [21]. These characteristics limit the ability of CA19-9 as an independent and stable prognostic biomarker of resectable PDAC. In some studies, CEA have been confirmed to be related to the prognosis of PC, and have gradually been accepted as tumor biomarkers that should be routinely detected after CA199 [5,22]. The role of preoperative CEA as a prognostic risk factor for patients undergoing radical resection of PDAC may vary with different patient cohort sizes or different stages.

Interestingly, in our study, the early recurrence of postoperative patients was related to preoperative ^{mut}KRAS ctDNA, clinical stage and postoperative pathology (vascular cancer embolus). In addition, the long-term survival of patients with radical PDAC was closely related to ^{mut}KRAS ctDNA and tumor size. PDAC is a multi-step, multi-gene involved complex process involving mutations in multiple genes, such as *CDKN2A*, *SMAD4*, *TP53* or *KRAS* [23–25]. *KRAS* point mutations in somatic cells can lead to cell division disorders and promote tumorigenesis. It is common in PC (95%), lung cancer (50%) and colon cancer (40–50%), which often associated with poor prognosis [11,26,27]. ^{mut}KRAS ctDNA can serve as a liquid biopsy, which can give new insight into the tumor progress and the prognosis in PC patients [28–30]. In this study, we focused on the use of preoperative status of ^{mut}KRAS ctDNA to screen the patient population who may relapsed early after surgery. This part of the population may benefit from preferential use of neoadjuvant chemotherapy. In terms of staging, we divided patients into two groups based on the presence or absence of lymph node metastasis and compared the survival of these two groups. This classification has proven to be more reasonable for prognostic grouping of patients [31]. In our study, the consistency between clinical staging and postoperative pathological staging was poor, which further verified the previous research results [17]. Our results showed that clinical staging was more beneficial in assessing postoperative recurrence.

