Emerging molecular biomarkers and treatment strategies in resectable pancreatic cancer

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Printed by GVO drukkers & vormgevers, Ede

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Proefschrift

ter verkrijging van
de graad van doctor aan de Universiteit Leiden,
op gezag van rector magnificus prof.dr.ir.H. Bijl,
volgens besluit van het college voor promoties
te verdedigen op dinsdag 9 maart 2021
klokke 13:45 uur

door

Susanna Willemina Leuntje de Geus

Promotor:	Prof. dr. C.J.H van de Velde
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Chapter 1

General introduction and thesis outline

INTRODUCTION

The last 25 years have witnessed monumental advancements in cancer care. Oncologic surgery has become safer and less invasive, more effective chemo- and immunotherapeutic agents have become available, and tremendous strides have been made in our understanding of the underlying tumor biology. In addition, several cancer screening programs have successfully been introduced and the resolution of cross linear imaging has increased significantly, allowing for earlier diagnosis. As a result, cancer-related death has decreased significantly across the board.[1] Unfortunately, for pancreatic cancer the pace of progress has lagged behind.[2] Current treatment strategies provide minor improvements in survival, with a 5-year survival of less than 9%.[3] Consequently, pancreatic cancer is projected to surpass colorectal cancer to become the second leading cause of cancer-related death by 2030.[1]

Complete surgical resection offers the only hope for long-term survival in patients with this dismal disease.[4-6] However, even among the fortunate to undergo curative-intent resection, recurrence rates remains high, indicating that there is a subgroup of patient who already harbor microscopic metastases at diagnosis.[7-12] These “resectable” patients with biologically aggressive disease may not benefit from upfront surgery. Prognostic markers may aid in the early identification of patients with rapidly progressing disease, sparing these patients potentially ineffective surgery, and guiding them towards alternative strategies, such as neoadjuvant therapy, that allow for early treatment of micro-metastatic disease.[10, 13-16] In addition, these biomarkers could be used to appropriately direct patient expectations, and inform shared decision making.[16] This is of critical importance consideration the high postoperative morbidity and mortality associated with pancreatic surgery.[17, 18] At present, preoperative radiographic studies or even careful intraoperative assessment have not yet been able to identify this subgroup of patients.[17] Therefore, biomarkers that are able to preoperatively identify patients with radiographically resectable disease, but unfavorable tumor biology, are in high demand.[10]

MOLECULAR BIOMARKERS

Strong evidence exists that the variation in the molecular pathology of otherwise indistinguishable cancers markedly impact the clinical characteristics of the disease.[19] Molecular subtypes currently guide clinical decision making for numerous malignancies.[19] Predicting the optimal treatment strategy ahead of treatment improves patient outcomes, minimizing treatment related morbidity and cost.[19] For example, in colorectal cancer KRAS sequencing and micro-satellite instability guide treatment plans.[16, 20-22] In breast cancer, hormone receptor status determines the necessity for endocrine therapy, while human epidermal growth factor receptor 2 (HER2) expression direct the use of trastuzumab.[16, 23, 24] In contrast to other cancers, molecular subtyping of pancreatic cancer is in its infancy and

clinically relevant molecular subtypes to guide clinical decision making have not yet been established.[19]

Over the past decades, knowledge of the molecular pathophysiology of pancreatic cancer has grown exponentially, rapidly surpassing our ability to translate these findings into clinical practice, widening the gap between scientific discovery and clinical utility.[19] Currently, cancer antigen 19-9 (CA 19-9) is the only serum markers for pancreatic cancer that has been approved by the Food and Drug Administration (FDA).[5] However, CA 19-9 has a relatively low sensitivity, is not expressed in Lewis negative patients, and is increased during cholestasis.[25] In addition, carcinoembryonic antigen (CEA) is routinely obtained at many institutions, but its use has not yet been recommended by the National Comprehensive Cancer Network (NCCN) guideline, due to lack of conclusive evidence.[26] Many large prognostic biomarkers studies, improved prognostic capabilities, have been performed in various cancer types, including prostate, colon, lung, and breast cancer. However, biomarker studies of comparable magnitude in pancreatic cancer patients are scarce, primarily due to the lack of tissue, as pancreatic cancer is relatively rare.[16]

