Evaluation of Matrix Metalloproteinase 7 in Plasma and Pancreatic Juice as a Biomarker for Pancreatic Cancer

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Abstract

Differentiating between periampullary carcinoma and chronic pancreatitis with an inflammatory mass is difficult. Consequently, 6% to 9% of pancreatic resections for suspected carcinoma are done inappropriately for chronic pancreatitis. Here, we test if matrix metalloproteinase 7 (MMP-7), a secreted protease frequently expressed in pancreatic carcinoma, can be measured in plasma, pancreatic, and duodenal juice, and if it can distinguish between periampullary carcinoma and chronic pancreatitis. Ninety-four patients who underwent pancreatic surgery for a (peri)pancreatic neoplasm (n = 63) or chronic pancreatitis (n = 31) were analyzed. Median plasma MMP-7 levels were significantly higher in carcinoma (1.95 ng/mL; interquartile range, 0.81-3.22 ng/mL) compared with chronic pancreatitis and benign disease (0.83 ng/mL; interquartile range, 0.25-1.21 ng/mL; P < 0.01). MMP-7 levels in pancreatic juice were higher, although not significantly, in carcinoma (62 ng/mg protein; interquartile range, 18-241 ng/mg protein) compared with chronic pancreatitis and benign disease (23 ng/mg protein; interquartile range, 8.5-99 ng/mg protein; P = 0.17). MMP-7 levels in duodenal juice were universally low. At an arbitrary cutoff of 1.5 ng/mL in plasma, positive and negative predictive values were 83% and 57%, respectively, values comparable to those of today's most common pancreatic tumor marker, carbohydrate antigen 19-9 (CA19-9; 83% and 53%, respectively). Positive and negative likelihood ratios for plasma MMP-7 were 3.35 and 0.52, respectively. The area under the receiver operating characteristic curve for MMP-7 was 0.73 (95% confidence interval, 0.63-0.84) and for CA19-9, 0.75 (95% confidence interval, 0.64-0.85). Combined MMP-7 and CA19-9 assessment gave a positive predictive value of 100%. Thus, plasma MMP-7 levels discriminated between patients with carcinoma and those with chronic pancreatitis or benign disease. The diagnostic accuracy of plasma MMP-7 alone is not sufficient to determine treatment strategy in patients with a periampullary mass, but combined evaluation of plasma MMP-7 with CA19-9 and other markers may be clinically useful. (Cancer Epidemiol Biomarkers Prev 2007;16(5):886–91)

Introduction

The preoperative differentiation between periampullary (including pancreatic) carcinoma and chronic pancreatitis with an inflammatory mass is difficult. Both diseases can present with similar symptoms such as pain and obstructive jaundice and can show a double duct sign at endoscopic retrograde cholangiopancreaticography. However, imaging techniques such as computed tomography and endosonography lack discriminative power between a malignant and an inflammatory pancreatic mass. The differential diagnosis is even more complicated because periampullary carcinoma is frequently accompanied by pancreatitis and periampullary carcinoma has a higher incidence in patients with chronic pancreatitis.

As a consequence, 6% to 9% of pancreatic resections for suspected carcinoma are done inappropriately for chronic pancreatitis (1-3). Although pylorus-preserving pancreaticoduodenectomy has become a procedure with low mortality and acceptable morbidity, patients with chronic pancreatitis who need surgical intervention are not optimally treated or overtreated with this procedure (4). Randomized trials have shown that patients have less pain and a better quality of life after a duodenum preserving pancreatic head resection (Beger's or Frey's procedure) compared with those after pancreaticoduodenectomy (5, 6).

Because of this diagnostic dilemma, the accuracy of many diagnostic methods has been evaluated. Imaging techniques such as ultrasound, computed tomography, magnetic resonance imaging, and positron emission tomography all have their limitation in distinguishing between pancreatic cancer and pancreatitis. Differentiation with endoscopic ultrasound is operator dependent, although in combination with fine needle aspiration cytology it can provide cytologic confirmation of the malignancy (7). Sampling error due to surrounding pancreatitis lowers its sensitivity; the same holds for brush cytology during endoscopic retrograde cholangiopancreaticography (8). Serologic tumor markers have been studied extensively to assess their usefulness in the diagnosis of pancreatic cancer, but at present there are no reliable serum markers for pancreatic cancer. The best available markers thus far are carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen, with CA19-9 having the best diagnostic value (9, 10). CA19-9 has a sensitivity of ~80% and a specificity of 60% to 70% (11, 12). If elevated, it can be used to assess response to treatment, but it is not helpful to differentiate between pancreatic cancer and pancreatitis (13).

