Research Article

Preoperative CTC-detection by Cell-Search® is associated with early distant metastasis and impaired survival in resected pancreatic cancer

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Simple Summary: The survival after surgical removal of pancreatic cancer is remaining dismal with frequent cases of treatment failure by early cancer recurrence. There are currently no means to preoperatively identify those at risk for early recurrence. Circulating tumour cells (CTCs) have been shown to identify high-risk individuals in a variety of cancer types. We did explore the impact of CTC detection with the FDA-approved CellSearch test on relapse and survival after removal of pancreatic cancer. CTCs were detected in about 7% of patients. The presence of CTCs in samples taken before the operation was associated with earlier cancer metastasis and shorter survival. The survival impact of CTCs was comparable to or exceeded that of risk-factors available after operation like spread to lymph nodes or aggressive tissue growth patterns. We conclude that analysis for CTCs by this method warrants further exploration of its clinical application in presumed operable pancreatic cancer.

Abstract: In patients with presumed pancreatic ductal adenocarcinoma (PDAC), biomarkers that may open for personalised, risk-adapted treatment are lacking. The study analysed the impact of circulating tumour cells (CTCs) on the patterns of recurrence and survival in 98 patients resected for PDAC with 5-10 years of follow-up. Preoperative samples were analysed by the CellSearch® system for EpCAM+/DAPI+/CK+/CD45- CTCs. CTCs were detected in 7 of the 98 patients. CTCs predicted a significantly shorter median DFS of 3.3 vs. 9.2 months and a median CSS of 6.3 vs. 18.5 months. Relapse status was confirmed by imaging for 87 patients. Of these, 58 developed distant metastases (DM) and 29 cases isolated local recurrence (ILR) as first event. All patients with CTCs experienced DM. pN-status and histological grade >2 were other independent risk factors for DM, but only CTCs predicted significantly shorter cancer-specific, disease-free and post-recurrence survival. We conclude that CTC presence in resected PDAC patients predicted early distant metastasis and impaired survival. The impact of CTCs was comparable to that of histopathological risk factors and exceeded the effect size of other preoperative parameters. Thus, preoperative CTCs alone or in combination with histopathological factors may guide initial treatment decisions in patients with resectable PDAC in the future.

Keywords: Pancreatic cancer, recurrence, metastasis, surgical resection, circulating tumour cells, CTC, CellSearch, prognostication

1. Introduction

The prognosis of pancreatic ductal adenocarcinoma (PDAC) is dismal. The mortality to incidence ratio is 0.96, one of the highest among solid tumours [1,2]. Patient outcomes have remained virtually unchanged in the last decades[3,4], despite improvements in chemotherapy, radiation and operative technique. PDAC is the 4th leading cause of cancer mortality in the Western world and projected to become the 2nd leading cause within the next two decades[5,6]. Surgical resection in cases of localised tumours is the only treatment offering potential cure, although the 5-year survival rate is between 5-9% in unselected cohorts [3]. About half of the patients experience recurrence within the first year [7,8]. There is no curative treatment option in the case of PDAC recurrence, although aggressive treatment may still prolong survival, especially in cases of isolated locoregional recurrence (9,10). Currently, risk assessment from preoperatively available factors has shown limited ability to predict survival[9], but postoperative staging based on histopathological parameters can better predict impaired survival [10-12]. While prognostic factors available after surgery may be of value for better care, the current lack of individualised up-front treatment stratification by preoperative risk assessment is a major hindrance for improved treatment results of patients with presumed resectable PDAC. The preoperative presence of even one single circulating tumour cell (CTC) per sample in peripheral blood detected by the CellSearch system® has been shown to be associated with metastatic recurrence and impaired survival in several cancer types [13,14]. Previous studies of PDAC-patients have shown CTCs to be a risk factor for impaired survival [15-18]. CTCs in the portal venous blood have been associated with liver metastasis in PDAC[19-21], but evidence linking CTCs from peripheral blood to a specific recurrence type is missing. The scope of the present study was to identify risk-factors for the type of recurrence and survival among patients that underwent a curative resection for PDAC with special focus on CTC-status.

2. Results

2.1. Patient group characteristics

Of the 98 patients in the study group, 95 patients had pT3 tumours, 73 were lymph node positive, 61 had R1 resections performed and 97 were of the pancreaticobililary histological subtype. Further details on clinical features, surgical procedures and systemic treatment of the study group are summarised in Table 1.

