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ORIGINAL ARTICLE

Preoperative serum ADAM12 levels as a stromal marker for overall survival and benefit of adjuvant therapy in patients with resected pancreatic and periampullary cancer

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Abstract

Background: We evaluated the stroma marker A Disintegrin And Metalloprotease 12 (ADAM12) as a preoperative prognostic and treatment-predictive marker for overall survival (OS) in pancreatic ductal adenocarcinoma (PDAC) and periampullary cancers.

Methods: Materials were derived from the prospective nationwide Dutch Pancreas Biobank (2015–2017). We included patients who underwent resection because of PDAC/periampullary cancer or non-invasive IPMN (control group) and had a preoperative serum sample available. ADAM12 levels were dichotomized using a pre-defined cut-off (316 pg/mL). Univariable and multivariable Cox regression analyses (backward selection) were performed.

Results: Median ADAM12 levels were 161 (IQR 79–352) pg/mL in 215 PDAC and periampullary adenocarcinomas. High ADAM12 levels (>316 pg/mL) predicted poor OS in the total group of pancreatic and periampullary adenocarcinomas (P = 0.04), but not after adjustment. In distal cholangiocarcinoma (n = 33), high ADAM12 levels predicted poor OS in univariable analysis (P = 0.02), but not in PDAC (P = 0.63). PDAC patients (n = 135) with high ADAM12 levels benefited from adjuvant treatment (median OS 27 vs 14 months, P = 0.02), whereas those with low levels did not (21 vs 21 months, P = 0.87).

Conclusion: High circulating ADAM12 levels, as a proxy for activated stroma, predict survival benefit from adjuvant chemotherapy in PDAC, requiring validation in future studies.

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Introduction

A typical feature of gastrointestinal adenocarcinomas is an abundance of stroma surrounding the cancer cells. This stroma comprises of extracellular matrix proteins, fibroblasts, endothelium, and immune cells, and holds tumor-promoting as well as tumor-restraining properties.[1] Stromal components are thought to exert mechanical force and thereby decrease vascularization and impair delivery of oxygen and cytotoxic agents.[1,2] It has been shown that stroma and the resultant hypoxia activate tumor cell pathways that promote cancer cell survival and metastasis.[1,3] In contrast, other work has demonstrated that stroma encapsulates cancer cells, and subsequently prevents them from metastasizing.[1]

A Disintegrin And Metalloprotease 12 (ADAM12) derives from cancer-associated fibroblasts (CAFs), the main constituents of the stroma and responsible for the bulk of extracellular matrix synthesis. We have previously shown that ADAM12 correlates with the activation status of CAFs (i.e. the degree to which these cells are instructed by tumor cells by for instance TGF-b secretion) and as such, informs on activated stroma, rather stromal content.[3] Previous research in patients with pancreatic ductal adenocarcinoma (PDAC) has shown that ADAM12 is upregulated specifically in the tumor stroma, and that high expression of ADAM12 is associated with worse survival.[3] In subsequent studies, serum ADAM12 levels showed prognostic value in patients with gastrointestinal adenocarcinomas.[4] In metastatic PDAC, it was shown that only patients with low circulating ADAM12 levels benefited from the addition of nab-paclitaxel to the standard of care gemcitabine.[3] This could possibly be explained by the mechanical barrier function of stroma preventing delivery of nab-paclitaxel, but needs to be explored further. The role of stroma and ADAM12 in patients with resected PDAC, and the interaction between stroma and response to chemotherapy is currently unclear.

Preoperatively, differentiating PDAC from periampullary cancers (i.e. distal cholangiocarcinoma, ampullary cancer, and duodenal cancer) poses challenges. However, for all these tumor types, resection with or without (neo)adjuvant chemotherapy, is the treatment of choice if the tumor is deemed resectable. Most current prognostic factors in PDAC and periampullary cancers are histopathological parameters and therefore only available *after* surgery. In the current era of emerging neoadjuvant treatment as a possible new treatment strategy, biomarkers should ideally be available *pre*operatively to predict treatment response. Moreover, these biomarkers need to perform well in both PDAC and periampullary tumors because the exact origin of the tumor is often not certain in the preoperative setting. Therefore, this study evaluated the prognostic value of circulating ADAM12 in PDAC and periampullary cancers and explored its predictive value for the survival benefit of adjuvant chemotherapy.