Matrix metalloproteinase 7 (MMP-7, matrilysin, EC 3.4.24.23) is a secreted member of the MMP family of zinc-dependent extracellular proteases. It can degrade some components of the extracellular matrix as well as an array of plasma membrane substrates and has been shown to have a role at many stages of tumor progression, including tumor formation, growth, invasion, and metastasis. Immunohistochemical analysis of patient samples has shown that MMP-7 is

Received 9/14/06; revised 1/12/07; accepted 2/28/07.

Grant support: NIH grant R01 CA100126 (H.C. Crawford).

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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doi:10.1158/1055-9965.EPI-06-0779

expressed in tumor cells in 98% of pancreatic ductal adenocarcinomas, at all stages of progression, and in metaplastic duct lesions associated with both pancreatic cancer and chronic pancreatitis (14-16). However, the usefulness of MMP-7 as an accessible marker of these diseases has not been assessed. Therefore, the aim of this study was to expand on these findings and test whether MMP-7 can be detected in plasma, pancreatic juice, or duodenal juice and whether these levels can be used to distinguish between periampullary carcinoma and benign periampullary disease. CA19-9 plasma levels were assessed to be able to compare the diagnostic strength of plasma MMP-7 to that of the current standard biomarker for pancreatic cancer.

Materials and Methods

Patients. Samples were obtained from 94 consecutive patients who underwent surgery for pancreatic and periampullary disease in the Academic Medical Center of the University of Amsterdam. Approval was obtained from the institute's Medical Ethical Committee.

Sample Collection. Blood samples were obtained during surgery, centrifuged, and stored at −80°C. Pancreatic juice was harvested during surgery by cannulation followed by aspiration of the pancreatic duct. Duodenal juice was harvested from the fresh resection specimen by needle aspiration. Pancreatic and duodenal juice was snap-frozen in the operating room and stored at −80°C until analysis.

MMP-7 Analysis. MMP-7 levels in plasma and juice samples were assayed by ELISA (Quantikine, R&D Systems) after dilution (1:50, 1:10, 1:2) according to the manufacturer's directions. Values that fell on the standard curve were adjusted for dilution and averaged. Protein quantitation was done using the BCA Protein Assay Kit (Pierce).

CA19-9 Analysis. CA19-9 concentrations were determined in a total volume of 25 μL of plasma using a microparticle enzyme immunoassay kit (AxSYM analyzer, Abbott Laboratories).

MMP-7 Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue was obtained from the Department of Pathology. MMP-7 immunohistochemistry was done as previously described (17), with strict attention paid to uniformity of processing. Two observers (J.O. and F.K.) scored all sections independently. The intensity of the immunostaining of the tumor epithelium, tumor stroma, and pancreatitis and of normal pancreas was graded on a semiquantitative basis as negative, weak, moderate, and strong. To facilitate interpretation and comparison of heterogeneous tissue staining, whole sections were assigned scores according to the strongest intensity staining on a section, provided that >10% of the specified cell type on the section stained positive. In cases where <10% of cells of a specific type were stained, the section was assigned as negative. Differences in assigned scores were resolved by simultaneous reexamination by both scorers to achieve a consensus. In none of the cases the assigned scores differed by more than 1 grade.

Statistics. Pathology results were taken as the gold standard in testing the discriminative power of MMP-7 levels in plasma, pancreatic, and duodenal juice. Differences between groups were analyzed statistically by means of Student's t test (if normality test was passed) or Mann-Whitney test. Receiver operating characteristic curves of plasma MMP-7 and CA19-9 with their areas under the curve were calculated. These analyses were done using Statistical Package for the Social Sciences (SPSS) version 12.0.1 (SPSS, Inc.). Statistical significance was considered if $P < 0.05$.