Table 1. Clinicopathological parameters

	Resected PDAC (n=98)	
Demographics	n	(%)
Age, median [years]	68	(34-80)
Sex, male	50	(51.0%)
Preoperative Risk Factors		
CTCs ≥1/7.5ml	7	(7.1%)
CA19-9 ≥ 200kU/l	31/74	(41.9%) *24 missing*
Tumour size on imaging> 25mm	39/97	(40.2%) *1 missing*
Bilirubin > 50μmol/L	75/94	(79.8%) *4 missing*
Treatment		
Neoadj. chemotherapy:		

GEMZ	3	(3.0%)
FOLFIRINOX	1	(1.0%)
Operation:		
PPPD	77	(78.6%)
PD	13	(13.3%)
total pancreatectomy	8	(8.2%)
Venous resection	36	(36.7%)
Adjuvant chemotherapy: FLV		
GEMZ	53	(54.1%)
FLOX	6	(6.1%)
none	3	(3.1%)
	36	(36.7%)

Histopathologic results		
Pancreatobiliary type	97	(100%)
Intestinal type	1	(1.0%)
UICC-stage (V7): Ib	2	(2.0%)
IIa	23	(23.5%)
IIb	73	(74.5%)
pN1-status	73	(74,5%)
R1-status	61	(62.2%)
Vascular infiltration	65	(66.3%)
Perineural infiltration	91	(92.9%)

FLV, GEMZ, FLOX, FOLFIRINOX: chemotherapy regimen

PD: pancreatico- duodenectomy; **PPPD:** pylorus preserving PD.

The 90-day mortality was 2.0% (2/98), both cases due to early cancer recurrence. The median observation time was 96 months (range 63-126).
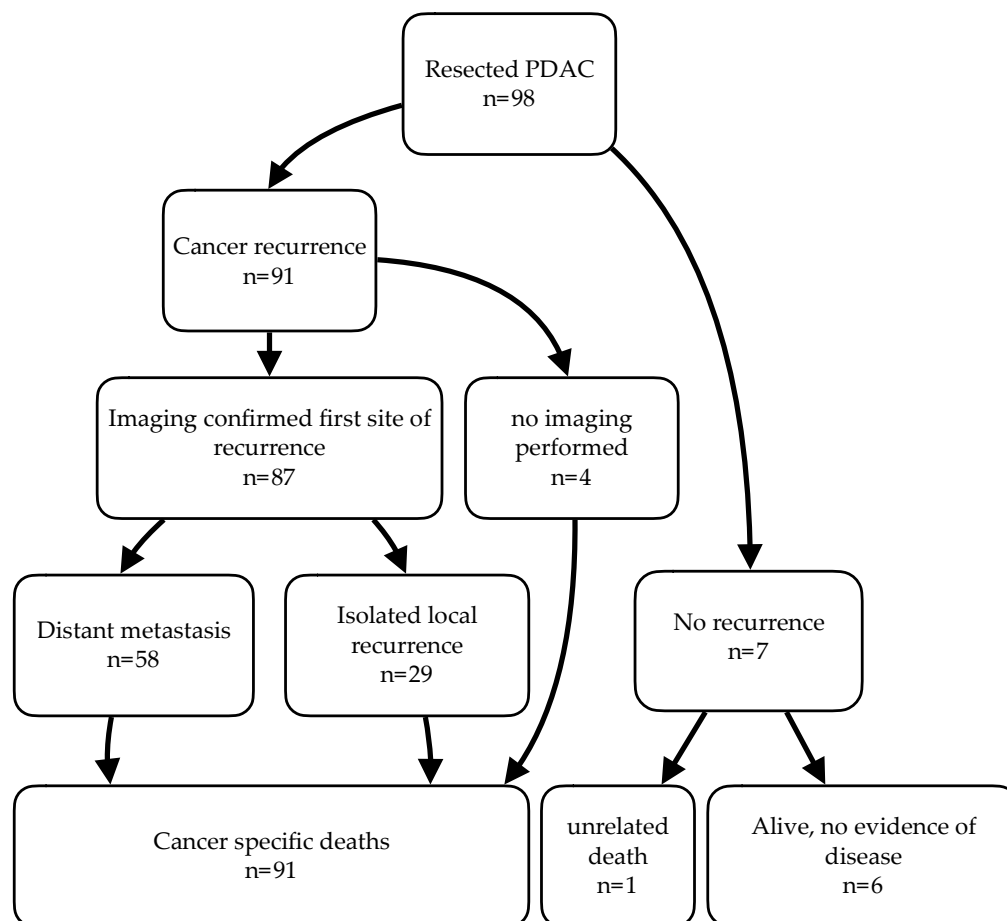
Figure 1. Overview of clinical events for the patient cohort, starting at the time of operation until the outcome stated in the last row at the end-point of observation was reached. **PDAC**: pancreatic ductal adenocarcinoma.

Figure 1 summarises the recurrence events including death during the follow-up period. Six (6.1%) patients were alive at the end of the study. Four patients died of cancer progression without the confirmation of the site of first recurrence, one patient died of pneumonia without signs of cancer recurrence. Among the 87 cases with imaging-confirmed recurrence, distant metastasis (DM) was detected first in 58 (67%) cases, 16 of whom had simultaneous local recurrence. Twenty-nine patients (33%) presented with isolated local recurrence (ILR).