Methods

Dutch Pancreas Biobank

This is a retrospective analysis of samples and data derived from the prospective nationwide Dutch Pancreas Biobank (PancreasParel). Design and structure of the Dutch Pancreas Biobank have been described previously.[5] In summary, the biobank is incorporated in the Parelsnoer Institute (https://www.health-ri.nl/parelsnoer), which is a nationwide organization providing logistics, infrastructure, and the legal and ethical frameworks for nationwide biobank projects. All patients undergoing pancreatic resection in participating centers are eligible for inclusion, regardless of indication (e.g. PDAC, distal cholangiocarcinoma, ampullary cancer, duodenal cancer, intraductal papillary mucinous neoplasms (IPMNs), mucinous cystic neoplasms, neuroendocrine tumors, chronic pancreatitis). The first center started inclusions in March 2015; currently 13 of the 16 centers performing pancreatic surgery in the Netherlands participate in the project and as of May 2020 over 1500 patients have been included. All included patients provide informed consent. During the study period, serum, EDTA plasma, and genomic DNA were collected prior to surgery (in 2019 the protocol has been changed for patients undergoing neoadjuvant treatment; for these patients a sample before start of neoadjuvant treatment and a sample after neoadjuvant/prior to surgery are now collected). During surgery, tumor and normal tissue are sampled from the resected specimen. During follow-up, serum and EDTA plasma samples are collected at the first postoperative visit to the out-patient clinic, 6 months, 12 months, and 24 months postoperatively, and at recurrence. Data are collected in conjunction with the Dutch Pancreatic Cancer Audit[6] and via Castor Electronic Data Capture (Amsterdam, the Netherlands). Researchers can submit a study proposal for use of the samples to the scientific committee (pancreasparel@dpcg.nl) of the Dutch Pancreatic Cancer Group.[7]

Study design

We included patients who underwent resection because of non-metastatic PDAC or periampullary cancer (final pathology diagnosis) between March 2015 and December 2017, of whom a serum sample was collected less than 30 days before resection (after neoadjuvant/induction therapy if applicable). Patients with ninety-day mortality were excluded, as it was assumed that these deaths were due to postoperative complications. A control group of patients with non-invasive IPMN (both low or high grade) was included in order to confirm the association between increased ADAM12 and adenocarcinoma. The study was approved by the Dutch Pancreas Biobank scientific committee.[7] Both the biobank program and the current use of the samples were approved by the Biobank Ethics Committee or the Medical Ethics Committee of all participating centers [Amsterdam UMC, location AMC 2014_180, Erasmus Medical Center, Rotterdam MEC-2015-085 and MEC-2017-1205, University Medical Center Utrecht 14/

512 and 17/088, Leiden University Medical Center 2014–01 and 2017–01, Catharina hospital, Eindhoven 2016–49]. The REMARK criteria were followed.[8,9]

Biomarker analyses

Each center participating in the Dutch Pancreas Biobank follows the standard operating procedures provided by the Parelsnoer Institute. Blood is centrifuged at 1500–2500 g for 10 min at room temperature or 4 °C to obtain serum. Serum is stored at −80 °C. ADAM12 levels were determined in mono in 80 µl of serum, using a commercially available ADAM12 DuoSet ELISA kit (R&D Systems, Minneapolis, MN), according to the manufacturer's instructions and as previously described.[4] The analyses were performed by a technician, blinded for outcomes. Carbohydrate antigen (CA) 19–9 and bilirubin levels were measured in 50 µl serum using an immunochemical assay on the Roche e602 (Roche Diagnostics, Almere, The Netherlands) integrated in a Cobas c8000 system (Roche Diagnostics).

Definitions

The majority of the clinical data (age, sex, comorbidity, BMI, ASA, WHO performance status, tumor diameter at pathology, residual disease, lymph node status, perineural growth, angioinvasion, adjuvant chemotherapy, time to recurrence, type of recurrence, pathology confirmation of recurrence, and overall survival (OS)) were selected from the associated clinical data available in the Dutch Pancreas Biobank. For this study additional data regarding preoperative imaging and biomarker characteristics (location of the tumor, tumor diameter, clinical lymph node status, and venous and arterial involvement on imaging, and bilirubin level in clinical laboratory) were obtained via the Dutch Pancreatic Cancer Audit.[6] All data were retrieved from medical records by a trained research nurse or MD. Cholestasis was defined as a bilirubin level >34 µmol/L. Vascular involvement was reported based on preoperative imaging. Arterial involvement was defined as any involvement of the coeliac trunk, hepatic artery, or superior mesenteric artery, and venous involvement as any involvement of the portal vein or superior mesenteric vein. R0 resection was defined as absence of cancer cells within 1 mm of each microscopically assessed margin and R1 resection as the presence of residual cancer cells by microscopic assessment, following the definition of the UK Royal College of Pathologists.[10] Administration of chemotherapy was defined as at least one cycle of chemotherapy. Standard treatment in the Netherlands during the study period was upfront surgery followed by adjuvant gemcitabine. In a subset of the patients with (borderline) resectable disease, neoadjuvant chemoradiotherapy was administered within the PREOPANC-1 trial and consisted of gemcitabine and 15 fractions of 2.4 gray.[11] In patients with locally advanced disease at diagnosis who eventually underwent resection after induction chemotherapy, both induction chemotherapy and adjuvant chemotherapy (if administered) consisted of FOLFIRINOX, gemcitabine/nab-paclitaxel or gemcitabine monotherapy with or without stereotactic radiotherapy. Recurrence was defined as a new lesion highly suspect for recurrence or metastasis; lesions were not always pathologically confirmed. Local recurrence was defined as recurrence at the resection bed or local lymph nodes. All other lesions, including those in distant lymph nodes, were considered distant metastases.