Results

Patients. Grouping of patients was based on pathology results. Thirty-one patients were found to have chronic pancreatitis, most of whom underwent a pancreaticojejunostomy. Twenty-nine patients were found to have pancreatic adenocarcinoma, 11 distal cholangiocarcinoma, 9 ampullary carcinoma, and 5 other (peri)pancreatic carcinomas (duodenum carcinoma, $n = 1$; neuroendocrine carcinoma, $n = 3$; and cystic papillary tumor, $n = 1$). Nine patients were found to have benign (peri)pancreatic neoplasms (mainly pancreatic, papillary, and duodenal adenomas). Most patients undergoing surgery for (peri)pancreatic carcinoma or benign neoplasms underwent a pancreaticoduodenectomy with a curative intention or a palliative gastric and biliary bypass. Patient characteristics are presented in Table 1. We attempted to collect pancreatic juice from all patients. Plasma and duodenal juice was collected from 86 patients.

MMP-7 Levels in Plasma. Median MMP-7 levels in plasma from patients with pancreatic cancer, distal cholangiocarcinoma, and ampullary carcinoma were 2- to 3-fold higher compared with those in patients with chronic pancreatitis and other benign disease (Table 2; $P < 0.01$ and $P < 0.05$, respectively). MMP-7 plasma levels in patients with noninflammatory benign disease or with other carcinoma without exocrine ductal involvement were not significantly different

Table 1. Patient characteristics according to pathologic diagnosis

	Chronic pancreatitis ($n = 31$)	Pancreatic carcinoma ($n = 29$)	Distal cholangiocarcinoma ($n = 11$)	Ampullary carcinoma ($n = 9$)	Carcinoma other ($n = 5$)	Benign disease ($n = 9$)
Gender						
Male	20	12	5	7	3	3
Female	11	17	6	2	2	6
Mean age (range), y	49 (21-72)	67 (47-79)	65 (52-78)	62 (33-79)	57 (23-75)	62 (33-84)
Presenting symptoms						
Pain (abdominal and/or back)	28	13	5	1	4	4
Jaundice	8	24	10	8	2	3
Weight loss	10	17	9	5	1	1
Surgical procedure						
Pancreaticojejunostomy	21	—	—	—	—	—
Pancreaticoduodenectomy	5	19	7	9	5	5
Double bypass	—	9	4	—	—	—
Pancreatic tail resection	3	1	—	—	—	—
Duodenum-preserving pancreatic head resection	2	—	—	—	—	—
Pancreas-preserving duodenum resection	—	—	—	—	—	3
Pancreatic corpus resection	—	—	—	—	—	1

Table 2. MMP-7 levels in plasma, pancreatic juice, and duodenal juice

Pathologic diagnosis	Plasma				Pancreatic juice				Duodenal juice			
	N	Median MMP-7 (ng/mL)	IQR	*P*	N	Median MMP-7 (ng/mg protein)	IQR	*P*	N	Median MMP-7 (ng/mg protein)	IQR	*P*
Chronic pancreatitis	27	0.83	0.60-1.21		25	24	9.1-144	†	24	4.0	0.2-17.0	
Pancreatic carcinoma	28	1.99	1.25-3.43	* ‡	17	143	43-456		19	2.6	0.4-10.0	
Distal cholangiocarcinoma	10	1.83	0.81-4.07		6	39	5.4-438		8	0.9	0.2-1.5	
Ampullary carcinoma	9	1.99	0.66-2.76	§	8	25	3.7-46		9	2.0	0.3-4.9	
Carcinoma other	4	0.48	0.29-0.79‖		4	133	4.8-1080‖		4	0.3	0.2-0.6‖	
Benign disease	8	0.79	0.71-1.54		5	18	1.0-63‖		6	1.0	0.2-1.7	
Carcinoma	51	1.95	0.81-3.22	¶	35	62	18-241		40	1.3	0.3-4.6	
No carcinoma	35	0.83	0.25-1.21		30	23	8.5-99		29	2.6	0.2-13.1	
PD; CP at pathology	5	1.16	0.79-2.16‖		3	24	5.7-826‖		4	13.3	0.2-25.5‖	

Abbreviations: IQR, interquartile range (25-75%); PD, pancreaticoduodenectomy; CP, chronic pancreatitis.
*Compared with chronic pancreatitis ($P < 0.01$) and with benign disease ($P < 0.047$).
†Compared with chronic pancreatitis ($P = 0.04$) and with benign disease ($P = 0.03$).
‡Compared with chronic pancreatitis ($P < 0.01$).
§Compared with chronic pancreatitis ($P = 0.03$).
‖Full range.
¶Compared with no carcinoma ($P < 0.01$).

from patients with chronic pancreatitis. MMP-7 levels of all patients with carcinoma were significantly higher compared with those of patients with benign disease ($P < 0.01$). MMP-7 plasma levels of these two groups are also depicted per patient in a scatter plot (Fig. 1A).

