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Combination of Tumour Markers CEA and CA19-9 Improves the Prognostic Prediction in Patients With Pancreatic Cancer

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Abstract and Introduction

Abstract

Aims Tumour markers including carcinoembryonic antigen (CEA) or carbohydrate antigen 19-9 (CA19-9) are frequently determined at the time of diagnosis in patients with pancreatic cancer. Several studies indicate a prognostic relevance of these markers in pancreatic cancer, but space for improvement with regard to the predictive accuracy and ability is given. In this work, the main focus is on mathematical combinations of these two tumour markers in order to validate an improvement of prognostic test results in terms of sensitivity and specificity.

Methods This retrospective study includes 393 patients with pancreatic cancer, who were treated between the years 2005 and 2012 at the Division of Oncology, Medical University of Graz, Austria. The goal of this study was to explore whether an appropriate combination of two tumour markers leads to a statistically significant improvement of the prognostic prediction.

Results Receiver operating characteristic curves comparison analyses with the classification variable cancer-specific survival showed that the mathematical product of two tumour markers ($TM_{product}$= (CEA×CA19-9); area under the curve (AUC)=0.727; 95% CI 0.680 to 0.770) is significantly better than CEA alone (AUC=0.644; 95% CI 0.594 to 0.691; p=0.003) but not significant compared with CA19-9 (AUC=0.710; 95% CI 0.662 to 0.754; p=0.1215). A linear combination of CEA and CA19-9 (TM_{linear}= (85×CEA+CA19-9); AUC=0.748; 95% CI 0.702 to 0.790) is significantly better than CEA (p<0.0001) as well as CA19-9 alone (p=0.0304).

Conclusions Mathematical combinations of pretherapeutic tumour markers CEA and CA19-9 are feasible and can significantly improve the prognostic prediction in patients with pancreatic cancer.

Introduction

Pancreatic cancer (PC) is a disease with a dismal prognosis that shows almost constant incidence and mortality. Five-year survival rates over the last three decades have only slightly changed and are overall <6%.[1,2] At the time of diagnosis, most patients are in an advanced stage of disease often exhibiting distant metastases.[3] A main reason for that is the absence of specific related symptoms in early stages. Surgical resection is the only curative treatment in PC since adjuvant chemotherapy is not very effective; however, <20% of patients are potentially surgical candidates. Therefore, in many cases, therapy options are restricted to palliative chemotherapy or novel treatment protocols.[1,4]

In clinical practice, tumour markers including carcinoembryonic antigen (CEA) or carbohydrate antigen 19-9 (CA19-9) are commonly determined at the time of diagnosis before treatment initiation. Concerning diagnostic meaning, meta-analyses for CEA and CA19-9 indicate a sensitivity of 44.2% and 78.2% (specificity: 84.8% and 82.8%).[5] Although >2000 biomarker studies are present, serum CA19-9 is still the only recommended biomarker in the routine management for PC.[6–8] Many studies suggest that these pretherapeutic tumour marker values also seem to yield a prognostic significance in patients with PC, but in terms of declaring independent prognostic factors publications often provide controversial results for CEA as well as CA19-9. Some studies only see CEA as prognostic independent,[9,10] other ones both markers,[11–13] while still others none of them.[14] One general problem to determine whether tumour markers imply a prognostic meaning in patients with PC is the narrowband survival distribution combined with the relative rareness of this disease in contrast to more common tumour entities. Therefore, a high number of patients is needed in a study cohort in order to facilitate statistical significant and reliable conclusions.[15]

Recently, the idea was generated to use aside from single markers a combination of tumour markers CEA and CA19-9 to make a prognostic prediction in patients with PC. The authors multiplied the perioperative tumour marker values and proved that this new index improves the prognostic validity.[16] The aim of this study was to explore mathematical combinations of these two pretherapeutic tumour marker values and their results in order to verify improvements relating prognostic accuracy in a large cohort of patients with PC.

Materials and Methods

This retrospective study included 393 patients with PC, who were treated between the years 2005 and 2012 at the Division of Clinical Oncology, Medical University of Graz, Austria. All patients had histological confirmed ductal adenocarcinoma of the pancreas, and the clinicopathological data were retrieved from medical records at the Division of Clinical Oncology, as well as from pathology records from the Institute of Pathology at the same institution.[17] Since the TNM classification system for PC changed

during the study period, tumour stages were uniformly adjusted according to the seventh edition of this system. Other documented clinicopathological variables included administration of chemotherapy with gemcitabine, surgery, Karnofsky index, gender and age. Follow-up evaluations were performed every three months within the first three years, 6 months for 5 years and annually thereafter for curative resected tumour stages as previously described.[18] Follow-up investigations included clinical check-up, laboratory including CEA and CA19-9, and radiological assessment. For deceased patients, dates of death were obtained from the central registry of the Austrian Bureau of Statistics.

Statistical Analyses

A descriptive statistical analysis was performed with absolute and relative incidences of clinicopathological variables. None of the outcome variables in this work was normally distributed, so we used the quartiles to describe them. A goal of this study was to explore whether an appropriate combination of two tumour markers leads to a statistically significant improvement of the prognostic prediction compared with single tumour markers. To verify this argument, the area under the curve (AUC) of receiver operating characteristic (ROC) analyses was compared with each other. The method by DeLong *et al*[19] was used for comparing ROC curves. The p value of a hypothesis test for equality indicated whether there was a significant difference between compared AUCs. Cancer-specific survival (CSS) was used as classification variable for ROC analyses, and it was defined as the time (in months) from date of surgery or date of histologically proven diagnosis to cancer-related death.

Furthermore, different cut-off values for an optimal test result in terms of sensitivity and specificity were calculated, so the values above and below the cut-off level could be arranged in two groups. In order to get an optimal test result, Youden's index, which is defined as 'sensitivity+specificity–1' should be as high as possible.[20] The optimal weight factor in the linear combination of tumour markers for CEA was determined by looking for maximum Youden's index for different weight factor values. A linear combination in the general mathematical form with two variables looks like this: $z=a\cdot x+b\cdot y$. Now we assume that x is the CEA value and y is the value of CA19-9 (concentrations in units per litre). If the equation is divided by 'b', we get: $z/b=(a/b)\cdot CEA+1\cdot CA19\text{-}9$. So we managed to eliminate one of the two factors since the weight factor for CA19-9 has a value of 1 now. It would also be possible to divide the original equation by 'a', then the weight factor for CEA would have been eliminated. Of course, then the scale and furthermore the cut-off values would be different but there would not be a change in the further analyses. Contingency tables with χ^2 independence tests were performed in order to see whether a dependency between the dichotomised tumour marker groups and clinicopathological variables was statistically significant. Survival analyses were carried out using the Kaplan–Meier method, and differences were validated through a log-rank test and comparing the p value with the significance level.

