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Pathologic Markers Of Prognosis In Ampullary Carcinoma

Rachna T. Shroff

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by

Rachna T. Shroff, MD

APPROVED:

_____
James L. Abbruzzese, MD

_____
Karin Hahn, MD, MS

_____
Robert A. Wolff, MD

_____
Michael Overman, MD

_____
Gary Gallick, PhD

APPROVED:

_____
Dean, The University of Texas
Health Science Center at Houston
Graduate School of Biomedical Sciences

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Rachna T. Shroff, MD

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Dedication

This thesis is dedicated to my husband, Puneet Shroff, to my parents, Deven and Amita Trivedi, and to my mentors, Robert A. Wolff and James L. Abbruzzese, who have each supported me in different ways over the years and made this possible.

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Pathologic Markers of Prognosis in Ampullary Carcinoma

Rachna T. Shroff, MD

Supervisory Professor: James L. Abbruzzese, MD

Ampullary cancer is a rare gastrointestinal malignancy that can be curable with surgical resection of localized disease. The benefit of adjuvant therapy, however, remains unknown in these patients partly because of difficulty in stratifying which patients are at high risk for recurrence. To better identify those patients who may benefit from adjuvant therapy, I conducted a retrospective analysis the pathology reports from 176 patients with surgically resected ampullary cancer who had not received any neoadjuvant therapy, the systemic therapy given, and the patient outcomes. A tissue microarray (TMA) of 95 surgically resected ampullary specimens was also constructed to examine whether there is a correlation between classical immunohistochemical profiles for intestinal and pancreaticobiliary tumors and their histologic classification. In this study, I confirmed the prognostic value of advanced T-stage, nodal metastases, and lymphovascular invasion. Patients whose tumors had “high risk” features had a significantly worse overall survival (p=.002). Furthermore, my research highlighted the importance of histology and its impact on survival, with pancreaticobiliary-like features being a negative prognostic factor (p=0.001). Importantly, patients whose tumors have pancreaticobiliary histology appear to benefit from adjuvant therapy, further implicating histology as an important pathologic marker (p=0.053). In addition, the TMA confirmed a

correlation between classical immunohistochemical profiles for intestinal and pancreaticobiliary tumors and histologic classification. My research findings suggest that histology subtypes, T-stage, nodal metastases, and lymphovascular invasion should all be taken into consideration when determining which patients with ampullary cancer may benefit from further adjuvant therapy.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1

INTRODUCTION

Ampullary cancer. The ampulla of Vater is located at the junction of the main pancreatic duct and the distal common bile duct within the head of the pancreas. The ampulla opens into the duodenum and thus cancers arising in the ampulla of Vater are composed of three different types of epithelium: pancreas, biliary, and small intestine [1]. Ampullary cancers make up 0.5% of all gastrointestinal malignancies with 5,625 cases reported in the Surveillance, Epidemiology, and End Results (SEER) database between 1973 and 2005. There has been a rise in the incidence of ampullary cancer over the past twenty-five years and the disease afflicts men more than women. The five-year overall survival rate for localized disease is 45% compared to 4% for patients with metastatic disease [2]. The cancer-specific five-year survival for resected ampullary cancer is 47.3 months [1]. Ampullary cancer has a better prognosis than distal common bile duct tumors and pancreatic cancer. This could be due to the fact that patients present at an earlier stage with obstructive jaundice, but data also points to ampullary cancer having a different biology than pancreaticobiliary cancers. The curative surgery for localized ampullary cancer is the Whipple resection, or pancreaticoduodenectomy. However, even with surgery, patients whose tumors have certain pathologic features are at a high risk of recurrence of disease.

Risk factors for the development of ampullary carcinoma. The precise risk factors for ampullary cancer are not clearly identified; however there is an association between this disease and Familial Adenomatous Polyposis (FAP). FAP is a genetic syndrome, caused by an inherited mutation in the *APC* gene, in which patients develop hundreds to thousands of polyps throughout the bowel. Colon cancer is most commonly associated with this mutation, but the risk of developing ampullary cancer with FAP is increased by 100-200% with a prevalence of 3-12% [3]. Accordingly, routine endoscopic evaluation of the duodenum and ampulla of Vater is recommended in patients with FAP. The colon cancer that develops in those with FAP involves the classic progression of adenoma to carcinoma, and thus it is thought that this same progression may be of pivotal importance in ampullary carcinogenesis as well [4]. Adenomas have been identified in as many as 90% of surgical specimens of ampullary cancer, and it has been demonstrated that over half of adenoma specimens may include areas of invasive carcinoma [5,6].

Intestinal versus pancreaticobiliary histology. Ampullary cancer has more recently been identified as being a heterogeneous disease with the histologic patterns of each tumor varying between a spectrum of intestinal and pancreaticobiliary differentiation [7,8,9,10]. This spectrum was first described by Kimura and colleagues who analyzed the histologic features of 53 ampullary cancer specimens and found that pancreaticobiliary-type tumors had more frequent nodal metastases and a worse overall outcome [9]. Since then, further

immunohistochemical characterization has been suggested based on a variety of markers, including: CK7, CK20, CK17, CDX2, and MUC1 [7,8,10].

Prognostic factors and the definition of "high risk" subpopulations. A number of prognostic factors have been described in both localized and advanced ampullary cancer. The revised American Joint Committee on Cancer (AJCC) staging of ampullary cancer recognizes the importance of tumor invasion into the surrounding pancreas (T3 and T4 lesions) and lymph node metastases (N1) as two relevant prognostic factors in ampullary carcinoma [11]. For example, a single institution study of 127 patients with ampullary cancer demonstrated a better median survival in patients with Tis-T2 tumors compared to those with invasion into the pancreas (60 months versus 26 months, p=0.025) [12]. Furthermore, a review of 1301 cases of resected ampullary cancer in the SEER database showed that the 5-year cancer-specific survivals for patients without and with lymph node metastases were 59.4% and 28.4%, respectively, highlighting the importance of lymph node status [13]. Other prognostic factors reported in the literature include poorly differentiated tumors, positive margins at the time of resection, and lymphovascular invasion [12,13,14,15,16]. More recently, the histology classifications of pancreaticobiliary versus intestinal have been suggested as prognostic, with ampullary cancers falling into the pancreaticobiliary subtype demonstrating a worse overall survival [14,17].