Five patients underwent pancreaticoduodenectomy for a suspected carcinoma, which turned out to be chronic pancreatitis with an inflammatory mass after pathologic workup. Median plasma MMP-7 levels in these five patients (1.16 ng/mL; range, 0.79-2.16 ng/mL) were comparable to those with chronic pancreatitis (0.83 ng/mL; $P = 0.56$).

MMP-7 Levels in Pancreatic Juice. In 65 of 94 patients, it was possible to collect pancreatic juice. Median MMP-7 levels in pancreatic juice were elevated in patients with pancreatic cancer compared with those with chronic pancreatitis, although variability was significant (Table 2). Patients with distal cholangiocarcinoma and ampullary carcinoma did not show elevated MMP-7 levels in pancreatic juice. Variability could be a result of the considerable differences in viscosity and clearness that were found between samples, which can interfere with both ELISA and protein quantification assay.

MMP-7 Levels in Duodenal Juice. In 70 of 86 patients, it was possible to collect duodenal juice. MMP-7 levels in duodenal juice were low, variable, and sometimes below the detection limit (Table 2). The median levels were >10-fold lower than those in pancreatic juice. No differences in duodenal MMP-7 levels were found between groups.

CA19-9 Levels in Plasma. CA19-9 plasma levels were measured to assess the diagnostic strength of the current standard biomarker for pancreatic cancer. The median CA19-9 level in all patients with carcinoma was 28 units/mL (interquartile range, 0-169 units/mL) versus 0 units/mL (interquartile range, 0-5.5 units/mL) in those with no carcinoma, which was significantly different ($P < 0.01$). At the commonly used CA19-9 cutoff of 37 units/mL (18), 17 of 28 (61%) patients with pancreatic cancer had elevated CA19-9. In patients with cholangiocarcinoma and ampullary carcinoma, this was 7 of 10 (70%) patients and 1 of 9 (11%) patients, respectively. In 30 of 34 (88%) patients with

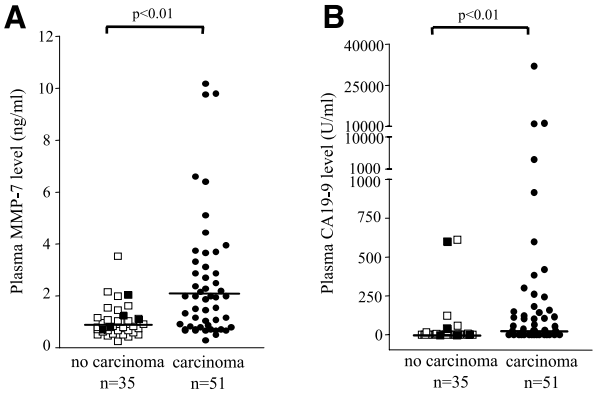

Figure 1. Plasma MMP-7 (**A**) and plasma CA19-9 (**B**) levels in patients with (peri)pancreatic carcinoma (●) compared with those with benign disease (□) according to pathology. ■, patients who underwent pancreatic resection for suspected malignancy, which turned out to be pancreatitis at pathology. Line, median plasma MMP-7 or CA19-9 level. Median and interquartile range of plasma MMP-7 in patients with (peri)pancreatic carcinoma versus those with benign disease was 1.95 ng/mL (interquartile range, 0.81-3.22 ng/mL) versus 0.83 ng/mL (interquartile range, 0.25-1.21 ng/mL), and of plasma CA19-9, 28 units/mL (interquartile range, 0-169 units/mL) versus 0 units/mL (interquartile range, 0-5.5 units/mL), respectively.