2.2. Distribution of CTCs and site of first recurrence

The frequency of CTCs in the entire group was 7.1% (7/98) (Table 1). As shown in Figure 2, all CTC-positive patients developed distant metastasis first. Furthermore, six of seven CTC-positive patients developed liver metastasis. Two patients with CTCs had both distant metastasis and local recurrence concurrently. No CTCs were observed in patients with ILR.
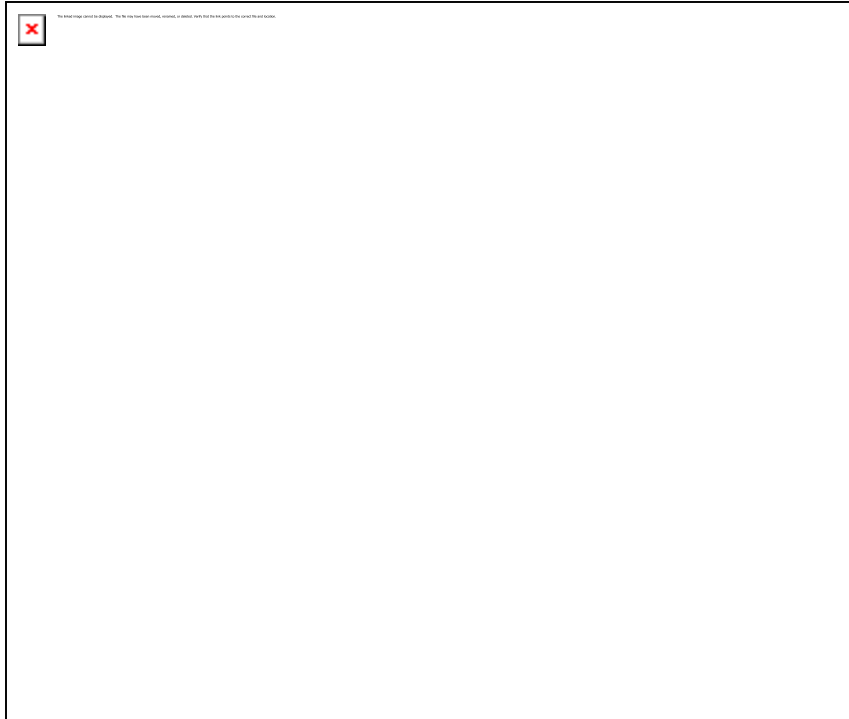
2.3. Recurrence and survival analysis

While the incidence of distant metastasis was twice the incidence of ILR (58 vs 29 patients), the time to either type of recurrence was not significantly different (TDM 6.2 months vs. TILR 9.2 months p=.886; Fig 3a). Thirty-seven patients experienced metastases to the liver, which was the most frequent localisation of recurrence. Liver metastasis was associated with a significantly shorter CSS compared to distant metastases at other sites (13.1 vs. 24.2 months, HR 1.9 p=.005; Figure 3b). The differences in survival between any of these two groups and patients with ILR did not reach statistical significance (Fig 3b). Finally, CTCs had strong impact on CSS (6.6 vs. 18.5 months, HR 4.6 p<.001; Fig 3c) and DFS (3.3 vs. 9.2 months, HR 2.8 p=.008; Fig 3d)
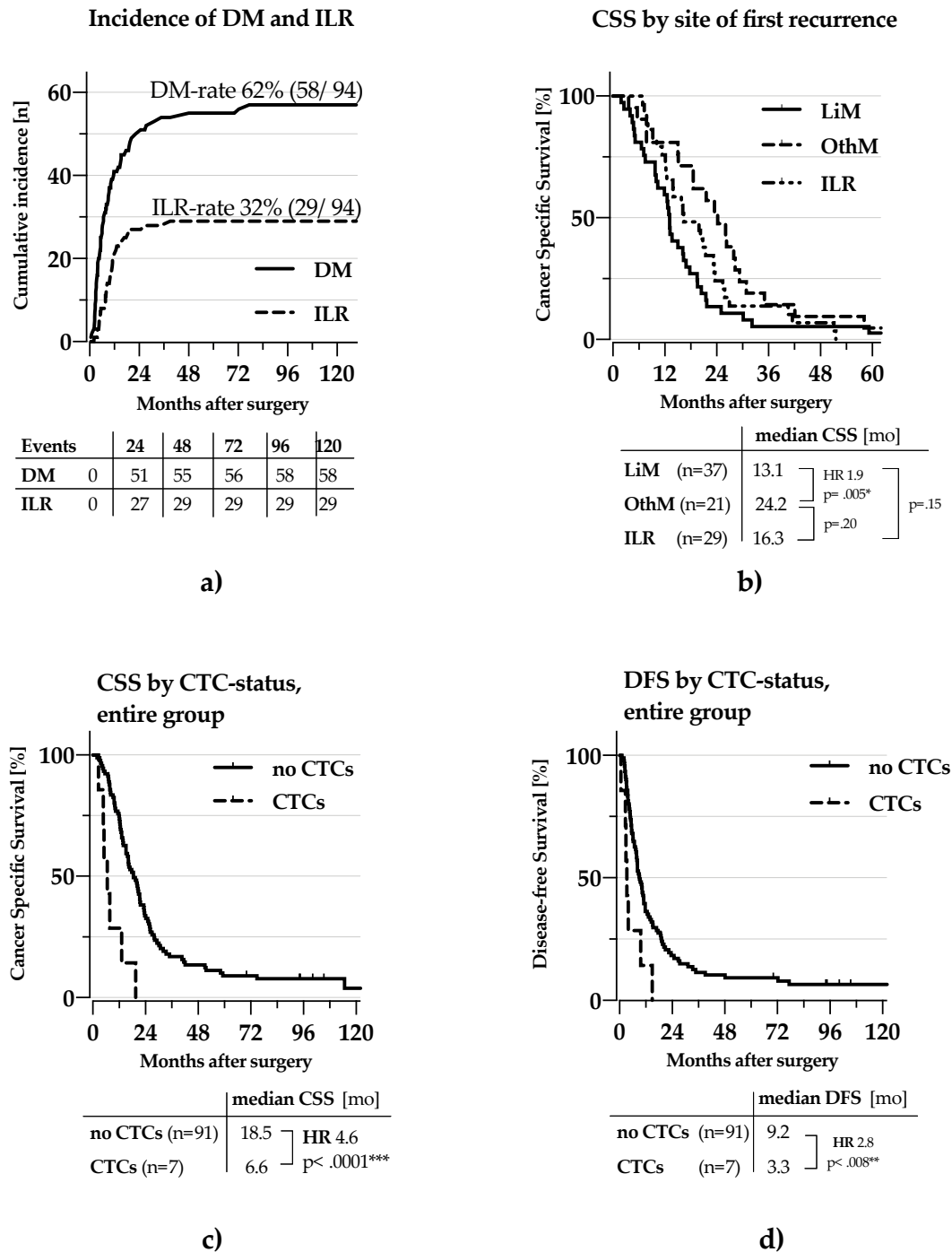
Figure 3: Recurrence and Survival patterns for resected PDAC Kaplan-Meier curves for survival and time to recurrence. **a)** Incidence by site of first recurrence **b)** Cancer specific survival by site of first recurrence. **c)** CSS by CTC-status for all patients **d)** DFS by CTC-status for all patients **CSS**: cancer-free survival, **DFS**: disease-free survival, **DM:** distant metastasis **ILR:** islolated local recurrence, **LiM:** Liver metastasis, alone or in combination with others, **OthM:** any other type of metastasis. **HR:** Hazard ratio. * Statistical significance assumed for p<0.05