In patients with resected ampullary cancer, risk of recurrence correlates with the presence of these previously mentioned adverse factors. These prognostic

variables help define a “high risk” group in whom further adjuvant chemotherapy and/or chemoradiation may provide an absolute benefit. A number of different prognostic factors have been incorporated in this definition, but most retrospective studies include T3/T4 tumors, positive lymph nodes, poorly differentiated histology, lymphovascular invasion, and positive resection margins as indicators of patients at “high risk” for recurrence [18,19,20].

Approach to adjuvant therapy in localized disease. Adjuvant therapy in resected ampullary carcinoma is typically reserved for patients at “high risk” for relapse, but the actual role of chemotherapy versus chemoradiation remains largely undefined. Most studies addressing this question are single-institution, retrospective analyses, thereby limiting the ability to draw definitive conclusions. Radiation therapy has played a role in adjuvant therapy because of the historically high rate of locoregional recurrence [21,22]. Adjuvant radiation has historically been reserved for patients whose tumors have poor prognostic features including T stage, nodal metastases, and positive margins of resection [18,20,23,24]. Sytemic 5-fluorouracil or capecitabine are typically given with radiation. However, as the R0 resection rate after pancreaticoduodenectomy has improved, the rate of distant relapse has remained a continued problem, suggesting the need for more effective systemic chemotherapy either alone or in conjunction with chemoradiation [16,25].
While 5-fluorouracil-based chemoradiation has been widely accepted for adjuvant therapy, the precise chemotherapies that provide maximum benefit remain unclear. Historically, ampullary cancers have been grouped under biliary tract cancers,

making gemcitabine the standard backbone of chemotherapy in advanced disease. However, a phase II study at MD Anderson Cancer Center (MDACC) of capecitabine and oxaliplatin in advanced small bowel and ampullary cancers demonstrated an improved response rate and overall survival when compared to historical controls [26]. Interestingly, the response rate in ampullary cancers was lower than that in small bowel cancers (33% versus 61%, respectively). Our group at MDACC hypothesized that this may have been due to differences in tumor histology among the ampullary cancer patients, with those tumors not responding to a 5-fluorouracil-based regimen possibly having a pancreaticobiliary subtype. We analyzed the role of chemotherapy in the adjuvant setting in this retrospective review, but did not see a survival improvement when chemotherapy was added to chemoradiation. The true benefit of chemotherapy may be magnified, however, when patients are stratified by histology.

Ampullary cancer is treated with surgical resection followed by adjuvant therapy in patients who are at “high risk” for recurrence. The precise pathologic markers that define “high risk” have been debated in the literature. Furthermore, ampullary cancers with pancreaticobiliary histology have a worse prognosis, but this factor has not been routinely included in the typical “high risk” definition. In addition, it remains unclear what benefit adjuvant therapy offers patients who have these “high risk” features, specifically because the components of what should constitute adjuvant therapy is controversial.

Summary. Ampullary cancer is a rare malignancy for which the role of adjuvant therapy remains unclear. As more is understood about the classification of these tumors into intestinal and pancreaticobiliary subtypes, it is possible that the benefit of adjuvant therapy may depend not only on the presence of specific prognostic features, but also on histology.

For my Master's research project, I performed a retrospective analysis of 176 patients with resected ampullary cancer who were treated at MDACC between 1990 and 2009 to determine which tumor factors impacted upon overall survival and possibly predict for the benefit of adjuvant therapy. My research confirmed that T3/T4, nodal metastases, lymphovascular invasion, and pancreaticobiliary histology were associated with a worse overall survival. Furthermore, in a multivariate analysis of the "high risk" patients, I found a survival benefit seen with adjuvant therapy. We also demonstrated a possible benefit to adjuvant therapy in the pancreaticobiliary subtype of ampullary cancer, but this needs to be confirmed in a larger patient population.

Chapter 2

METHODS

Retrospective chart review:

This retrospective chart review was approved by the Institutional Review Board at MDACC. The MDACC tumor registry was queried for patients seen at our institution from 1990 to 2009 with a diagnosis of ampullary cancer. From this group, we collected further clinical information only on patients who specifically had a resectable ampullary cancer and who underwent a pancreaticoduodenectomy at MDACC or at an outside institution. This retrospective review included 176 cases of resected ampullary cancer after excluding patients who received neoadjuvant therapy.

Basic demographic information was collected on all 176 patients including age and gender. Additionally, the date of surgery and any chemotherapy or chemoradiation given in the adjuvant setting were recorded. Surgical pathology features were noted, including: T stage by AJCC staging, lymph node metastases present and the lymph node ratio (number of positive lymph nodes divided by the total number of lymph nodes sampled), presence of adenoma, histologic differentiation (well, moderately, poorly), positive or negative margin of resection, and lymphovascular invasion. Based upon previous literature, the patients were stratified as “high risk” of recurrence if they had T3/T4 tumors, nodal metastases, poorly differentiated

histology, lymphovascular invasion, or positive surgical margins [11,12,13,14,15,16].

Preparation of tissue microarray of resected ampullary cancers:

We collected available tissue specimens on 95 of the 176 patients who underwent pancreaticoduodenectomy at MDACC between 1995 and 2009. None of these patients had received neoadjuvant chemotherapy and/or radiation. These 95 tissue specimens were examined by our pathologist collaborating on this study (Dr. Huamin Wang) and categorized as either pancreaticobiliary, intestinal, or mixed histology.

To construct the ampullary tissue microarray used in this study, formalin-fixed, paraffin-embedded archival tissue blocks and their matching hematoxylin and eosin-stained (H & E) slides were retrieved, reviewed and screened for representative tumor regions by our pathologist (Dr. Wang). For each patient, two cores of tumor were sampled from the tissue blocks from representative areas using a 1.0-mm punch. Under the direction of Dr. Wang, the tissue microarray was constructed with a tissue microarrayer (Beecher Instruments, Sun Prairie, WI) as described previously [27].

Histology and immunohistochemistry analysis of tissue microarray:

In Dr. Wang's lab, the tissue microarray (TMA) prepared for each tumor was stained for two cytokeratin markers, CK7 and CK20, which typically represent a pancreaticobiliary and intestinal histology, respectively. The TMA also tested for

staining of caudal type homeobox 2 (CDX2) which has previously been described as a pathologic prognostic marker in ampullary cancer and has been hypothesized to be more indicative of an intestinal-type tumor in this disease [8].