In the last step, univariable and multivariable Cox regression models with CSS for all clinicopathological variables were performed and HRs with their 95% CI and the p value were calculated. The Cox proportional hazard assumption was checked with Schoenfeld residual plots.[21] For all statistical analyses, a significance level of $\alpha=0.05$ was defined. We used two (statistical) software tools for our analyses: MedCalc Software (Acacialaan 22, B-8400 Ostend, Belgium) and MATLAB and Statistics Toolbox Release 2010a (The MathWorks, Natick, Massachusetts, USA).

Results

Descriptive Analysis

Our study cohort included 393 (213 men and 180 women) patients with an age at diagnosis between 35 and 86 years and a median age of 65 years ((Q_{25}; Q_{75})=(57; 72)). Pretherapeutic tumour marker values for CEA were located between 0.4 and 1511.7 with a median value of 4.1 U/L ((Q_{25}; Q_{75})=(2.18; 11.13)). CA19-9 serum values were distributed in a range between 1.9 and 586998.1 with a median of 761.7 U/l ((Q_{25}; Q_{75})=(116.7; 5359)). CSS for all patients showed a median survival time of 7 months ((Q_{25}; Q_{75})=(3; 14)). gives an overview including absolute and relative incidences for different clinicopathological variables.

Table 1. Descriptive clinicopathological variables of the study cohort (n=393)

Variable	No. (%)
Age at diagnosis (years)	
Mean±SD	64.4±10.4 years
Median	65.0 (35.0–86.0) years
<65	118 (48.6%)
≥65	125 (51.4%)
Gender	
Female	180 (45.8%)
Male	213 (54.2%)
Stage	
I–II	87 (22.1%)

III	33 (8.4%)
IV	273 (69.5%)
Grade	
G1+G2	234 (59.5%)
G3+G4	159 (40.5%)
Surgical resection	
No	288 (73.3%)
Yes	105 (26.7%)
Chemotherapy	
No	109 (27.7%)
Yes	283 (72%)
Neoadjuvant	1 (0.3%)
Adjuvant	62 (15.8%)
Palliative	208 (52.9%)
Neoadjuvant/adjuvant	12 (3.1%)
Missing	1 (0.3%)
Karnofsky index	
≤80	232 (59%)
90–100	157 (39.9%)
Missing	4 (1%)

ROC Analyses

ROC analyses were performed for the tumour markers CEA and CA19-9, as well as their mathematical combinations using classification variable CSS (figure 1); the corresponding variables AUC and test performance (sensitivity and specificity) for an optimal cut-off value are shown in .

Table 2. ROC curves variables with optimal points' cut-off level and test performance using CSS status as classification variable (n=393)

Variable	AUC (95% CI)	Cut-off value (Youden's index)	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
CEA	0.644 (0.594 to 0.691)	6.9 (0.2264)	37.16 (31.9 to 42.6)	85.48 (74.2 to 93.1)
CA19-9	0.710 (0.662 to 0.754)	931.4 (0.3633)	52.57 (47.0 to 58.1)	83.87 (72.3 to 92.0)
CEA×CA19-9	0.727 (0.680 to 0.770)	1976.5 (0.3875)	61.33 (55.8 to 66.6)	77.42 (65.0 to 87.1)
85×CEA+CA19-9	0.748 (0.702 to 0.790)	845 (0.4559)	69.79 (64.5 to 74.7)	75.81 (63.3 to 85.8)

AUC, area under the curve; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CSS, cancer-specific survival; ROC, receiver operating characteristic.

