Published in final edited form as:

J Gastrointest Surg. 2016 May ; 20(5): 899–904. doi:10.1007/s11605-015-3057-z.

A Pilot Study Evaluating Serum MMP7 as a Preoperative Prognostic Marker for Pancreatic Ductal Adenocarcinoma Patients

Sam C. Wang¹, Justin R. Parekh¹, Matthew R. Porembka¹, Hari Nathan², Michael I. D'Angelica³, Ronald P. DeMatteo³, Yuman Fong⁴, T. Peter Kingham³, William R. Jarnagin³, and Peter J. Allen³

¹ Department of Surgery, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-8548, USA

² Department of Surgery, University of Michigan, Ann Arbor, MI, USA

³ Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA

⁴ Department of Surgery, City of Hope Medical Center, Duarte, CA, USA

Abstract

Background—Current preoperative risk stratification modalities for pancreatic ductal adenocarcinoma (PDA) patients are inadequate. Elevated serum matrix metalloproteinase 7 (MMP7) is associated with metastatic PDA. We evaluated preoperative MMP7 level as a prognostic marker in patients with resectable PDA.

Methods—From a prospectively maintained database, we identified PDA patients who underwent operation with curative intent from 2004 to 2008 and had serum collected preoperatively. MMP7 was measured by enzyme-linked immunosorbent assay. Patients were defined as having advanced disease if they were found to be unresectable at the time of operation or had nodal involvement on final pathology.

Results—Preoperative serum samples were available for 134 patients. Using a cutoff of 13.5 ng/mL, MMP7 was highly predictive for advanced disease. For patients who underwent R0 resection, MMP7 > 13.5 ng/mL was strongly associated with N1 status, T3/T4 stage, moderate/poor differentiation, and perineural invasion. The median recurrence-free survival was 5.0 months in patients with MMP7 > 13.5 ng/mL versus 9.9 months for patients with lower values ($P = 0.004$).

Conclusions—Very elevated serum MMP7 was highly predictive of unresectable disease and nodal involvement despite favorable preoperative cross-sectional imaging. MMP7 should be further evaluated as a biomarker to risk-stratify PDA patients prior to operation.

Sam C. Wang sam.wang@utsouthwestern.edu.

Presented at the 2014 SSO Annual Cancer Symposium in Phoenix, AZ, as a Poster.

Compliance with Ethical Standards

Disclosures The authors have no disclosures to make.

Keywords

Pancreas cancer; MMP7; CA19-9; Biomarker

Introduction

Despite the use of modern imaging modalities, 15–25 % of pancreatic ductal adenocarcinoma (PDA) patients with putatively resectable tumors are found to have unresectable disease at the time of exploration either due to local invasion or occult metastasis.^{1,2} For patients who undergo R0 resections, the median survival is less than 2 years, which suggests that a significant number of patients had occult distant disease at the time of presentation and accentuated the inadequacy of current staging modalities.³ Improved risk stratification methods are needed to identify patients who will not benefit from surgical resection as first-line therapy. Since lymph node involvement is one of the strongest prognostic variables for PDA, the ability to predict nodal disease will significantly improve risk stratification accuracy.

Matrix metalloproteinase 7 (MMP7) is an epithelium-derived member of a family of zinc-dependent endopeptidases that is overexpressed in PDA. Elevated serum MMP7 level is associated with stage IV disease in PDA patients.⁴ Increased MMP7 has also been found to be associated with malignant diagnosis or worse outcomes in patients with prostate,⁵ bladder,⁶ renal cell,^{7,8} ovarian,^{9,10} and bile duct cancer.¹¹ We hypothesized that preoperative serum MMP7 can identify patients with advanced disease despite favorable preoperative radiographic findings.

Materials and Methods

The prospectively maintained Memorial Sloan Kettering Cancer Center database was queried for patients who underwent operation for PDA from 2004 to 2008. All patients were consented to our disease-site serum and tissue banking protocol. A waiver of authorization was obtained from our Institutional Review Board prior to review of any patient information.

