HHS Public Access

Author manuscript

Pancreas. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Pancreas. 2016 January ; 45(1): 123–126. doi:10.1097/MPA.0000000000000423.

Impact of intratumoral inflammation on survival after pancreatic cancer resection

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Abstract

Objectives—Pancreatic cancer (PaC) frequently results in death despite surgical resection. We sought to evaluate whether inflammation in the primary tumor was associated with early death following surgical resection.

Methods—In this case-control study we identified 21 individuals who died <12 months after surgery for PaC and 42 controls who survived >36 months after surgery. Differences in the composition of host inflammatory response between the groups were evaluated with univariate comparisons and odds ratios for early death were calculated using logistic regression modeling.

Results—Cases were more likely to have a high tumor grade (90.5% vs. 26.2%, p<0.01). The odds of early death were increased in those with a high-grade tumor (unadjusted OR 26.77, 95% CI 5.35–134.07, p<0.01).. Conversely, the density (high vs. low) of inflammatory cells in tumors was similar between the groups and odds of early death were not associated with any inflammatory marker.

Conclusions—High tumor grade, but not altered density of inflammatory cells in the intratumoral compartment, is associated with increased odds of early death after PaC resection. Future studies evaluating the host response in multiple tumor compartments with advanced histologic techniques is needed to further elucidate the role of inflammation in PaC.

INTRODUCTION

Pancreatic cancer (PaC) is the fourth most common cause of cancer mortality in the United States, resulting in almost 40,000 deaths per year[1]. The 5 year survival rate remains dismal (~5%), and reflects a combination of presentation with advanced disease and ineffective treatment options[1]. The best chance for long-term survival accrues to patients receiving surgical resection for early stage cancer. Unfortunately, the median survival even in this scenario is still less than two years. It is also troubling that approximately 20% of patients

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Conflicts of interest/disclosures: No conflicts of interest exist.

undergoing pancreatic resection with curative intent die within one year of surgery[2]. For patients experiencing early death following surgery, cancer recurrence is usually the cause of death. Thus, greater understanding of factors associated with early death after cancer resection will improve our understanding of tumorigenesis and potentially direct future investigations into novel therapeutic approaches.

Chronic inflammatory conditions have been linked with increased risk for a variety of malignancies including gastric cancer (*H. pylori* infection), colon cancer (inflammatory bowel disease), hepatocellular carcinoma (chronic viral hepatitis), and esophageal adenocarcinoma (Barrett's esophagus). PaC is no exception; multiple inflammatory states have been identified as risk factors including hereditary pancreatitis, chronic pancreatitis, cigarette smoking, and type 2 diabetes mellitus[3]. Regardless of whether or not a premalignant inflammatory state is identified, PaC itself is histologically characterized by a marked host inflammatory response. Despite longstanding recognition of this phenomenon, the role of the various components of the host response remains incompletely understood.

Recent studies have demonstrated improved postoperative survival in those with varying densities and subtypes of tumor infiltrating lymphocytes[4, 5]. We hypothesized that increased density of other immune infiltrates, particularly mast cells and macrophages in the tumor (i.e., intratumoral compartment) are associated with early death after PaC resection. We compared specific components of the intratumoral host response in subjects who experienced early death following PaC resection to those with prolonged postoperative survival.

METHODS

This study protocol was approved by the Mayo Clinic Institutional Review Board, and informed consent for participation in future research was obtained from all subjects at the time of enrollment.

The Mayo Clinic Pancreas Cancer SPORE database was reviewed to identify all individuals who had undergone surgical resection for pancreatic cancer between 2000 and 2010. Patients given neoadjuvant radiation or chemotherapy were not included, to minimize confounding from previous treatment response. Operative reports were reviewed to exclude subjects who had either a palliative or aborted (due to the presence of distant disease) procedure. "Early death" was defined as a patient death within 12 months from the date of initial surgery. Subjects who died within 30 days from the surgical date, during the initial hospitalization, or due to a non-disease related cause (e.g., motor vehicle accident) were excluded (n=10). The remaining cases of early death were matched with two subjects who had a prolonged survival (>36 months) following cancer surgery in a consecutive manner solely based on duration of survival starting with those with a survival closest to 36 months. Vital status of subjects was collected using multiple sources as part of routine research followup, which included periodic mailings, medical records, tumor registry, and death indices from online services.