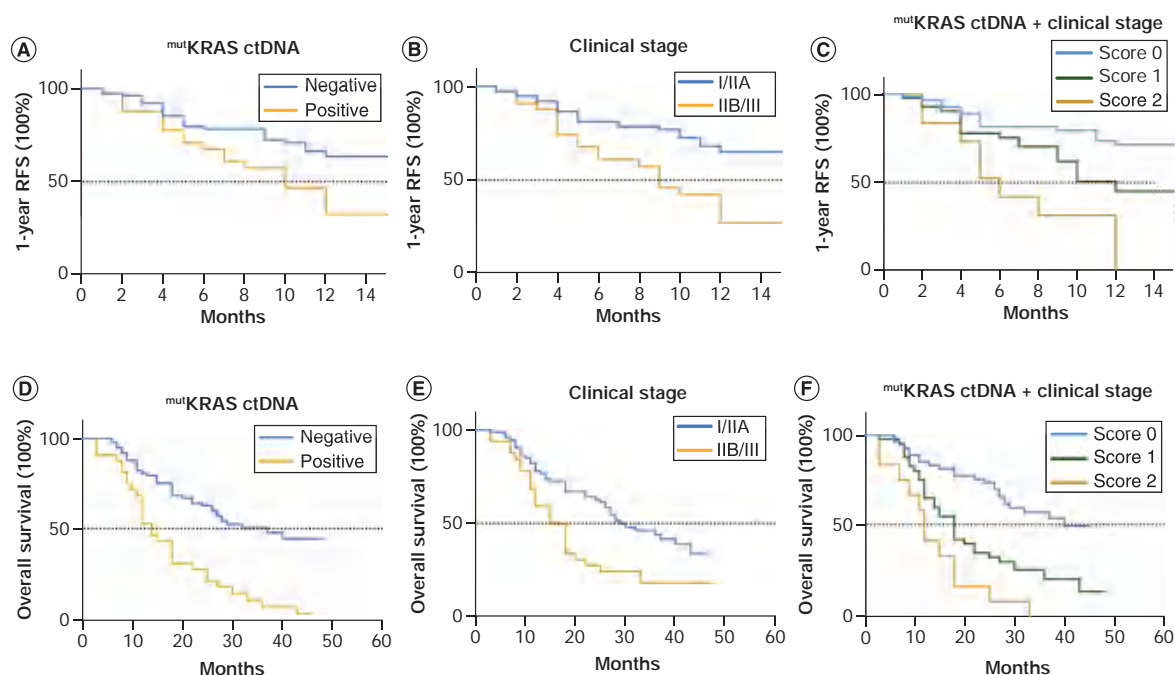


Figure 4. Kaplan-Meier method showed the 1-year relapse free survival and overall survival in patients with resectable pancreatic cancer based on the stratification of $mutKRAS$ ctDNA, clinical stage and the scoring system. Kaplan-Meier method showed the 1-year RFS curves of 105 patients with resectable pancreatic cancer based on the stratification of (A) $mutKRAS$ ctDNA, (B) clinical stage and (C) the scoring system. Kaplan-Meier method showed OS curves based on the stratification of (D) $mutKRAS$ ctDNA, (E) clinical stage and (F) the scoring system. ctDNA: Circulating tumor DNA.

Zhang *et al.* proposed to add liquid biopsy (B) based on TNM staging to develop TNMB staging system that makes cancer diagnosis, treatment and prognosis of individual patients more accurate, but no population-based cohort study has yet been used to verify this hypothesis [15]. In this study, $mutKRAS$ ctDNA and TNM staging played an important role in the early recurrence prediction of patients with resectable PDAC. After combining the two indicators, patients with higher scores were more likely to have early recurrence after surgery, and their prognosis was worse. This will accurately stratify the risk of prognosis in resectable patients with same clinical stage. Therefore, it was recommended to combine TNM staging and liquid biopsy before surgery to screen out patients who may be prone to early recurrence after surgery.

Several limitations exist in this study. First, this study evaluated the prognostic stratification of patients with resectable PDAC. The stage III patients included in this study were only a part of patients who have not invaded the blood vessels, and they were not representative of all stage III patients, which may cause partial bias of results. It should also be noted that because $KRAS$ mutations are almost always limited to codon 12 (G12A, G12V, G12S, G12R, G12C, G12D), followed by codon 13 (mainly G13D) [32,33], although the kit used to detect $KRAS$ in this study was economical and convenient, only these main seven mutation sites were detected, which may cause low detection rates. In addition, whether patients with possible early recurrence screened out before surgery will benefit from neoadjuvant therapy needs to be further verified by expanding the cohort size in the future.

Conclusion

In this study, the results confirmed that the scoring system, combining preoperative $mutKRAS$ ctDNA and TNM staging, can further accurately predicted early postoperative recurrence in resectable patients with PDAC. In assessing postoperative recurrence, the clinical stage was superior to the pathological stage. In short, the cohort of patients with resectable PDAC should be expanded, and multi-center prospective studies should be conducted in the future to screen patients who are prone to early recurrence and metastasis after radical surgery. For these patients with poor tumor biology or occult metastasis, we can try to carry out preoperative neoadjuvant chemotherapy

related clinical trials in the future to provide new evidence for whether patients are suitable for preoperative neoadjuvant chemotherapy.

Future perspective

In recent years, neoadjuvant therapy has become a new treatment method for PC. At present, the evaluation of neoadjuvant therapy for PC usually adopts retrospective research analysis, which may cause bias in the selection of research subjects. The main goal of future research is to determine a biomarker to exclude patients who cannot benefit from direct resection by preoperative screening. These patients are expected to undergo neoadjuvant treatment before surgery. Using individualized precision medicine methods, ideally, some resectable patients will be screened into the early postoperative recurrence risk group before surgery and undergo neoadjuvant treatment to rule out the resectable patients with undetectable occult metastases or rapid recurrence, so as to better choose patients. Future research should consider performing imaging combined with liquid biopsy (^{mut}KRAS ctDNA) indicators for patients before surgery, so as to more accurately perform individualized assessment of patients and select treatment options.

