Postoperative serum CEA and CA125 levels are supplementary to perioperative CA19-9 levels in predicting surgical outcomes of pancreatic ductal adenocarcinoma.

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Funding: This work was supported by grants from the National Natural Science Foundation of China (81472670, 81172005, 81402397, 81402398, and 81172276), the National Natural Science Foundation of Shanghai (14ZR1407600), and the “Yang-Fan” Plan for Young Scientists of Shanghai (14YF1401100). The funding agencies had no role in study design, data collection and analyses, decision to publish, or preparation of the manuscript.

Running title: Postoperative CA125 and CEA supplementary to CA199

ABSTRACT

Background: CA19-9 has been a well-established marker to monitor disease status following resection of pancreatic cancer. However, few serum markers have been reported to improve the prognostic ability of postoperative CA19-9, especially in patients with normal postoperative CA19-9.

Methods: A total of 353 patients with pancreatic ductal adenocarcinoma treated by radical resection were retrospectively reviewed, and a prospective cohort including 142 patients with resectable pancreatic head carcinoma was analyzed as a validation cohort. Perioperative CA19-9 and postoperative serum markers (CEA, CA242, CA72-4, CA50, CA125, CA153, and AFP) were investigated.

Results: Patients with postoperative normalization of CA19-9 had an improved survival times (RFS: 11.9 months; OS: 22.5 months) compared to those with decreased but still elevated postoperative CA19-9 (RFS: 6.8 months, $P < 0.001$; OS: 13.5 months, $P < 0.001$) or those with increased postoperative CA19-9 (RFS: 3.5 months, $P < 0.001$; OS: 7.9 months, $P < 0.001$), which was similar to those with consistently normal CA19-9 during perioperative periods (RFS: 10.6 months, $P = 0.799$; OS: 24.1 months, $P = 0.756$). Normal postoperative CA19-9 levels were an independent indicator for a positive outcome after surgery, regardless of preoperative CA19-9 levels. Elevated postoperative CEA and CA125 were further identified as independent risk factors for patients with normal postoperative CA19-9, while elevated postoperative CA125 and nondecreased postoperative CA19-9 were independent prognostic markers for patients with elevated postoperative CA19-9.

Conclusion: The postoperative monitoring of CEA and CA125 provided prognostic significance to the measurement of CA19-9 in pancreatic cancer following resection.

Keywords: postoperative CA19-9, postoperative CEA, postoperative CA125, serum tumour markers, prognosis, pancreatic cancer

INTRODUCTION

Pancreatic cancer has a poor prognosis, with a 5-year survival rate less than 5%.¹ Radical resection is the only treatment option for long-time survival.² However, the 5-year survival percentage after radical surgery is still approximately 20%: a high postoperative recurrence rate is the main reason for the short survival time.³ A marker system that could accurately predict postoperative recurrence and survival would therefore be informative and useful for the selection of subsequent therapies.

Carbohydrate antigen 19-9 (CA19-9) is the only well-established serum marker to predict the prognosis of pancreatic cancer.^{4, 5} Preoperative serum CA19-9 was shown to be positively associated with tumor burden, and its elevation predicted early recurrence and short survival.⁶ However, patients could still have improved survival if the elevated serum CA19-9 decreased during postoperative periods.^{7, 8} Postoperative serum CA19-9 therefore provides advantages compared with preoperative serum CA19-9 in the prediction of recurrence and survival following radical resection.^{8, 9} The monitoring of serum CA19-9 levels and their postoperative alterations are crucial in the follow-up of pancreatic cancer after resection. However, serum CA19-9 alone is not sufficient to predict patient survival, especially for patients with normal postoperative serum CA19-9. False negatives and false positives also decrease its prognostic value. Thus, additional serum markers are urgently needed to improve the prognosis using serum CA19-9.

Among the eight serum markers usually used in gastrointestinal cancer (CA19-9, CA242, CA50, CEA, CA125, CA724, CA153, and AFP), baseline serum CEA and CA125 improved the diagnosis and prognosis prediction of patients with serum CA19-9.^{10, 11} In patients with extremely high serum CA19-9 ($\geq 1,000$ U/mL), serum CEA and CA125 levels

preoperatively helped to identify a subgroup of patients with poor outcome to surgery.¹⁰ Serum CEA and CA125 at baseline were also proposed as potential markers to improve the diagnosis of pancreatic cancer in CA19-9 seronegative patients (Lewis negative).¹¹ In this study, we determined whether these serum markers could postoperatively improve the prediction of prognosis based on postoperative CA19-9 levels.

