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BIOMARKERS
2020, VOL. 25, NO. 2, 186–193
https://doi.org/10.1080/1354750X.2020.1725786

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ORIGINAL ARTICLE

Elevated CEA and CA19-9 serum levels independently predict advanced pancreatic cancer at diagnosis

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ABSTRACT

Purpose: It is suggested that tumour markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) could be used to predict the stage of pancreatic cancer. However, optimal cut-off values for CEA and CA19-9 are disputable. This study aimed to assess the value of CEA and CA19-9 serum levels at diagnosis of pancreatic ductal adenocarcinoma (PDAC) as predictors for the advanced stage of PDAC in patients discussed at pancreatic multidisciplinary team (MDT) meetings.

Methods: Patients with suspected PDAC discussed at MDT meetings from 2013 to 2017 were reviewed, in order to determine optimal cut-off values of both CEA and CA19-9.

Results: In total, 375 patients were included. Optimal cut-off values for predicting advanced PDAC were 7.0 ng/ml for CEA and 305.0 U/ml for CA19-9, resulting in positive predictive values of 83.3%, 73.6%, and 91.4% for CEA, CA19-9 and combined, respectively. Both tumour markers were independent predictors of advanced PDAC, demonstrated by an odds ratio of 4.21 (95% CI:1.85–9.56; $p = 0.001$) for CEA and 2.58 for CA19-9 (95% CI:1.30–5.14; $p = 0.007$).

Conclusions: CEA appears to be a more robust predictor of advanced PDAC than CA19-9. Implementing CEA and CA19-9 serum levels during MDT meetings as an additional tool for establishing tumour resectability is worthwhile for tailored diagnostics.

ARTICLE HISTORY
Received 8 January 2019
Accepted 26 January 2020

KEYWORDS
Carcinoembryonic antigen; carbohydrate antigen 19-9; pancreatic cancer; CEA; CA19-9; diagnosis

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the leading causes of cancer-related death (Siegel *et al.* 2017). Most patients present with metastatic disease, and a minority (20–25%) presents with localised disease eligible for curative-intended surgery (Alexakis *et al.* 2015). Computed Tomography (CT) is currently the predominant imaging modality for diagnosis and preoperative staging of PDAC. Additional diagnostics include Magnetic Resonance Imaging (MRI), Positron Emission Tomography, endoscopic ultrasound-guided fine-needle aspiration and diagnostic laparoscopy (Best *et al.* 2017). However even with currently available predominant imaging modalities, in 12–18% of surgical explorations, a resection cannot be performed due to unexpected locally advanced disease or occult metastases. The high rate of this futile explorations, which have an associated morbidity up to 30% and a hospital mortality of 2% in open setting, highlights the importance of correct preoperative staging in PDAC (Zamboni *et al.* 2007, Kim *et al.* 2015).

It is suggested that serum tumour markers could be used in clinical practice as an additional tool for screening, tumour staging, prediction of prognosis and even surveillance of treatment in several cancer types (Zhang *et al.* 2007, Adamo *et al.* 2017, Lin *et al.* 2018). Carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are the most studied serum biomarkers for establishing both diagnosis and prognosis in pancreatic cancer patients (Goonetilleke and Siriwardena 2007, Meng *et al.* 2017). The role of CEA and CA19-9 in predicting advanced PDAC, defined here as either metastatic or locally advanced PDAC, has scarcely been studied and only performed in patients undergoing surgical exploration (Fujioka *et al.* 2007, De Rosa *et al.* 2016). To our knowledge, no reports are available about the role of CEA and CA19-9 in preoperative multidisciplinary decision making.

We hypothesised that CEA and CA19-9 serum levels at diagnosis are valuable tools for the prediction of advanced PDAC. This study aimed to assess the value of CEA and CA19-9 serum levels at diagnosis as predictors for advanced

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Supplemental data for this article can be accessed here.

#Labrinus van Manen, Jesse V. Groen, and Hein Putter are responsible for statistical design and analysis. E-mail: l.van_manen@lumc.nl (L. van Manen); j.v.groen@lumc.nl (J. V. Groen); h.putter@lumc.nl (H. Putter)

PDAC in patients discussed at the pancreatic multidisciplinary team (MDT) meetings in a tertiary referral centre.

Clinical significance

- The role of CEA and CA19-9 in multidisciplinary decision making for prediction of advanced PDAC has scarcely been studied.
- Our study results showed that both CEA and CA19-9 serum levels independently predict advanced disease in PDAC patients discussed at multidisciplinary team meetings.
- This indicates that patients with increased CEA (>7.0 ng/ml) and CA19-9 (>305.0 U/ml) levels should undergo meticulous preoperative staging focussing on both locoregional status and detection of distant metastases.
- Implementation of routine CEA and CA19-9 sampling might aid in tailored diagnostics.