We retrieved all relevant clinicopathologic and survival data for patients who had preoperatively collected serum samples available for MMP7 measurement and recorded recurrence-free survival and disease-specific survival. The charts of these patients were reviewed to ensure that it was the opinion of the attending surgeon that the planned operation was for curative intent. Patients were defined as having an advanced disease if they had an unresectable disease found at the time of operation or if they had positive lymph node involvement in the resected specimen.

Serum MMP7 was measured by a sandwich enzyme-linked immunosorbent assay method according to the manufacturer's instructions (Quantikine Human Total MMP-7 Immunoassay, R&D Systems, Inc, Minneapolis, MN). Samples were stored at –80 °C since collection and no prior freeze-thaw cycles had been performed. The samples were thawed and diluted by 1:4 ratios and assayed in duplicates with two separate runs. The dilution ratio was altered as needed to ensure that the readings were within the linear proportion of the

standard curve. The assay was repeated when standard deviation between duplicates was greater than 10 % or if there was a greater than 10 % difference between runs. The mean of all four samples was used as the reported value.

The Mann–Whitney *U* or Kruskal–Wallis test was used to compare the median MMP7 between cohorts. Fisher's exact test was used to determine association of elevated biomarker level and a given variable. A receiver operating characteristic (ROC) curve was generated to evaluate MMP7 or CA19-9 as a diagnostic test for advanced disease. Univariate logistic regression was employed to analyze the association between demographic and clinical factors and our primary endpoint. Survival data were analyzed via the Kaplan–Meier method, and the curves were compared by the log-rank test.

Results

Clinicopathologic Characteristics and Serum MMP7

One-hundred and thirty-four patients had serum samples collected preoperatively, and their characteristics are presented in Table 1. At the operation, 36 patients (27 %) had unresectable disease due to locally advanced tumors ($n = 17$, 13 %) or occult metastatic disease ($n = 19$, 14 %). Of the 98 patients who underwent complete resection, 74 (76 %) had N1 disease. Thus, a total of 110 patients had advanced disease. With a median follow-up of 18 months, 73 % of patients had died from disease, 10 % had no evidence of disease, 9 % were alive with disease, and 8 % had died of unknown or unrelated causes.

The median MMP7 value was 7.0 ng/mL, with a range of 1.7 to 33.0 ng/mL and an interquartile range of 5.0 to 10.7 ng/mL. MMP7 was significantly higher in patients who had higher T stage ($P = 0.04$), nodal involvement ($P = 0.02$), vascular invasion ($P = 0.006$), moderate/poor differentiation ($P = 0.04$), and advanced disease ($P = 0.006$).

Highly Elevated MMP7 Was Specific for Advanced Disease

A receiver operating characteristic (ROC) curve was constructed to evaluate serum MMP7 as a diagnostic tool to predict unresectable disease and nodal involvement. The area under curve (AUC) was 0.68 (95 % confidence interval (CI) 0.57 to 0.79), which makes MMP7 a modestly useful diagnostic assay to predict advanced disease. However, we noted that at the very elevated value of 13.5 ng/mL, MMP7 was highly predictive for unresectable disease or nodal involvement (PPV = 100 %), albeit at a very low sensitivity of 18 % and a negative predictive value (NPV) of 21 % (Fig. 1a). We also constructed a ROC curve using CA19-9 and found that in our cohort, CA19-9 was not predictive of advanced disease (AUC = 0.57, 95 % CI 0.40–0.73, Fig. 1b).

Of the 20 patients (15 %) who had MMP7 ≥ 13.5 ng/mL, three had occult metastases and five had locally unresectable disease. All 12 patients who underwent resection and had MMP7 greater than 13.5 ng/mL had nodal involvement on final pathologic evaluation (Table 2). Importantly, MMP7 > 13.5 ng/mL was correlated with pathologic findings that are known to be associated with poor outcomes such as nodal involvement ($P = 0.04$) and vascular invasion ($P = 0.03$) (Table 2). Unfavorable outcome was correlated with highly elevated MMP7 ($P = 0.02$), as expected based on the ROC curve. We also used logistical

regression to evaluate the association between highly elevated MMP7 and these pathologic variables. We found that serum MMP7 ≥ 13.5 ng/mL was highly predictive for nodal involvement, T3/T4 stage, moderate/poor differentiation, and perineural involvement. Multivariate analysis of these factors was not possible as they are highly correlated and/or already perfectly predictive.