MATERIALS AND METHODS

Patients

From January 2010 to January 2014, a total of 402 patients who received radical surgery for pancreatic ductal adenocarcinoma at Shanghai Cancer Center were retrospectively studied. A total of 353 patients were selected based on the following exclusion criteria: (a) incomplete clinicopathological and follow-up data (n = 7), (b) any antitumor treatment before surgery (n = 34), and (c) any history of malignant tumor (n = 8). All clinicopathological and outcome data of patient were registered in the pancreatic cancer database of our institution as previously described.^{10, 12, 13} A prospective dataset from a clinical trial (ClinicalTrials.gov; identifier: NCT01731821) at our institution was previously reported¹³ and retrospectively analyzed as a validation cohort. The dataset contained 142 patients with pancreatic head carcinoma who underwent pancreaticoduodenectomy between November 2012 and December 2014. This study was reviewed and approved by the Human Research Ethics Committee of Shanghai Cancer Center and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all patients according to the committee's guidelines.

Patient follow-up

All patients were routinely followed until death for disease progression by a standardized follow-up protocol as described in our previous study.^{10, 12, 13} Briefly, each patient was closely monitored every 3 months or more often by clinical and laboratory examinations, including the eight serum markers mostly used in gastrointestinal cancer (CA19-9, CA242, CA50, CEA, CA125, CA724, CA153 and AFP). Image examinations (computed tomography scans, magnetic resonance imaging, bone scans, and positron emission tomography/computed tomography) were selectively conducted when local recurrence or metastatic disease was suspected. Overall survival (OS) was defined as the interval from the time at which pancreatic adenocarcinoma was pathologically confirmed until death or the last follow-up visit. Recurrence free survival (RFS) was defined as the interval from the time at which pancreatic adenocarcinoma was pathologically confirmed until tumor recurrence or the last follow-up visit.

Serum tumor marker measurements

The levels of eight serum tumor markers (CA19-9, CEA, CA242, CA72-4, CA50, CA125, CA153 and AFP) were determined using radioimmunoassay kits from Abbott Laboratories (Chicago, IL, USA). Preoperative levels of these tumor markers were determined within 1 week before resection. A postoperative level of a serum marker was defined as the lowest level within 3 months after surgery. The normal upper limits of serum tumor markers were adopted as follows: CA19-9 (37 U/mL), CEA (5.2 ng/mL), CA242 (20 U/mL), CA72-4 (6.9 U/mL), CA50 (25 U/mL), CA125 (35 U/mL), CA153 (25 U/mL), and AFP (10 ng/mL).

Statistical analysis

Qualitative variables were analyzed by Pearson's χ^2 or Fisher's exact test. Kaplan-Meier curves were used to analyze the distribution of OS and RFS by groups. Comparison of patient survival among subgroups was analyzed by the

log-rank test. Independent association of all clinicopathological features with survival was evaluated by multivariate survival analyses using Cox proportional hazard models, and the strength of association was measured by the hazard ratio (HR). All statistical analyses were performed with SPSS software, version 13.0 for Windows (SPSS, Chicago, IL, USA) and were two-sided. *P*-values < 0.05 were considered statistically significant.

RESULTS

Clinicopathological characteristics and survival

Detailed clinicopathological characteristics of 353 patients are summarized in Table 1. The median age of all patients was 61 years (range, 33–82 years). The mean tumor size was 4.13 ± 1.60 cm. Metastasis to lymph nodes was pathologically confirmed in 162 patients. Sixty-six patients had pathological vascular invasion and 301 patients had nerve invasion. A majority of patients [82.4% (291/353)] received adjuvant chemotherapy and 31.7% of patients (112/353) underwent adjuvant chemoradiotherapy. A total of 275 of 353 patients presented with a preoperative elevated level of serum CA19-9, and 151 patients presented with a postoperative elevated serum CA19-9 level. The levels of postoperative CA125, CEA, CA242, CA50, CA72-4, CA153, and AFP were elevated in 105 patients, 86 patients, 126 patients, 119 patients, 50 patients, 30 patients and 5 patients respectively. All patients were followed-up until May 2015. At last follow-up, 215 patients had died of the disease. The median OS time was 18.1 months, and the OS percentages at 1 and 3 years were 62.2% and 27.1% respectively. Tumor recurrence after surgery was detected in 286 patients. The median RFS time was 9.2 months, and the percentages of RFS at 1 and 3 years were 35.3% and 13.2% respectively.