Uni- and multivariable analysis of risk-factors for either TDM, TILR or CSS identified several independent risk factors (Table 2).

Table 2: Univariate and multivariable analyses of time to type of first recurrence and cancer specific survival

Potential Risk Factors	Level	n	TMR Univariate HR (95% CI)	p	TMR Multivariable HR (95% CI)	p	TILR Univariate HR (95% CI)	p	TILR Multivariable HR (95% CI)	p	CSS Univariate HR (95% CI)	p	CSS Multivariable HR (95% CI)	p
Age	≤70	58	1.2 (0.7-2.1)	0.602			1.3 (0.6-2.8)	0.449			1.4 (0.9-2.1)	0.156		
	>70	40												
Sex	male	50	0.7 (0.4-1.2)	0.127			0.8 (0.4-1.7)	0.627			0.8 (0.5-1.2)	196		
	female	48												
CA19-9 (24 missing)	≥200	43	1.2 (0.6-2.2)	0.623			1.0 (0.5-2.3)	0.939			1.1 (0.7-1.8)	0.601		
	<200	31												
Tumour size on CT (1 missing)	≥25mm	39	1.4 (0.8-2.3)	0.272			2.0 (1.0-4.)	0.057			1.3 (0.8-2.0)	0.241		
	<25mm	58												
CTC	≥1	7	**3.9 (1.7-8.8)**	**0.001**	**2.9 (1.3-6.6)**	**0.010**	All censored				**4.4 (2.0-9.8)**	**<0.001**	**3.7 (1.7-8.3)**	**0.001**
	none	91												
Neoadjuvant chemotherapy	yes	4	1.2 (0.3-5.0)	0.928			2.0 (0.5-8.7)	0.333			1.0 (0.4-3.7)	0.964		
	no	94												
Venous resection	yes	36	**0.5 (0.3-0.9)**	**0.017**	eliminated		1.6 (0.8-3.3)	0.209	eliminated		0.8 (0.5-1.2)	0.326	eliminated	
	no	62												
Adjuvant chemotherapy	yes	62	0.8 (0.5-1.4)	0.466			0.9 (0.4-1.9)	0.735			0.7 (0.7-1.1)	0.124		
	no	36												
pT	1,2	3	0.7 (0.2-2.7)	0.557			All censored				0.5 (0.1-1.9)	0.300		
	3,4	95												
pN	1	73	**3.6 (1.7-7.3)**	**0.001**	**3.0 (1.5-6.3)**	**0.003**	1.6 (0.7-3.6)	0.226	eliminated		**2.1 (1.2-3.5)**	**0.004**	**2.0 (1.2-3.4)**	**0.009**
	0	25												
Grade	G3,4	28	**2.1 (1.2-3.6)**	**0.007**	**1.8 (1.1-3.1)**	**0.030**	0.6 (0.2-1.7)	0.336	eliminated		1.4 (0.9-2.1)	0.184	eliminated	
	G1,2	70												
R	1	61	1.4 (0.8-2.3)	0.212	eliminated		**2.3 (1.0-5.0)**	**0.040**	**2.3 (1.0-5.0)**	**0.040**	1.4 (0.9-2.2)	0.128	eliminated	
	0	37												
Vascular infiltration	1	65	1.7 (0.9-3.1)	0.075	eliminated		0.9 (0.4-1.9)	0.790	eliminated		**1.6 (1.0-2.6)**	**0.034**	eliminated	
	0	33												
Perineural infiltration	1	91	1.3 (0.5-3.7)	0.588			2.7 (0.4-19.7)	0.334			1.8 (0.7-4.6)	0.185		
	0	7												