IMMUNE MARKERS

The immune system represents a key player in the regulation of tumor growth and metastatic dissemination, and as such evading immune destruction has been incorporated in the hallmarks of cancer as one of the essential traits that enable cells to become tumorigenic and ultimately malignant.[27] The interplay between the immune system and tumor cells is complex, as the immune system has not solely halts tumor development and progression by attacking and destroying tumor cells, but under certain circumstances also may create favorable conditions for tumor growth.[28, 29] Since the introduction of immunotherapy, immune evasion has gained interest as the sources of resistance to single checkpoint immunotherapies and cancer vaccines.[30] Tumor cells can employ multiple strategies to avoid the host immune defense, including increasing resistance to apoptosis by expressing immunoregulatory molecules, secreting immunosuppressive cytokines, and avoiding recognition by the host immune cells.[30] Human leukocyte antigen (HLA) expression has shown to play a critical role in the ability of tumor cells to escape detection by immune surveillance.[30, 31] Consequently, HLA expression has been associated with survival in pancreatic cancer and may represent a promising biomarker. However, the sample size of previous studies was small and more robust data are necessary.[32-35]

ANGIOGENESIS MARKERS

Another hallmark that has been extensive studied in pancreatic cancer, as well as in other malignancies, is angiogenesis, the formation of new capillaries from pre-existing blood vessels to maintain appropriate oxygen and nutritional supply once the tumor exceeds

1-2 mm in size, which represents an important event in tumor growth and the formation of hematogenous metastasis.[36, 37] Compared to other types of cancer, pancreatic cancer is characterized by high microvascular density, impaired integrity of tumor capillaries, and excessive dense extracellular matrix deposition associated with vasculature collapse and poor perfusion.[38, 39] There is accumulating evidence to indicate that the vascularity of pancreatic cancer strongly contribute to the clinical features of this disease.[40] In addition, the extend of angiogenesis is heterogeneous between different subtypes of pancreatic cancer, making it a promising prognostic target.[38, 39] The prognostic value of angiogenesis inducing factors, like integrin's, and vascular endothelial growth factor (VEGF), has frequently been described for various gastrointestinal tumors, as well as for pancreatic cancer.[41-44] However, few of these studies have been independently validated and none have been adopted in clinical practice. Therefore, further assessment of their clinical relevance in pancreatic cancer is critical.

NEOADJUVANT THERAPY

Next to the appropriate selection of surgical candidates, multimodal therapy, including chemotherapy and radiation, plays a pivotal role in pancreatic cancer care. Especially, considering the high metastatic potential of this disease.[7-9, 45, 46] Traditionally, upfront surgery followed by adjuvant therapy was considered the standard of care for potentially resectable patients.[5, 47] Unfortunately, serious postoperative complications and early disease progression are common after seemingly successful pancreatic resection – the latter being particularly disheartening.[47-49] Consequently, a sizeable number of patients fail to receive adjuvant therapy (up to 50%), or experience substantial treatment delay.[14, 49-51] Neoadjuvant therapy has been proposed as a means to ensure the delivery of all components of multimodal therapy, and to allow for early treatment of micro-metastatic disease.[47-49, 52] In addition, contemporary combination chemotherapy, including FOLFIRINOX and gemcitabine/nab-paclitaxel, has shown to decrease tumor bulk and vessel involvement, thereby increasing resectability and negative resection rates.[53] Furthermore, neoadjuvant therapy allows patients with either rapidly progressive disease, or low "physiologic resilience," to declare themselves on restaging, and spare them the risks of a highly morbid operation.[13, 14]