Clinical and laboratory details were abstracted from the electronic medical record at study entry. Diabetes mellitus (DM) was defined as those patients with a fasting blood glucose (FBG) level >126 mg/dL or, for those without FBGs, a self-report of DM on a standardized study questionnaire.

Histopathologic data

A GI pathologist (TCS) blinded to clinical outcomes reviewed the original tumor specimens for standard variables including tumor size, grade, margin status, and lymph node status. The tumor block with the most viable tumor was selected and a variety of inflammatory parameters were evaluated in the intratumoral compartment. Parameters scored as high or low by semiquantitative assessment included collagen deposition (scored twice, using hematoxylin & eosin (H&E) stain and trichrome stain), stromal cellularity, α-smooth muscle actin density, lymphocytes, macrophage density (as marked by CD68 immunohistochemistry) and polymorphonuclear cells (PMN) (scored twice, as stromal and intraluminal/intratumoral). Mast cells were recorded as number of tryptase-positive cells in one 400× microscopic field in the most densely involved area. The host inflammatory response was scored intratumorally within the pancreatic tissue, rather than at the advancing edge of tumor. Even with that restriction, there was still wide variation in density within individual cases. Slides were scanned at low power to find areas with the most intense response for each parameter, then evaluated in that area. The supplemental figures 1–7 show representative examples of host response categorized as high or low. Immunohistochemistry for alpha smooth muscle actin seemed to yield two different staining patterns, one with thin wavy cytoplasm and another with wider, paler cytoplasm (supplemental figure 8).

Statistical analyses

For the primary endpoint, differences in the inflammatory density between PaC subjects experiencing early death and those with prolonged survival, univariate comparisons were performed using either a Pearson chi-square test for categorical variables or a Kruskal-Wallis Rank Sum test for continuous variables.. For the purposes of analysis tumor grade was dichotomized into low- (well-differentiated and moderately differentiated) and high-grade (undifferentiated and poorly differentiated). Odds ratios (OR) for early death were calculated using logistic regression modeling. OR were also adjusted for tumor size, margin status (R0, R1, or R2), lymph node status (negative vs. positive), and preoperative DM status (present vs. absent). Statistical significance was $P<0.05$. All analyses were performed using SAS software, version 9.1.3 [SAS Institute, Cary, NC].

RESULTS

Clinical data

For this case-control study a total of 21 subjects with early death (<12 months) following surgery for PaC were identified and matched to 42 controls. These groups were similar in regards to gender and median age at diagnosis (Table 1). Aside from postoperative survival (which defined the two groups) and tumor size, other clinical factors were similar.

Histologic data

The density (high vs. low) of collagen, stroma, α-smooth muscle actin, lymphocytes, PMNs, macrophages, and mast cells in tumor specimens was similar between the two groups (Table 2). However, high tumor grade was significantly more common in the early death group.

Odds ratio

The odds of early death were not increased in subjects with high density of inflammation for each of the findings (Table 3), with the exception of those with a high-grade tumor (unadjusted OR 26.77, 95% CI 5.35–134.07, $p<0.01$). These comparisons were unchanged even after adjusting for tumor size, margin status, lymph node status, and preoperative DM status (Table 3).

DISCUSSION

In this study comparing individuals with early death from pancreatic cancer (<12 months) after surgery to those who survived at least 36 months, an increased density of inflammation in the intratumoral compartment was not associated with increased odds of early death. On the other hand, a high-grade tumor (undifferentiated or poorly differentiated) was associated with early death, even after adjusting for tumor size, margin status, and lymph node status. We were unable to demonstrate that the composition of the inflammatory microenvironment within the tumor explains why certain individuals die earlier than others.

Since clinical outcomes of PaC are generally poor and current treatments have limited efficacy, opportunities to better understand the mechanisms of tumor development, growth, and spread represent an important chance to identify potential therapeutic targets. The tumor microenvironment is of particular interest in PaC considering the characteristic, dense desmoplastic reaction. This environment contains a wide range of immune cells, including fibroblasts and endothelial cells, and is felt to promote tumor angiogenesis, metastasis, and contribute to treatment resistance [6]. Previous human studies investigating the prognostic significance of immune infiltrates are of particular interest since they have previously been associated with differences in survival. Important considerations in determining the pro- vs. anti-neoplastic activity of immune cells include the distribution, density, and function[6].