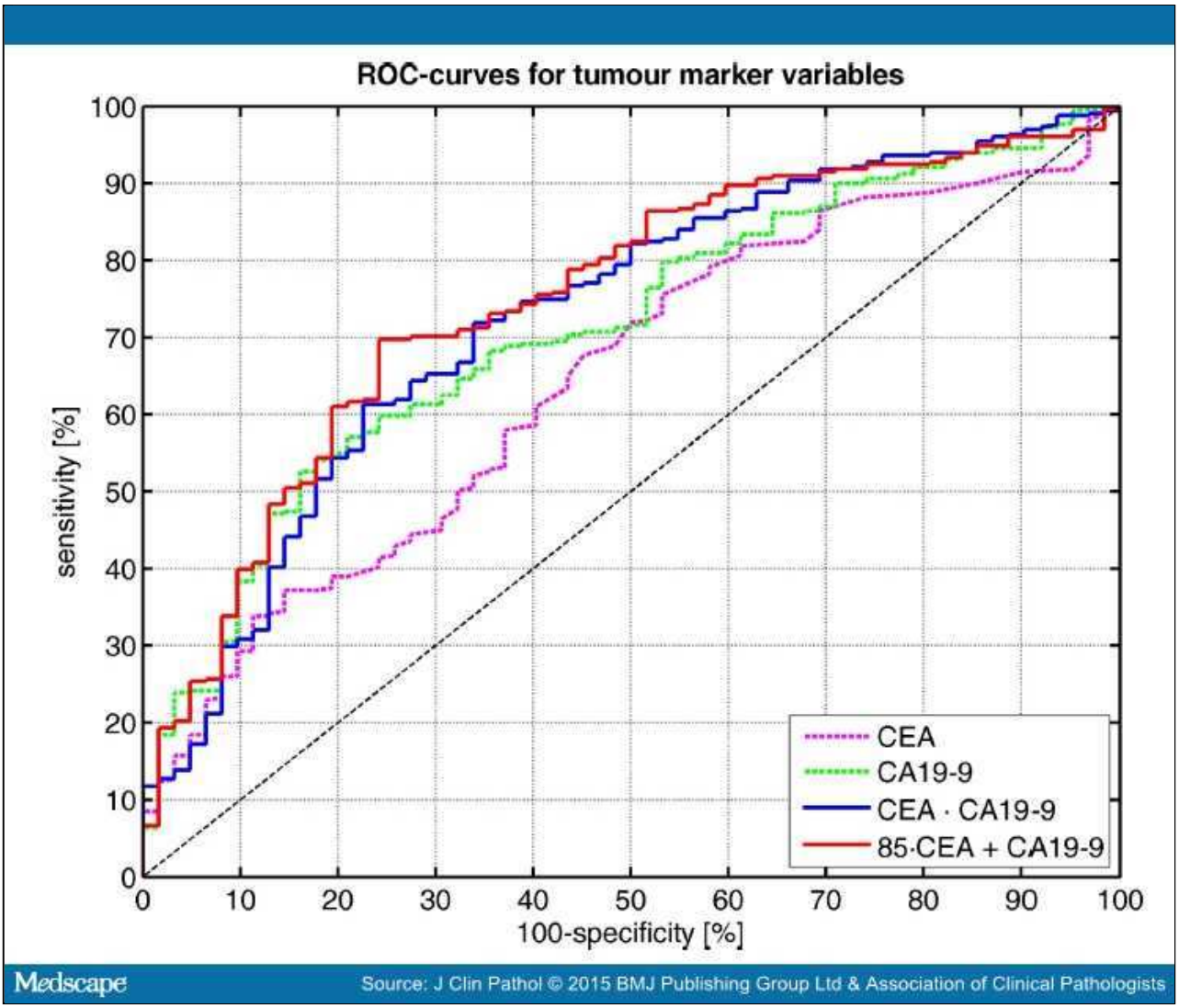

Figure 1.

Receiver operating characteristic (ROC) curves showing the results of tumour markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9), as well as their mathematical combinations (product and linear); cancer-specific survival status is used as classification variable.

In a direct comparison, the linear combination of CEA and CA19-9 indicates the best ROC result with the highest AUC (AUC=0.748) of all and also a maximal Youden's index of 0.4559, which yields to the highest sensitivity level of almost 70% during maintaining a specificity of >75%. An optimal weight factor in the linear combination for CEA ('85') in terms of maximal sensitivity and specificity could be determined by searching for maximal Youden's index for different values, and we chose the weight factors' median value of range between 81 and 89 (figure 2).

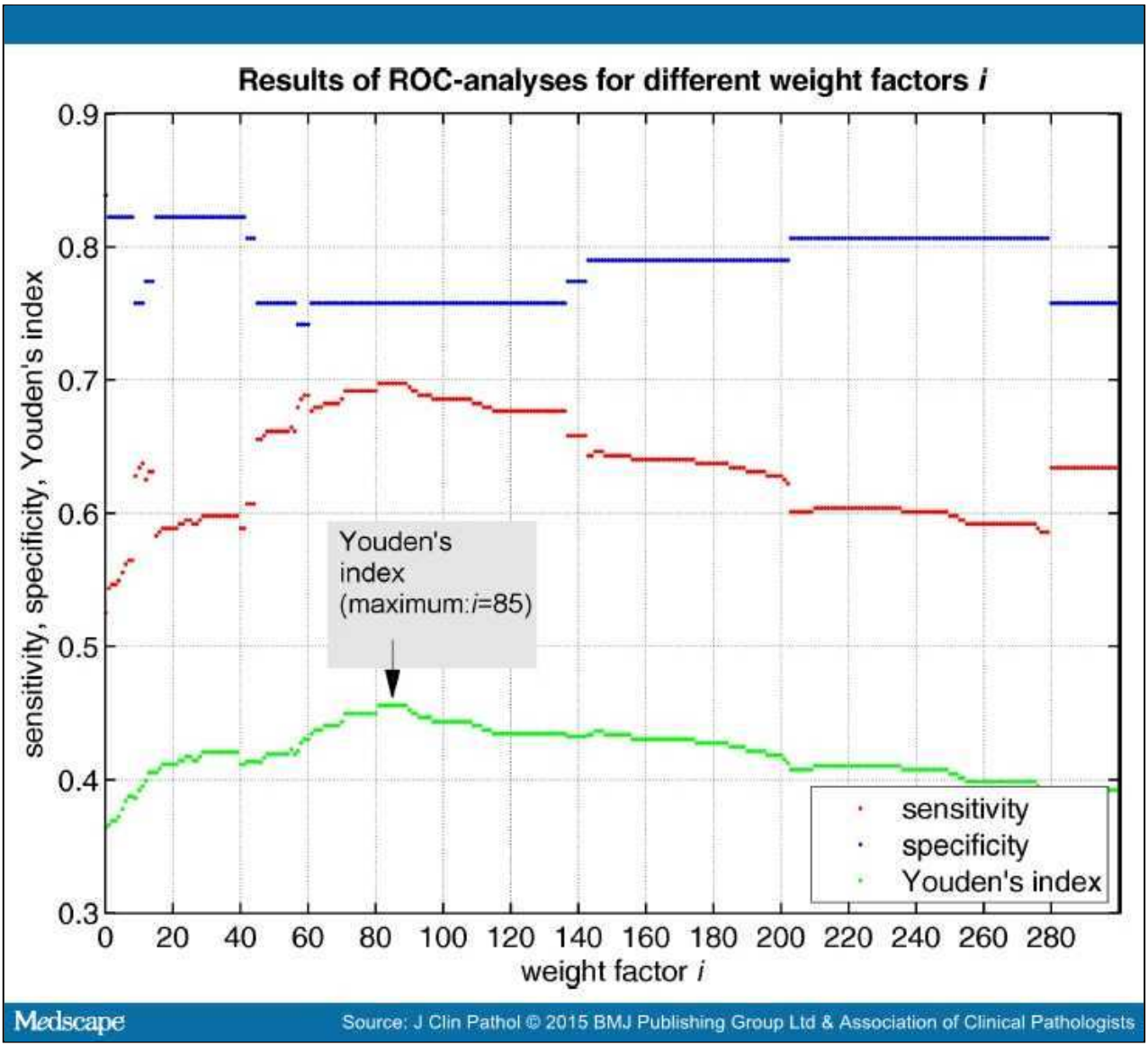

Figure 2.

Plot indicating optimal weight factor 'i' (maximal Youden's index: i=85) for carcinoembryonic antigen (CEA) in the linear combination of tumour markers CEA and carbohydrate antigen 19-9 (CA19-9) (i×CEA+CA19-9); the corresponding sensitivity and specificity for different factors is also shown. ROC, receiver operating characteristic.

ROC curves comparison analyses show () that the mathematical product of two tumour markers ($TM_{product}$=(CEA×CA19-9)) is significantly better than CEA alone (p=0.003), but not significant compared with CA19-9 (p=0.1215). A linear combination of CEA and CA19-9 (TM_{linear}=(85×CEA+CA19-9)) is significantly better than CEA (p<0.001) as well as CA19-9 (p=0.0304).