Patients and methods

Patient selection

Four-hundred twenty consecutive patients with suspected PDAC were discussed at pancreatic MDT meetings from January 2013 through December 2017 at the Leiden University Medical Centre, a tertiary referral centre for pancreatic cancer patients, were retrospectively reviewed. The diagnosis of PDAC was confirmed either after analysing of tumour tissue acquired during endoscopic ultrasound-guided fine-needle aspiration, endoscopic retrograde cholangiopancreatography, direct biopsy of a target lesion, the resected specimen or in case there was no suspicion of another pathology on preoperative imaging. Forty-five patients with resectable disease, who did not want to undergo surgery or were unfit for surgery (as decided by the MDT) were excluded from the analyses because resectability could not be proven by surgical exploration. One hundred sixty-one patients were excluded from analyses involving CEA and CA19-9 serum levels (Figure 1). Approval of the local Medical Research and Ethical Committee was obtained for this retrospective cohort study.

Definitions

Laboratory findings (CEA, CA19-9, and total bilirubin) were defined as the last measured value before an MDT meeting. Tumour markers were not regularly measured during the course of neoadjuvant therapy; only in patients participating in the PREOPANC trial (registered at Dutch Trial Register: NL3525). At MDT meetings, at least one medical specialist of the following departments was present: Medical Oncology, Radiology, Hepatopancreaticobiliary Surgery, Gastroenterology and Pathology. All patients underwent CT or MRI scanning in order to assess the resectability of the tumour, whereas tumour marker levels were not taken into consideration. Locally advanced pancreatic cancer (LAPC) patients, who had stable disease or partial response on neoadjuvant therapy, were considered for surgical exploration. Tumour size was determined as the largest diameter in the transversal direction on preoperative CT or MRI. Preoperative staging of PDAC was performed according to the American Joint Committee on Cancer

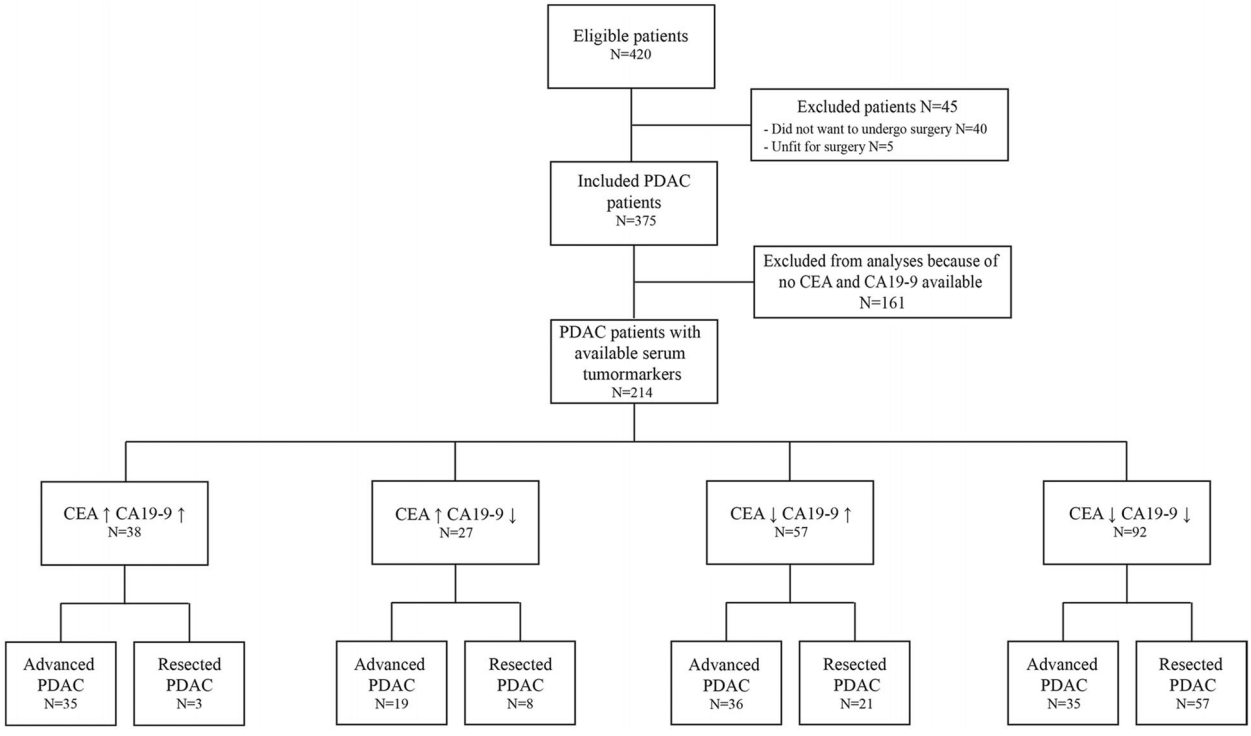


Figure 1. Study flow chart of patient inclusion. Abbreviations: CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; PDAC: pancreatic ductal adenocarcinoma.