The methodology for the TMA has been detailed in a prior manuscript [28]. To summarize, immunohistochemical stains were performed on 5 μm unstained sections from the tissue microarray blocks using three antibodies listed in Table 1. To retrieve the antigenicity, the tissue sections were treated at 100 °C in a steamer containing 10 mmol citrate buffer (pH 6.0) for 60 min. The sections were then immersed in methanol containing 0.3% hydrogen peroxidase for 20 min to block the endogenous peroxidase activity and were incubated in 2.5% blocking serum to reduce nonspecific binding. Sections were incubated for 90 min at 37 °C with primary antibodies at the dilutions specified in Table 1. Standard avidin–biotin immunohistochemical analysis of the sections was performed according to the manufacturer's recommendations (Vector Laboratories, Burlingame, CA, USA). Diaminobenzidine tetrahydrochloride was used as a chromogen, and haematoxylin was used for counterstaining.

Table 1. Antibodies used for tissue microarray analysis

Antibody	Clone	Titre	Company
Anti-CDX2	CDX-88	1:50	Biogenex, San Ramon, CA, USA
Anti-CK7	OVT-TL 12/30	1:100	Dako, Carpinteria, CA, USA
Anti-CK20	KS20.8	1:4000	Dako, Carpinteria, CA, USA

Statistical Analysis:

Summary statistics were used to describe the clinical and demographic characteristics of the study population. We used Pearson chi-square test (or Fisher's exact test) to assess differences between patients with different histologies and immunohistochemical markers.

For overall survival, the time to death or censoring was calculated in months since date of surgery for each patient. Overall survival was censored at the date of last follow-up if death was not observed. For recurrence-free survival, the time to recurrence was calculated in months since date of surgery for each patient. Recurrence-free survival was censored at the date of last follow-up if recurrence or death was not observed. Univariate Cox proportional hazards regression was used to model the association between each potential prognostic factor and overall survival and recurrence-free survival [29]. A p-value of <0.05 was determined to be statistically significant. The Kaplan-Meier product limit method was used to estimate the median overall survival and relapse-free survival [30].

The multivariate proportional hazard model for overall survival showed a full model with all the variables that were significant in the univariate analysis and a reduced model after selecting variables through backwards selection methods. The histology variables of intestinal/mixed and pancreaticobiliary were each further analyzed using univariate Cox proportional hazards regression to model an association between histology and any adjuvant therapy.

Statistical analysis was performed using STATA/SE version 12.0 statistical software (Stata Corp. LP, College Station, TX).

Chapter 3

RESULTS

Clinical and pathologic characteristics of patients

Table 2 summarizes the clinical and pathologic characteristics of the patients. Of the 176 patients reviewed, the mean age of diagnosis was 60.6 years. With regard to “high risk” tumor features, 52.8% of patients had nodal metastases, 43.8% of patients had T3/T4 tumors, 35.3% had tumors with poorly differentiated histology, 39.2% had tumors with lymphovascular invasion, and 4% had tumors with positive margins of resection. While 69.3% of patients were deemed “high risk” after resection (n=122), only 50% of all patients received adjuvant chemotherapy, radiation therapy or chemotherapy plus radiation (n=88). The remainder entered surveillance after surgery. Among all patients in this series, radiation therapy was given to 40.9% of patients and only 30.1% of patients received systemic chemotherapy either alone or in conjunction with radiation.

Table 2. Clinical/pathologic characteristics in 176 patients with ampullary cancer

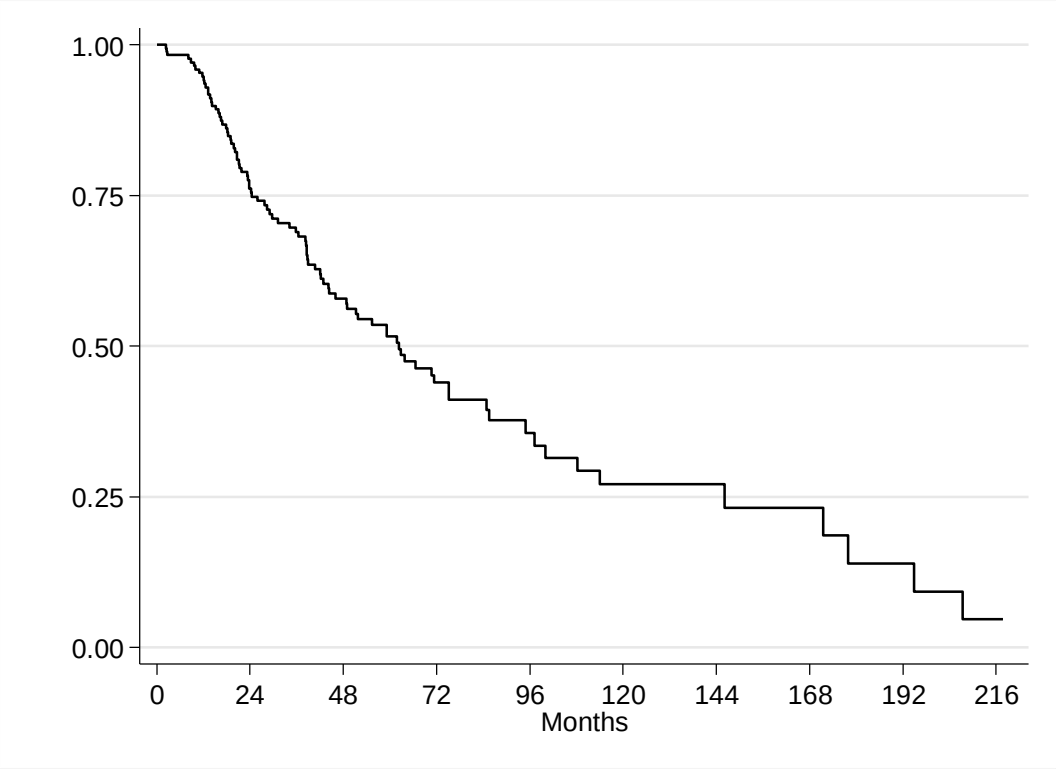
Age at diagnosis		
N	176	
Mean (SD)	60.6 (12.3)	
	N	**%**
Pathologic Stage		
1	1	0.7
2	58	39.2
3	89	60.1
Pathologic T stage		
T0 - T2	99	56.3
T3 - T4	77	43.8
Grade		
Unknown	2	1.2
Well	17	9.8
Moderate	93	53.8
Poor	61	35.3
Lymphovascular Invasion (LVI)		
No	107	60.8
Yes	69	39.2
Lymph Node (LN) Involvement		
No	83	47.2
Yes	93	52.8
LN Ratio Groups		
0	83	47.4
0-0.2	50	28.6
>0.2	42	24.0
Surgical Margins		
Negative	169	96.0
Positive	7	4.0
High Risk		
No	54	30.7
Yes	122	69.3
Adjuvant		
No	88	50.0
Yes	88	50.0
Systemic Chemotherapy		
None	123	69.9
5-fluorouracil	38	21.6
Gemcitabine	12	6.8
5-fluorouracil+Gemcitabine	3	1.7
	N	**%**