Table 3. ROC curves comparison analyses showing AUC difference and equality hypothesis test result (n=393)

Compared ROC curves	Δ AUC =AUC_2–AUC_1	SD (95% CI)	p Value
CEA~CEA×CA19-9	0.0827	0.0279 (0.028 to 0.137)	0.003
CEA~85×CEA+CA19-9	0.104	0.0222 (0.061 to 0.148)	<0.001
CA19-9~CEA×CA19-9	0.0168	0.0109 (−0.005 to 0.038)	0.1215
CA19-9~85×CEA+CA19-9	0.0384	0.0177 (0.004 to 0.073)	0.0304

AUC, area under the curve; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; ROC, receiver operating characteristic.

Contingency tables for the dichotomised tumour marker values and different clinicopathological variables with the result of χ^2 independence tests (p value) are shown in .

Table 4. Relation between clinicopathological variables and the pretreatment tumour marker values of patients with pancreatic cancer (n=393)

	CEA			CA19-9		
Characteristics	**≤6.9**	**>6.9**	**p Value**	**≤931.4**	**>931.4**	**p Value**
Age at diagnosis (years)						
<65	130	58	0.271	103	85	0.48
≥65	131	74		105	100	
Gender						
Female	116	64	0.448	88	92	0.141
Male	145	68		120	93	
Tumour stage						
I–II	80	7		67	20	
III	26	7	<0.001	22	11	<0.001
IV	155	118		119	154	
Tumour grade						
G1+G2	157	77	0.728	133	101	0.06
G3+G4	104	55		75	84	
Surgical resection						
No	166	122	<0.001	132	156	<0.001
Yes	95	10		76	29	
Chemotherapy						
No	64	45	0.041	50	59	0.077
Yes	197	86		158	127	
Karnofsky index						
≤80	147	85		110	122	
90–100	112	45	0.001	96	61	0.029
Missing	2	2		2	2	
CEA						
≤6.9	–	–	–	167	94	<0.001
>6.9				41	91	
CA19-9						
≤931.4	167	41	<0.001	–	–	–
>931.4	94	91				

CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen.

For the CEA subgroups based on the p value (significance level: 0.05), the null hypothesis (independence) is rejected for the following variables: tumour stage, surgical resection, chemotherapy, Karnofsky index and CA19-9 value. CA19-9 subgroups reject the null hypothesis for the variables tumour stage, surgical resection, Karnofsky index and CEA value.

Kaplan–Meier Analyses

gives an overview of the mean and median CSS times using Kaplan–Meier analyses.

Table 5. Kaplan–Meier analyses based on cut-off values for the tumour markers and their combinations showing mean and median survival (n=331, censored by CSS status)

Variable	Number of events (%)	Mean survival±SE (95% CI) (months)	Median survival (95% CI) (months)
CEA			
≤6.9	208 (79.7%)	14.8±1.0 (12.7 to 16.8)	10 (8 to 12)
>6.9	123 (93.2%)	6.6±0.6 (5.4 to 7.8)	4 (3 to 5)
CA19-9			
≤931.4	157 (75.5%)	16.3±1.3 (13.7 to 18.9)	10 (9 to 13)
>931.4	174 (94.1%)	7.6±0.6 (6.4 to 8.8)	5 (4 to 6)
CEA×CA19-9			
≤1976.5	128 (72.7%)	17.5±1.5 (14.6 to 20.4)	12 (10 to 16)
>1976.5	203 (93.6%)	8.0±0.6 (6.8 to 9.2)	5 (4 to 6)
85×CEA+CA19-9			
≤845	100 (68.0%)	19.4±1.8 (16.0 to 22.9)	13 (10 to 18)
>845	231 (93.9%)	8.1±0.6 (7.0 to 9.2)	5 (4 to 6)

CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CSS, cancer-specific survival.

Log-rank tests performed for all four variables were statistically significant ($p<0.001$), which demonstrated relevant differences between the survival distributions. As an example, figure 3 shows the Kaplan–Meier curves for CSS of the linear combination of tumour markers CEA and CA19-9 and reveals that patients with PC with values ≤845 U/L have a significantly better prognosis ($p<0.001$, log-rank test) than patients exhibiting higher index values. Accordingly, the median survival time is 13 (10–18) vs 5 (4–6) months in this case. Among the 393 patients with PC, death occurred in 100 of 147 (68.0%) patients with values ≤845 U/L and in 231 of 246 (93.9%) patients with values >845 U/L.