Table 3. Immunohistochemical staining of tissue

(Peri)Pancreatic disease	Staining intensity	Carcinoma or pancreatitis	Normal pancreas
Chronic pancreatitis ($n = 7$)	0	2 (29)	3 (75)
Normal pancreas ($n = 4$)	1	1 (14)	1 (25)
	2	3 (43)	0 (0)
	3	1 (14)	0 (0)
Pancreatic carcinoma ($n = 27$)	0	3 (11)	12 (71)
Normal pancreas ($n = 17$)	1	5 (19)	4 (24)
	2	9 (33)	1 (6)
	3	10 (37)	0 (0)
Distal cholangiocarcinoma ($n = 8$)	0	0 (0)	2 (40)
Normal pancreas ($n = 5$)	1	1 (13)	1 (20)
	2	2 (25)	2 (40)
	3	5 (63)	0 (0)
Ampullary carcinoma ($n = 9$)	0	3 (33)	4 (50)
Normal pancreas ($n = 8$)	1	2 (22)	3 (38)
	2	2 (22)	1 (13)
	3	2 (22)	0 (0)
Carcinoma other ($n = 5$)	0	4 (80)	1 (50)
Normal pancreas ($n = 2$)	1	1 (20)	1 (50)
	2	0 (0)	0 (0)
	3	0 (0)	0 (0)
Benign ($n = 8$)	0	4 (50)	4 (80)
Normal pancreas ($n = 5$)	1	4 (50)	1 (20)
	2	0 (0)	0 (0)
	3	0 (0)	0 (0)

chronic pancreatitis and benign disease, CA19-9 levels were <37 units/mL. A scatter plot of CA19-9 levels of patients with carcinoma and benign disease is shown in Fig. 1B.

Immunohistochemistry. To verify by histology that MMP-7 is indeed highly expressed in pancreatic carcinoma and thus is the likely source of MMP-7 in these patients, we analyzed the MMP-7 expression in the available patient samples by immunohistochemistry (Supplementary Fig. S1; results summarized in Table 3). We could confirm earlier reports that MMP-7 is highly expressed in pancreatic cancer (14-16). Only seven samples of patients with chronic pancreatitis were available. Three had negative or weak, four had moderate, and one had strong immunohistochemical staining intensity for MMP-7 in the inflamed epithelium (data not shown). In all patients, normal pancreatic tissue showed no or weak MMP-7 expression. Whereas we could confirm that the majority of areas of diseased pancreata did produce MMP-7, no clear relationship between plasma MMP-7 levels and staining intensity in individual patients was found.

Differential Diagnostic Strength of Plasma MMP-7 and CA19-9. Diagnostic values were determined to analyze whether MMP-7 can be used to differentiate between patients with cancer and those with benign disease. Plasma MMP-7 levels, at a cutoff of 1.5 ng/mL, revealed a sensitivity of 57%, compared with 47% for CA19-9. The likelihood ratio of a positive test result was comparable between MMP-7 and CA19-9 (3.35 and 3.36, respectively). At a cutoff of 1.0 ng/mL, sensitivity of MMP-7 increases to 69%, but with a concomitant drop in specificity to 60%. The positive predictive value was 83% and 71% at a cutoff of 1.5 and 1.0 ng/mL, respectively, for MMP-7 and 83% for CA19-9, making MMP-7 assessment no improvement over CA19-9. Figure 2 shows the receiver operative characteristics curves to compare plasma MMP-7 and CA19-9 in both patient groups. The area under the curve for MMP-7 was 0.73 [95% confidence interval (95% CI), 0.63-0.84] and for CA19-9, 0.75 (95% CI, 0.64-0.85). These data, taken together, suggest that the potential usefulness of plasma MMP-7 alone as a pancreatic cancer marker is comparable to that of CA19-9.

Given these results, we then analyzed whether the diagnostic value improves when MMP-7 was combined with CA19-9, with MMP-7 at a cutoff of 1.5 ng/mL and a CA19-9 cutoff of 37 units/mL. The positive predictive value increased to 100%, but with a concomitant drop in sensitivity to 27%. Diagnostic values are given in Table 4.