Adjuvant Treatment		
None	88	50.0
Chemotherapy Only	16	9.1
Chemotherapy + Radiation	37	21.0
Radiation only	35	19.9
Recurrence		
No	90	51.4
Yes	85	48.6
Vital Status		
Alive	86	48.9
Dead	90	51.1
Recurrence		
Early ≤12months	64	36.4
Late >12months	112	63.6
First Recurrence		
None	88	50.0
Local Recurrence	15	8.5
Metastasis	71	40.3
Unknown	2	1.1

Survival analysis

Median follow-up for all 176 patients was 38.1 months. The median overall survival of the entire group was 62.3 months (Figure 1). As summarized in Table 2, 48.9% of patients were still alive at the time of analysis, and importantly, of the 48.6% of patients who had recurrent disease, close to two-thirds of them were late recurrences (> 12 months after surgery). Recurrent disease was primarily metastatic disease rather than locoregional recurrence.

Figure 1. Overall survival of 176 patients with resected ampullary cancer



"High risk" features correlate with overall survival and recurrence

122 of the patients in this analysis were categorized as "high risk" based on the previously described pathologic characteristics. Tables 3 and 4 summarize the impact of these "high risk" features on overall survival and relapse-free survival (RFS), respectively, by univariate analysis.

Table 3. Univariate proportional hazards regression model - Overall survival

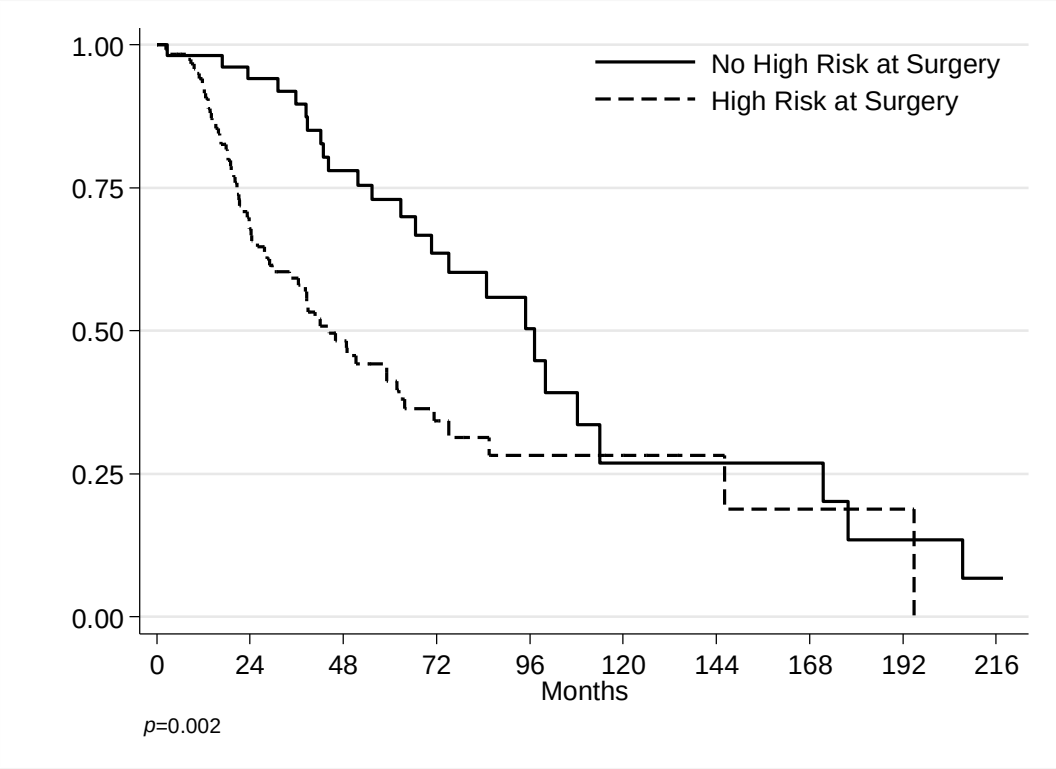
	N	No. of deaths	Median time (months)	Hazard Ratio (HR)	95% CI for HR	HR p-value	Log-rank p-value
T Stage							
T0-T2	99	49	84.80				
T3/T4	77	41	38.64	2.17	(1.40, 3.36)	0.001	<0.001
Grade							
Well	17	13	66.60				
Moderate	93	44	62.75	0.75	(0.40, 1.41)	0.379	0.394
Poor	61	32	38.93	1.01	(0.53, 1.95)	0.971	
LVI							
No	107	52	84.80				
Yes	69	38	42.09	2.15	(1.38, 3.34)	0.001	0.001
LN Positive							
No	83	41	84.80				
Yes	93	49	45.93	1.54	(1.01, 2.34)	0.046	0.044
LN Ratio							
0	83	41	84.80				
0-0.2	50	19	75.10	0.91	(0.53, 1.58)	0.739	<0.001
>0.2	42	29	24.41	2.82	(1.73, 4.61)	<0.001	
Surgical Margins							
Negative	169	85	62.75				
Positive	7	5	21.85	2.13	(0.86, 5.28)	0.102	0.094
High Risk							
No	54	25	97.15				
Yes	122	65	44.42	2.11	(1.31, 3.41)	0.002	0.002

Table 4. Univariate proportional hazards regression model – RFS

	N	No. of events	Median time (months)	Hazard Ratio (HR)	95% CI for HR	HR p-value	Log-rank p-value
T Stage							
T0/T2	99	59	40.71				
T3/T4	77	51	18.20	1.70	(1.16, 2.49)	0.007	0.006
Grade							
Well	17	15	16.82				
Moderate	93	52	41.79	0.58	(0.33, 1.03)	0.064	0.034
Poor	61	41	20.01	0.93	(0.52, 1.69)	0.823	
LVI							
No	107	63	37.68				
Yes	69	47	20.01	1.66	(1.13, 2.45)	0.010	0.009
LN Positive							
No	83	49	36.53				
Yes	93	61	23.98	1.34	(0.92, 1.95)	0.129	0.127
LN Ratio							
0	83	49	36.53				
0-0.2	50	22	45.08	0.72	(0.44, 1.20)	0.205	<0.001
>0.2	42	38	11.07	2.72	(1.77, 4.19)	<0.001	
Surgical Margins							
Negative	169	105	29.27				
Positive	7	5	13.31	1.95	(0.79, 4.81)	0.148	0.140
High Risk							
No	54	29	54.51				
Yes	122	81	20.67	1.81	(1.18, 2.77)	0.007	0.006

Patients who were defined as “high risk” had a significantly decreased overall survival (p=0.002, Figure 2) and relapse-free survival (p=0.006) compared to those patients who did not have advanced T-stage, nodal metastases, poorly differentiated histology, lymphovascular invasion, or positive surgical margins.