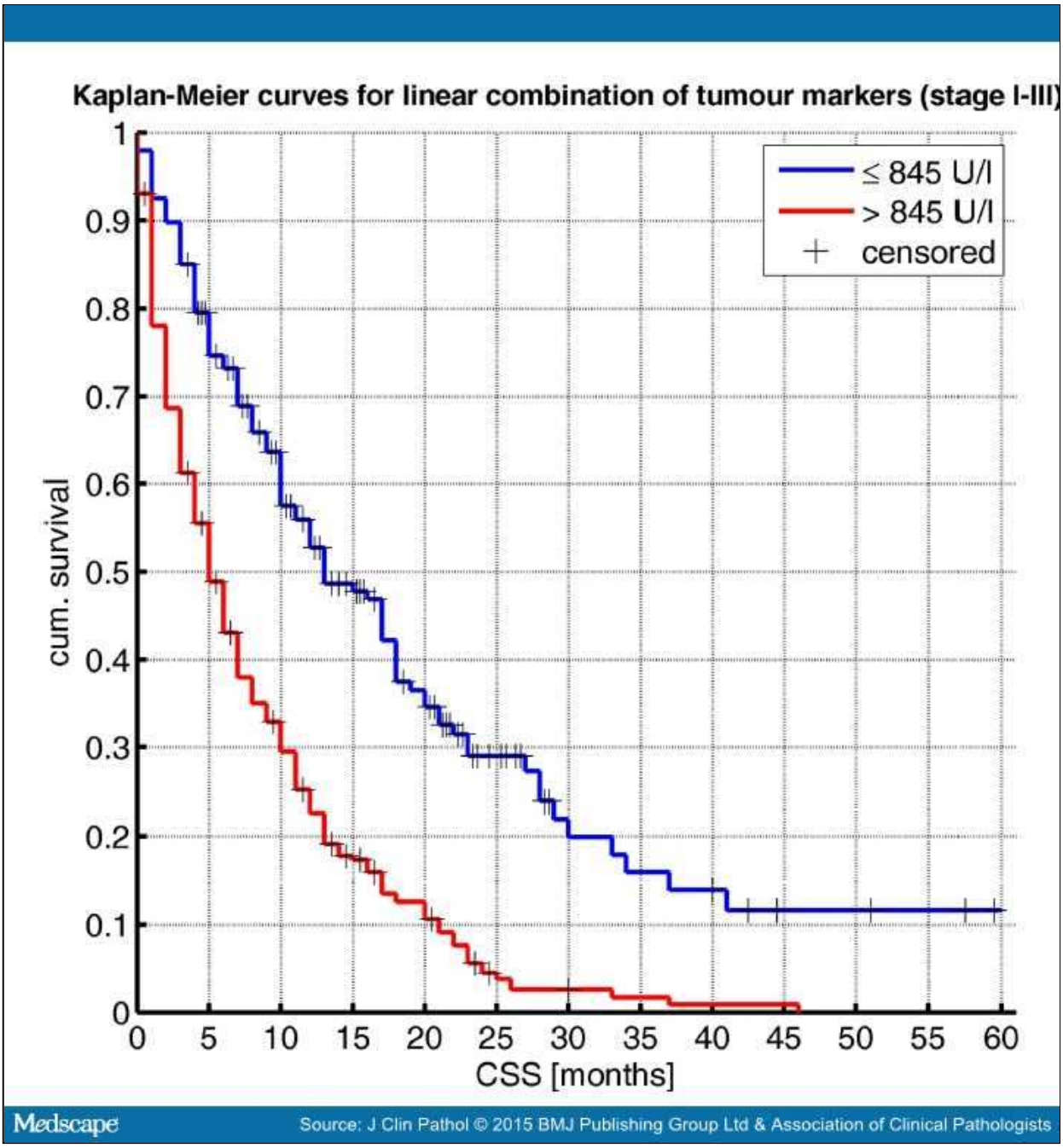

Figure 3.

Kaplan–Meier curves categorised by the optimal cut-off value (based on receiver operating characteristic analysis result: 845 U/L) of the linear combination of carcinoembryonic antigen and carbohydrate antigen 19-9 for the whole study cohort (log-rank test: p<0.001). CSS, cancer-specific survival.

Cox Analyses

To investigate whether clinicopathological variables are associated with clinical outcome of patients with PC, we calculated univariable and multivariable models for CSS ().

Table 6. Univariable and multivariable Cox analyses of clinicopathological variables for the prediction of CSS in patients with pancreatic cancer (n=393)

	Univariable analysis		Multivariable analysis*	
Variable	**HR (95% CI)**	**p Value**	**HR (95% CI)**	**p Value**
Age at diagnosis (years)				

<65	1 (reference)	0.153	1 (reference)	0.991
≥65	1.17 (0.94 to 1.46)		1.00 (0.80 to 1.25)	
Gender				
Female	1 (reference)	0.394	1 (reference)	0.243
Male	1.11 (0.89 to 1.38)		1.14 (0.91 to 1.43)	
Tumour stage				
I–III	1 (reference)	<0.001	1 (reference)	0.002
IV	1.51 (1.37 to 1.66)		1.37 (1.12 to 1.66)	
Tumour grade				
G1+G2	1 (reference)	0.008	1 (reference)	<0.001
G3+G4	1.34 (1.08 to 1.67)		1.73 (1.38 to 2.18)	
Surgical resection				
No	1 (reference)	<0.001	1 (reference)	0.206
Yes	0.33 (0.25 to 0.43)		0.69 (0.39 to 1.22)	
Chemotherapy				
No	1 (reference)	<0.001	1 (reference)	<0.001
Yes	0.43 (0.34 to 0.55)		0.33 (0.26 to 0.43)	
Karnofsky index				
≤80	1 (reference)	0.137	1 (reference)	0.775
90–100	0.95 (0.89 to 1.02)		0.99 (0.91 to 1.08)	
CEA (U/L)				
≤6.9	1 (reference)	<0.001	1 (reference)	0.054
>6.9	2.05 (1.63 to 2.58)		1.27 (1.00 to 1.61)	
CA19-9 (U/L)				
≤931.4	1 (reference)	<0.001	1 (reference)	0.007
>931.4	2.01 (1.62 to 2.51)		1.38 (1.09 to 1.74)	
CEA×CA19-9 (U^2/L^2)				
≤1976.5	1 (reference)	<0.001	ND	ND
>1976.5	2.11 (1.69 to 2.64)		(dependency)	
85×CEA+CA19-9 (U/L)				
≤845	1 (reference)	<0.001	ND	ND
>845	2.33 (1.84 to 2.96)		(dependency)	

*Covariates: age, gender, tumour stage, tumour grade, surgical resection, chemotherapy, Karnofsky index, CEA and CA19-9. CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CSS, cancer-specific survival; ND, not done.

Univariable Cox analyses identified tumour stage (stage I–III vs stage IV, p<0.001), tumour grade (G1+G2 vs G3+G4, p=0.008), surgical resection (no vs yes, p<0.001), chemotherapy (no vs yes, p<0.001), CEA (≤6.9 vs >6.9, p<0.001), CA19-9 (≤931.4 vs >931.4, p<0.001), $TM_{product}$ (≤1976.5 vs >1976.5, p<0.001) and TM_{linear} (≤845 vs >845, p<0.001) as statistically significant prognostic factors for CSS. Age, gender and Karnofsky index were not significantly associated with clinical outcome. also indicates that the linear combination of the tumour markers CEA and CA19-9 with the cut-off value '845' leads to the highest HR (HR 2.33; 95% CI 1.84 to 2.96) of all clinicopathological variables.