An elevated level of postoperative serum CA19-9 was an independent risk factor for outcomes after radical resection

Preoperative serum CA19-9 levels were significantly associated with tumor size ($r = 0.157$, $P = 0.003$), whereas elevated postoperative CA19-9 serum levels were significantly associated with a large tumor size ($P = 0.019$), presence of lymph node metastasis ($P = 0.003$), and high tumor stage ($P = 0.011$). An elevated preoperative or postoperative level of serum CA19-9 was correlated with poor outcomes after surgery. The median times of RFS and OS were significantly shorter in patients with elevated levels of preoperative serum CA19-9 compared with those with normal levels (RFS, 8.0 versus 10.4 months, $P = 0.021$; OS, 16.7 versus 24.1 months, $P = 0.027$). Likewise, patients with elevated levels of postoperative serum CA19-9 were associated with a shorter median RFS times (5.3 versus 11.0 months, $P < 0.001$) and OS times (10.9 versus 22.5 months, $P < 0.001$) compared with those with normal levels. Using multivariate Cox regression analyses involving all the clinicopathological features showed that an elevated level of serum CA19-9, postoperatively but not preoperatively, was an independent risk factor for recurrence and survival (Table S1 and S2).

Patients with normalization of postoperative serum CA19-9 obtained a survival comparable to those with persistently normal perioperative serum CA19-9 levels

The comparison on the categorizations of preoperative and postoperative CA19-9 was performed (Table S3). A majority of (76/78) patients with normal preoperative levels of serum CA19-9 had normal postoperative levels of serum CA19-9. Whereas, among 275 patients with elevated preoperative levels of serum CA19-9, 210 patients showed decreased postoperative serum CA19-9 levels. Among patients with elevated preoperative levels of serum CA19-9, the median times of RFS and OS in patients with decreased serum CA19-9 levels were significantly longer than those for patients without a decrease (RFS, 10.0 versus 3.5 months, $P < 0.001$; OS, 19.3 versus 7.9 months, $P < 0.001$; Figure 1A and B). More importantly, among these 210 patients with decreased postoperative serum CA19-9 levels, 126 patients had decreased serum CA19-9 levels in the normal range whereas 84 patients still had elevated serum CA19-9 levels.

Subgroup Kaplan-Meier survival analyses showed that the median times of RFS and OS in patients whose serum CA19-9 levels decreased to the normal range were significantly longer than those in patients whose serum CA19-9 levels decreased but were still above the normal level (RFS, 11.9 versus 6.8 months, $P < 0.001$; OS, 22.5 versus 13.5 months, $P < 0.001$; Figure 1C and D). Notably, the median times of RFS and OS in patients who underwent a decrease in serum CA19-9 to normal levels were comparable to patients who consistently retained normal levels of serum CA19-9 from the preoperative to the postoperative periods (RFS, 11.9 versus 10.6 months, respectively, $P = 0.799$; OS, 22.5 versus 24.1 months, respectively, $P = 0.756$; Figure 1E and F).

Postoperative elevated levels of serum CA125 and CEA were independent risk factors for prognoses in patients with a normal level of postoperative serum CA19-9

To identify potential serum tumor markers that could further predict survival in patients with normal postoperative serum CA19-9 levels, the other seven commonly used serum markers were analyzed. Multivariate Cox regression analyses including all clinicopathologic features confirmed that elevated postoperative levels of serum CA125 and CEA were independent risk factors for both RFS and OS in patients with normal postoperative CA19-9 levels (Table 2). The median times of RFS were 7.0 and 7.5 months in patients with elevated levels of postoperative serum CA125 and CEA, respectively, which were significantly shorter than those with normal levels (12.1 and 12.0 months, $P = 0.004$ and $P = 0.001$, respectively; Figure 2A and C). Similarly, the median times of OS (16.8 and 14.0 months) in patients with elevated levels of postoperative serum CA125 and CEA were significantly poorer compared with those with normal levels (25.0 and 25.0 months, $P = 0.006$ and $P < 0.001$, respectively; Figure 2B and D). Among 202 patients with normal levels of postoperative CA19-9, 10 patients showed elevated levels of both postoperative serum CA125 and CEA, 50 patients had elevated normal levels of either postoperative serum CA125 or CEA, and 142 patients showed

normal levels of both postoperative serum CA125 and CEA. Kaplan-Meier analyses showed that patients with elevated levels of postoperative serum CA125 and CEA had the shortest median RFS time (4.8 months) and OS time (7.5 months), whereas patients with normal levels of postoperative serum CA125 and CEA had the longest median RFS time (12.1 months, $P < 0.001$; Figure 2E) and OS time (25.3 months, $P < 0.001$; Figure 2F).