For patients with MMP7 ≥ 13.5 ng/mL, the median time to recurrence was 5.0 months as compared to 9.9 months in patients with lower MMP7 ($P = 0.004$, Fig. 2a). Interestingly, disease-specific survival was not different between the two groups (17.7 months for high MMP7 and 25.5 months for low MMP7, NS, Fig. 2b).

We also found that MMP7 identified a subset of patients who had advanced disease despite relatively low CA19-9. Six of the 43 patients (14 %) with CA19-9 < 150 U/mL, three of the 13 patients (23 %) with normal CA19-9 (< 37 U/mL), and one of the six patients (16 %) who did not secrete CA19-9 had MMP7 ≥ 13.5 ng/mL and correspondingly advanced disease.

Discussion

Prognosis for pancreatic ductal adenocarcinoma patients is dismal. Even for patients who undergo R0 resections, the median survival is less than 2 years, which suggests that a significant proportion of these patients have advanced disease burden at the time of operation.³ The short survival interval also emphasizes the inadequacy of current risk stratification modalities. Improved preoperative risk stratification to identify patients with aggressive tumor biology despite favorable staging studies will help avoid futile operations. In addition, avoiding unnecessary operations will expedite the initiation of systemic therapies, which may improve quality of care and prolong survival.

In this pilot study, we evaluated serum MMP7 as a prognostic biomarker for patients with putatively resectable pancreatic cancer. Because previous work has shown that elevated serum MMP7 is associated with metastatic PDA,⁴ we hypothesized that it may predict occult advanced disease in patients with seemingly resectable tumors. We found that MMP7 is of only modest utility as a diagnostic tool to predict unresectability and nodal involvement (AUC = 0.68) due to its poor sensitivity. However, we noted that for patients who had levels above 13.5 ng/mL, which was approximately 15 % of the cohort, MMP7 was highly predictive for unresectable disease or nodal involvement.

Obtaining this powerful prognostic information at the pre-operative time point can rationally guide clinical recommendations for patients with putatively resectable disease. While unresectability clearly precludes operative intervention (with curative intent), the ability to predict nodal involvement may also alter recommendation for upfront operation since lymph node disease is strongly associated with worse outcomes. According to the SEER database, patients with N1 disease have only 10.7 % 5-year survival, as compared to 27.1 months for patients without nodal involvement (<http://seer.cancer.gov/statfacts/html/pancreas.html>). In our cohort, median recurrence-free survival for patients with nodal involvement was 8.5 months as compared to 48 months for patients with N0 disease ($P < 0.001$). The fact that highly elevated MMP7 was associated with vascular invasion and strongly predictive of N1

status, advanced T stage, moderate/poor differentiation, and perineural invasion is consistent with the conclusion that highly elevated MMP7 is a marker for advanced disease.

Based on our data, we estimate that approximately one in five patients with tumors judged to be resectable based on pre-operative staging studies will actually have unresectable disease or nodal involvement that can be identified using MMP7 testing. If validated, preoperative MMP7 levels may be useful to stratify patients to upfront systemic treatment despite favorable cross-sectional imaging. Our data also suggests that MMP7 can be used in conjunction with CA19-9, as MMP7 was highly elevated in 14–23 % of patients who had relatively low or normal CA19-9 and correctly predicted advanced disease.

The median time to recurrence for patients with highly elevated MMP7 who underwent R0 resection was approximately 5 months, as compared to 10 months in patients with lower MMP7. The rapid recurrence in patients with high MMP7 is consistent with the association of highly elevated MMP7 to pathologic findings associated with worse outcomes. Interestingly, there was no significant difference in overall survival when patients are stratified by MMP7. This may be due to our relatively small sample size, a reflection of the poor sensitivity of MMP7 (i.e., there were still a large number of patient with poor biology in the low MMP7 group), or underlying biology in patients with highly elevated MMP7 where despite early recurrence, they are able to achieve relatively long overall survival. Future studies will be needed to distinguish between these possibilities.