Primary outcome was OS, defined as time from surgery until death from any cause (excluding deaths within the 90 days which were assumed to result from postoperative complications), or censored at date of last follow-up. Secondary outcome was recurrence-free survival (RFS), defined as time from surgery until recurrence (local recurrence or distant metastases). Patients were censored for RFS at last follow-up focused on PDAC/periampullary cancer recurrence symptoms (e.g. at outpatient visit to surgeon, gastroenterologist, or medical oncologist), as no routine imaging during follow-up is performed in the Netherlands.

Statistical analysis

ADAM12 was dichotomized based on the previously established cut-off value in resected PDAC of 316 pg/mL.[4] ADAM12 levels were compared between subgroup of adenocarcinoma and the control group of non-invasive IPMN. Differences in clinicopathological characteristics between high and low ADAM12 were analyzed using a Mann–Whitney U test in the total group of patients with PDAC and periampullary adenocarcinomas (unpaired, not-normally distributed variables) and $\chi 2$ test/Fisher's exact test (unpaired, categorical data).

Subsequently, the prognostic value of ADAM12 in the total group of adenocarcinomas, PDAC subgroup and distal cholangiocarcinoma subgroup was analyzed. Univariable and multivariable Cox regression analysis was performed including known predictors and factors that were of (borderline) significance ($P < 0.10$) in univariable analysis. Per group median OS with 95% confidence interval (CI) was estimated using Kaplan–Meier analysis and compared with log-rank test. Parameters for adjustment were selected using backward selection with a $P > 0.10$ for removal.

Lastly, the predictive value of ADAM12 for benefit of adjuvant treatment in PDAC patients (as this is the only subgroup for whom adjuvant treatment is standard of care in the Netherlands) was analyzed; both in the total group of PDAC patients, as well as in the subgroup of PDAC patients after exclusion of those who received neoadjuvant treatment. Data were analyzed using IBM SPSS Statistics for Windows version 24.0 (IBM corp., Armonk, NY). A $P < 0.05$ was considered statistically significant.

Results

Cohort description

In total, 226 patients underwent resection because of PDAC or periampullary cancer, of whom 11 were excluded because of 90-

day mortality (4.9%), leaving 215 patients eligible for analysis. Of the 215 adenocarcinomas, 135 were non-IPMN associated PDAC, 14 IPMN-associated PDAC, 33 distal cholangiocarcinoma, 26 ampullary cancer, and 7 duodenal cancer. A group of 20 non-invasive IPMNs (seven high grade, 13 low grade dysplasia) were included as controls. Median ADAM12 levels differed per subtype of adenocarcinoma ($P = 0.04$; Table 1). This difference, however, appeared to be caused by the lower ADAM12 levels in the group of IPMNs with invasive carcinomas. When combining the group of invasive IPMN with non-IPMN associated PDAC (for which the treatment is the same), there was no significant difference in ADAM12 levels between the subgroups of adenocarcinomas ($P = 0.34$). Median ADAM12 concentrations were significantly higher in patients with adenocarcinoma compared to non-invasive IPMN control patients (161 (IQR 79–352) pg/mL vs 58 (IQR 0–152) pg/mL, $P = 0.002$), but could not be used to discriminate on an individual patient level between IPMN with dysplasia only and IPMN with invasive carcinoma.

During follow-up 129 patients deceased (60%), and OS was 25 months (95% CI 22–27) for all patients with adenocarcinoma. Median follow-up for patients alive at last follow-up was 27 (interquartile range (IQR) 12–36) months. OS was 21 (95% CI 17–25) months for non-IPMN associated PDAC, 32 (95% CI 22–41) months for IPMN-associated PDAC, and 25 (95% CI 18–31) months for distal cholangiocarcinoma. For duodenal cancer and ampullary cancer, median OS was not reached. During follow-up, 122 recurrences were detected (data available for 207 patients); 46.2% of recurrences were pathologically confirmed. RFS (available for 205 adenocarcinomas, 120 events (58.5%)) was 15 (95% CI 12–18) months for all adenocarcinomas, 13 (95% CI 11–15) months for PDAC and 17 (95% CI 11–23) months for distal cholangiocarcinoma.

In the total group of adenocarcinomas, high ADAM12 levels (>316 pg/mL) were more frequent in patients with PDAC than other types of cancer ($P = 0.04$), in patients of male sex ($P = 0.004$), with higher CA19-9 ($P = 0.05$ for continuous variable, $P = 0.02$ when dichotomized by median), cholestasis ($P = 0.02$), positive lymph nodes on preoperative imaging or at final pathology assessment (both $P = 0.02$), any venous contact on preoperative imaging ($P = 0.02$), and perineural growth ($P = 0.005$; Table 2).