An increase of postoperative serum CA19-9 and serum CA125 were independent risk factors for prognosis in patients with elevated postoperative serum CA19-9 levels

Multivariate Cox regression analyses of the seven serum markers at postoperative times, the changes of postoperative serum CA19-9 and all clinicopathologic features were performed in patients with elevated postoperative serum CA19-9

levels. A nondecrease of postoperative serum CA19-9 levels and an elevated level of postoperative serum CA125 were determined as independent risk factors for RFS and OS, respectively (Table 3). The median times of RFS and OS in patients with elevated postoperative levels of serum CA125 were significantly shorter than those without (RFS, 4.0 versus 6.6 months, $P < 0.001$; OS, 7.9 versus 12.8 months, $P < 0.001$; Figure 3A and B). In addition, the median times of RFS and OS in patients who underwent a nondecrease of postoperative serum CA19-9 levels were significantly shorter compared with those with a decrease of postoperative serum CA19-9 levels (RFS, 3.5 versus 6.8 months, $P < 0.001$; OS, 7.9 versus 13.5 months, $P < 0.001$; Figure 3C and D). A combination of these two markers was analyzed in 151 patients with an elevated level of postoperative serum CA19-9. Forty-two patients had elevated postoperative levels of serum CA125 and a nondecrease of postoperative serum CA19-9 levels, 48 patients had either elevated postoperative levels of serum CA125 or a nondecrease of postoperative serum CA19-9 levels, and 61 patients had both normal postoperative levels of serum CA125 and decreased postoperative serum CA19-9 levels. Subgroup survival analyses showed that patients who had elevated postoperative levels of serum CA125 and a nondecrease of postoperative serum

CA19-9 levels had the shortest median RFS times (2.8 months) and OS times (7.0 months), whereas patients with normal postoperative levels of serum CA125 and decreased postoperative serum CA19-9 levels had the longest median RFS times (6.8 months, $P < 0.001$; Figure 3E) and OS times (18.4 months, $P < 0.001$; Figure 3F).

The prognostic significance of postoperative serum CA19-9, CA125, and CEA were validated in a prospective dataset

We verified the prognostic role of postoperative serum levels of CA19-9, CA125, and CEA using independent prospective data from one of our clinical trials (ClinicalTrials.gov identifier: NCT01731821). Patients with a decrease in postoperative serum CA19-9 survived longer than those with an increase in postoperative serum CA19-9 (Figure S1A and B). Moreover, the median OS and RFS times in patients with decreased postoperative serum CA19-9 levels down to the normal range were longer than those in patients with a decrease of postoperative serum CA19-9 levels that were still above normal levels (Figure S1C and D). They were even comparable with the median times of OS and RFS in patients with serum CA19-9 levels consistently within the normal range (Figure S1E and F). Subgroup analyses further confirmed that elevated levels of postoperative serum CA125 and CEA were associated with better survival in patients with a normal level of postoperative serum CA19-9 (Figure S2A-F), whereas an elevated level of postoperative serum CA125 and an increase in postoperative serum CA19-9 level were associated with a poorer survival in patients with postoperative elevated serum CA19-9 levels (Figure S3A-F).

DISCUSSION

The survival of pancreatic cancer patients after surgery, even during early stages, is variable. It is therefore essential to find markers, especially preoperative and postoperative serum markers, to provide prognostic indicators that would help

identify patients who might benefit from an intensive therapy following surgery. In this study, we analyzed the prognostic value of perioperative serum CA19-9 levels that are most commonly used in pancreatic cancer.⁴ Although we found that preoperative and postoperative serum CA19-9 levels were both significantly associated with survival after pancreatectomy, a high level of postoperative, but not preoperative, serum CA19-9 level was an independent risk factor for poor surgical outcomes of patients (Table S1 and S2). And when we investigated the prognostic role of preoperative and postoperative serum CA19-9 levels by the exclusion of 22 patients with Lewis antigen negative, similar results were also observed (Table S4 and S5). Although they had elevated preoperative CA19-9 levels, patients with decreased postoperative CA19-9 levels experienced a significantly improved median survival time. These results were validated in a prospective cohort (NCT01731821), and were consistent with previous studies.^{8, 9} Accordingly, although we and others previously reported that extremely high baseline levels of CA19-9 were associated with a poor median survival of approximately 12 months, and no 5-year survival,^{8, 10, 14} Hartwig et al. still proposed that these patients should not be precluded from pancreatectomy.¹⁵ On this basis, we showed that a high percentage of patients with high baseline serum CA19-9 levels showed decreased CA19-9 levels after pancreatectomy with a significantly longer survival time, with more than half of the patients with a decrease in postoperative CA19-9 showing normalization of CA19-9 levels. The survival of these patients with normalization of postoperative CA19-9 levels was comparable with patients with consistently normal preoperative and postoperative CA19-9 levels. These results were consistent with previous reports,^{9, 16} and notably confirmed that a normal postoperative level of serum CA19-9 is an indicator for a positive outcome after surgery, regardless of high baseline levels of serum CA19-9.