Summary points

- The combination of liquid biopsy (^{mut}KRAS ctDNA) and imaging before surgery can be used to screen patients with resectable pancreatic cancer with occult metastasis or rapid recurrence.
- About 43.8% (46/105) of patients experienced early recurrence after radical resection.
- The clinical stage and pathological stage of patients with radical resection are not consistent.
- Preoperative clinical staging is more conducive to evaluating postoperative recurrence than pathological staging.
- Both preoperative ^{mut}KRAS ctDNA and clinical stage are independent risk factors for early recurrence in resectable patients.
- In the comprehensive scoring system, the higher the score, the higher the risk of early recurrence and the worse the prognosis.
- Currently, there is a lack of clinical biomarkers for screening beneficiaries of neoadjuvant chemotherapy.
- Clinically, we should consider applying a comprehensive scoring system to screen out resectable patients with occult metastasis or rapid relapse before surgery and neoadjuvant chemotherapy should be given priority.

Author contributions

S Li and X Li contributed to the research experiments. Y Xu participated in the data acquisition. S Li, G Zhang and X Li contributed to the data analysis and interpretation. G Zhang and X Li contributed to the statistical analysis. S Li contributed to the drafting of manuscript. XB Chen participated in the revision of this manuscript and the formulation of technical standards. H Ren contributed to the manuscript revision and the study supervision.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that this study was approved by the Ethics Committee of Tianjin Cancer Institute and Hospital and have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, all participants involved signed the informed consent.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J. Clin.* 70(1), 7–30 (2020).
2. Kommalapati A, Tella SH, Goyal G, Ma WW, Mahipal A. Contemporary management of localized resectable pancreatic cancer. *Cancers (Basel)* 10(1), 24 (2018).