(AJCC; 7th edition) (Edge and Compton 2010). Advanced PDAC was defined as either LAPC or the presence of distant metastases (M+). According to the guidelines of the Dutch Pancreatic Cancer Group (DPCG, 2012), the following criteria were applicable for LAPC:

1. Tumour abutment of the superior mesenteric artery, coeliac axis or common hepatic artery >90° of the circumference of the vessel wall.
2. Tumour involvement of the superior mesenteric vein/portal vein vessel wall resulting in occlusion or >270° contact.

Overall survival was calculated from the date of the first suspicion of pancreatic cancer on CT or MRI to the date of death (event) or last follow-up (censored).

Statistical analysis

First, patient- and tumour characteristics were compared between the preoperatively advanced PDAC group (determined during MDT meetings), the intraoperatively advanced PDAC group (determined during explorative surgery) and the resected group (underwent resection). Continuous variables are presented as mean (standard deviation [SD]) in normal distributed data or median (interquartile range [IQR]) in non-normal distributed data. Categorical variables are presented as absolute numbers and percentages. Chi-Squared test, One-Way ANOVA, and Kruskal–Wallis test were used to compare the patients and tumour characteristics. A Kaplan–Meier curve was used to determine the median survival.

Second, the predictive value of CEA and CA19-9 serum levels at diagnosis for advanced PDAC was evaluated by comparing the advanced PDAC group (preoperatively and intraoperatively) with the resected group. Subgroup analyses were performed to investigate if serum CEA and CA19-9 levels can differentiate between patients with LAPC, M + or both (LAPC & M+). Receiver-operating characteristic (ROC) curve analyses and the Youden-Index were used to determine the optimal cut-off values for CEA and CA19-9, after which the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using the cut-off values.

Lastly, a multivariable logistic regression analysis using clinically relevant parameters and the optimal cut-off values, was carried out to assess the independence of CEA and CA19-9 as predictors of advanced PDAC.

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY). A p-value below 0.05 (two-sided) was considered as statistically significant.

Results

Patient characteristics

Patient and tumour characteristics are summarised in Table 1. Out of the included 375 PDAC patients, 151 (40%) patients underwent resection, 58 (16%) patients were intraoperatively classified as advanced PDAC and 166 (44%) patients were classified as preoperatively advanced PDAC. The mean (SD) tumour size differed significantly ($p < 0.001$) between the groups: 28.3 (12.8) mm in the resected group, 35.6 (15.7) mm in the intraoperative advanced PDAC group and 40.9 (17.9) mm in the preoperative advanced PDAC group. Overall survival differed significantly ($p < 0.001$) between the three patient groups: median (95% CI) survival was 5.0 (4.2–5.8) months in the preoperative advanced PDAC group, 7.0 (5.9–8.1) months in the intraoperative advanced PDAC group and 21.0 (16.5–25.5) months in the resected group. The median (IQR) CEA serum level differed significantly ($p < 0.001$) between the groups with the lowest level in the resected PDAC group (3.2; 2.0–4.8) compared to the intraoperative advanced (5.2; 3.3–16.3) and preoperative advanced PDAC group (5.7; 2.6–14.6). The median (IQR) CA19-9 serum level also differed significantly ($p < 0.001$) between the resected group (153.0; 30.5–520.8), intraoperative advanced PDAC group (243.5; 66.8–678.3) and preoperative advanced PDAC group (476.3; 107.9–2145.3). Subgroup analysis in LAPC patients showed that median CEA was significantly higher in LAPC patients who were not considered for surgery than patients found to have LAPC at exploration (3.4 vs 7.7; $p = 0.014$), although median CA19-9 was similar (374.3 vs 315.3; $p = 0.732$). Furthermore, six LAPC patients received neoadjuvant chemotherapy, of which four of them had stable disease or tumour regression and therefore underwent successful surgical exploration. Median CEA levels, determined in two out of two patients in the unresected group, was 15.2 [3.3–27.1] ng/ml compared to 6.4 ng/ml in the resected group, although this was only determined in one out of four patients. Median CA19-9 levels, which were determined in all patients, was 129.8 [30.7–228.8] U/ml in the unresected group and 36.3 [0.6–261.8] U/ml in the resected group, respectively.