Various studies reported different cutoff values for postoperative CA19-9 levels as a predictor of OS, including 200 U/mL by Ferrone et al.⁸ and 180 U/mL by Montgomery et al.⁹. The upper normal limit of 37 U/mL was suggested by

other studies as the best cutoff value for postoperative serum CA19-9 in predicting survival.^{14, 17} In the present study, 37 U/mL was also used as the cutoff value for postoperative CA19-9 levels because it is commonly used in clinical settings and conforms to a theoretical basis. High levels of CA19-9 reflect a heavy tumor burden. CA19-9 has a half-life of only 14 hours and its levels postoperatively return to normal levels within a few weeks when cancer cells are radically removed by surgery.¹⁸ The normalization in postoperative CA19-9 levels 3 months after surgery was largely the result of an effective reduction in tumor burden associated with the best survival. In contrast, postoperative levels of CA19-9 remained above the normal range or even increased, resulting from the remaining residual tumor or from disease recurrence, suggesting a worse surgical outcome.¹⁶ The normalization of postoperative CA19-9 levels is therefore a marker for a good response to radical surgery, while patients with elevated postoperative levels of postoperative CA19-9 require intensive adjuvant therapies such as chemoradiotherapy because of tumor residues or a tumor recurrence. It was supported by the different responses to chemoradiotherapy between postoperative CA19-9 subgroups. Adjuvant chemoradiotherapy significantly prolonged the survival times in patients with elevated postoperative CA19-9, but provided only a limited survival benefit in patients with normal postoperative CA19-9 (Figure S4). Thus, postoperative CA19-9 could serve as a surrogate marker for response to the intensive adjuvant chemoradiotherapy.

Most patients who had normal preoperative levels of serum CA19-9 had normal postoperative levels of serum CA19-9. Another subgroup of patients had elevated preoperative levels of serum CA19-9, but experienced decreased postoperative levels down to normal ranges. The monitoring of postoperative serum CA19-9 levels alone may have a limited prognostic value for these two groups of patients, especially for patients with steady and normal perioperative CA19-9 levels within the normal range. Additional markers were therefore needed to classify the survival of these patients. We showed that postoperative serum CEA and CA125 levels could help identify a subgroup of patients with

normalization of postoperative CA19-9 levels who had a higher risk of recurrence and mortality. This differs from previous studies showing the preoperative prognostic significance of serum CEA and CA125 levels in pancreatic cancer.¹⁰ Serum CEA is the second most used biomarker for pancreatic cancer. We previously showed that increases in CEA serum levels in patients with pancreatic cancer were independent of CA19-9, and its combination with CA19-9 could significantly enhance the prognostic value of CA19-9.¹⁰ Serum CA125 has been widely used as a biomarker for ovarian cancer. Its screening was recently reported to improve the early diagnosis of pancreatic cancer (even 2–3 years earlier), particularly in patients that were CA19-9 seronegative.¹⁹ We recently reported that CA125 had superior efficiency compared with CA19-9 in predicting the resectability of pancreatic cancer,²⁰ and further reported that baseline CA125 levels positively correlated with the metastasis-associated burden of pancreatic cancer.¹³ In another study, we proposed that baseline CEA and CA125 levels, together with extremely high levels of CA19-9 (> 1,000 U/mL), were able to better identify a subgroup of patients with poor response to radical resection.¹⁰ In CA19-9 seronegative patients (Lewis negative patients) with pancreatic cancer, baseline CEA and CA125 have the potential to be used as biomarkers for diagnosis and determination of the metastatic burden of pancreatic cancer.¹¹ Based upon these possibilities, we first identified postoperative CEA and CA125 serum levels as independent predictors for surgical survival in patients with normal levels of postoperative serum CA19-9. These levels could determine survival in patients who had normal postoperative CA19-9 serum levels. Simultaneous elevation of postoperative CA125 and CEA levels were associated with a significantly shorter median survival of only 7.5 months in patients who had normal postoperative levels of serum CA19-9, compared with 25.3 months in patients with normal postoperative CA125 and CEA and normalization of postoperative CA19-9. Thus, patients with simultaneously elevated postoperative CA125 and CEA levels need intensive therapy even if their postoperative serum CA19-9 levels are within the normal range.