3. Groot VP, Gemenetzis G, Blair AB *et al.* Defining and predicting early recurrence in 957 patients with resected pancreatic ductal adenocarcinoma. *Ann. Surg.* 269(6), 1154–1162 (2019).
4. Hadano N, Murakami Y, Uemura K *et al.* Prognostic value of circulating tumour DNA in patients undergoing curative resection for pancreatic cancer. *Br. J. Cancer* 115(1), 59–65 (2016).
5. Ni XG, Bai XF, Mao YL *et al.* The clinical value of serum CEA, CA19-9, and CA242 in the diagnosis and prognosis of pancreatic cancer. *Eur. J. Surg. Oncol.* 31(2), 164–169 (2005).
6. Jung K, Fleischhacker M, Rabien A. Cell-free DNA in the blood as a solid tumor biomarker—a critical appraisal of the literature. *Clin. Chim. Acta* 411(21–22), 1611–1624 (2010).
7. Chu D, Paoletti C, Gersch C *et al.* ESR1 mutations in circulating plasma tumor dna from metastatic breast cancer patients. *Clin. Cancer Res.* 22(4), 993–999 (2016).
8. El Messaoudi S, Moulriere F, Du Manoir S *et al.* Circulating DNA as a strong multimarker prognostic tool for metastatic colorectal cancer patient management care. *Clin. Cancer Res.* 22(12), 3067–3077 (2016).
9. Camps C, Jantus-Lewintre E, Cabrera A *et al.* The identification of KRAS mutations at codon 12 in plasma DNA is not a prognostic factor in advanced non-small cell lung cancer patients. *Lung Cancer* 72(3), 365–369 (2011).
10. Iqbal S, Vishnubhatla S, Raina V *et al.* Circulating cell-free DNA and its integrity as a prognostic marker for breast cancer. *Springerplus* 4, 265 (2015).
11. Bournet B, Buscail C, Muscari F, Cordelier P, Buscail L. Targeting KRAS for diagnosis, prognosis, and treatment of pancreatic cancer: hopes and realities. *Eur. J. Cancer* 54, 75–83 (2016).
12. Karapetis CS, Khambata-Ford S, Jonker DJ *et al.* K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N. Engl. J. Med.* 359(17), 1757–1765 (2008).
13. Gerlinger M, Rowan AJ, Horswell S *et al.* Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* 366(10), 883–892 (2012).
14. Zhang J, Fujimoto J, Zhang J *et al.* Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. *Science* 346(6206), 256–259 (2014).
15. Yang M, Forbes ME, Bitting RL *et al.* Incorporating blood-based liquid biopsy information into cancer staging: time for a TNMB system? *Ann. Oncol.* 29(2), 311–323 (2018).
16. Frenel JS, Carreira S, Goodall J *et al.* Serial next-generation sequencing of circulating cell-free DNA evaluating tumor clone response to molecularly targeted drug administration. *Clin. Cancer Res.* 21(20), 4586–4596 (2015).
17. Mirkin KA, Greenleaf EK, Hollenbeak CS, Wong J. Correlation of clinical and pathological staging and response to neoadjuvant therapy in resected pancreatic cancer. *Int. J. Surg.* 52, 221–228 (2018).
18. Chang JC, Kundranda M. Novel diagnostic and predictive biomarkers in pancreatic adenocarcinoma. *Int. J. Mol. Sci.* 18(3), 667 (2017).
19. Duffy MJ, Sturgeon C, Lamerz R *et al.* Tumor markers in pancreatic cancer: a European Group on tumor markers (EGTM) status report. *Ann. Oncol.* 21(3), 441–447 (2010).
20. Capello M, Bantis LE, Scelo G *et al.* Sequential validation of blood-based protein biomarker candidates for early-stage pancreatic cancer. *J. Natl Cancer Inst.* 109(4), (2017).
21. Bilici A. Prognostic factors related with survival in patients with pancreatic adenocarcinoma. *World J. Gastroenterol.* 20(31), 10802–10812 (2014).
22. Luo G, Liu C, Guo M *et al.* Potential biomarkers in lewis negative patients with pancreatic cancer. *Ann. Surg.* 265(4), 800–805 (2017).
23. Hong SM, Park JY, Hruban RH, Goggins M. Molecular signatures of pancreatic cancer. *Arch. Pathol. Lab. Med.* 135(6), 716–727 (2011).
24. Waddell N, Pajic M, Patch AM *et al.* Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* 518(7540), 495–501 (2015).
25. Di Magliano MP, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. *Gastroenterology* 144(6), 1220–1229 (2013).
26. Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. *Cancer Res.* 72(10), 2457–2467 (2012).
27. Jancik S, Drabek J, Radzich D, Hajdych M. Clinical relevance of KRAS in human cancers. *J. Biomed. Biotechnol.* 2010, 150960 (2010).
28. Kruger S, Heinemann V, Ross C *et al.* Repeated mutKRAS ctDNA measurements represent a novel and promising tool for early response prediction and therapy monitoring in advanced pancreatic cancer. *Ann. Oncol.* 29(12), 2348–2355 (2018).
29. Bernard V, Kim DU, San Lucas FA *et al.* Circulating nucleic acids are associated with outcomes of patients with pancreatic cancer. *Gastroenterology* 156(1), 108–118 e104 (2019).
30. Pietrasz D, Pecuchet N, Garlan F *et al.* Plasma circulating tumor DNA in pancreatic cancer patients is a prognostic marker. *Clin. Cancer Res.* 23(1), 116–123 (2017).

31. Kamarajah SK, Burns WR, Frankel TL, Cho CS, Nathan H. Validation of the American Joint Commission on Cancer (AJCC) 8th edition staging system for patients with pancreatic adenocarcinoma: a surveillance, epidemiology and end results (SEER) analysis. *Ann. Surg. Oncol.* 24(7), 2023–2030 (2017).
32. Maire F, Micard S, Hammel P *et al.* Differential diagnosis between chronic pancreatitis and pancreatic cancer: value of the detection of KRAS2 mutations in circulating DNA. *Br. J. Cancer* 87(5), 551–554 (2002).
33. Alcaide M, Cheung M, Bushell K *et al.* A novel multiplex droplet digital PCR assay to identify and quantify KRAS mutations in clinical specimens. *J. Mol. Diagn.* 21(2), 214–227 (2018).