Thirty-four out of 35 metastatic cases found during exploration had primary resectable tumour. Median CEA levels were similar between metastasised patients who were primary resectable and primary irresectable (primary resectable: 4.4 [2.6–10.6] ng/ml and primary irresectable: 7.6 [3.1–25.0] ng/ml; $p = 0.173$), although median CA19-9 was significantly higher (813.4 [157.0–3941.0] U/ml vs. 206.1 [65.3–518.4] U/ml; $p = 0.006$) in patients who were primary irresectable.

Determination of optimal cut-off value of CEA and CA19-9

No significant difference in CEA and CA19-9 serum levels were detected between the LAPC, M + and LAPC & M + group (CEA: $p = 0.562$; CA19-9: $p = 0.177$). Median (IQR) CEA and CA19-9 levels were 5.2 (2.7–12.0) and 367.1 (79.6–1149.0) for the LAPC group, 6.0 (3.2–24.7) and 320.4 (128.8–1215.0) for the M + group and 5.6 (2.6–14.6) and 528.0 (180.0–3033.0) for LAPC & M + group. Serum CEA and CA19-9 levels differed significantly between the resected and advanced PDAC patients, with an Area Under the Curve of 0.66 (95% CI: 0.59-0.74; $p < 0.001$) for CEA and 0.68 (95% CI:

Table 1. Patient and tumour characteristics of patients discussed at the multidisciplinary team meetings.

	Resected ($N = 151$)	Intraoperative advanced PDAC ($N = 58$)	Preoperative advanced PDAC ($N = 166$)	p
Age (y), mean (SD)	64.8 (9.6)	66.1 (10.1)	67.8 (9.6)	0.021
Sex, *n* (%)				0.206
Male	80 (53.0)	25 (43.1)	94 (56.6)	
Female	71 (47.0)	33 (56.9)	72 (43.4)	
ASA score, *n* (%)				0.360
1	21 (13.9)	7 (12.1)	34 (20.5)	
2	99 (65.6)	39 (67.2)	95 (57.2)	
≥3	31 (20.5)	12 (20.7)	37 (22.3)	
Bilirubin (μmol/L), mean (SD)	114.9 (129.8)	89.1 (120.2)	85.7 (127.5)	0.143
Tumour location, *n* (%)				0.044
Head	119 (78.8)	44 (75.9)	106 (63.9)	
Body	17 (11.3)	9 (15.5)	36 (21.7)	
Tail	15 (9.9)	5 (8.6)	24 (14.5)	
Tumour size (mm), mean (SD)	28.3 (12.8)	35.6 (15.7)	40.9 (17.9)	<0.001
Radiologic tumour stadium at diagnosis, *n* (%)				<0.001
Ia	23 (15.2)	4 (6.9)	0	
Ib	34 (22.5)	7 (12.1)	0	
IIa	74 (49.0)	33 (56.9)	7 (4.2)	
IIb	18 (11.9)	12 (20.7)	2 (1.2)	
III	2 (1.3)	2 (3.4)	65 (39.2)	
IV	0	0	92 (55.4)	
Neoadjuvant therapy with curative intend, *n* (%)	7 (4.6)	0	6 (3.8)[a]	
Diagnostic laparoscopy before surgery, *n* (%)	53 (35.1)	26 (44.8)	–	0.194
Reason why no resection, *n* (%)[b]				0.261
Locally advanced	–	23 (39.7)	80 (48.2)	
Involvement SMA	–	9	59	
Involvement CA	–	1	37	
Involvement CHA	–	8	65	
Involvement SMV/PV	–	18	118	
Metastases	–	35 (60.3)	86 (51.8)	
Lung	–	0	14	
Liver	–	22	66	
Peritoneum	–	12	16	
Lymph nodes	–	7	32	
Other	–	0	4	
Median survival (months, 95% CI)	21.0 (16.5–25.5)	7.0 (5.9–8.1)	5.0 (4.2–5.8)	<0.001
Tumour markers available, *n* (%)				
CEA	90 (59.6)	41 (70.7)	86 (51.8)	0.037
CA19-9	121 (80.1)	50 (86.2)	130 (78.3)	0.431
Tumour markers, median (IQR)				
CEA (ng/ml)	3.2 (2.0–4.8)	5.2 (3.3–16.3)	5.7 (2.6–14.6)	<0.001
CA19-9 (U/ml)	153.0 (30.5–520.8)	243.5 (66.8–678.3)	476.3 (107.9–2145.3)	<0.001

PDAC: pancreatic ductal adenocarcinoma; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; SMA: superior mesenterial artery; CA: coeliac axis; CHA: common hepatic artery; SMV: superior mesenterial vein; PV: portal vein.
[a]4/6 LAPC patients who received neoadjuvant chemotherapy were finally explored and underwent successful tumour resection.
[b]In general, more than one structures were involved in case of locally advanced and metastatic disease. In addition, if both metastases and locally advanced PDAC was found, we considered metastases as the reason of irresectability.