Prognostic value

In an univariable analysis, increased ADAM12 was associated with worse OS in the total group of invasive carcinomas (n = 215) using the predetermined cut-off of 316 pg/mL (Table 3; Fig. 1a). ADAM12 also predicted RFS using the cut-off of 316 pg/mL (HR 1.57, 95% CI 1.07–2.30, $P = 0.02$). In multivariable analysis, increased ADAM12 was not significantly ($P = 0.10$) associated with OS after adjustment for other relevant *pre*operatively available predictors (i.e. CA19-9, WHO performance, and venous involvement; tumor location, and arterial involvement were removed in backward selection). In PDAC only, ADAM12 was not prognostic in univariable analysis (Fig. 1b). In distal cholangiocarcinoma, high ADAM12 predicted poor OS in univariable Cox analysis (dichotomized HR 3.39, 95% CI 1.21–8.98, $P = 0.02$; OS curve with log-rank test in Fig. 1c). The limited number of events (19 of 33 patients) precluded multivariable analysis.

Predictive value

We also analyzed the group of PDAC (n = 135) patients separately, in relation to different treatment strategies. ADAM12 levels were lower after neoadjuvant/induction chemotherapy; median concentration was 123 (IQR 58–165) pg/mL in 22 patients who underwent neoadjuvant treatment and 213 (IQR 104–430) pg/mL in 193 treatment-naive patients ($P = 0.03$). Interestingly, PDAC patients with high ADAM12 levels that received adjuvant therapy had a significantly better OS compared

Table 1 ADAM12 levels in patients undergoing resection because of pancreatic or periampullary cancer or IPMN (n = 235).

Diagnosis	N	ADAM12 concentration, median (IQR)	P	
All adenocarcinomas	215	161 (79-352)		
Pancreatic ductal adenocarcinoma (non-IPMN)	135	171 (84-421)		
IPMN with invasive carcinoma	14	67 (42-177)		
Distal cholangiocarcinoma	33	187 (63-370)	0.04[a]	
Ampullary carcinoma	26	153 (65-236)		0.002[b]
Duodenal carcinoma	7	115 (81-155)		
IPMN non-invasive, all	20	58 (0-152)		
High grade dysplasia	7	70 (0-266)	0.60	
Low grade dysplasia	13	40 (0-127)		

a When IPMN-associated carcinomas are grouped with pancreatic ductal adenocarcinoma P = 0.34. b All adenocarcinomas (n = 215) versus non-invasive IPMN (n = 20)

Table 2 High vs low preoperative serum ADAM12 levels in patients with pancreatic ductal adenocarcinoma and periampullary adenocarcinomas (n = 215)

Characteristics	All (n = 215)	Low ADAM12 (n = 154)	High ADAM12 (n = 61)	*P*
Final pathology diagnosis				0.04
Pancreatic cancer (non-IPMN associated)	135 (62.8)	89 (57.8)	46 (75.4)	
IPMN with invasive carcinoma	14 (6.5)	13 (8.4)	1 (1.6)	
Distal cholangiocarcinoma	33 (15.3)	23 (14.9)	10 (16.4)	
Ampullary cancer	26 (12.1)	22 (14.3)	4 (6.5)	
Duodenal cancer	7 (3.3)	7 (4.5)	0	
Age (n = 214)	69 (60–75)	69 (61–75)	68 (59–75)	0.71
Sex, male	129 (60.0)	83 (53.9)	46 (75.4)	0.004
Comorbidity, yes				
Cardiac	44 (20.5)	30 (19.5)	14 (23.0)	0.57
Pulmonary	28 (13.0)	20 (13.0)	8 (13.1)	0.98
Diabetes mellitus	50 (23.3)	31 (20.1)	19 (31.1)	0.09
Family history of pancreatic/periampullary cancer	16 (7.6)	12 (7.8)	4 (6.9)	0.82
BMI (n = 212)	25 (22–28)	25 (22–28)	24 (22–27)	0.73
ASA				0.26
ASA 1	35 (16.4)	29 (19.0)	6 (9.8)	
ASA 2	129 (60.3)	89 (58.2)	40 (65.6)	
ASA 3	50 (23.4)	35 (22.9)	15 (24.6)	
Missing	1			
WHO performance status				0.34
WHO 0	101 (50.8)	75 (52.8)	26 (45.6)	
WHO 1	81 (40.7)	55 (38.7)	26 (45.6)	
WHO 2	14 (7.0)	11 (7.7)	3 (5.3)	
WHO 3	3 (1.5)	1 (0.7)	2 (3.5)	
Missing	16	12	4	
CA19.9 (kU/L)	64 (20–268)	56 (16–212)	120 (28–348)	0.05
CA19.9 (dichotomized by median)				0.02
Low	107 (49.8)	85 (55.2)	23 (37.7)	
High	108 (50.2)	69 (44.8)	38 (62.3)	
Bilirubin	16 (8–48)	12 (8–42)	20 (8–50)	0.50
Cholestasis (bilirubin >34 μmol/L), yes	67 (31.2)	41 (26.6)	26 (42.6)	0.02
Location on preoperative imaging				0.89
Pancreatic head	119 (69.6)	80 (65.6)	39 (79.6)	
Pancreatic corpus	7 (4.1)	5 (4.1)	2 (4.1)	
Pancreatic tail	10 (5.8)	8 (6.6 (	2 (4.1)	
Periampullary	30 (17.5)	24 (19.7)	6 (12.2)	
Duodenal	5 (2.9)	5 (4.1)	0	
Missing	44	32	12	
Tumor diameter on preoperative imaging (mm, n = 161)	25 (20–30)	25 (20–30)	25 (20–34)	0.15
Positive lymph nodes on preoperative imaging, yes (n = 208)	30 (14.0)	16 (10.9)	14 (23.0)	0.02
Venous involvement on preoperative imaging, yes (n = 212)	79 (37.3)	49 (32.5)	30 (49.2)	0.02
Arterial involvement on preoperative imaging, yes (n = 208)	21 (10.1)	15 (10.1)	6 (10.0)	0.59
Involvement structures on preoperative imaging, yes (n = 209)	16 (7.7)	9 (6.0)	7 (11.7)	0.17