Uni- and multivariable analysis of prognostic factors for TDMR/ TILR of resected PDAC patients. **Univariate HR**: Cox regression using single factors; **Multivariable HR**: Cox regression model with manual backwards elimination. Statistical significance assumed for p<0.05 **HR**: Hassard ratio. **CI**: Confidence interval. **TDMR**: time to distant metastasis. **TILR**: time to isolated local recurrence.

CTC-status, pN and histological grade>2 were independent risk factors for TDM. R-status was the sole risk factor to predict TILR. In addition, CTC-status and pN were independent risk factors for CSS (Table 2).
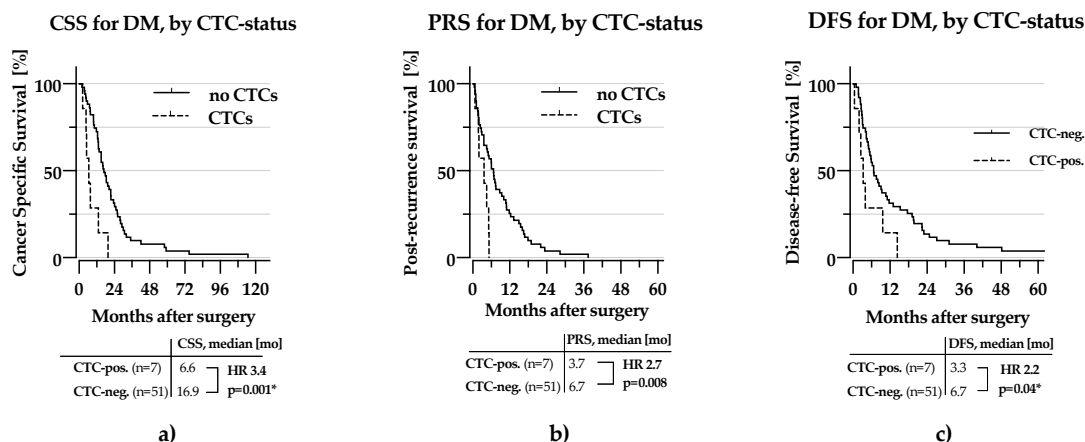

Figure 4: KM-curves for patients with DM as site of first metastasis **A)** CSS by CTC-status **B)** PRS by CTC-status **C)** DFS by CTC-status **CTC:** Circulating tumour cell, **CSS:** Cancer-free survival, **PRS:** Post-recurrence survival, **DFS:** Disease-free survival, **HR:** Hazard ratio. Statistical significance assumed for p<0.05

Figure 4 presents the isolated survival results for patients who developed distant metastasis during the observation period, showing reduced CSS and DFS for CTC-positive patients (Fig 4a,4c). Also, the post-recurrence survival was severely affected by CTC-status (HR 2.7, p=.008, Fig 4b).

2.4. CSS according to subgroups by combining risk-factors for distant metastasis

When combining the three independent unfavorable risk factors for metastatic recurrence (CTC-positivity, node-positivity and high Grade), patients having all three factors had a dismal CSS compared to those positive for just one or two of the factors (5.1 months vs. 16.4 months, HR 3.1, p=.001, Fig. 5).
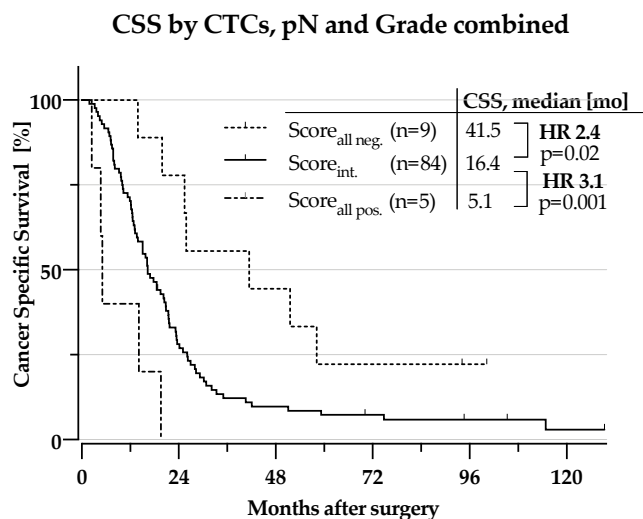