Figure 2. Patients with "high risk" features have a worse overall survival



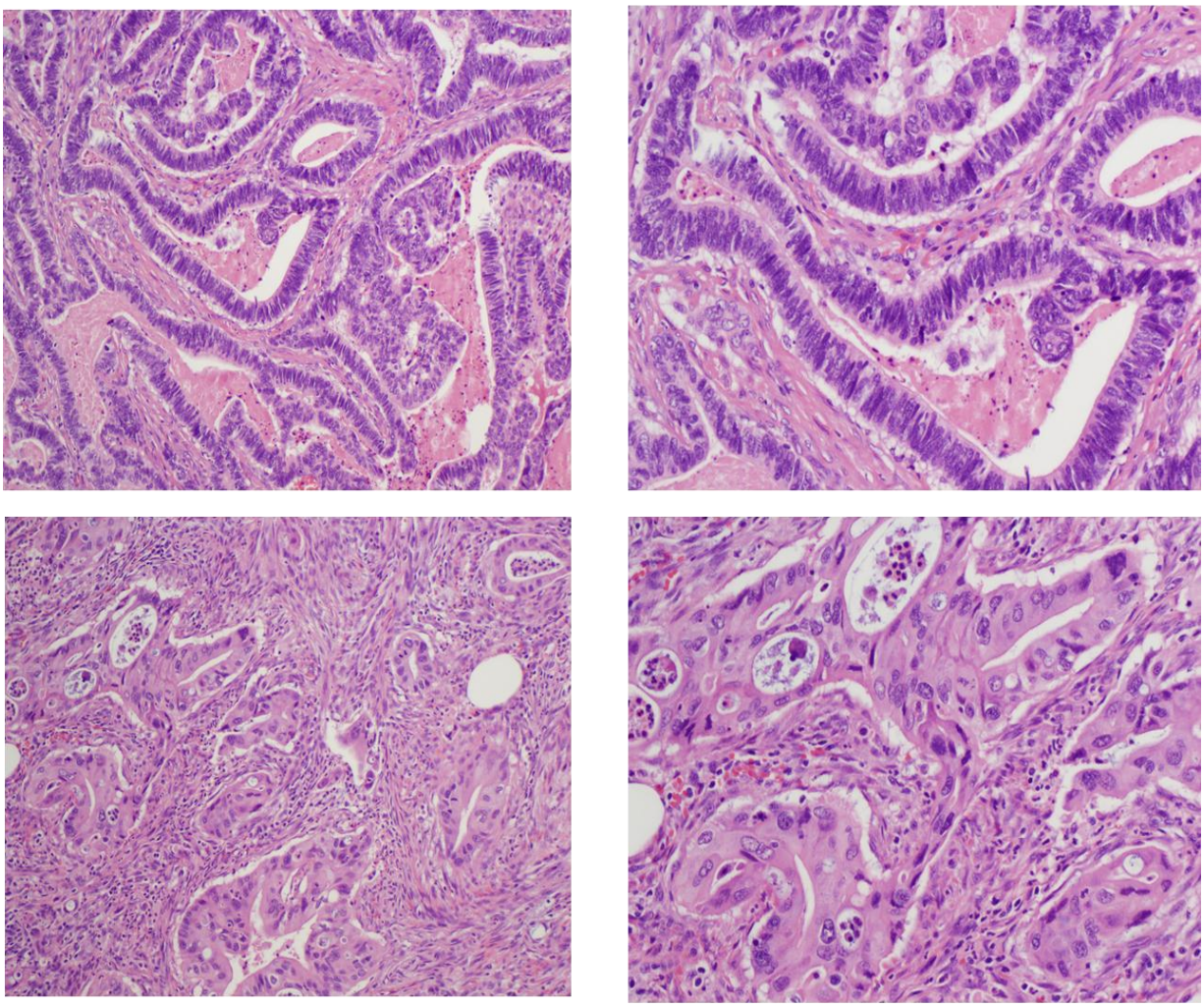
The most notable predictors for survival and recurrence included T stage, lymph node ratio, and lymphovascular invasion. Patients with T3 or T4 tumors had a median overall survival of 38.6 months versus 84.8 months for patients with T0-T2 lesions (HR 2.17, p<0.001). Lymph node ratio has been previously described by our group at MDACC as being a better predictor of survival in small bowel adenocarcinoma than the total number of positive lymph nodes [31]. Similarly, in our cohort of patients, lymph node ratio was strongly correlated with survival and recurrence. In patients with a lymph node ratio of >0.2, median overall survival was only 24.4 months compared to 84.8 months in patients with no positive lymph nodes (HR 2.82, p<0.001). Additionally, lymphovascular invasion increased risk of death and relapse, with a HR of 2.15 for overall survival (p=0.001).

Interestingly, poorly differentiated histology and positive surgical margins did not significantly impact survival or recurrence, although the lack of impact of a positive surgical margin may be reflective of the small number of patients with this (n=7). It is important to note, however, that these results are different than what has been previously reported in the literature.