Table 2 (*continued*)

Characteristics	All (n = 215)	Low ADAM12 (n = 154)	High ADAM12 (n = 61)	*P*
Neoadjuvant treatment, yes	22 (10.2)	19 (12.3)	3 (4.9)	0.11
Chemotherapy	11 (5.1)	10 (55.6)	1 (33.3)	
Chemoradiotherapy	10 (4.7)	8 (44.4)	2 (66.7)	
Missing	1			
Tumor diameter at pathology (mm, n = 201)	28 (20–35)	25 (19–35)	30 (22–35)	0.05
Resection margins				0.08
R0	143 (67.5)	108 (71.1)	35 (58.3)	
R1	69 (32.5)	44 (28.9)	25 (41.7)	
R2	0			
Unknown	3			
Differentiation grade				0.23
Well differentiated	12 (6.1)	10 (7.2)	2 (3.4)	
Moderately differentiated	132 (67.3)	88 (63.8)	44 (75.9)	
Poorly differentiated	52 (26.5)	40 (29.0)	12 (20.7)	
Unknown	19			
No of positive lymph nodes	1 (0–5)	1 (0–4)	2 (0–6)	0.02
No of examined lymph nodes	16 (12–20)	15 (12–20)	17 (12–21)	0.34
pN status				
N0	81 (37.7)	65 (42.2)	16 (26.2)	0.07
N1	65 (30.2)	45 (29.2)	20 (32.8)	
N2	69 (32.1)	44 (28.6)	25 (41.0)	
Lymph node ratio	0.11 (0–0.28)	0.08 (0–0.26)	0.16 (0–0.33)	0.06
Perineural growth, yes (n = 190)	136 (71.6)	88 (65.7)	48 (85.7)	0.005
Angio-invasion, yes (n = 193)	100 (51.8)	68 (49.6)	32 (57.1)	0.34
Adjuvant chemotherapy, yes (n = 214)	112 (52.3)	77 (50.3)	35 (57.4)	0.35
Outcome				
Relapse detected, yes (n = 207)	122 (58.9)	81 (55.1)	41 (68.3)	0.08
Relapse type (total of 122 recurrences)				
Local	36 (29.8)	23 (28.7)	13 (31.7)	0.34
Distant	85 (70.2)	57 (71.3)	28 (68.3)	
Unknown	1	1		
Recurrence pathologically confirmed				
Yes	55 (46.2)	40 (51.3)	15 (36.6)	0.13
No	64 (53.8)	38 (48.7)	26 (63.4)	
Unknown	3	3		

to those with high ADAM12 levels but did not receive adjuvant chemotherapy (27 (95% CI 16–37) months vs 14 (95% CI 5–22) months, $P = 0.02$; Fig. 2a). In the low ADAM12 group, adjuvant chemotherapy was not associated with longer OS (21 months (95%CI 16–26) vs 21 months (95%CI 17–25) for no adjuvant therapy received, $P = 0.87$). Prognosis of PDAC patients with high ADAM12 receiving adjuvant treatment was similar to those with low ADAM12 levels. Of note, this effect was seen in both the total group as well as after exclusion of those patients who received neoadjuvant treatment (Fig. 2b).