The exact role of mast cells in cancer remains undefined, but infiltration has been demonstrated in multiple human malignancies[7]. Mast cell infiltration is more prominent in PaC tumors compared to normal pancreatic tissue, and higher densities of infiltration have been previously associated with increased tumor grade and worse survival[8–10]. Also, there may be clinical relevance in the observation that higher mast cell density in the juxtatumoral compartment is less problematic. For example, in a study evaluating mass cell density in four different compartments of tumor and peritumoral tissue, there were increased densities in mast cells at the border between the two areas, but only increased mast cell levels on the tumor side of the border zone were associated with a difference in the overall survival[8]. Thus, our inability to confirm that higher counts of mast cells in the intratumoral region were associated with early death following PaC resection may have been partially the consequence of not accounting for zonal distribution within the intratumoral compartment.

There is also recent interest in the role of macrophages in pancreatic diseases. For example, in chronic pancreatitis, macrophages (CD68+) (as well as mast cells), are closely associated with activated pancreatic stellate cells, which in turn lead to fibrosis[11]. Understanding the exact role of macrophages is complicated because of their functional variety, however some have proposed that macrophages may play a role in tumor progression, lymphangiogenesis, and lymphatic metastasis. An example of the functional variety is that some macrophages are activated by T helper 1 cytokines (resulting in CD68+ receptors) while others are activated by T helper 2 cytokines (resulting in CD 163+ and CD204+ receptors). This heterogeneity likely explains the mixed results that have been demonstrated in regards to the influence of tumor-associated macrophages in PaC clinical outcomes. For example, previous investigations have shown that increased numbers of M2 macrophages (bearing CD 163+ or CD204+ receptors), particularly located at the tumor periphery, are associated with increased tumor size, density of lymphatic vessels, and worse survival in PaC[5, 12, 13]. Conversely, levels of the M1 macrophages (bearing only CD68+ receptors) in the tumor center or periphery were not associated with meaningful differences in clinical outcomes, as seen in our study[12, 13].

Activated pancreatic stellate cells express α-smooth muscle actin and are felt to be an important component of the desmoplastic stroma in pancreatic cancer[14–16]. High stromal turnover (suggested by an increased stroma:collagen ratio) has been associated with decreased survival[17]. Our efforts to quantify this population of cells included an overall assessment of stromal cellularity, scoring the density of smooth muscle actin as documented by immunohistochemistry, and subdividing positive staining on the basis of the appearance of the actin-positive cytoplasm. However, none of these parameters correlated with outcomes in our series.

The strongest factor associated with early death in our study of histologic features of the intratumoral region is tumor grade. It should be noted the large magnitude of effect in our adjusted OR for high tumor grade is in part a reflection of the statistically small sample size. Nevertheless, our observation adds to a growing body of literature from other single-center studies and analyses of population-based registries (e.g., SEER database) demonstrating survival following surgical resection is worse in individuals with high-grade tumors[18–21] To this end, some have advocated incorporating tumor grade into the current TNM cancer staging system for PaC[18].

Considering the strictly defined study groups the sample size, although clinically modest, was statistically small, which may limit our conclusions. Multiple areas were examined in the intratumoral compartment in an effort to address the potential for heterogeneous inflammation. As discussed above, it will helpful to further examine each of the inflammatory components in the various tumor compartments (including juxtatumoral), to determine the influence of the inflammatory distribution (as demonstrated for TIL's).

In this analysis of subjects who underwent surgical resection of PaC, the odds of early death were not increased in those with a high density of inflammation in the intratumoral compartment. A detailed histologic evaluation of this compartment does not identify patients at risk for early death after surgery, with the exception of a high-grade tumor. This adds to

previous literature suggesting this may be a relevant variable to consider in PaC staging. Future studies analyzing inflammation in the various tumor compartments simultaneously are needed to better understand the inflammatory response in this malignancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant Support: Dr Chari's research was funded by a grant from the Mayo Clinic Pancreas Cancer SPORE (P50 CA 102701) and an internal grant from the Mayo Clinic Department of Internal Medicine.