Previous pathologic analysis showed that PDA overexpresses MMP7, which is normally expressed only in the small intestine and the cycling uterus.¹² One possible explanation for why elevated MMP7 is a marker of advanced disease is that as cancer cells establish distant colonies in locoregional lymph nodes or end organs, the amount of circulating MMP7 increases in proportion to the number of cancer cells with access to the circulation. This is consistent with our finding that elevated MMP7 was associated with nodal or vascular involvement.

The current report has several limitations. As a pilot study, we evaluated a relatively small number of patients. In addition, the single-institution retrospective nature of the study predisposes the results to selection bias. Thus, the utility of preoperative serum MMP7 as a prognostic marker needs to be validated in a prospective manner in larger cohorts.

Conclusions

For patients with very elevated MMP7 levels, advanced disease was common despite favorable cross-sectional imaging or relatively low CA19-9. Serum MMP7 should be further evaluated as a potential prognostic biomarker for patients with pancreatic cancer as it may improve preoperative risk stratification.

Acknowledgments

We thank Frances Weis-Garcia and Olivera Grbovic of the Memorial Sloan Kettering Cancer Center Antibody & Bioresource Core Facility for the optimization and performance of the MMP7 ELISA assays.

This study was supported in part by NIH/NCI Cancer Center Support Grant P30 CA008748.

References

1. Mayo SC, Austin DF, Sheppard BC, Mori M, Shipley DK, Billingsley KG. Evolving Preoperative Evaluation of Patients with Pancreatic Cancer: Does Laparoscopy Have a Role in the Current Era? *Journal of the American College of Surgeons*. Jan; 2009 208(1):87–95. [PubMed: 19228509]
2. White R, Winston C, Gönen M, D'Angelica M, Jarnagin W, Fong Y, et al. Current Utility of Staging Laparoscopy for Pancreatic and Peripancreatic Neoplasms. *Journal of the American College of Surgeons*. Mar; 2008 206(3):445–50. [PubMed: 18308214]
3. Winter JM, Brennan MF, Tang LH, D'Angelica MI, DeMatteo RP, Fong Y, et al. Survival after Resection of Pancreatic Adenocarcinoma: Results from a Single Institution over Three Decades. *Ann Surg Oncol*. Jul 15; 2011 19(1):169–75. [PubMed: 21761104]
4. Fukuda A, Wang SC, Morris JP, Folias AE, Liou A, Kim GE, et al. Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. *Cancer Cell*. Apr 12; 2011 19(4):441–55. [PubMed: 21481787]
5. Szarvas T, Becker M, Dorp vom F, Meschede J, Scherag A, Bánkfalvi Á, et al. Elevated serum matrix metalloproteinase 7 levels predict poor prognosis after radical prostatectomy. *Int J Cancer*. Jan 28; 2011 128(6):1486–92. [PubMed: 20473942]
6. Szarvas T, Becker M, Dorp vom F, Gethmann C, Tötsch M, Bánkfalvi Á, et al. Matrix metalloproteinase-7 as a marker of metastasis and predictor of poor survival in bladder cancer. *Cancer Science*. May; 2010 101(5):1300–8. [PubMed: 20180812]
7. Sarkissian G, Fergelot P, Lamy PJ, Patard JJ, Culine S, Jouin P, et al. Identification of Pro-MMP-7 as a Serum Marker for Renal Cell Carcinoma by Use of Proteomic Analysis. *Clinical Chemistry*. Mar 1; 2008 54(3):574–81. [PubMed: 18202161]
8. Ramankulov A, Lein M, Johannsen M, Schrader M, Miller K, Jung K. Plasma matrix metalloproteinase-7 as a metastatic marker and survival predictor in patients with renal cell carcinomas. *Cancer Science*. Jun; 2008 99(6):1188–94. [PubMed: 18422740]
9. Meinhold-Heerlein I, Bauerschlag D, Zhou Y, Sapinoso LM, Ching K, Frierson H, et al. An Integrated Clinical-Genomics Approach Identifies a Candidate Multi-Analyte Blood Test for Serous Ovarian Carcinoma. *Clinical Cancer Research*. Jan 15; 2007 13(2):458–66. [PubMed: 17255266]
10. Acar, A.; Onan, A.; Coskun, U.; Uner, A.; Bagriacik, U.; Atalay, F., et al. *Med Oncol*. Vol. 25. Humana Press Inc; 2008. Clinical significance of serum MMP-2 and MMP-7 in patients with ovarian cancer.; p. 279-83.
11. Leelawat K, Narong S, Wannaprasert J, Ratanashu-ek T. Prospective study of MMP7 serum levels in the diagnosis of cholangiocarcinoma. *World J Gastroenterol*. Oct 7; 2010 16(37):4697–703. [PubMed: 20872971]
12. Wilson CL, Heppner KJ, Rudolph LA, Matrisian LM. The metal-loproteinase matrilysin is preferentially expressed by epithelial cells in a tissue-restricted pattern in the mouse. *Mol Biol Cell*. Jul; 1995 6(7):851–69. [PubMed: 7579699]