Intestinal histology predicts for improved overall survival

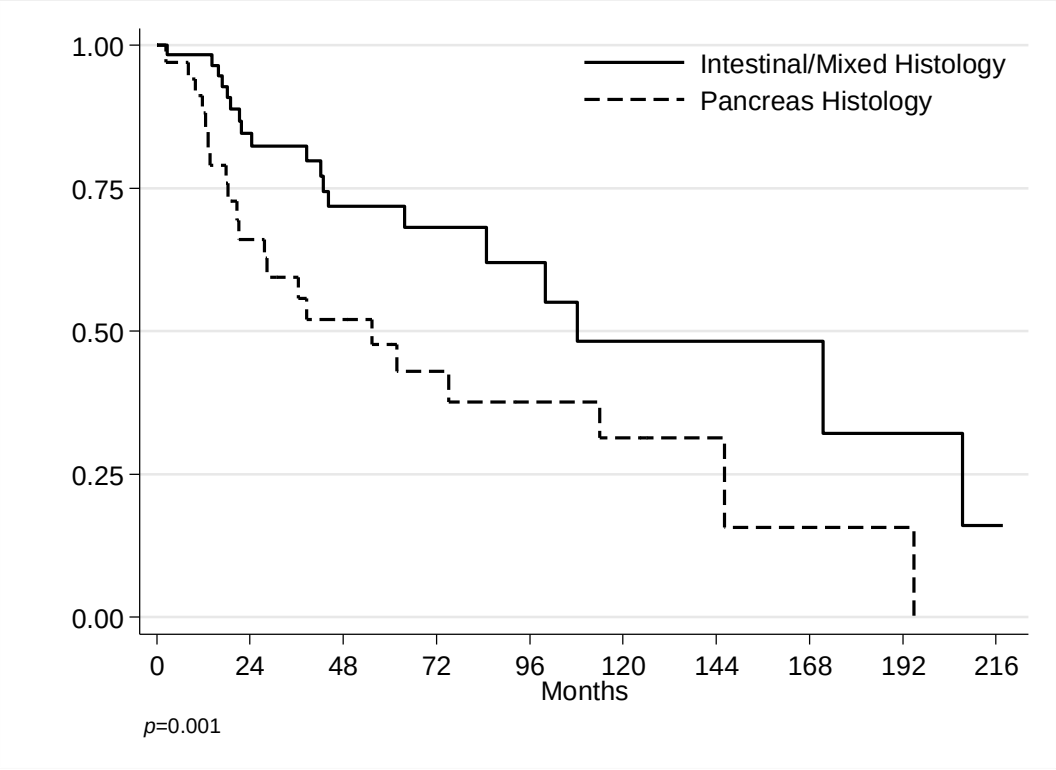
Representative slides of intestinal/mixed and pancreaticobiliary histologies are shown in Figure 3. Univariate analysis demonstrated that patients with an intestinal or mixed histology (n=60) had a better overall survival compared to patients with a pancreaticobiliary-like tumor (n=34) (Figure 4; 108.3 months versus 55.4 months, HR 2.23, p=0.001). Our findings validate previous reports, but in a larger set of patients.

Figure 3. Histologic subtypes



Clockwise from top left: Intestinal histology, 100x; Intestinal histology, 200x; Pancreaticobiliary histology, 100x; Pancreaticobiliary, 200x

Figure 4. Intestinal/mixed histology has a better overall survival than pancreaticobiliary



Relapse-free survival was also improved in the intestinal histology group, but this difference did not meet statistical significance (data not shown).

Multivariate analysis confirmed the prognostic value of histology as well. Patients with a pancreaticobiliary histology had a hazard ratio of 3.37 for death relative to intestinal-like tumors (p=0.012). This stratification was equally striking in terms of risk of recurrence with a pancreaticobiliary histology compared to an intestinal one (HR 8.87, p=0.003).

Multivariate analysis of "high risk" patients demonstrates benefit to adjuvant therapy

To better assess the benefit of adjuvant therapy in our "high risk" population of patients, we performed a multivariate analysis involving all of the prognostic variables that were significant by univariate analysis. In the 122 "high risk" patients, there is a survival benefit to adjuvant therapy, but this did not reach statistical significance (HR 0.39, p=0.071). A similar trend was seen for relapse-free survival in this same cohort (HR 0.37, p=0.06). When looking at the entire population of 176 patients, there was also a trend towards a benefit in overall survival with adjuvant therapy, but interestingly, this was in patients who received adjuvant chemotherapy or chemoradiation therapy alone (n=51; HR 0.34, p=0.059). Patients receiving chemotherapy followed by chemoradiation in the adjuvant setting did not demonstrate an improvement in overall survival (n=37; HR 0.52, p=0.351).

Tissue microarray analysis

The ampullary tissue microarray included tumor tissue from 95 of the 176 resected ampullary patients. We correlated patients' histology subtypes with the following immunohistochemical markers: CK7, CK20, CDX2. Our findings are summarized in Table 5. While the percentage of surgical specimens that was CK7 positive were relatively equal, only 5.7% of the tumors with a pancreaticobiliary histology were CK20 positive, compared to 51.7% in the intestinal/mixed tumors (p<0.001). Tumors that were CK7 positive and CK20 negative, the classic immunohistochemical profile for upper gastrointestinal tract cancers, were seen more prominently in specimens with a pancreaticobiliary histology (p=0.011). In addition, CDX2 positivity, typically

seen in intestinal cancers, was seen significantly more frequently in the intestinal/mixed histology tumors (p=0.028). Importantly, though these immunohistochemical markers correlated with histology, they were not independently associated with survival or recurrence by univariate analysis.

Table 5. Tissue microarray analysis

	All Patients		TMA Histology				p-value
			Intestinal/Mixed		Pancreas		
	N	%	N	%	N	%	
TMA CK7							0.782[a]
Negative	26	27.4	17	28.3	9	25.7	
Positive	69	72.6	43	71.7	26	74.3	
TMA CK20							<0.001[b]
Negative	62	65.3	29	48.3	33	94.3	
Positive	33	34.7	31	51.7	2	5.7	
TMA CK7+ CK20-							0.011[a]
Negative	46	48.4	35	58.3	11	31.4	
Positive	49	51.6	25	41.7	24	68.6	
TMA CDX2							0.028[a]
Negative	54	56.8	29	48.3	25	71.4	
Positive	41	43.2	31	51.7	10	28.6	