Discussion

This multicenter study demonstrated that ADAM12 predicted prognosis in patients with pancreatic and periampullary adenocarcinomas in univariable analysis, but not after adjustment for CA19-9, WHO performance status, and venous involvement. ADAM12 was a prognostic factor for poor OS in the subgroup of distal cholangiocarcinoma, but not in PDAC. Several mechanisms may explain these findings. The presence of abundant stroma decreases tumor perfusion, and the resultant hypoxia has

Table 3 Preoperative predictors for overall survival in patients with resected pancreatic and periampullary adenocarcinoma (n = 215)

Characteristic	Univariable		Multivariable	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Biomarkers				
ADAM12; per 100 pg/mL increase	1.04 (1.00–1.08)	0.04	1.03 (0.99–1.07)	0.10
ADAM12			[a]	
≤316 pg/mL	1			
>316 pg/mL	1.44 (1.00–2.07)	0.05		
Bilirubin; per 100 units increase	1.02 (0.83–1.26)	0.83	–	
Cholestasis (bilirubin >34 μmol/L)	1.34 (0.93–1.93)	0.11	–	
CA19.9; per 100 kU/L increase	1.03 (1.02–1.05)	<0.001	1.03 (1.01–1.04)	0.001
CA19.9/bilirubin ratio; per 100 units increase	1.81 (1.26–2.59)	0.001	[a]	
Clinical factors				
Age; per 10 years	1.08 (0.92–1.26)	0.37	–	
Sex, male	1.21 (0.84–1.73)	0.30	–	
Comorbidity, yes				
Cardiac	0.97 (0.63–1.49)	0.88	–	
Pulmonary	1.29 (0.79–2.11)	0.30		
Diabetes mellitus	1.32 (0.90–1.95)	0.16		
Family history of pancreatic/periampullary cancer, yes	1.10 (0.58–2.11)	0.77	–	
BMI; per unit increase	0.97 (0.93–1.02)	0.20	–	
ASA		0.59		
ASA 1	1		–	
ASA 2	0.88 (0.56–1.39)			
ASA 3	1.08 (0.64–1.83)			
WHO performance status		0.07		0.20
WHO 0	1		1	
WHO 1	1.41 (0.97–2.06)		1.42 (0.97–2.09)	
WHO 2 or 3	1.79 (0.98–3.27)		2.27 (1.22–4.22)	
Location on imaging		0.003		
Pancreatic head	1		[b]	
Pancreatic corpus or tail	0.82 (0.44–1.54)			
Periampullary or duodenal	0.37 (0.21–0.66)			
Tumor diameter on imaging; per 1 cm increase	1.00 (0.88–1.13)	0.94	–	
Positive lymph nodes on imaging, yes	0.93 (0.81–1.07)	0.32	–	
Venous involvement, yes	2.56 (1.80–3.64)	<0.001	2.86 (1.78–3.74)	0.02
Arterial involvement, yes	1.79 (1.07–3.00)	0.03	[b]	
Involvement structures, yes	0.96 (0.50–1.83)	0.90	–	
Neoadjuvant treatment				
No	1		–	
Yes	1.06 (0.60–1.89)	0.84		
Chemotherapy	1			
Chemoradiotherapy	0.57 (0.17–1.90)	0.36		

[a] Not included to avoid multicollinearity.
[b] Removed in backward selection.

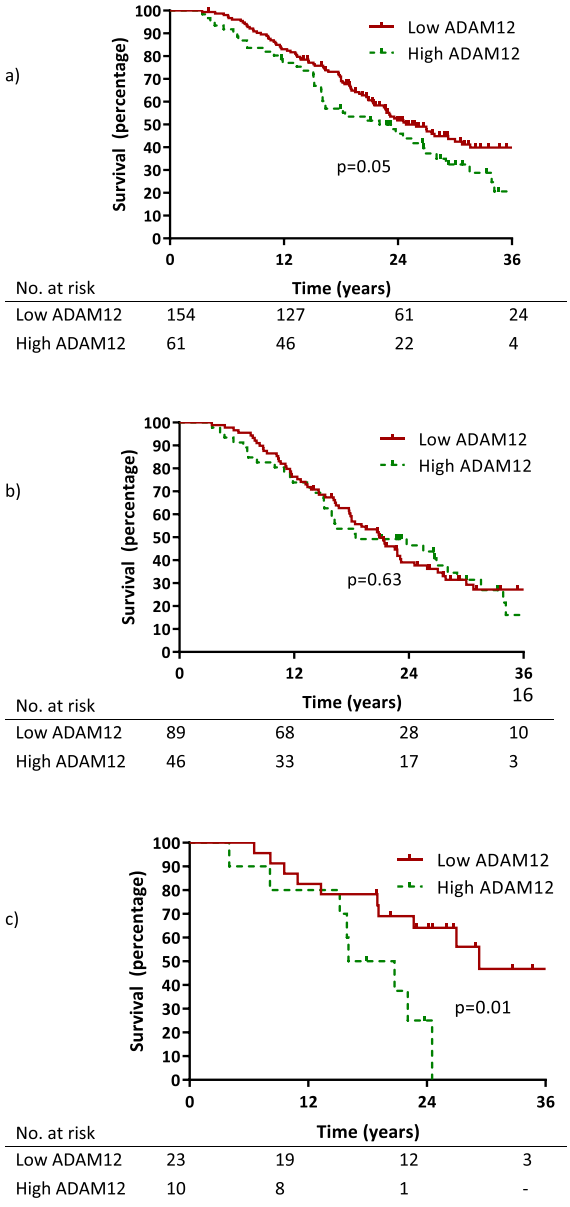
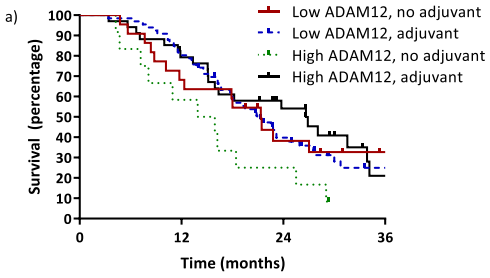