We also identified elevated postoperative serum CA125 levels as an independent risk factor for survival in patients with increases in postoperative CA19-9 levels. A previous study reported that postoperative serum CEA levels could help determine survival, especially in patients with elevated postoperative CA19-9 levels.²¹ However, the present study used multivariate Cox regression analyses to show that postoperative serum CA125 levels were more important than postoperative serum CEA levels for prognosis of patients with elevated postoperative CA19-9 levels. A combination of elevated postoperative serum CA125 levels with the nondecrease of postoperative serum CA19-9 levels predicted the poorest survival with median RFS and OS times of only 2.8 and 6.8 months, respectively. We therefore propose that patients with elevated postoperative levels of serum CA19-9, especially those with elevated postoperative CA125 levels and nondecreased postoperative CA19-9, need urgent intensive adjuvant chemotherapy after surgery.

In summary, patients with postoperative normalization of CA19-9 levels had a good prognosis after pancreatectomy, which is comparable to patients with steady perioperative CA19-9 levels within the normal range. Postoperative normalization of CA19-9 could serve as a potential biomarker for a good response to surgery. Postoperative elevated serum CEA and CA125 levels were further identified as serum markers to identify high risk patients with normal postoperative serum CA19-9 for intensive adjuvant therapy. The follow-up examinations of CEA and CA125 levels in serum samples cost only RMB 87 yuan (about 13.1 dollars). But the results could help discriminate 29.7% of the patients (60/202) with high risk among the patients with normal postoperative CA19-9 levels. The results could also classify 59.6% of the patients (90/151) with worse survivals among the patients with elevated postoperative CA19-9 levels. Thus, the measurement of serum CEA and CA125 is inexpensive, convenient, and necessary for the monitoring of disease status and survival.

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ACKNOWLEDGEMENTS:

We thank Huanyu Xia for assistance in collecting patient data.

FIGURES LEGENDS

Figure 1. Recurrence-free survival (RFS) and overall survival (OS) of patients with elevated preoperative CA19-9 were stratified by decrease/nondecrease of postoperative CA19-9 (A-B). Patients with postoperative normalization of CA19-9 had the longest RFS and OS times compared to those with decreased but still elevated postoperative CA19-9 or those with nondecreased postoperative CA19-9 (C-D), which was similar to those with consistently normal CA19-9 during perioperative periods (E-F).

Figure 2. Recurrence-free survival (RFS) and overall survival (OS) of patients were stratified by postoperative CA125 (A-B) and CEA (C-D) in patients with normal postoperative CA19-9. Patients with elevated postoperative CA125 and CEA had the shortest RFS and OS times in patients with normal postoperative CA19-9 (E-F).

Figure 3. Recurrence-free survival (RFS) and overall survival (OS) of patients were stratified by postoperative CA125 (A-B) and postoperative nondecrease of CA19-9 (C-D) in patients with elevated postoperative CA19-9. Patients with elevated postoperative CA125 and nondecrease of postoperative CA19-9 had the shortest RFS and OS times in patients with elevated postoperative CA19-9 (E-F).

TABLES

Table 1. Clinicopathological characteristics of patients with pancreatic ductal carcinoma following resection

Features	n = 353
Age [years, median (range)]	61 (33 - 82)
Gender (male/female)	201/152
Tumour location (head/body, tail)	203/150
Preoperative CA19-9 (> 37 versus ≤ 37 U/mL)	275/78
Postoperative CA19-9 (> 37 versus ≤ 37 U/mL)	151/202
Tumour size (cm, mean ± SD)	4.13 ± 1.60
Lymph node metastasis (yes/no)	162/191
TNM stage (I/IIA/IIB)	86/105/162
Differentiation (well/moderate/poor)	225/128
Neural invasion (yes/no)	301/52
Microvascular invasion (yes/no)	66/287
Adjuvant chemotherapy (yes/no)	291/62
Adjuvant chemoradiotherapy (yes/no)	112/241
Preoperative bilirubin (< 2 mg/dL / ≥ 2 mg/dL)	247/106