CONSIDERATIONS

On the other hand, there are some important considerations connected to the use of neoadjuvant therapy for pancreatic cancer. Administration of neoadjuvant therapy often requires stent placement to relieve biliary obstruction. This puts the patient at risk for stent occlusion, which may interrupt therapy and can result in life-threatening infection.[54, 55] Patients who undergo upfront surgery often do not require stent placement, since the biliary

obstruction is relieved by removal of the tumor during surgery. In addition, the use of neoadjuvant therapy requires a tissue diagnosis, which introduces risk related to biopsy procedure (bleeding and/or pancreatitis). It also can be challenging to obtain a reliable tissue sample, as pancreatic tumors often have low cellularity with high stromal content. Furthermore, neoadjuvant therapy could induce an inflammatory tissue reaction, which may increase surgical risk and possibly post-operative outcomes.[56] Finally, neoadjuvant approaches are not always easily endorsed both by patients and institutions, especially in the setting of highly competitive health care systems.[9]

Although previous studies on neoadjuvant therapy have shown ambiguous results, there is growing evidence favoring the use of neoadjuvant therapy for pancreatic cancer.[57-59] In particular, emerging combinations of chemotherapeutic agents, such FOLFIRINOX and gemcitabine/nab-paclitaxel, and novel radiation strategies, have shown potential to tip the scale.[53] At present, most centers do not yet recommend neoadjuvant therapy for clearly resectable pancreatic cancer, but American and European treatment guidelines support the use of neoadjuvant therapy for patients with borderline resectable pancreatic cancer.[5, 57] However, robust data underpinning the use of neoadjuvant therapy currently remains scant, as randomized trials were inconclusive or are still ongoing.[60, 61]

CURRENT CHALLENGES

Pancreatic cancer has a dismal prognosis and micro-metastases are often present at diagnosis, even among patients with radiographically clearly resectable disease. Novel molecular biomarkers are necessary to identify these patients and allow for early systemic treatment. Molecular subtyping for pancreatic cancer is still in its early stages, yet considerable progress has been made over the past decades in our understanding of the underlying tumor biology. Further clinical validation of potential tumor markers is necessary to define a meaningful and clinically applicable molecular taxonomy that could inform clinical decisions. Alongside identification of patients at high risk for rapidly progressively disease, early treatment of potential micro-metastasis is key. There is growing consensus in favor of the use of neoadjuvant therapy for locally advanced, borderline resectable, and even upfront resectable pancreatic cancer patients. However, conclusive evidence remains scant. Therefore, further studies are needed to further delineate the value of neoadjuvant therapy for non-metastatic pancreatic cancer, especially for patients with clearly resectable disease.

THESIS OUTLINE

This thesis is divided in two parts; Part I focuses on the exploration of clinically valuable molecular biomarkers that can be used in addition to current staging strategies to identify radiographically resectable patients with rapidly progressing pancreatic cancer. Part II

describes the potential role for neoadjuvant therapy compared to upfront surgery in non-metastatic pancreatic cancer patients.

Chapter 2 assesses the role and prognostic value of HLA expression in pancreatic cancer patients. **Chapter 3** investigates the clinical impact of angiogenic growth factors in pancreatic cancer. **Chapter 4** evaluates the clinical significance of urokinase plasminogen activator receptor expression in pancreatic cancer. **Chapter 5** identifies potential molecular targets for tumor-specific imaging of pancreatic adenocarcinoma.