0.60–0.75; $p < 0.001$) for CA19-9 (Figure 2). The optimal cut-off value, calculated using the Youden-Index, was 7.0 ng/ml for CEA and 305.0 U/ml for CA19-9. A CEA > 7.0 ng/ml had a PPV of 83.3% and a CA19-9 > 305.0 U/ml had a PPV of 73.6% for the presence of advanced PDAC (Table 2). The distribution of CEA and CA19-9 serum levels at diagnosis and the percentages of patients above and under the optimal cut-off values are illustrated in a boxplot (Figure 3). Diagnostic accuracy was calculated for other clinically relevant cut-off values for CEA and CA19-9 (Table S1). Furthermore, optimal cut-off values for CEA and CA19-9 serum levels were applied in the subgroups (LAPC, M + and combined), of which the distribution per group is depicted in Figure S1.

Combined value of CEA and CA19-9 levels

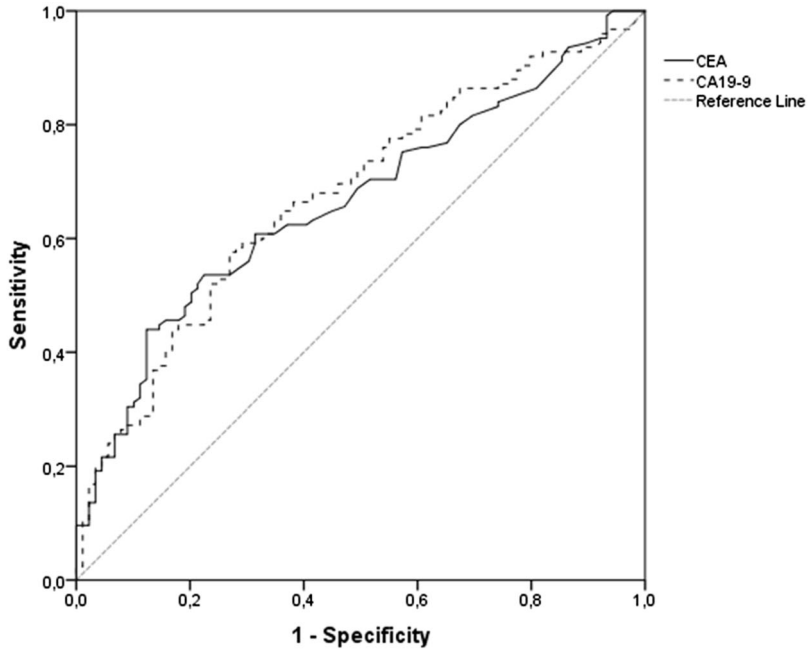
Combining both optimal cut-off values yielded a 91.4% PPV for the prediction of advanced PDAC (Table 2). Resection rates in the four groups were 7.9% (both elevated), 29.6% (solely elevated CEA), 36.8% (solely elevated CA19-9), and 62.0% (both not elevated). Other relevant combinations of cut-off values for CEA and CA19-9 are added in Table S1.

Multivariable analysis of predictive factors

At multivariable analysis, elevated CEA and CA19-9, female sex, age, tumour size increment were independent predictive factors for advanced PDAC (Table 3). CEA >7.0 ng/ml showed a higher odds ratio (OR) (OR: 4.18; 95% CI: 1.83–9.56; $p = 0.001$) than CA19-9 > 305.0 U/ml (OR: 2.66; 95% CI: 1.33–5.33; $p = 0.006$).

Discussion

The aim of this study was to evaluate the value of CEA and CA19-9 serum levels in predicting advanced PDAC in patients with suspected PDAC discussed at MDT meetings. Median



	AUC (95%CI)	Optimal cut-off value	Youden-Index	P
CEA	0.66 (0.59 - 0.74)	7.0	0.316	<0.001
CA19-9	0.68 (0.60 - 0.75)	305.0	0.306	<0.001

Figure 2. ROC curves CEA & CA19-9 for prediction of advanced PDAC. Abbreviations: ROC: receiver operating characteristic; PDAC: pancreatic ductal adenocarcinoma; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; AUC: area under the curve.

Table 2. Diagnostic value of optimal cut-off value of CEA and CA19-9 at diagnosis.