Figure 1 Overall survival for patients with high and low circulating ADAM12 levels (cut-off value 316 pg/mL) a) All adenocarcinomas (n = 215, *P* = 0.05); b) Pancreatic ductal adenocarcinoma (n = 135, *P* = 0.63); c) Distal cholangiocarcinoma (n = 33, *P* = 0.01)

been shown to promote stem cell related cell properties such as self-renewal, infinite replication potential, and therapy resistance.[1,12] Additionally, stroma is thought to promote epithelial-to-mesenchymal transition (EMT) and subsequently contribute to tumor cell invasiveness and the presence of circulating tumor cells.[1,13] ADAM12 is upregulated specifically in the tumor stroma; this could explain an association between ADAM12 and worse survival. In addition, tumor cell-secreted transforming growth factor beta (TGF-β) has been show to activate stroma, but also to drive EMT in tumor cells themselves. ADAM12 has been described to be upregulated by TGF-β in for example liver cancer.[14] Also in human breast cancer it has been shown that ADAM12 is associated with TGF-β induced EMT.[15] In agreement, TGF-β predicts prognosis in unresectable PDAC patients treated with FOLFIRINOX.[16] Therefore, ADAM12 could be proposed to indirectly report on the autocrine effects of tumor cell TGF- β expression, which further contributes to a negative prognostic effect. Of course, it should be noted that after adjustment for other preoperative factors, ADAM12 was not a predictor for survival; it supports the hypothesis that ADAM12 is related to several processes in cancer, and that other factors have strong impacts on survival.

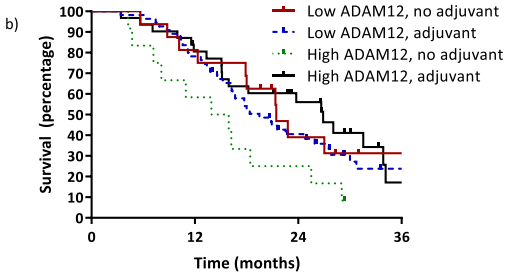
In our study, ADAM12 levels were significantly higher in patients with PDAC and periampullary adenocarcinoma than in controls with non-invasive IPMN. This can be explained by the hypothesis that cysts do not comprise activated stroma. The invasive PDAC component in an IPMN will often be smaller than a non-IPMN associated PDAC, possible explaining lower ADAM12 levels in IPMN with invasive component.

We found that patients with PDAC and high ADAM12 levels had more benefit from adjuvant chemotherapy than PDAC patients with low ADAM12 levels, although sample sizes were small. This is relevant as adjuvant chemotherapy improves survival after resection for PDAC, but at the cost of toxicity; better selection of patients could improve outcomes.[17,18] It is now well-recognized that clinically relevant subgroups of gastrointestinal cancers can be identified based on the classification of gene expression data. In most gastrointestinal adenocarcinomas including esophageal, colon, and PDAC, molecular subtypes such as an epithelial, and a mesenchymal/basal like subtype have been identified. Moreover, these subtypes bear high similarity to each other across organs.[19–21] In PDAC, the mesenchymal/basal like subtype is associated with increased stroma infiltration and poorest prognosis, and high tissue ADAM12 expression is associated with this poor prognostic mesenchymal/basal-like subtype.[3,21] In line with our results, Moffitt et al.[21] found that patients with the mesenchymal/basal-like subtype tumor have substantial benefit from adjuvant treatment, while patients with the classical subtype tumor had no significant benefit. It could be hypothesized that after resection especially the patients with previously high ADAM12 (i.e. increased stroma), poor prognostic mesenchymal/basal-like subtype have increased risk of recurrence, due to increased EMT of the cancer cells and already higher tumor cell invasiveness and metastatic properties preoperatively. Systematic adjuvant treatment could possibly limit the effect of the already spreading tumor cells. Therefore, we propose that ADAM12 is a potential biomarker to identify those patients with a particularly aggressive tumor, and who seem to benefit from additional therapy. Thus far, there are no specific and FDA-approved ADAM12 inhibitors available and we foresee that ADAM12 will be used as a biomarker rather than a druggable target.