Abbreviations

DM	Diabetes mellitus
FBG	Fasting blood glucose
H&E	Hematoxylin & Eosin
IQR	Interquartile range
PaC	Pancreas cancer
PMN	Polymorphonuclear cell
PV	Portal vein
SMV	Superior mesenteric vein

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Table 1

Baseline demographics and operative tumor data for the study population.

Clinical variable	Early death (<12m), n=21	Prolonged survival (>36m), n=42	*P*-value
Median age at diagnosis (y), (IQR)	69.0, (58.0–75.4)	64.2, (54.0–72.8)	0.22
Male gender	8 (38.1)	23 (54.8)	0.21
Preoperative diabetes mellitus	11 (52.4)	13 (31.0)	0.10
Median tumor size (cm), (IQR)	4.0, (3.5–4.8)	3.0, (2.5–3.5)	<0.01
R0 margin status	17 (81.0)	37 (88.1)	0.58
Venous (PV and/or SMV) resection/reconstruction	6 (28.6)	6 (14.3)	0.17
Metastasis to lymph nodes	14 (66.7)	17 (40.5)	0.05
Postoperative survival (m); range	1.6–5.5	36.0–54.2	<0.01

Unless otherwise indicated, data are presented as n (%). IQR, interquartile range; PV, portal vein; SMV, superior mesenteric vein

Table 2

Histologic features of intratumoral inflammation in subjects undergoing pancreatic cancer resection who died within one year postoperatively compared to those who survived more than three years.

Histologic variable	Early death (<12m), n=21	Prolonged survival (>36m), n=41	p-value
Tumor grade			**<0.01**
High-grade	19 (90.5%)	11 (26.2%)	
Collagen (H&E)			0.86
High	12 (57.1%)	25 (59.5%)	
Collagen (trichome) *			0.85
High	12 (60.0%)	25 (62.5%)	
Stroma			0.33
High	8 (38.1%)	11 (26.2%)	
Smactin density *			0.27
High	11 (55.0%)	16 (40.0%)	
Smactin pattern *			0.10
Wide/mixed (vs. thin)	13 (65.0%)	17 (42.5%)	
Lymphocytes			0.21
High	2 (9.5%)	1 (2.4%)	
PMN density (lumen)			0.63
High	4 (19.0%)	6 (14.3%)	
PMN density (stroma)			0.50
High	3 (14.3%)	9 (21.4%)	
Macrophage density *			0.40
High	10 (50.0%)	24 (61.5%)	
Mast cells (per hpf) *			0.62
Median, (IQR)	14.0, (9.0–24.0)	12.0, (8.0–18.0)	

Unless otherwise indicated, data are presented as n (%).

* There was inadequate tissue to evaluate collagen (trichrome), α-smooth muscle actin, macrophage, and mast cells for 1 subject from the early death and 2 subjects in the prolonged survival groups, respectively. One other subject in the long-surviving group had inadequate tissue to evaluate macrophage and mast cell densities.

Table 3

Odds ratios for early death following pancreatic cancer resection in those with high density of inflammation for each of the findings.

Histologic Variable	Level	Unadjusted OR	95% CI		p-value	Adjusted	95% CI		p-value
Grade	**High**	**26.77**	**5.35**	**134.07**	**<0.01**	**213.94**	**9.56**	**∞**	**<0.01**
Collagen (H&E)	High	0.91	0.31	2.62	0.86	0.91	0.27	3.14	0.89
Collagen (trichome)	High	0.90	0.30	2.70	0.85	0.89	0.26	3.11	0.86
Stroma	High	1.73	0.57	5.30	0.33	2.28	0.61	8.62	0.22
Smactin (density)	High	1.83	0.62	5.42	0.27	1.37	0.41	4.58	0.61
Smactin (pattern)	Wide	2.51	0.83	7.64	0.10	2.34	0.68	8.05	0.18
Lymphocytes	High	4.32	0.37	50.58	0.24	5.25	0.30	91.50	0.26
PMN (lumen)	High	1.41	0.35	5.67	0.63	1.21	0.24	6.03	0.82
PMN (stroma)	High	0.61	0.15	2.55	0.50	0.78	0.16	3.81	0.76
Macrophages	High	0.63	0.21	1.86	0.40	0.52	0.14	1.87	0.31
Mast cells, #/hpf	--	1.02	0.96	1.08	0.49	1.03	0.97	1.11	0.33

*Adjusted for tumor size, margin status, lymph node status, and preoperative DM status.