Figure 5: Kaplan-Meier curves for survival times: Cancer specific survival by summation of CTC-pos., pN1 and Grade>2 for the entire cohort. **Score$_{all neg.}$:** all three negative, **Score$_{int.}$:** one or two factors positive, **Score$_{all pos.}$:** all three positive, **CSS:** cancer-free survival, **CTC:** circulating tumour cell, **HR:** Hazard ratio. Statistical significance assumed for p<0.05

Interestingly, those with none of the risk factors present had a markedly improved CSS compared to those with one or two factors present (41,5 months vs. 16.4 months, HR 2.4, p=.02, Fig 5).

3. Discussion

This report presents results from a well-characterised single institution study of patients resected for PDAC with an extended observation time. To the best of our knowledge, the cohort represents currently the largest group of resected PDAC patients analysed for CTCs by CellSearch. The study gives insight into the association between preoperative CTC-status, other clinical and histopathological risk factors and the patterns of recurrence and patient survival. CTCs predicted earlier metastasis and impaired survival following potentially curative surgery. The relatively low frequency of CTCs of 7.1% for the 98 patients in the study group is consistent with other reports [19,22-24] utilising the same detection method. While a correlation of CTCs in the portal venous blood with earlier liver metastasis was reported from two smaller cohorts[19,25], we are not aware of any reported study of the association between CTCs from peripheral blood and recurrence types. The presented association of CTCs with DM as the first site of recurrence and the prediction of a markedly shorter TDM underscore the usefulness of CTCs for prediction of early metastatic events in the clinic. In addition, of all risk-factors examined, only CTCs had a significant impact also on post-recurrence survival, which further corroborates the severe impact of CTC-presence for the subsequent CSS.

Of the other risk factors with impact on DM, pN-status and high histological grade, only pN-status correlated with CSS and none with PRS. Still, the combination of the three factors could further differentiate the survival among the patients. Those, with absence of all three risk factors had the longest CSS (41.5 months) while the survival of patients positive for all three factors was detrimental (5.1 months). No CTCs were detected in patients with ILR and R-status was the only risk-factor for ILR.

The ability to preoperatively predict the likelihood for long-term survival from factors identified in the initial work-up would be of potential clinical benefit for patients currently scheduled for up-front surgical resection. Recently, a predictive nomogram, based solely on preoperative variables has been created from several factors (CA 19-9, neoadjuvant therapy, tumour size, age, centre volume, Charlson-Deyo score, primary site, sex) that were independently associated with overall survival in 7849 patients from the National Cancer Database in the US[9]. Combined in a score derived from the regression parameters they were able to classify patients into three groups of favorable (>2.5y median OS), intermediate (1.5-2.5y median OS) and poor prognosis (<1.5y median OS). It has to be noted that the HRs of the single risk factors were very low, ranging from 1.07-1.37. Due to these weak correlations, a cohort size of nearly 8000 patients was necessary to establish statistical significance[9]. In contrast, in the present study, the HR for CTCs was 3.7 (95% CI 1.7-8.3, p=.001), exhibiting a strength of correlation commonly found for histopathologial factors[26,27]. Comparing the prediction strength, patients falling into the high-risk group of the PDAC-nomogram had a predicted median OS of less than 1.5 years, while the present study shows a median CSS of 6.63 months for CTC-positive patients, indicating a stronger survival impact by CTCs. However, "in-between study comparisons" should be interpreted with caution. For postoperative prediction, the Amsterdam model[27], with its recent update[26] achieves excellent survival prediction. This was obtained by combining histopathological factors together with information about the completion of adjuvant therapy. While of interest, the delayed availability of the prediction by the Amsterdam model, i.e. 6 months after operation, may limit the clinical utility of this score.

The current study confirms the prognostic impact of histopathological factors as lymph node status, margin status and histological grade, but adds important information on the potential use of preoperative CTC-analysis for prognostic classification of PDAC. With the currently available techniques for CTC-detection and treatment modalities in PDAC the most promising use of CTC-analysis probably would seemingly be to support surgical treatment decisions.

When comparing published results on the effects of CTCs in cohorts of resected PDAC-patients, one should be aware of the the non-interchangeable nature of CTC-results by different detection methods [28,29]. In several medium-sized cohorts of resected PDAC, detection rates vary from 7-39% and the DFS/RFS ranges from 3.3-14 months for patients with CTC-presence to 10-31 months for patients without CTCs[15,18,30].