No. at risk				
Low ADAM12, no adjuvant treatment	22	15	7	3
Low ADAM12, adjuvant treatment	66	53	21	7
High ADAM12, no adjuvant treatment	12	7	3	-
High ADAM12, adjuvant treatment	34	26	14	3

Low ADA12: adjuvant (n=66) vs no adjuvant treatment (n=22), *P*=0.87
High ADAM12: adjuvant (n=34) vs no adjuvant treatment (n=12), *P*=0.02



No. at risk				
Low ADAM12, no adjuvant treatment	16	13	5	2
Low ADAM12, adjuvant treatment	55	43	18	6
High ADAM12, no adjuvant treatment	12	7	3	-
High ADAM12, adjuvant treatment	31	24	13	2

Low ADA12: adjuvant (n=55) vs no adjuvant treatment (n=16), *P*=0.59
High ADAM12: adjuvant (n=31) vs no adjuvant treatment (n=12), *P*=0.02

Figure 2 Predictive value of ADAM12 in patients with resected pancreatic ductal adenocarcinoma. a) All patients (n = 134); b) After exclusion of patients undergoing neoadjuvant treatment (n = 114)

Interestingly, previous research from our group in *metastatic* PDAC showed that only patients with low circulating ADAM12 levels benefited from the addition of nab-paclitaxel to the standard of care gemcitabine. These results were in line with, for example, results from the COMPASS trial which showed that the classical subtype responded better to first-line chemotherapy than patients with basal-like tumors. It seems that in the different stages of disease (resected tumor versus metastatic tumor in situ) also different features of stroma play key roles (e.g. increased tumor spreading mechanisms in resectable disease, versus mechanical barrier function in metastatic disease).

We observed that patients treated with neoadjuvant chemo (radio)therapy had lower preoperative ADAM12 levels in the PDAC group. Although we did not show the direct effect of the therapy (no samples before start of neoadjuvant treatment were available), this is remarkable as such therapies supposedly target the tumor cells and not stromal cells. We take this to support our previous findings that ADAM12 is a marker of *activated* stroma[3]; and that tumor cells are required for this activation. In other words; if tumor cells are killed by neoadjuvant therapy, the levels of *activated* stroma and circulating ADAM12 levels will decrease. Moreover, this argues against ADAM12 being a marker of fibrotic tissue in general, as chemo (radio)therapy leads to necrosis, inflammation and eventually increased fibrosis (in this case, ADAM12 levels would be higher after neoadjuvant treatment which was not observed).[22,23] It would be of interest to study the differences in ADAM12 levels before and after neoadjuvant treatment in order to see if ADAM12 could be used to monitor response. If changes in ADAM12 levels could indicate response to neoadjuvant treatment, this would be very helpful in clinical practice. This, as the preoperative evaluation of the tumor and its vascular involvement after neoadjuvant treatment is very difficult, because of the challenges in the discrimination between tumor and neoadjuvant therapy-induced fibrotic tissue.[24]

The results of this study should be interpreted in light of several limitations. Firstly, the retrospective and exploratory design of this study (including possible selection of patients with better performance for adjuvant chemotherapy), and secondly the lack of a validation cohort. These limitations prevent definitive conclusions about the predictive value of ADAM12 for benefit of adjuvant treatment. Thirdly, this study was conducted with adjuvant chemotherapy that mainly consisted of gemcitabine monotherapy. However, nowadays FOLFIRINOX is increasingly used as adjuvant treatment; the results may not be generalizable to other adjuvant treatments. Of note, as FOLFIRINOX improves survival compared to gemcitabine, the effect could be even stronger. Strengths of this study include the uniform and prospective collection of the samples within the nation-wide Dutch Pancreas Biobank,[5] and the elaborate dataset of preoperatively and postoperatively available factors.

Future prospective studies on ADAM12 and other stromal markers should investigate the role of stroma in specific stages of disease (e.g. resectable vs non-resectable), and in relation to different treatment strategies. Moreover, if additional stroma-targeting agents become available in randomized controlled trials, ADAM12 could be used to stratify patients.

Previous communication

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Conflict of interest

MAB has received research support from Johnson&Johnson, Acelity/KCI, Bard, Ipsen, New Compliance, and Mylan, and acts as a consultant, instructor or speaker for Johnson&Johnson, Acelity/KCI, Bard, Gore, and Smith&Nephew

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NHM has acted as a consultant for BMS, Merck, Eli Lilly and Servier.

JWW has acted as a consultant for Celgene and Servier and has received grants from Amgen, Celgene, Novartis, Nordic Pharma Group, Servier and Novartis.

HWL has acted as a consultant for Celgene, Eli Lilly and Company, Nordic Pharma Group, Merck, Novartis, Servier and Philips, has received research grants from Amgen, Bayer Schering Pharma AG, Celgene, Eli Lilly and Company, GlaxoSmithKline Pharmaceuticals, Merck, Nordic Pharma Group, Philips, Roche Pharmaceuticals and Servier.

MFB has received research funding from Celgene and acted as a consultant for Servier. MFB is inventor on a patent application describing the use of serum ADAM12 levels in gastrointestinal cancers.

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