Chapter 6 used a Markov decision analysis model to compare the (quality-adjusted) life expectancy of neoadjuvant therapy to conventional upfront surgical strategies for pancreatic cancer. **Chapter 7** shows the stage-dependent survival impact of neoadjuvant therapy in resectable pancreatic cancer patients. **Chapter 8** evaluates the value of additional postoperative therapy in patients who already received neoadjuvant therapy followed by surgery. **Chapter 9** reveals that while neoadjuvant therapy may decrease positive resection margins rates, it does not abrogate the poor prognostic impact of residual disease after pancreatic cancer surgery. **Chapter 10** validates the 8th edition American Joint Commission on Cancer staging paradigm in pancreatic cancer patients who underwent neoadjuvant therapy. **Chapter 11** demonstrates the significant survival impacted of stereotactic body radiation therapy in unresected pancreatic cancer patients. **Chapter 12** investigates the international differences and impact of adjuvant chemoradiation use after pancreatic surgery. **Chapter 13** describes the role of large dataset studies in the implementation of new treatment strategies.

Finally, **chapter 14,** summarizes all results and outlines future research perspectives.

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Part I

Molecular biomarkers

Chapter 2

Identifying immune resistance mechanisms in pancreatic cancer: the role of human leukocyte antigen G expression

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Submitted

ABSTRACT

Background: Human leucocyte antigen (HLA) -G expression is known for its role in immune evasion and represents also, like the other HLA molecules, a strong prognostic factor for the clinical course of many malignancies. The purpose of this study was to investigate the prognostic value of classical (-A, -B, -C), and non-classical (-E, -G) HLA class I expression in pancreatic cancer patients.

Methods: Classical HLA class I (using monolclonal antibodies HCA2 and HC10), HLA-E (MEM-E/02), and HLA-G (4H84) expression was determined by immunohistochemistry in 130 resected pancreatic adenocarcinomas. Survival analysis was performed using Cox proportional hazard analysis.

Results: Classical HLA class I, HLA-G and HLA-E expression was observed in respectively 78%, 21%, and 96% on tumor cells of the pancreatic adenocarcinomas. On multivariate analysis, HLA-G expression was significantly associated with decreased overall survival (median overall survival, 11 vs. 18 months; HR, 1.863; 95% CI, 1.124 – 3.090; P=0.016). Multivariate analyses did not identify classical HLA class I and HLA-E expression as independent predictive factors for overall survival.

Conclusions: HLA-G expression was significantly associated with adverse overall survival in pancreatic adenocarcinoma and provides further evidence for the immunogenic character of pancreatic cancer and subsequent potential for therapeutic strategies targeting the immune system.

INTRODUCTION

Pancreatic cancer ranks the third leading cause of cancer-related mortality in the Western World, but is projected to be the second leading cause by 2030[1, 2]. Surgery constitutes the cornerstone of pancreatic cancer care, representing the only realistic chance for long-term survival. Unfortunately, the majority of pancreatic cancer patients presents with unresectable disease [3-5]. In addition, chemotherapy plays a central role in disease control, considering the early systemic nature of this disease. [6]. However, commonly used chemotherapeutic agents, including FOLFIRINOX (folinic acid, 5-fluorouracil, irinotecan, and oxaliplatin), gemcitabine, and nab-paclitaxel have shown only modest impact on clinical outcomes[7, 8].

Immune checkpoint inhibitors targeting cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) , programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PDL-1) have demonstrated encouraging results in various malignancies, including melanoma and lung cancer. Unfortunately, the success of these therapeutic agents is restricted to approximately 30% of patients[9-12]. In addition, regardless of preclinical rationale and favorable results in other types of cancer, clinical trials with immune checkpoint inhibitors have shown minimal survival benefit in pancreatic cancer[13, 14]. These findings have enkindled new interest in the role of cancer immune surveillance in the control of tumor growth in this cancer type.

Several cancer immune escape mechanisms have been identified, including defects in human leukocyte antigens (HLA)[15-17]. HLA-G is a frequently investigated tolerogenic molecule, which protects (tumor) cells from destruction by natural killer (NK) cells, similar to the shielding of the fetal cytotophoblasts from NK cell-mediated rejection during pregnancy[18]. In addition, HLA-G expression provides protection from immune recognition and destruction by inducing the differentiation of CD4+ and CD8+ T cells into regulatory T cells [19, 20]. Tumor cell expression of HLA-G has found to be inversely correlated with survival in most malignancies[21-24]. However, the results in pancreatic cancer remain ambiguous[25-27]. Therefore, the present study investigates the impact of classical and non-classical HLA class I expression on survival of pancreatic cancer patients.