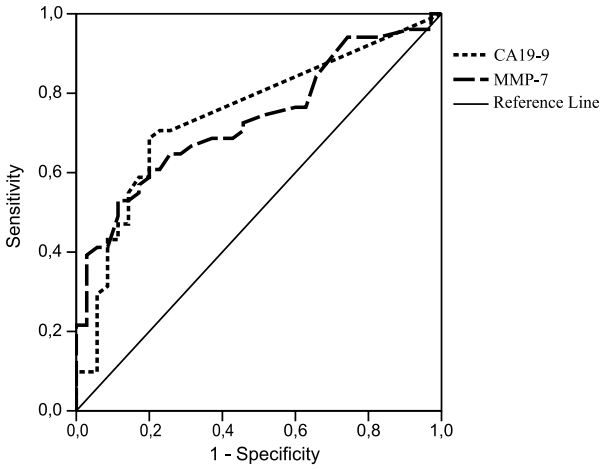

Figure 2. Receiver operating characteristic curve of plasma MMP-7 and CA19-9 for the detection of (peri)pancreatic carcinoma. Area under the curve for MMP-7 is 0.73 (95% CI, 0.63-0.84) and for CA19-9, 0.75 (95% CI, 0.64-0.85).

Discussion

The present study shows that MMP-7 levels can be detected in plasma and that plasma MMP-7 levels in patients with periampullary carcinoma are significantly higher than in those with chronic pancreatitis. Thus, plasma MMP-7 could be useful as a diagnostic marker to distinguish between periampullary carcinoma and chronic pancreatitis with an inflammatory mass. Whereas animal studies have suggested otherwise,[4] MMP-7 measurement in pancreatic and duodenal juice in a clinical setting was not useful. The levels in pancreatic juice had a wide range and those in duodenal juice were hardly detectable.

MMP-7 has been reported to have multiple functions in inflammation and cancer. Like many MMPs, it has been shown to facilitate tumor invasion through the extracellular matrix (19, 20). However, MMP-7 activity has been shown to contribute to early intestinal tumor formation (21) and to the desmoplastic response in intestinal and gastric cancers (22, 23). Its determined functions in inflamed tissue have ranged from the control of the desmoplastic response and release of chemotactic factors in models of lung injury (24, 25) to the induction of acinar cell apoptosis in pancreatic inflammation (14). The contribution of MMP-7 to early tumor progression and inflammatory disease has most commonly been associated with its ability to process non–extracellular matrix proteins, including tumor necrosis factor α (26), Fas ligand (14, 27), heparin-binding epidermal growth factor (28), syndecan-1 (25), and insulin-like growth factor binding protein 5 (22, 23).

MMP-7 expression has been identified repeatedly as up-regulated in pancreatic cancer in large-scale differential expression studies, with the degree of up-regulation usually ~10-fold (29, 30). By immunohistochemistry, MMP-7 is expressed in the neoplastic epithelium of most patients with pancreatic tumors (14, 15). This is the first study to show that differences in MMP-7 levels are evident in plasma and are statistically correlative to disease status. Given that MMP-7 expression has been identified in inflammatory cells, it is important to emphasize the use of plasma rather than serum in these studies to decrease background. Curiously, former studies show MMP-7 expression at high levels in the epithelial lesions of patients with chronic pancreatitis and early-stage neoplasia (14). In the current study, this elevated protein production in the tissue did not translate to significantly higher plasma MMP-7 levels. This could be due to many factors, such as differential access to the circulation, the nonquantitative nature of immunohistochemistry, or the limitations of a single section representing the complete lesion. It is also possible that the source of plasma MMP-7 protein is not the tissue at all, but cells that constitutively express MMP-7 that have been shed into the circulation. Significant numbers of such cells are much more likely to be found in advanced cancer patients than in those with precancerous disease.

Multiple histologic studies have shown a relation between MMP-7 expression and survival in patients with pancreatic cancer (15, 16). These studies showed that patients with negative or weak immunohistochemical staining for MMP-7 survived significantly longer than those with moderate or strong expression. However, in the present study, we were not able to confirm these findings. When the correlation of plasma MMP-7 levels and survival was analyzed in patients with pancreatic cancer, we found a significantly decreased survival of patients with a plasma MMP-7 level in the highest quartile compared with those with levels in the three lowest quartiles (P = 0.01). However, in the highest quartile, more patients

[4] Personal observations.