	CEA > 7.0 (ng/ml)	CA19-9 > 305.0 (U/ml)	CEA >7.0 (ng/ml) and CA19-9 > 305.0 (U/ml)
Sensitivity (95% CI) (%)	43.3 (34.6–52.4)	57.2 (49.7–64.6)	27.1 (19.3–36.1)
Specificity (95% CI) (%)	87.8 (79.2–93.7)	69.4 (60.4–77.5)	96.4 (89.9–99.3)
PPV (95% CI) (%)	83.3 (73.5–90.0)	73.6 (67.4–78.9)	91.4 (77.2–97.1)
NPV (95% CI) (%)	52.3 (48.1–56.5)	52.2 (47.0–57.3)	48.5 (45.6–62.9)

CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; PPV: positive predictive value; NPV: negative predictive value.

CEA and CA19-9 differed significantly between the resected, intraoperative advanced PDAC and preoperative advanced PDAC patient groups ($p < 0.001$). The optimal cut-off values for predicting advanced PDAC were 7.0 ng/ml for CEA and 305.0 U/ml for CA19-9, resulting in a positive predictive value of 83.3%, 73.6%, and 91.4% for elevated CEA, CA19-9 and combined, respectively. Both tumour markers were independent predictors of advanced PDAC; however, the numerical difference between CEA (OR: 4.18) and CA19-9 (OR: 2.66) could indicate that CEA appears to be a more robust factor.

Previous studies showed optimal cut-off values of CA19-9 varying between 92.77 U/ml and 353.15 U/ml, resulting in PPV varying from 79 to 95% for advanced PDAC during staging laparoscopy or laparotomy as recently reviewed by De Rosa *et al.* (2016). Hartwig *et al.* (2013) reported the predictive value for resectability and survival of CA19-9 levels in 1543 patients. They reported resection rates below 70% in case of preoperative CA19-9 levels >500 U/ml, which is therefore included in the definition of borderline resectable PDAC as a biological factor (Isaji *et al.* 2018).

Schlieman *et al.* (2003) valuated the role of CEA as a predictor of resectability and found no significant difference in preoperative CEA levels between the resected and non-resected group. Most studies used a combination of CEA and CA19-9 levels to determine the prognosis (Tsavaris *et al.* 2009, Distler *et al.* 2013, Lee *et al.* 2013, Kanda *et al.* 2014, Reitz *et al.* 2015, Wu *et al.* 2015), however, two studies evaluated a combination of those two tumour markers for prediction of resectability (Fujioka *et al.* 2007, Kim *et al.* 2009). Fujioka *et al.* combined CEA and CA19-9 levels, yielding a NPV for resectability of 88% in 244 patients by using 5.5 ng/ml as an optimal cut-off value for CEA and 157 U/ml for CA19-9 (Fujioka *et al.* 2007). Remarkably, CA19-9 was only associated with the presence of metastases (both liver and peritoneal) and not significantly associated with LAPC, whereas CEA was associated with LAPC and the presence of liver metastases. However, no comparison was made between median CEA and CA19-9 serum levels in LAPC and the metastasised patient group. Kim *et al.* demonstrated an 86.6% NPV for resectability after combining optimal cut-off values for CEA (2.47 ng/ml) and CA19-9 (92.77 U/ml) levels (Kim *et al.* 2009). The large variety of optimal cut-off values could be explained by the difference in the definition of (ir)resectability, the racial diversity and size of the study population. Our study included all patients discussed at MDT-meetings, which makes comparison with current literature somewhat complicated; but is clinically more relevant. Nevertheless, our cut-off values for CEA and CA19-9 were