Table 2. Multivariate cox regression analyses of overall survival and recurrence-free survival in patients with normal postoperative serum CA19-9 levels

Serum Markers	Overall Survival						Recurrence-Free Survival					
	Univariate Analyses			Multivariate Analyses			Univariate Analyses			Multivariate Analyses		
	HR	95 % CI	P	HR	95 % CI	P	HR	95 % CI	P	HR	95 % CI	P
Age [years, median (range)]	0.929	0.626-1.379	0.715			NS	0.957	0.695-1.319	0.789			NS
Gender (male/female)	1.311	0.870-1.977	0.196			NS	1.010	0.729-1.399	0.954			NS
Tumour location (head/body, tail)	0.761	0.502-1.154	0.198			NS	0.709	0.504-0.999	0.049			NS
Tumour size (cm, mean $\pm$ SD)	1.442	0.964-2.155	0.075			NS	1.204	0.872-1.660	0.259			NS
T stage (T1-2/T3)	1.791	1.162-2.763	0.008			NS	1.447	1.033-2.027	0.032	1.566	1.105-2.220	0.012
Lymph node metastasis (yes/no)	1.647	1.107-2.450	0.014			NS	1.252	0.905-1.734	0.175			NS
TNM stage (I/IIA/IIB)	1.523	1.179-1.967	0.001			NS	1.194	0.979-1.457	0.080			NS
Differentiation (well/moderate/poor)	1.219	0.806-1.842	0.348			NS	1.051	0.747-1.479	0.776			NS
Neural invasion (yes/no)	1.089	0.637-1.861	0.755			NS	1.214	0.764-1.927	0.412			NS
Microvascular invasion (yes/no)	1.303	0.773-2.197	0.321			NS	1.153	0.745-1.785	0.523			NS
Preoperative bilirubin (< 2 mg/dL / $\geq$ 2 mg/dL)	1.384	0.925-2.070	0.114			NS	1.321	0.949-1.840	0.099			NS
Adjuvant Chemotherapy (yes/no)	0.504	0.313-0.811	0.005			NS	0.789	0.513-1.214	0.281			NS
Adjuvant Chemoradiotherapy (yes/no)	0.685	0.448-1.046	0.080	0.619	0.401-0.955	0.030	0.761	0.540-1.072	0.119			NS
Postoperative CA125 (> 35 versus $\leq$ 35 U/mL)	1.912	1.194-3.061	0.007	1.900	1.164-3.101	0.010	1.740	1.181-2.565	0.005	1.699	1.151-2.508	0.008
Postoperative CEA (> 5.2 versus $\leq$ 5.2 ng/mL)	2.654	1.643-4.289	< 0.001	2.146	1.296-3.553	0.003	2.036	1.328-3.120	0.001	1.990	1.296-3.057	0.002
Postoperative CA242 (> 20 versus $\leq$ 20 U/mL)	1.585	0.823-3.052	0.168			NS	1.435	0.795-2.592	0.231			NS
Postoperative CA50 (> 25 versus $\leq$ 25 U/mL)	1.012	0.488-2.099	0.975			NS	0.809	0.411-1.590	0.538			NS
Postoperative CA72-4 (> 6.9 versus $\leq$ 6.9 U/mL)	1.609	0.927-2.794	0.091			NS	1.861	1.134-3.052	0.014			NS
Postoperative CA153 (> 25 versus $\leq$ 25 U/mL)	1.348	0.547-3.321	0.516			NS	1.653	0.810-3.377	0.167			NS
Postoperative AFP (> 10 versus $\leq$ 10 ng/mL)	0.049	0-554871	0.716			NS	5.410	0.741-39.527	0.096			NS

Table 3. Multivariate cox regression analyses of overall survival and recurrence-free survival in patients with elevated postoperative serum CA19-9 levels