MATERIALS AND METHODS

Patient selection

Patients who underwent primary surgical treatment for non-metastatic pancreatic adenocarcinoma at the Leiden University Medical Center, Leiden, The Netherlands, between 2003 and 2013 were identified from a local retrospective database. Resectability was evaluated according the guidelines proposed by the International Study Group of Pancreatic Surgery[40]. Patients were excluded if they deceased within 30 days of surgery

or received chemo-radiotherapy prior to surgery. Pancreatic adenocarcinoma diagnosis was confirmed histopathologically by a gastrointestinal pathologist (H. Morreau) according to the American Joint Committee on Cancer (8th Edition) and the WHO classification system[41, 42]. Clinicopathologic data were based on chart review and included patient age, gender, tumor location, pathological primary tumor stage (pT-stage), pathological lymph node stage (pN-stage), group stage, tumor size, number of lymph nodes examined, number of positive lymph nodes, tumor differentiation, perineural invasion, vascular invasion, resection margin status, and receipt of adjuvant gemcitabine. This study was designed and performed in line with Strengthening the reporting of observational studies in epidemiology (STROBE) criteria[43]. Patient confidentiality was maintained according to the Health Insurance Portability and Accountability Act of 1996. Institutional Review Board approval was obtained.

Immunohistochemistry

Classical HLA class I, and non-classical HLA-G, and HLA-E expression was assessed in the tumor epithelial cells by immunohistochemistry. Tissue microarrays were constructed from representative areas of neoplastic epithelial cells marked by a gastrointestinal pathologist (H. Morreau), as previously described[44]. Each patient's tissue was represented on the tissue array by triplicate 2.0 mm cores of pancreatic adenocarcinoma tissue. Tissue microarray blocks were cut into 5 μm sections, deparaffinized and rehydrated in graded alcohol. All slides were stained simultaneously to avoid inter-assay variation. Endogenous peroxidase was blocked for 20 min. in hydrogen peroxide-methanol. For HCA2 and HC10 immunohistochemical staining, antigen retrieval was performed with 0.01 mol/L citrate buffer (pH 6.0) for 10 min at maximum power in a microwave oven. Antigen retrieval for MEM-E/02 (Abcam, Cambridge, U.K.) and 4H84 (Nuclilab, Ede, The Netherlands) staining was achieved using 0.01 M Trizma EDTA buffer (pH 6) for 10 min at maximum power in a microwave oven. Sections were incubated overnight with HCA2, HC10, MEM-E/02 (Abcam, Cambridge, U.K.), and 4H84 at room temperature using predetermined optimal concentration. The reactivity spectrum of HCA2 is composed of all HLA-A chains (except HLA-A24), as well as some HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G chains.[45, 46] HC10 reacts mostly with HLA-B and HLA-C heavy chains and some HLA-A (HLA-A10, HLA-A28, HLA-A29, HLA-A30, HLA-A31, HLA-A32, HLA-A33)[47, 48]. MEM-E/02 reacts specifically with the denatured H chain of human HLA-E24. The 4H84 Ab recognizes denatured HLA-G molecules and has been described to cross-react with classical HLA class I molecules[49-51]. After incubation with the secondary antibodies envision anti-mouse (K4001; Dako Cytomation Glostrup, Denmark), sections were visualized using 3,3'-diaminobenzide solution (25 ml 3,3'-diaminobenzidine in 225 ml 0.05 mol/L Tris-HCI). Tissue sections were counterstained with hematoxyline, dehydrated, and mounted in malinol. For the HCA2 and HC10 staining, placenta served as a positive control and normal structures (i.e., lymphoid and endothelial

cells) were used as internal control to evaluate staining intensity of malignant cells[52]. For HLA-E and HLA-G reactivity, tonsil tissue served as a positive control. For all immunohistochemical stainings, negative control tissue micro array sections were processed with omission of the primary antibodies.