Table 6. Univariable and multivariable Cox analyses of clinicopathological variables for the prediction of CSS in patients with pancreatic cancer (n=393)

	Univariable analysis		Multivariable analysis*	
Variable	**HR (95% CI)**	**p Value**	**HR (95% CI)**	**p Value**

Age at diagnosis (years)				
<65	1 (reference)	0.153	1 (reference)	0.991
≥65	1.17 (0.94 to 1.46)		1.00 (0.80 to 1.25)	
Gender				
Female	1 (reference)	0.394	1 (reference)	0.243
Male	1.11 (0.89 to 1.38)		1.14 (0.91 to 1.43)	
Tumour stage				
I–III	1 (reference)	<0.001	1 (reference)	0.002
IV	1.51 (1.37 to 1.66)		1.37 (1.12 to 1.66)	
Tumour grade				
G1+G2	1 (reference)	0.008	1 (reference)	<0.001
G3+G4	1.34 (1.08 to 1.67)		1.73 (1.38 to 2.18)	
Surgical resection				
No	1 (reference)	<0.001	1 (reference)	0.206
Yes	0.33 (0.25 to 0.43)		0.69 (0.39 to 1.22)	
Chemotherapy				
No	1 (reference)	<0.001	1 (reference)	<0.001
Yes	0.43 (0.34 to 0.55)		0.33 (0.26 to 0.43)	
Karnofsky index				
≤80	1 (reference)	0.137	1 (reference)	0.775
90–100	0.95 (0.89 to 1.02)		0.99 (0.91 to 1.08)	
CEA (U/L)				
≤6.9	1 (reference)	<0.001	1 (reference)	0.054
>6.9	2.05 (1.63 to 2.58)		1.27 (1.00 to 1.61)	
CA19-9 (U/L)				
≤931.4	1 (reference)	<0.001	1 (reference)	0.007
>931.4	2.01 (1.62 to 2.51)		1.38 (1.09 to 1.74)	
CEA×CA19-9 (U^2/L^2)				
≤1976.5	1 (reference)	<0.001	ND	ND
>1976.5	2.11 (1.69 to 2.64)		(dependency)	
85×CEA+CA19-9 (U/L)				
≤845	1 (reference)	<0.001	ND	ND
>845	2.33 (1.84 to 2.96)		(dependency)	

*Covariates: age, gender, tumour stage, tumour grade, surgical resection, chemotherapy, Karnofsky index, CEA and CA19-9.
CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CSS, cancer-specific survival; ND, not done.

In multivariable analysis (selected covariates: age, gender, tumour stage, tumour grade, surgical resection, chemotherapy, Karnofsky index, CEA, CA19-9) using Cox proportional hazard model to find independent prognostic factors for CSS among the two tumour markers, only CA19-9 could be identified as prognostic independent (HR 1.38; 95% CI 1.09 to 1.74; p=0.007), CEA narrowly missed the significance level (HR 1.27; 95% CI 1.00 to 1.61; p=0.054). Based on the inherent dependency of the mathematical combinations with the single tumour markers, they were not added to the multivariable analysis model at this time. Other independent prognostic factors for CSS were tumour stage (p=0.002), tumour grade (p<0.001) and chemotherapy (p<0.001).

Furthermore, to verify an optimal Cox model, we applied a 'stepwise' algorithm that sequentially added significant variables into the model (initially selected covariates: age, gender, tumour stage, tumour grade, surgical resection, chemotherapy, Karnofsky index, CEA, CA19-9, CEA×CA19-9, 85×CEA+CA19-9); after every single step, it checked the new model and possibly removed non-significant variables, so finally of all the clinicopathological variables shown in only tumour stage (HR 1.53; 95% CI 1.38 to 1.70;

p<0.001), tumour grade (HR 1.68; 95% CI 1.34 to 2.11; p<0.001), chemotherapy (HR 0.34; 95% CI 0.26 to 0.43; p<0.001) and the linear combination of CEA and CA19-9 (HR 1.61; 95% CI 1.25 to 2.07; p<0.001) remained.

Table 6. Univariable and multivariable Cox analyses of clinicopathological variables for the prediction of CSS in patients with pancreatic cancer (n=393)

	Univariable analysis		Multivariable analysis*	
Variable	**HR (95% CI)**	**p Value**	**HR (95% CI)**	**p Value**
Age at diagnosis (years)				
<65	1 (reference)	0.153	1 (reference)	0.991
≥65	1.17 (0.94 to 1.46)		1.00 (0.80 to 1.25)	
Gender				
Female	1 (reference)	0.394	1 (reference)	0.243
Male	1.11 (0.89 to 1.38)		1.14 (0.91 to 1.43)	
Tumour stage				
I–III	1 (reference)	<0.001	1 (reference)	0.002
IV	1.51 (1.37 to 1.66)		1.37 (1.12 to 1.66)	
Tumour grade				
G1+G2	1 (reference)	0.008	1 (reference)	<0.001
G3+G4	1.34 (1.08 to 1.67)		1.73 (1.38 to 2.18)	
Surgical resection				
No	1 (reference)	<0.001	1 (reference)	0.206
Yes	0.33 (0.25 to 0.43)		0.69 (0.39 to 1.22)	
Chemotherapy				
No	1 (reference)	<0.001	1 (reference)	<0.001
Yes	0.43 (0.34 to 0.55)		0.33 (0.26 to 0.43)	
Karnofsky index				
≤80	1 (reference)	0.137	1 (reference)	0.775
90–100	0.95 (0.89 to 1.02)		0.99 (0.91 to 1.08)	
CEA (U/L)				
≤6.9	1 (reference)	<0.001	1 (reference)	0.054
>6.9	2.05 (1.63 to 2.58)		1.27 (1.00 to 1.61)	
CA19-9 (U/L)				
≤931.4	1 (reference)	<0.001	1 (reference)	0.007
>931.4	2.01 (1.62 to 2.51)		1.38 (1.09 to 1.74)	
CEA×CA19-9 (U^2/L^2)				
≤1976.5	1 (reference)	<0.001	ND	ND
>1976.5	2.11 (1.69 to 2.64)		(dependency)	
85×CEA+CA19-9 (U/L)				
≤845	1 (reference)	<0.001	ND	ND
>845	2.33 (1.84 to 2.96)		(dependency)	

*Covariates: age, gender, tumour stage, tumour grade, surgical resection, chemotherapy, Karnofsky index, CEA and CA19-9.
CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CSS, cancer-specific survival; ND, not done.