Table 4. Diagnostic value of plasma MMP-7 and CA19-9

Marker	Carcinoma (n = 51) versus no carcinoma (n = 35)					
	Sens. (%)	Spec. (%)	PPV (%)	NPV (%)	+LR	−LR
MMP-7 (≥1.5 ng/mL)	57	83	83	57	3.35	0.52
MMP-7 (≥1.0 ng/mL)	69	60	71	57	1.73	0.52
CA19-9 (>37 units/mL)	47	86	83	53	3.36	0.61
MMP-7 (≥1.5 ng/mL) and CA19-9 (>37 units/mL)	27	100	100	49	∞	0.73
MMP-7 (≥1.0 ng/mL) and CA19-9 (>37 units/mL)	39	97	95	52	13	0.63

Abbreviations: Sens., sensitivity; Spec., specificity; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; −LR, negative likelihood ratio.

underwent a bypass, a palliative intervention that correlates with poorer survival compared with pancreatic resection (31). On subgroup analyses of more homogeneous patient groups (bypass alone and pancreatic resection alone), there was also no significant difference in survival between patients in the highest quartile and those in the lowest three.

Plasma MMP-7 levels in patients with cancer and benign disease were used to address its diagnostic value. At an arbitrary cutoff of 1.5 ng/mL, a sensitivity of 57% and a specificity of 83% were found. The sensitivity and specificity of CA19-9, today's most commonly used pancreatic tumor marker, were 47% and 86%, respectively. Plasma MMP-7 alone is as good, but not significantly better than CA19-9 alone, as a diagnostic tool. When MMP-7 at a cutoff of 1.5 ng/mL was combined with CA19-9, the specificity increased to 100% with an infinite positive likelihood ratio (i.e., pathognomonic for the presence of a malignancy). Sensitivity dropped to 27% and a negative predictive value of 49% using this combination, meaning that almost half of patients with cancer will have a negative test result. Thus, MMP-7 and CA19-9 assessed together have no strong predictive value. However, the data from this study encourage further exploration of the diagnostic accuracy of MMP-7 as a periampullary tumor marker in combination with other tumor markers. With this in mind, we find it potentially interesting that MMP-7 has well-established relationships with other proteins that have been suggested as pancreatic tumor markers. Tissue inhibitor of metalloproteinase-1 (TIMP-1) has been shown to be up-regulated in pancreatic cancer (16). Thus, testing plasma or pancreatic juice for the MMP-7/TIMP-1 complex, an indication of coexpression of both proteins in the same tissue, may eliminate false positivity for either potential marker alone. Potentially more interesting would be to assay for unique products of MMP-7 activity, such as fragmented osteopontin (32). Osteopontin has been shown to be a MMP-7 substrate *in vivo* and yields unique cleavage products compared with other proteases (33). Antibodies designed to recognize these unique osteopontin products would enhance not only the specificity of both markers but also their sensitivity because one MMP-7 molecule should be able to process multiple molecules of osteopontin. Furthermore, because there are powerful inhibitors of MMPs in the circulation, the possibility of false positives is decreased because spurious substrate processing outside of the local tissue environment is highly unlikely (34).

In the current study, five patients underwent a pancreatic resection for suspected malignancy, which turned out to be pancreatitis with an inflammatory mass at pathology. Plasma MMP-7 levels in these patients were consistent with those in patients with chronic pancreatitis and benign disease. However, this observation must be interpreted carefully, in light of the small patient number. More patients with suspected carcinoma must be analyzed to confirm the possible usefulness of these initial findings. Furthermore, not all patients with pancreatitis with inflammatory mass were tested, but only those undergoing surgery, creating a selection bias that precludes definite conclusion in that respect. However, the very patients that are scheduled for surgery because of the suspicion of malignancy form the target group to be tested. Moreover, including patients without surgery would have caused verification bias.

In conclusion, we have shown that MMP-7 can be detected in plasma and pancreatic juice but not in duodenal juice. Plasma MMP-7 levels are significantly higher in periampullary carcinoma compared with chronic pancreatitis and benign disease. Although the diagnostic accuracy of plasma MMP-7 alone is comparable to that of CA19-9, it is not high enough to determine treatment strategy in patients with a periampullary mass. However, given the body of knowledge that exists with regard to the biology and functions of MMP-7, we believe that the combined evaluation of plasma MMP-7 with CA19-9 and other tumor markers could have significant clinical potential for the diagnosis of pancreatic cancer.

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Cancer Epidemiol Biomarkers Prev 2007;16:886-891.