Evaluation of immunostaining

Microscopic investigation of HC10, HCA2, 4H84, and MEM-E/02 expression was performed by two independent investigators (S.W.L. de Geus, 100%; H.A.J.M. Prevoo, 30%) blinded to clinical outcomes. In case of inter-observer difference (<5%), a consensus was reached by simultaneous evaluation. For the HCA2 and HC10 staining, normal structures (i.e., lymphoid and endothelial cells) were used as internal control to evaluate staining intensity of malignant cells[52]. Classical HLA class I expression status was determined according to the standard set by the International HLA and Immunogenetics Workshop, 2007[53]. According to this standard, loss of HLA class I antigen was defined as less than 5% expression of both HCA2 and HC10, down regulation was specified as less than 5% expressing of either HCA2 or HC10, and HLA class I antigen expression was characterized as 5% or more expression of HCA2 and HC10[21, 22, 54]. For the definitive analyses loss and down regulation were combined, due to small sample size. HLA-G and HLA-E were scored in a binary manner, considering any specific staining of tumor cells as positive expression and no staining as no expression[21]. For all immunohistochemical stainings, the scores of the three 2.0 mm cores were averaged.

Statistical analysis

All statistical analyses were performed using the statistical package SPSS (version 23.0; IBM-SPSS, Chicago, IL). The Chi-square or Fisher's exact tests were used to evaluate associations between clinicopathological parameters and HLA class I, HLA-E and HLA-G expression. OS was defined from date of surgery until death or last follow-up. DFS was defined from date of surgery until recurrence, death or last follow-up, and measured based on surveillance imaging obtained at regular intervals after surgical resection. Survival analyses were performed using the Kaplan-Meier method and log-rank test. Multivariate analyses were undertaken by the Cox proportional hazard method. Factors examined on univariate analysis included age, sex, pT-stage, pN-stage, and resection margin status, classical HLA class I, HLA-E and HLA-G expression. Covariates associated (p<0.10) with OS or DFS on univariate analysis were included in the multivariate model, i.e. lymph node stage. p-value <0.05 was considered to indicate statistical significance.

RESULTS

Patient characteristics

In total, 130 patients with primary pancreatic cancer were included in the study. The patient characteristics are summarized in Table 1. Female patients constituted 51% (n=67) of the cohort, and the median patient age was 66 years (interquartile range, 60 – 72 years). Of the patients 19% (n=25) were diagnosed with stage I disease, 45% (n=59) with stage II, and 35% (n=46) with stage III. Lymph node metastases were present at initial surgery in 76% (n=99) of the patients. Positive tumor resection margins were observed in 34 % (n=44) of the patients and 42% (n=55) of the overall cohort received adjuvant chemotherapy.

Table 1a. Baseline characteristics of pancreatic cancer patients by HLA class I expression.

Characteristics	**HLA class I**		***p***
	Loss/down-regulation (n=22)	**Expression (n=80)**	
Age, n (%)			
<65 years	8 (36%)	39 (49%)	0.302
≥65 years	14 (64%)	41 (51%)	
Sex, n (%)			
Male	11 (50%)	41 (51%)	0.917
Female	11 (50%)	39 (49%)	
Tumor location, n (%)			
Caput	20 (91%)	77 (96%)	0.294
Other	2 (9%)	3 (4%)	
Tumor differentiation, n (%)			
Well differentiation	4 (23%)	6 (10%)	0.241
Moderately differentiation	5 (29%)	28 (48%)	
Poorly/undifferentiated	8 (47%)	25 (42%)	
Missing	5	21	
pT stage, n (%)			
pT1	8 (36%)	17 (21%)	0.081
pT2	6 (27%)	45 (56%)	
pT3	7 (32%)	13 (16%)	
pT4	1 (5%)	5 (6%)	
pN stage, n (%)			
pN0	6 (27%)	22 (25%)	0.470
pN1	11 (50%)	31 (39%)	
pN2	5 (23%)	29 (36%)	
Margin status, n (%)			
Negative	15 (68%)	54 (68%)	0.952
Positive	7 (32%)	26 (32%)	
Adjuvant therapy, n (%)			
No	15 (68%)	43 (54%)	0.226
Yes	7 (32%)	37 (46%)	