Serum Markers	Overall Survival						Recurrence-Free Survival					
	Univariate Analyses			Multivariate Analyses			Univariate Analyses			Multivariate Analyses		
	HR	95 % CI	P	HR	95 % CI	P	HR	95 % CI	P	HR	95 % CI	P
Age [years, median (range)]	1.209	0.838-1.745	0.310				1.260	0.900-1.766	0.179			
Gender (male/female)	1.029	0.713-1.487	0.877				1.150	0.819-1.614	0.420			
Tumour location (head/body, tail)	0.997	0.686-1.448	0.986				0.960	0.681-1.353	0.815			
Tumour size (cm, mean $\pm$ SD)	1.834	1.234-2.727	0.003	1.693	1.132-2.532	0.010	1.673	1.168-2.397	0.005	1.544	1.064-2.239	0.022
T stage (T1-2/T3)	1.112	0.747-1.657	0.601				1.088	0.764-1.549	0.640			
Lymph node metastasis (yes/no)	1.613	1.108-2.348	0.013				1.484	1.054-2.089	0.024			
TNM stage (I/IIA/IIIB)	1.364	1.058-1.759	0.017				1.269	1.013-1.590	0.038			
Differentiation (well/moderate/poor)	1.298	0.894-1.886	0.171				1.238	0.879-1.744	0.222			
Neural invasion (yes/no)	1.024	0.594-1.766	0.932				1.004	0.610-1.651	0.989			
Microvascular invasion (yes/no)	1.168	0.762-1.790	0.477				1.186	0.800-1.758	0.395			
Preoperative bilirubin (< 2 mg/dL / $\geq$ 2 mg/dL)	0.876	0.566-1.357	0.554				0.897	0.600-1.341	0.596			
Adjuvant Chemotherapy (yes/no)	0.595	0.375-0.943	0.027				0.745	0.482-1.153	0.187			
Adjuvant Chemoradiotherapy (yes/no)	0.509	0.334-0.775	0.002	0.541	0.351-0.835	0.006	0.524	0.359-0.764	0.001	0.473	0.317-0.706	< 0.001
Postoperative CA125 (> 35 versus $\leq$ 35 U/mL)	2.553	1.741-3.743	< 0.001	2.077	1.386-3.112	< 0.001	2.081	1.476-2.934	< 0.001	1.564	1.081-2.262	0.018
Postoperative CEA (> 5.2 versus $\leq$ 5.2 ng/mL)	2.150	1.477-3.130	< 0.001			NS	1.563	1.102-2.215	0.012			NS
Postoperative CA242 (> 20 versus $\leq$ 20 U/mL)	1.611	1.040-2.498	0.033			NS	1.206	0.820-1.772	0.342			NS
Postoperative CA50 (> 25 versus $\leq$ 25 U/mL)	1.823	1.195-2.781	0.005	1.849	1.203-2.843	0.005	1.651	1.129-2.414	0.010			NS
Postoperative CA72-4 (> 6.9 versus $\leq$ 6.9 U/mL)	1.396	0.883-2.207	0.154			NS	1.561	1.020-2.390	0.040			NS
Postoperative CA153 (> 25 versus $\leq$ 25 U/mL)	1.609	0.982-2.637	0.059			NS	1.659	1.028-2.680	0.038			NS
Postoperative AFP (> 10 versus $\leq$ 10 ng/mL)	0.994	0.314-3.147	0.992			NS	1.506	0.555-4.082	0.421			NS
CA19-9 Alteration (nondecrease versus decrease)	2.295	1.582-3.328	< 0.001	1.746	1.178-2.586	0.005	2.438	1.724-3.448	< 0.001	2.432	1.641-3.605	< 0.001

No. at risk

—	210	80	21	7	1	0
—	65	4	0

No. at risk

—	210	137	47	10	2	0
—	65	17	2	1	0

No. at risk

—	126	58	18	6	1	0
—	84	22	3	1	0	
—	65	4	0

No. at risk

—	126	93	36	9	2	0
—	84	44	11	1	0	
—	65	17	2	1	0

No. at risk

—	126	58	18	6	1	0
—	76	33	12	7	1	0

No. at risk

—	126	93	36	9	2	0
—	76	47	18	8	1	0

No. at risk

162	78	26	13	2	0
40	13	4	0

No. at risk

162	120	48	17	3	0
40	20	6	0

No. at risk

172	82	29	13	2	0
30	9	1	0

No. at risk

172	125	50	17	3	0
30	15	4	0

No. at risk

142	70	25	13	2	0
50	20	5	0		
10	1	0

No. at risk

142	108	45	17	3	0
50	29	8	0		
10	3	1	0

No. at risk

86	21	3	1
65	4	0

No. at risk

86	45	12	2
65	16	1	0

No. at risk

84	22	3	1
67	4	0

No. at risk

84	44	11	1
67	17	2	1

No. at risk

61	18	3	1
48	7	0	
42	1	0

No. at risk

61	35	10	1
48	19	3	1
42	7	0	