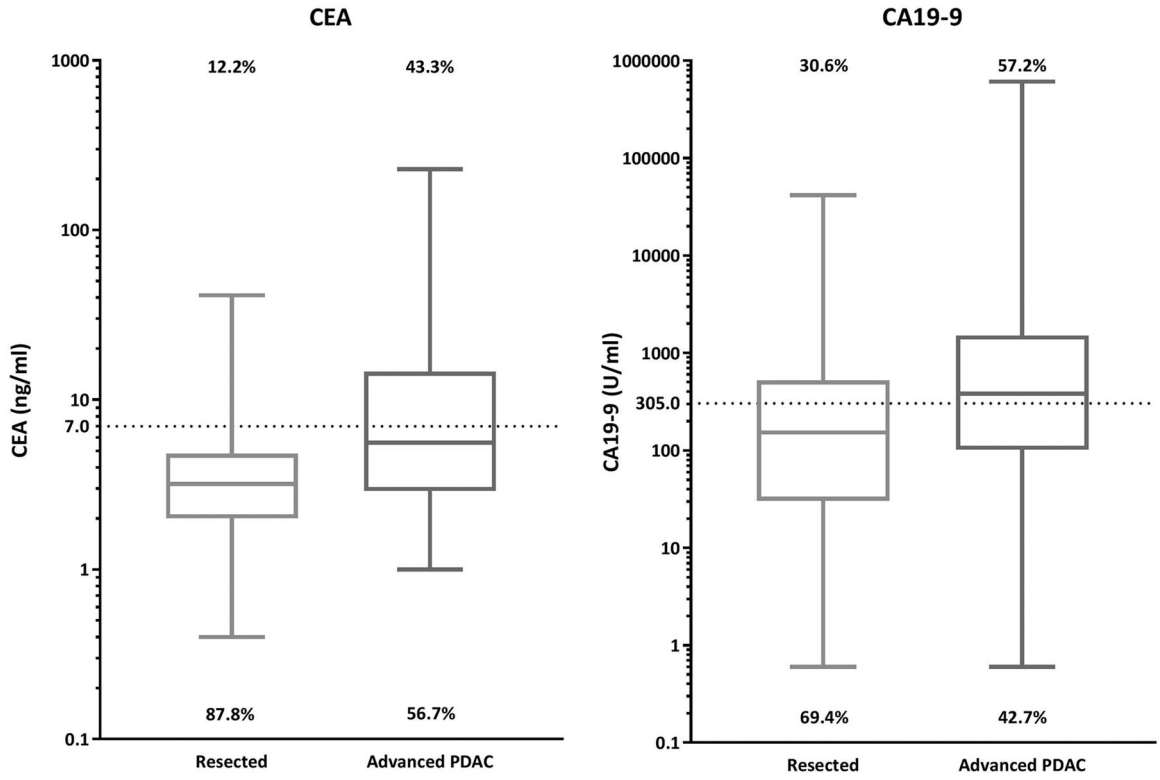


Figure 3. Boxplot presentation of the distribution of CEA & CA19-9 serum levels at diagnosis. Optimal cut-off values found by ROC analysis are illustrated as a horizontal dotted line. A logarithmic scale was used on the y-axis. The percentages indicate the proportion of patients per group above and below the optimal cut-off values. Abbreviations: CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9; PDAC: pancreatic ductal adenocarcinoma.

Table 3. Multivariable analysis of predictive factors for establishing advanced PDAC.

	Odds ratio	95% confidence interval	*p*
CEA > 7.0 (ng/ml)	4.18	1.83–9.56	0.001
CA19-9 > 305.0 (U/ml)	2.66	1.33–5.33	0.006
Bilirubin> 17 (μmol/L)	0.61	0.30–1.22	0.159
Age	1.05	1.01–1.09	0.013
Female	2.15	1.08–4.30	0.029
Tumour size (mm)	1.07	1.04–1.10	<0.001

PDAC: pancreatic ductal adenocarcinoma; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen 19-9.

substantially higher, resulting in much higher prediction values and lower resection rates.

This study has several limitations. The tumour markers CEA and CA19-9 were not measured in all patients. In patients who did not undergo surgical exploration CEA and CA19-9 levels were less often measured. In addition, our study included all PDAC patients discussed at MDT meetings, were other studies included solely patients who underwent surgical exploration, which states the need for external validation of the optimal cut-off values in a prospective cohort study. Sixty percent of the patients discussed at our MDT meetings underwent surgical exploration, which is significantly higher than the national average (27%) (van der Geest *et al.* 2017). Nevertheless, a 60% resection rate is still low for a tertiary referral centre, which could be explained by the fact that we included all PDAC patients discussed at MDT. Furthermore, it is to be expected that the use of neoadjuvant therapy will result in improved resection rates for LAPC patients; however, the resection rates could decrease in patients with (borderline) resectable PDAC as patients with aggressive tumours, unsensitive to chemotherapy, will become either locally unresectable or metastasise early during the neoadjuvant treatment period.

Another limitation is the small amount of patients, who underwent neoadjuvant chemotherapy in our population, as neoadjuvant treatment will become standard care. Not all patients treated with neoadjuvant chemotherapy will benefit from neoadjuvant treatment, therefore patient selection is key. Although in our cohort limited patients with LAPC underwent neoadjuvant chemotherapy, a small difference in CEA and CA19-9 was seen, which suggests that besides tumour anatomy, tumour biology has to be taken into account in the treatment of PDAC patients. Furthermore, it has been suggested that a decrease (>30%) of CA19-9 level in LAPC patients during the course of neoadjuvant therapy, resulted in improved resection rates during surgical exploration, which confirmed the relevance of tumour biology in patient selection for surgery (van Veldhuisen *et al.* 2018).