Abbreviations: HLA, Human Leukocyte Antigen

HLA expression

HLA class I, HLA-G, and HLA-E expression could be assessed in respectively 79% (n=102), 86% (n=112), and 86% (n=112) of the patient population. The missing ones were due to insufficient neoplastic cellularity within the TMA tissue punches. Representative images of immunohistochemical staining for classical HLA class I, HLA-G, and HLA-E, expression are shown in Figure 1.

Table 1b. Baseline characteristics of pancreatic adenocarcinoma patients by HLA-E and HLA-G expression.

Characteristics	HLA-G expression			HLA-E expression		
	Absent (n=88)	Present (n=24)	*p*	Absent (n=4)	Present (n=108)	*p*
Age, n (%)						
<65 years	43 (49%)	9 (38%)	0.322	4 (100%)	49 (45%)	0.047
≥65 years	45 (52%)	15 (62%)		0 (0%)	59 (55%)	
Sex, n (%)						
Male	47 (53%)	9 (38%)	0.167	2 (50%)	56 (52%)	>0.999
Female	41 (47%)	15 (62%)		2 (50%)	52 (48%)	
Tumor location, n (%)						
Caput	81 (92%)	24 (100%)	0.154	3 (75%)	102 (94%)	0.230
Other	7 (8%)	0 (0%)		1 (25%)	6 (6%)	
Tumor differentiation, n (%)						
Well differentiation	10 (15%)	2 (11%)	0.845	0 (0%)	12 (15%)	0.705
Moderately differentiation	28 (41%)	9 (47%)		2 (50%)	36 (44%)	
Poorly/undifferentiated	30 (44%)	8 (42%)		2 (50%)	33 (41%)	
Missing	20	5		0	27	
pT stage, n (%)						
pT1	22 (25%)	4 (17%)	0.035	0 (0%)	28 (26%)	0.582
pT2	39 (44%)	16 (67%)		3 (75%)	51 (47%)	
pT3	23 (26%)	1 (4%)		1 (25%)	22 (20%)	
pT4	4 (5%)	3 (12%)		0 (0%)	7 (7%)	
pN stage, n (%)						
pN0	25 (28%)	3 (13%)	0.115	1 (25%)	27 (25%)	0.698
pN1	37 (42%)	9 (37%)		1 (25%)	47 (43%)	
pN2	26 (30%)	12 (50%)		2 (50%)	34 (32%)	
Margin status, n (%)						
Negative	60 (68%)	14 (58%)	0.366	4 (100%)	70 (65%)	0.298
Positive	28 (32%)	10 (42%)		0 (0%)	38 (35%)	
Adjuvant therapy, n (%)						
No	52 (59%)	12 (50%)	0.425	2 (50%)	62 (57%)	>0.999
Yes	36 (41%)	12 (50%)		2 (50%)	46 (43%)	

Classical HLA class I (immunohistochemically positive staining for both HC10 and HCA2) expression was observed in 78% (n=80) of the patients, downregulation (either HCA2 or HC10 immunohistochemically positive) of HLA class expression in 19% (n=19), and loss (both, HCA2 and HC10 immunohistochemically negative) of HLA class I expression in 3% (n=3). HLA-G and HLA-E expression was identified in 21% (n=24) and 96% (n=108) of the patients, respectively. Absence of HLA-G expression was associated

with higher pT-stage (p=0.035). However, no other correlations were found between HLA class I, HLA-G, and HLA-E expression and any other patient characteristics (Table 1).