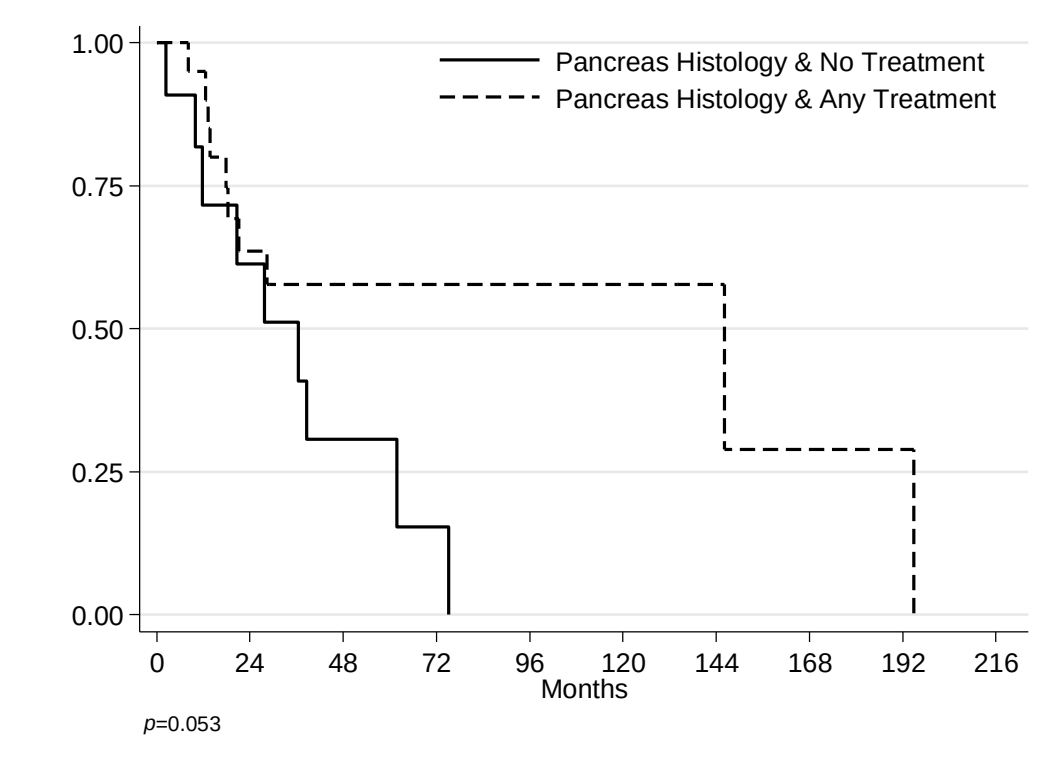
[a] Pearson chi-square
[b] Fisher's exact test

Pancreaticobiliary histology may benefit from adjuvant therapy

To assess if there were a correlation between adjuvant therapy and histology, we looked at the tumors of 95 patients with pancreaticobiliary and intestinal histologies and stratified them by treatment. These univariate analyses included a small number of patients, but there was a suggestion that adjuvant therapy may benefit patients with pancreaticobiliary histology. Figure 4 demonstrates the overall survival curves for patients with pancreaticobiliary histology who received any form of adjuvant therapy versus those who entered surveillance after surgery (n=20

patients who received adjuvant therapy and 14 who did not; p=0.053). Patients with an intestinal/mixed tumor did not see this same overall survival benefit (n=23 patients who received adjuvant therapy and 37 who did not; p=0.535). These findings have not been previously reported in the literature, and are clinically relevant as they suggest that all patients with pancreaticobiliary-like tumors should receive further therapy after surgery.

Figure 5. Pancreaticobiliary histology may benefit from adjuvant therapy



Chapter 4

DISCUSSION

Ampullary cancer is a rare malignancy of the gastrointestinal tract, and thus, there is little data on the role of adjuvant therapy (chemotherapy, radiation therapy or both). Retrospective analyses have identified a number of different prognostic factors that help classify patients as “high risk” for recurrence. These include: T3/T4 tumors, lymph node involvement, poorly differentiated histology, lymphovascular invasion, and positive surgical margins of resection. In this analysis, I reviewed MDACC’s experience with resected ampullary cancer in 176 patients seen at our institution from 1990-2009. We investigated the validity of the prognostic pathologic features that have been reported previously in the literature and explored other potential pathologic markers that may be of added value in determining which patients may benefit from adjuvant therapy after surgical resection of their ampullary cancer.

My analysis represents one of the largest reports on resected ampullary cancer in the literature. I identified the “high risk” features of advanced T-stage (T3/T4 tumors), nodal metastases, and lymphovascular invasion as being significantly associated with reduced overall survival and relapse-free survival. Additionally, I demonstrated that those patients who were classified as “high-risk” of recurrence based on pathologic features not only have a worse overall survival but they also benefit from adjuvant therapy after surgical resection. As has been described in a number of other series, lymph node involvement was one of the strongest predictors for recurrence [12,32].

My research did not, however, validate poorly differentiated histology and positive surgical margins as important prognostic markers. This was a striking observation given the previous findings in the literature of poorly differentiated histology as a poor prognostic factor [14]. This finding may be due to grade being a confounding variable; those patients with poorly differentiated tumors could be more likely to have nodal metastases, for instance. It is further important to note that only 7 patients had positive surgical margins, making it difficult to accurately measure margin status as it relates to survival. In the combined retrospective study done by Johns Hopkins and Mayo Clinic, a similar lack of association was seen due to small numbers of positive surgical margins [16]. However, the steady improvement in the R0 resection rate for pancreaticoduodenectomies may diminish the impact of margin status and locoregional recurrences in ampullary cancer patients and increase the importance of effective chemotherapy.

Interestingly, in our series, the majority of patients relapsed with distant metastases, suggesting a need for the use of adjuvant systemic therapy or better chemotherapy. This pattern of systemic rather than locoregional recurrence has been reported in other studies although the frequent use of chemoradiation in “high risk” patients may have decreased local recurrence rates [16,33,34]. Interestingly, chemotherapy followed by chemoradiation in our study did not show a survival benefit when compared to chemoradiation alone. This may stem from the fact that the patients who were selected to receive chemotherapy followed by chemoradiation had worse prognostic features after surgery, and thus received more aggressive adjuvant

therapy. The exact role of chemotherapy as an adjunct to chemoradiation remains unclear, but the high rate of distant failure in our study implies that ampullary cancer is a systemic disease for which better chemotherapy options are needed.

Using a TMA, my research, in collaboration with Dr. Wang's lab, further identified a correlation between histology subtype and overall survival with pancreaticobiliary-like tumors having a worse overall survival than intestinal/mixed tumors. This concurs with the findings reported in other studies [14,35]. In contrast, some other reports have implied that these differences in survival are not as striking [36,37]. Importantly, these studies did demonstrate that patients with pancreaticobiliary-like tumors have a worse prognosis, but these differences did not reach statistical significance.