Comparison of Subgroups: Stage I–III vs Stage IV

Our results for the original cohort were checked separately in more homogeneous subgroups. We applied ROC analyses in stage I–III, which showed similar results, but the optimal cut-off values were different (CEA: 1.8 (AUC=0.549; 95% CI 0.455 to 0.640;

p=0.373); CA19-9: 267.8 (AUC=0.688; 95% CI 0.597 to 0.768; p<0.001); $TM_{product}$=CEA×CA19-9: 303.6 (AUC=0.688; 95% CI 0.597 to 0.770; p<0.001)) with the exception of the linear combination that resulted in the same value as the original results (TM_{linear}=85×CEA+CA19-9; cut-off-value: 845 (AUC=0.708; 95% CI 0.618 to 0.787; p<0.001)). Furthermore, the optimal weight factor i for CEA in the linear combination could be proved to be the same as in the whole cohort (85). CEA did not show a significant AUC difference to 0.5 (p=0.3731) in the ROC analysis, as well as in the log-rank test in Kaplan–Meier analyses (p=0.2741), and it is therefore not recommended to be used. Kaplan–Meier analyses for CEA (cut-off value: 1.8) showed a median CSS time of 20 (95% CI 13–28) months (≤1.8) vs 17 (95% CI 13 to 23) months (>1.8), for CA19-9 (cut-off value: 267.8) a median survival time of 28 (95% CI 20 to 41) months (≤267.8) vs 12 (95% CI 8 to 16) months (>267.8), for $TM_{product}$=(CEA×CA19-9) (cut-off value: 303.6) a median survival time of 28 (95% CI 20 to 41) months (≤303.6) vs 13 (95% CI 8 to 18) months (>303.6) and for TM_{linear}=(85×CEA+CA19-9) (cut-off value: 845) a median survival time of 28 (95% CI 18 to 34) months (≤845) vs 11 (95% CI 7 to 17) months (>845). All log-rank tests were highly significant (p<0.001) with the exception of the CEA variable (p=0.2741). Regarding survival in stage I–III, the linear combination clearly demonstrated the same significant tendency to represent a prognostic variable (figure 4).

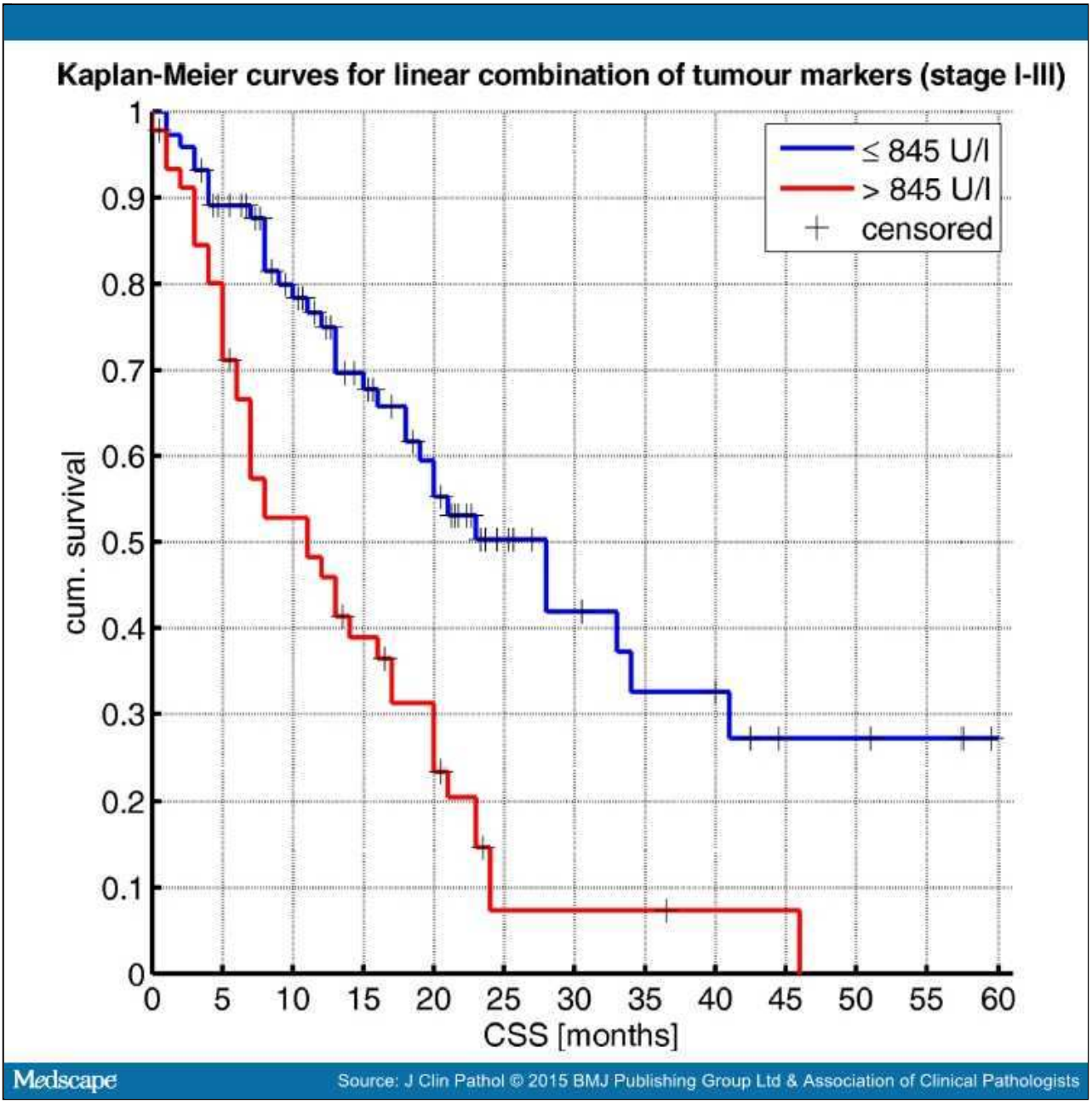

Figure 4.

Kaplan–Meier curves categorised by the optimal cut-off value (based on receiver operating characteristic analysis result: 845 U/L) of the linear combination of carcinoembryonic antigen and carbohydrate antigen 19-9 for patients in tumour stage I–III (log-rank test: p<0.001). CSS, cancer-specific survival.

In stage IV, it is recommended to use the results of the global cohort since AUC of our ROC analyses inside this subgroup was not significantly different to 0.5 for any of the four tumour marker variables (p>0.2). Additionally, in stage IV, it makes no big difference which of the four tumour marker variables is used relating to survival differences. Kaplan–Meier analyses showed for CEA (cut-off-value: 6.9) a median CSS time of 7 (95% CI 5 to 9) months (≤6.9) vs 4 (95% CI 3 to 5) months (>6.9), for CA19-9 (cut-off value: 931.4) a median survival time of 7 (95% CI 5 to 10) months (≤931.4) vs 4 (95% CI 3 to 5) months (>931.4), for $TM_{product}$= (CEA×CA19-9) (cut-off value: 1976.5) a median survival time of 8 (95% CI 6 to 10) months (≤1976.5) vs 4 (95% CI 3 to 5) months (>1976.5) and for TM_{linear}=(85×CEA+CA19-9) (cut-off value: 845) a median survival time of 9 (95% CI 5 to 10) months (≤845) vs 4 (95% CI 3 to 6) months (>845). All log-rank tests were highly significant (p<0.001).