Studies exploring the prognostic value of ctDNA in PDAC have the potential to substantially enhance the information from a preoperative blood sample[31-33]. So far, evidence indicates that the clinical impact of these test could complement the information from a CTC-test [34,35]. The use of CTCs or ctDNA for further molecular characterisation of the disease is also warranted. Most probably, future improvements in systemic treatment are dependent upon identification of the core molecular characteristics or driver mutations of the cancer cells.

Limitations of the present study are mainly due to the low detection frequency for CellSearch in the setting of presumed resectable PDAC. Even as the study presents the largest cohort of PDAC examined by this method, the low detection frequency of around 7% and a still low number of patients studied limits the generalisation of the results reported. An independent study confirmation of the prognostic value of CTCs by CellSearch would strongly support the use of CTCs as a biomarker in future clinical practice. Ideally, further studies of the liquid-biopsy paradigm would also include detection methods with potentially complementary properties like ctDNA and exosomes to help advance clinical care for this patient group.

4. Materials and Methods

4.1. Patients, Study design and Follow-up

The cohort in the present study was recruited among patients referred to the hepatobiliary unit at Oslo University Hospital with the clinical suspicion of a potentially resectable solid mass in the periampullary region. Patients who were evaluated in a multidisciplinary tumour board as potentially resectable[36] were offered participation in an observational study on the survival impact of tumour cells in the peripheral blood (CTC) or bone marrow (DTC). Properties of the study cohort and general results have been published previously[18,37].

Prospective recording of the clinical data was done in an Epi-Info 3.5.3 database (CDC; Atlanta, GA; USA). Follow-up of patients included a thoracoabdominal CT-scan and CA19-9 assessment twice a year on a voluntary basis, either at the study hospital or the local hospital as previously described[38].

The type of recurrence was defined by the first location of cancer relapse as detected by diagnostic imaging. When imaging findings were consistent with recurrence, bioptic verification was rarely performed. Isolated local recurrence (ILR) was defined as recurrence solely in the remnant pancreas or in the surgical bed, such as soft tissue along the celiac or superior mesenteric artery, aorta or around the pancreaticojejunostomy site. Distant mestastasis (DM) was defined as recurrence in any site other than ILR. In the case of concurrent metastatic and local recurrence, cases were assigned to the DM group due to the greater survival impact of metastases [39]. Starting point for the time-to-event variables TDM and TILR was the date of operation, until DM or ILR respectively. Mortality data were taken from the Norwegian Cause of Death Registry, provided by the Norwegian Institute of Public Health. Cancer Specific Survival (CSS) was defined the time from date of surgery until cancer related death, post-recurrence survival (PRS) as the time between recurrence until cancer related death and disease-free surivival (DFS) as the time from surgery until any kind of recurrence or death. Cases reaching the end of observation without event were censored for these variables[40]. Patient inclusion started from October 1st, 2009 ,patient inclusion stopped December 31st   2014 and observation ended July 31st, 2020. Analysis and reporting were done observing the STROBE(2014) and REMARK(2012) checklists.

4.2. Characteristics of the patient cohort

For the present study, 98 patients in whom a curative resection for PDAC was successfully performed and the CTC-status was determined were selected from a cohort of 277 patients with presumed resectable periampullary carcinoma (results previously published [18]).

Along with the CTC-status, the following clinical risk factors were analysed: age, gender, preoperative CA19-9 level, tumour size on CT-scan, AJCC/UICC-stage (7th ed.), pTNM-staging including resection margin, cancer origin, tumour grade, histological subtype (predominantly intestinal or pancreatobiliary), vascular and perineural infiltration. CA19-09 and tumour size were

dichotomized at the following thresholds: CA19-9 ≥ 200kU/L and the size of the tumour on CT-scan ≥ 25mm (for results, see Table 1).

4.3. CTC Detection

The detection of EpCAM-positive CTCs was performed on the FDA-approved CellSearch platform, utilising the Circulating Epithelial Cell Kit (Product Code: 7900000) and the Celltracks Analyzer II System (Product Code: 9555, both from Menarini Silicon Biosystems Inc., Castel Maggiore, Italy).

A blood sample of 7.5 mL was drawn into one CellSave® tube (Product Code: 7900005) just before surgery. The samples were kept at room temperature until processing within 72 hours at the Micrometastasis Laboratory, Oslo University Hospital, Norway. Candidate events that conformed to the DAPI+/ CK+/ CD45- criteria for potential CTCs by the CellSearch Analyser Software were reviewed by a certified technician (CS) and the diagnosis was set by an experienced pathologist (EB). A threshold of 1 CTC/7.5 ml was set for a patient sample to be considered CTC-positive in accordance with previously published reports[18]. Results were stored in the National Micrometastasis Register at the Micrometastasis Laboratory, Department of Pathology, Oslo University Hospital, and were not available to treating clinicians. Following closure of the observation period, CTC-results were extracted from the Micrometastasis register and combined with the clinical data.