It has been shown that biliary obstruction could disturb the CA19-9 serum levels, however, after correction for biliary serum levels, CA19-9 remained an independent predictor. Another factor influencing the CA19-9 level is the absence of a Lewis antigen, which is the case in approximately 4–7% of the population (Brockhaus *et al.* 1981, Tempero *et al.* 1987). CA19-9 is not expressed in patients missing the Lewis antigen, even in the presence of tumours. Although we did not asses the Lewis antigen in our study, it exemplifies that CA19-9 serum levels should always be interpreted with caution. Moreover, CA19-9 is also associated with many other

cancers, such as gastric or colorectal cancer (Filella *et al.* 1992, Victorzon *et al.* 1995). We hypothesised that CEA is associated with tumour size because CEA is shedded into blood from tumour cells. However, in this study an increased CEA remains an independent predictor of advanced PDAC, even after correction for tumour size. Although, CEA serum levels can also be increased in colorectal cancer or metastases, lung cancer or metastases, and nicotine abuses, which illustrates its non-specificity for pancreatic tumours (Pezzuto *et al.* 2013).

Besides CEA and CA19-9, several other promising serum markers for PDAC have been reported the last years. Most studies focussed on the capability of tumour marker to detect PDAC and therefore its potential for screening purposes (Ruckert *et al.* 2010, Petrushnko *et al.* 2016, Swords *et al.* 2016). For instance, carbohydrate antigen variants, such as CA242, showed good potential for early pancreatic cancer detection (Dong *et al.* 2018). Moreover, it was shown that another variant, CA125, had superior detection rates for irresectable disease in a cohort of 212 patients (Luo *et al.* 2013), although its application is limited because a substantial proportion of patients with pancreatitis and jaundice also have increased serum CA125 levels (Ruckert *et al.* 2010). During the last years, more research is spent on microRNA detection in serum and exosomes in order to detect pancreatic tumours in the early stage with promising results and high diagnostic accuracies (Liu *et al.* 2012, Melo *et al.* 2015). Further studies should be performed to evaluate the most sensitive microRNA markers and role of these markers in the prediction of advanced stage, i.e. irresectable, pancreatic cancer.

Clinical implications of our findings might be that specific patient groups need an intensified diagnostic multi-modality approach. In general, increased CEA and CA19-9 levels resulted in a high chance (91.4% PPV) of having advanced PDAC. Subgroup analysis showed that CEA and CA19-9 levels did not differ significantly between the LAPC, M + and LAPC & M + groups. This indicates that patients with increased CEA and CA19-9 levels should undergo meticulous preoperative staging focussing on both locoregional status and detection of distant metastases (Figure S1). Indeed, our study also included patients who were not explored and having higher tumour marker levels, because of the higher metastatic tumour burden, but even patients who were explored had significantly higher tumour marker levels. Therefore MRI for example, could play an important role in preoperative staging, i.e. preoperative detection of small (liver) metastases as it is more sensitive than CT, which is most commonly used (Bipat *et al.* 2005, Holzapfel *et al.* 2011, Kim *et al.* 2017, Jeon *et al.* 2018). Furthermore, diverse combinations of cut-off values and corresponding chances on having advanced disease are calculated based on our cohort, which could guide the MDT in the treatment process (Table S1). Moreover, in the case of negative CA19-9 levels, in patients who are missing the Lewis antigen, CEA turned out to be a complementary predictor of advanced PDAC. Although, it does not implicate that in case of elevated CEA and CA19-9 levels the MDT should not consider surgical treatment. In this situation, we recommend a staging or diagnostic laparoscopy before performing laparotomy, which is cost-effective and therefore the current standard of care in our and some other centres (Chang *et al.* 2009, Morris *et al.* 2015). Moreover, additional intraoperative imaging modalities (e.g. laparoscopic ultrasound or near-infrared fluorescence imaging) should also be considered in this patient category. Preliminary results showed that CEA-targeting near-infrared fluorescence imaging is promising for intraoperative evaluation of locoregional status and detection of distant metastases and further studies defining clinical benefit are ongoing (Gutowski *et al.* 2017, Hoogstins *et al.* 2018).

Conclusions

The results of this study showed that CEA and CA19-9 are independent prediction factors for the presence of advanced PDAC at diagnosis. Although CEA appears to be a more robust factor for the prediction of advanced PDAC in our study, combining CEA and CA19-9 cut-off values enhances the positive predictive value, which indicates that implementing these levels during MDT meetings could be worthwhile for tailored diagnostics. Further studies should focus on the role of tumour markers in LAPC patients treated with neoadjuvant therapy for determining the efficacy of treatment on resectability.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the Bas Mulder Award [grant UL2015-7665] from the Alpe d'HuZes foundation/Dutch Cancer Society.

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