Association Between Linear Combination of Tumour Markers and Recurrence

Fifty-six patients developed a cancer recurrence after pathological R0 resection surgery after a median time of 10.5 months (95% CI 8.0 to 12.9) during our observation period. We tried to dichotomise this patients based on the recurrence time using the median value. ROC analyses were performed based on the classification (below or above median time of recurrence) and lead to a cut-off value for the linear combination of tumour markers of 2769.5 U/L. Although the AUC value (AUC=0.617; 95% CI 0.477 to 0.744) was not significantly different to 0.5 (p=0.13), most likely due to the small number of cases, Kaplan–Meier analyses showed a significant difference in the log-rank test (p=0.002) for the linear combination: a recurrence was diagnosed after a median time of 12 months (95% CI 10 to 15) for values below the cut-off value vs 4 months (95% CI 3 to 10) for values above.

Discussion

In the present study, we investigated how combinations of tumour markers CEA and CA19-9 affect the prognostic accuracy in patients with PC. Inspired by Kanda *et al*,[16] who had multiplied these two pretreatment marker values using the new index as prognostic variable, we had the idea to try to find other possible better options. Our study cohort with 393 examined patients is adequately large and has a long follow-up period. Concerning descriptive analyses of clinicopathological variables, results are widely comparable to many other publications (eg, age distribution, survival time distribution).[9,22] Nevertheless, because of the retrospective study design, we cannot fully exclude a selection bias in our study cohort.

As far as we know from review, this seems to be the first study that tries to use a linear combination of tumour markers for prognostic reasons in patients with PC ever. In our case, the solution for an optimal linear combination was quite easy to find as there are only two markers joined together and only one weight factor variable must be optimised; however, if more biomarkers are combined, special methods may be needed to maximise AUC of ROC analyses.[23,24] Based on the results of our analyses, the linear combination of CEA and CA19-9 managed to give the best solutions in terms of prognostic usefulness of pretherapeutic tumour marker values compared with single markers. Multiplication was only significantly better than CEA alone though not better than CA19-9.

Most studies that tried to verify the prognostic prediction of pretherapeutic tumour markers in patients with PC did not use ROC analyses like we did to find optimal cut-off values; instead, they applied the median value of a group to realise a dichotomy. However, in many times this makes no big difference to get significant changes in the survival time between two groups.[25–27] Of course, to realise ROC analyses an additional classification variable like CSS status is needed. Certainly it should be taken into account when comparing results that sometimes just a simple dichotomy of elevated values is made (eg, the usual cut-off value between normal and abnormal range of CA19-9 is 37 U/L, for CEA 5 U/L[9]), which often leads automatically to circumstances that no feasible conclusions are possible. Ballehaninna *et al*[28,29] verified that preoperative patients with PC with CA19-9 values <37 U/L had a significant longer median survival compared with the group that had values above. Lee *et al* could not find a significant difference in log-rank testing for CA19-9 (>37 U/L), unlike CEA (>5 U/L). Additionally, univariable and multivariable analyses proved from the tumour marker side only for CEA a prognostic relevant meaning.[9] Haas *et al* observed patients with PC in an advanced stage of disease receiving palliative chemotherapy. Univariable analyses could only define pretreatment CA19-9 as prognostic significant for overall survival, but not CEA.[30] Another study explained that both markers showed in univariable as well as multivariable analyses a highly significant prognostic result.[31] Lundin *et al* tried to find the lowest cut-off value by graphical analysis of χ^2 values and looked whether log-rank test results fell below the significance level. For CA19-9, only patients in tumour stage II and III could offer a cut-off level of 370, CEA managed to define a level of 15, but only in tumour stage IV.[32] Mehta *et al*[33] described in a similar manner that elevated levels of CA19-9 and CEA (>2 times) predict increased chances of inoperability and poor survival in PC.

From the diagnostic point of view, publications show that combinations of tumour markers can significantly improve the diagnostic accuracy; as an example we mention two studies (Carpelan-Holmstrom *et al*[34] and Louhimo *et al*[35]) that used logistic regression models with tumour markers CEA and CA19-9 with either CA72-4 or hCGbeta for different kinds of gastrointestinal malignancies. Jiang *et al*[36] described especially for PC that parallel combined testing of tumour markers was able to increase the sensitivity to almost 90% and serial combined examination offered a specificity of over 92%. Louhimo *et al*[37] described in another study that hCGbeta and CA72-4 were even better prognostic factors in multivariable analysis than CEA and CA19-9 in patients with PC. Ni *et al*[38] showed in a combined test that if two or more tumour markers (CEA, CA19-9, CA242) exceeded the normal range, then prognosis was worse than if only one single or no marker was expressed.

In summary, it is an interesting fact that mathematical combinations of tumour markers are feasible and right combinations offer significantly better prognostic prediction results than a single marker for itself, although no further information is needed and therefore no additional costs are generated. Apart from the economic side, this allows yet other thoughts. It seems obvious that combinations of tumour markers may not only improve the prognostic accuracy in patients with PC but also in many other malignancies.

In our study cohort, the pretherapeutic serum CA19-9 value proved to be an independent prognostic factor in patients with PC, so aside from being a progression variable for treatment response and relapse diagnostic in the routine management CA19-9 offers information about the expected course of the disease. Finally, the most important result of this study is that a linear combination of CEA and CA19-9 is significantly better in the prognostic prediction compared with single tumour markers in all performed analyses. Large prospective studies are warranted to confirm the findings and, more importantly, to evaluate the value of the mathematical combination in early tumour stages.

Sidebar

Take Home Messages

- Tumour markers carcinoembryonic antigen and carbohydrate antigen 19-9 in pancreatic cancer can help to prognosticate individual risk assessment in different tumour stages.
- The linear mathematical combination of these tumour markers improves the prognostic power and might be helpful to stratify patients in clinical trials according to different risk groups.
- Mathematical combinations of tumour markers might be helpful to improve the prognostic accuracy in many other malignancies too.

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