4.4. Statistics

Analyses were carried out in SPSS, V26 (IBM Cooperation Analytics, Armonk, NY, USA) and STATA 16 (Stata Corp LLC, College Station, Texas, USA). Graphs were prepared in PRISM 8 (GraphPad Software Inc., La Jolla, CA, USA). Survival analyses were computed by the Kaplan-Meier method, the difference of curve pairs was assessed by the Log-rank test. The adjustment for confounders was performed by Cox Proportional Hazard regression models with a manual backward stepwise elimination procedure. Multivariable analyses were preceded by estimation of correlation between confounders. The Proportional Hazard assumptions was controlled by plotting the logarithm of the integrated hazards (log–log survival plots). The association between potential risk factors and survival metrics was quantified by hazard ratios (HR) with a 95% confidence interval (95% CI). For $p < .05$, statistical significance was assumed.

5. Conclusions

CTCs in resected PDAC predict early distant metastasis and markedly impaired survival. Preoperative CTC detection, alone or in combination with histopathological factors, may be used to guide risk-adapted treatment in patients with presumed resectable PDAC.

Appendix A

Appendix Table 1. Clinical parameters by site of first relapse

	Entire cohort (n=98)		Remission (n=6)		ILR (n=29)		DM (n=58)		No imaging (n=4)	
Demographics										
Age, median [years]	68	(34-80)	65	(57-75)	68	(51-78)	68	(34-79)	74	(68-80)
Sex, male	50	(51.0%)	2	(33.3%)	14	(48.3%)	33	(59.0%)	0	(0%)
Preoperative Risk Factors										
CTCs≥1	7	(7.1%)	none		none		7	(12.5%)	none	
CA19-9 ≥ 200kU/l	31/74	(41.9%)	2/4	(50%)	10/25	(40%)	18/42	(42.9%)	1/2	(50%)
	24 missing		*2 missing*		*2 missing*		*14 missing*		*2 missing*	
Tumour size on imaging> 25mm	39/97	(40.2%)	2	(33.3%)	15	(51.7%)	21/57	(36.8%)	1	(25%)
	1 missing						*1 missing*			
Bilirubin > 50µmol/L	75/94	(79.8%)	5	(83.3%)	20/27	(74.1%)	47/56	(82.4%)	2	(50%)
	4 missing				*2 missing*		*1 missing*			
Treatment										
Neoadjuvant therapy:										
GEMZ	3	(3.0%)	none		2	(6.9%)	1	(1.7%)	none	
FOLFIRINOX	1	(1.0%)	none		none		1	(1.7%)	none	
Operation:										
PPPD	77	(78.6%)	5	(83.3%)	23	(79.3%)	46	(79.3%)	2	(50%)
PD	13	(13.2%)	1	(16.7%)	4	(13.8%)	7	(12.1%)	1	(25%)
tot. Pancreatect.	8	(8.2%)	none		2	(6.9%)	5	(8.6%)	1	(25%)
Venous resection	36	(36.7%)	3	(50%)	16	(55.2%)	15	(25.9%)	1	(25%)
Adjuvant therapy:										
FLV:	53	(54.1%)	5	(83.3%)	14	(48.3%)	31	(53.4%)	2	(50%)
GEMZ:	6	(6.1%)	none		3	(10.3%)	3	(5.2%)	none	
FLOX	3	(3.1%)	none		1	(3.4%)	1	(1.7%)	none	
none:	36	(36.7%)	1	(16.7%)	11	(37.9%)	22	(37.9%)	2	(50%)
Histopathologic results										
Pancreatobiliary type	97	(99%)	6		28	(96.6%)	58	(100%)	4	(100%)
Intestinal type	1	(1%)		(100%)	1	(3.4%)				
UICC-stage (V7):										
Ib	2	(2.0%)	none		none		1	(1.7%)	0	(0%)
IIa	23	(23.5%)	4	(66.7%)	9	(31.0%)	8	(13.8%)	2	(50%)
IIb	73	(74.5%)	2	(33.3%)	20	(69.0%)	49	(84.5%)	2	(50%)
pN1-status	73	(74,5%)	2	(33.3%)	20	(69.0%)	49	(84.5%)	2	(50%)
R1-status	61	(62.2%)	3	(50%)	20	(69.0%)	35	(60.3%)	3	(75%)
Vascular infiltration	64	(65.3%)	2	(33.3%)	17	(55.1%)	42	(72.4%)	3	(75%)
Perineural infiltration	91	(92.9%)	4	(66.7%)	28	(96.6%)	54	(93.1%)	4	(100%)

Comparison of characteristics between different subgroups and the entire cohort. One single patient with death unrelated to cancer omitted from the subgroup presentation ILR: isolated locoregional recurrence, DM: distant metastasis, CTC: circulating tumour cell, GEMZ, FLV, FLOX, FOLFIRINOX: chemotherapy regimens; PD: pancreato-duodenectomy, PPPD: pylorus preserving PD

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