My results further showed that patients with the pancreaticobiliary subtype benefit from adjuvant therapy, though the small sample size precludes our ability to make definitive recommendations. This finding has not been previously reported by other groups, and is clinically relevant in our approach to resected ampullary patients. It merits further investigation in a larger set of patients. Tissue microarray analysis also showed that the histology classifications correspond to the typical immunohistochemical profiles seen for pancreaticobiliary and intestinal tumors. It is important to note, however, that the immunohistochemical markers did not correlate with survival, implying that histology is a more useful prognostic indicator for this disease. Taken together, our results demonstrate the role of histologic classification

in identifying those patients who are at a higher risk for recurrence and who may derive the greatest benefit from adjuvant therapy after surgical resection.

My retrospective review of MDACC's experience with surgically resected ampullary cancer confirms the prognostic value of specific pathologic features in this disease. As has been previously reported in the literature, surgical pathology and histology should be evaluated to determine which patients may benefit from adjuvant therapy. Our group did not validate the previously reported prognostic value of poorly differentiated histology or surgical margins. We did confirm advanced T-stage, nodal metastases, and lymphovascular invasion as relevant prognostic markers. We also demonstrated the importance of histology subtypes over immunohistochemical profiling when identifying "high risk" patients and highlighted the benefit of adjuvant therapy in patients with pancreaticobiliary-like tumors.

Our study does not clarify the question of whether chemotherapy and chemoradiation should both be given to decrease local recurrence and distant failure rates. This limitation is primarily due to the small sample size and the retrospective nature of this analysis. Further studies are needed to ascertain what components of adjuvant therapy improve overall survival in patients who are "high risk" for recurrence and to validate the role of histology in defining a "high risk" population.

Chapter 5

STRENGTHS AND WEAKNESSES

This retrospective analysis has clinical relevance for ampullary cancer because it is a disease for which prospective data is very limited. My study included a relatively large sample size in comparison to previous reports in the literature. The population I examined was homogeneous in that the ampullary cancers had been resected and the patients had received no prior neoadjuvant therapy. However, as with all retrospective analyses, this study has some limitations.

MDACC is a referral center for rare malignancies such as ampullary cancer. There could have been a bias in the patient population that was analyzed in that close to 70% of the patients were “high risk”, and this may not be reflective of the general population of resected ampullary cancer patients. Additionally, in my analysis of the benefit of adjuvant therapy in the “high risk” cohort, we included all pathologic features that were described in the literature as poor prognostic factors. However, in our final analysis, poorly differentiated histology and positive margins were not negative prognostic factors, and thus our assessment of the benefit of adjuvant therapy may have been skewed. Furthermore, given that all patients who receive adjuvant therapy are “high risk”, there is an inherent bias in prognosis that may have minimized the survival benefit of adjuvant therapy. In addition, this issue may have been more pronounced in patients who received chemotherapy followed by chemoradiation, as these patients may have been deemed especially “high risk”

based on the presence of multiple poor prognostic factors, thereby influencing the benefit of adding chemotherapy to chemoradiation.

While the multivariate analysis attempted to mitigate this inherent bias, it is difficult to include all the factors that play into a physician's decision tree when assessing the need for adjuvant therapy. For instance, data was lacking for performance status after surgery which can influence the decision to give adjuvant therapy and the type of treatment offered. Any delays in administering adjuvant therapy cannot be accounted for in this analysis as well.

My results indicate that histologic subtypes correlate with overall survival, with pancreaticobiliary-like tumors having a worse prognosis than intestinal or mixed histology. This pathologic classification is subject to inter-reader variation, making it difficult to standardize among pathologists. Lymphovascular invasion and tumor grade can also suffer from a similar bias [38,39]. This variability may have influenced which patients were labeled as "high risk" in this study. In future studies, if a "high risk" population is identified based on these pathologic criteria, it will be essential to standardize these assessments to ensure that the appropriate patients are captured.

Finally, our finding that pancreaticobiliary histology may benefit from adjuvant therapy is based on an exploratory analysis of a small number of patients. As a result, this correlation is hypothesis- generating and requires a more robust

evaluation with a larger sample size. Our findings do suggest, however, that histology may be a more useful prognostic pathologic marker than immunohistochemistry. Furthermore, elaborating on what constitutes adjuvant therapy will be essential to truly understanding the magnitude of benefit in this subpopulation of ampullary cancer patients.

Chapter 6

FUTURE DIRECTIONS

This retrospective analysis highlights the importance of pathological markers of prognosis in ampullary cancer. Future studies in our group at MDACC will be directed at confirming the relevance of these markers with regard to adjuvant therapy and survival and understanding the differences in biology between pancreaticobiliary-like tumors and intestinal ones. Our future research will attempt to identify those patients who would benefit most from adjuvant therapy and what types of therapies should make up adjuvant treatment.

My study results indicate that pancreaticobiliary subtypes of ampullary cancer benefit from adjuvant therapy. Our group's next step in this project will be to collaborate with other institutions to obtain a larger dataset of resected ampullary patients. By doing so, we hope to validate histology as a new "high risk" factor. Additionally, we will study whether we can stratify patients by histology subtype and chemotherapy received to assess if gemcitabine-based therapy is more beneficial in pancreaticobiliary-like ampullary tumors while 5-fluorouracil-baased chemotherapy should be reserved for intestinal or mixed histology tumors. Furthermore, we hope to better understand which factors may predict for local recurrences versus distant failures, so that we can tailor the appropriate adjuvant therapy regimen to each "high risk" patient.

The current study highlights the importance of pathological markers of prognosis in ampullary cancer. Future studies are needed to better delineate what specific markers should be assessed regularly in resected ampullary cancer patients. Additionally, clarifying what exactly should constitute adjuvant therapy will be of vital importance in improving outcomes in ampullary cancer.

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VITA

Rachna T. Shroff, MD was born in New York, NY on May 8, 1979 to Deven and Amita Trivedi. She moved to Tucson, AZ at a very young age and completed high school at St. Gregory College Preparatory in 1996. She then entered Brown University in Providence, RI where she received a Bachelor of Science with honors in Biochemistry in 2000. She then moved to Philadelphia, PA where she completed her medical doctorate degree in 2004. Dr. Shroff completed her internal medicine residency and chief residency at Washington University in St. Louis, MO from 2004-2007 and her medical oncology fellowship at M. D. Anderson Cancer Center in Houston, Texas from 2007-2010. She joined the Gastrointestinal Medical Oncology department as an Assistant Professor in May 2010 where she has a clinical and research focus in pancreatic and biliary cancers. In 2008, Dr. Shroff entered the University of Texas Health Science Center at the Houston Graduate School of Biomedical Sciences.