Fig. 1. Receiver operating characteristic curves for using serum **a** MMP7 or **b** CA19-9 to predict unresectable disease or nodal involvement. *AUC* area under curve. *CI* 95 % confidence interval

Fig. 2.
Kaplan–Meier curves for **a** recurrence-free survival and **b** disease-specific survival when patients are stratified based on MMP level of 13.5 ng/mL

Table 1

Clinicopathologic characteristics and serum MMP7

	<i>N</i>	%	Median MMP7 (ng/mL), (IQR)	<i>P</i> value
Cohort	134		7.0 (5.0 to 10.7)	
Gender				
Male	75	56	7.7 (5.7–10.9)	0.03 [*]
Female	59	44	5.9 (4.2–9.5)	
Age (years), median (range)	66 (44–86)			
OR findings				NS
Resectable	98	73	6.7 (4.7–10.1)	
Locally unresectable	17	13	9.2 (6.7–14.5)	
Metastatic	19	14	7.0 (4.0–12.3)	
Tstage				0.04 [*]
T1/T2	4	4	4.2	
T3/T4	94	96	6.9	
Nstage				0.02 [*]
N0	24	24	5.7 (3.6–7.4)	
N1	74	76	7.4 (5.0–10.9)	
Vascular invasion (microscopic)				0.006 [*]
No	36	38	5.5 (3.8–7.5)	
Yes	58	62	7.8 (5.9–10.9)	
Perineural invasion				
No	5	5	4.6 (4.5–5.4)	NS
Yes	90	95	7.0 (5.0–10.7)	
Differentiation			0.04	
Well	3	3	3.7	0.04 [*]
Moderate/poor	92	97	6.9	
Margin				NS
Negative	74	79	6.8 (4.6–10.0)	
Positive	20	21	7.0 (4.9–11.5)	
Outcomes				0.006 [*]
Early disease	24	18	5.7 (3.6–7.4)	
Advanced disease ^a	110	82	7.4 (5.1–11.4)	

The Mann-Whitney *U* or Kruskal-Wallis test was used to compare median MMP7 between each given cohort. *IQR* interquartile range (IQR could not calculated for certain variables due to the small sample size)

^{*} *P* < 0.05

^a Advanced disease is defined as unresectability or nodal involvement

Table 2

Very elevated MMP7 is associated with unfavorable pathologic characteristics

	MMP7 < 13.5 ng/mL	MMP7 13.5 ng/mL	P value
Gender			NS
Male	63	12	
Female	51	8	
Age			NS
T stage			NS
T1/T2	4	0	
T3/T4	82	12	
N stage			0.04 *
N0	24	0	
N1	62	12	
Margin			NS
Negative	66	8	
Positive	17	3	
Vascular invasion (microscopic)			0.03
No	35	1	
Yes	48	10	
Perineural invasion			NS
No	5	0	
Yes	78	12	
Differentiation			NS
Well	3	0	
Moderate/poor	80	12	
Outcome			0.02 *
Early disease	24	0	
Advanced disease ^a	90	20	

* $P < 0.05$ ^a Advanced disease is defined as unresectability or nodal involvement