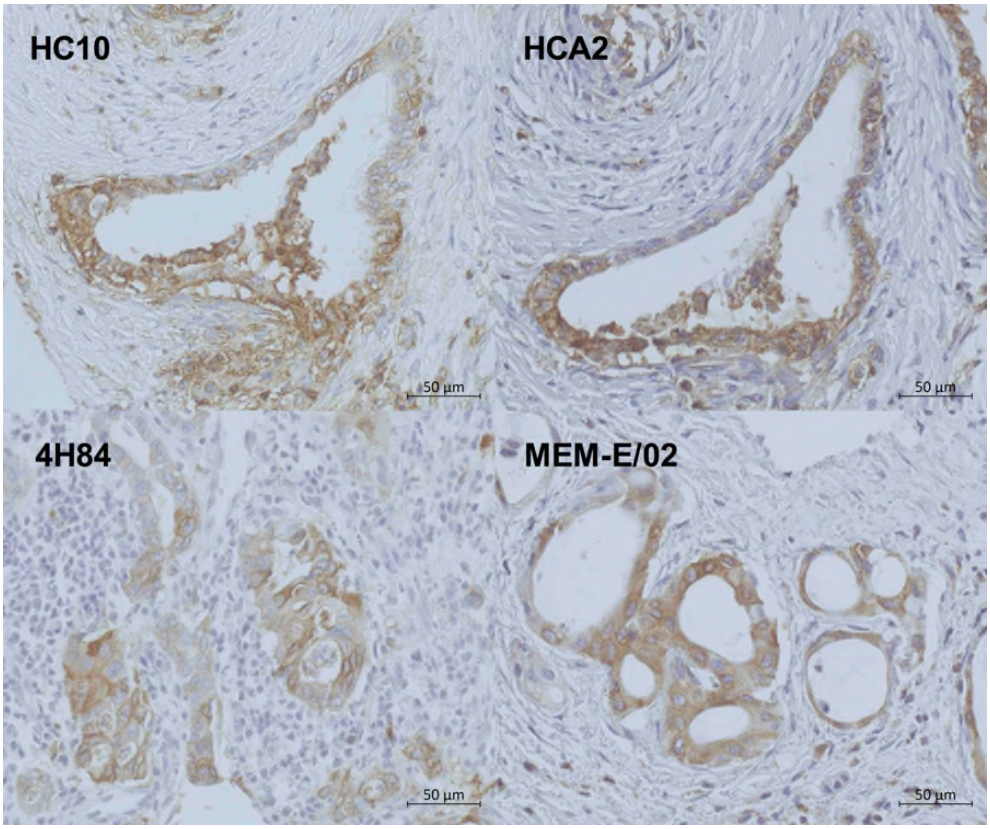

Figure 1. Representative images of predominantly cytoplasmic and membranous immunohistochemical staining using monoclonal antibodies HCA2 (HLA class I), HC10 (HLA class I), 4H84 (HLA-G), and MEM-E/02 (HLA-E) in pancreatic cancer.

Overall survival

At the time of analysis, 93% (n=121) of the patients had died and the median overall survival (OS) was 17 months. On univariate analyses, age, gender, pT-stage, resection margin status, classical HLA class I expression (median OS, 18 vs. 15 months; log-rank p=0.034; Fig 2), HLA-E expression (median OS, 16 vs. 17 months; log-rank p=0.357; Fig 2) were not significantly associated with OS (Table 3). HLA-G expression (median OS, 18 vs. 11 months; log-rank p=0.001; Fig 2) was significantly predictive for decreased OS on both univariate and multivariate analyses (Table 3).

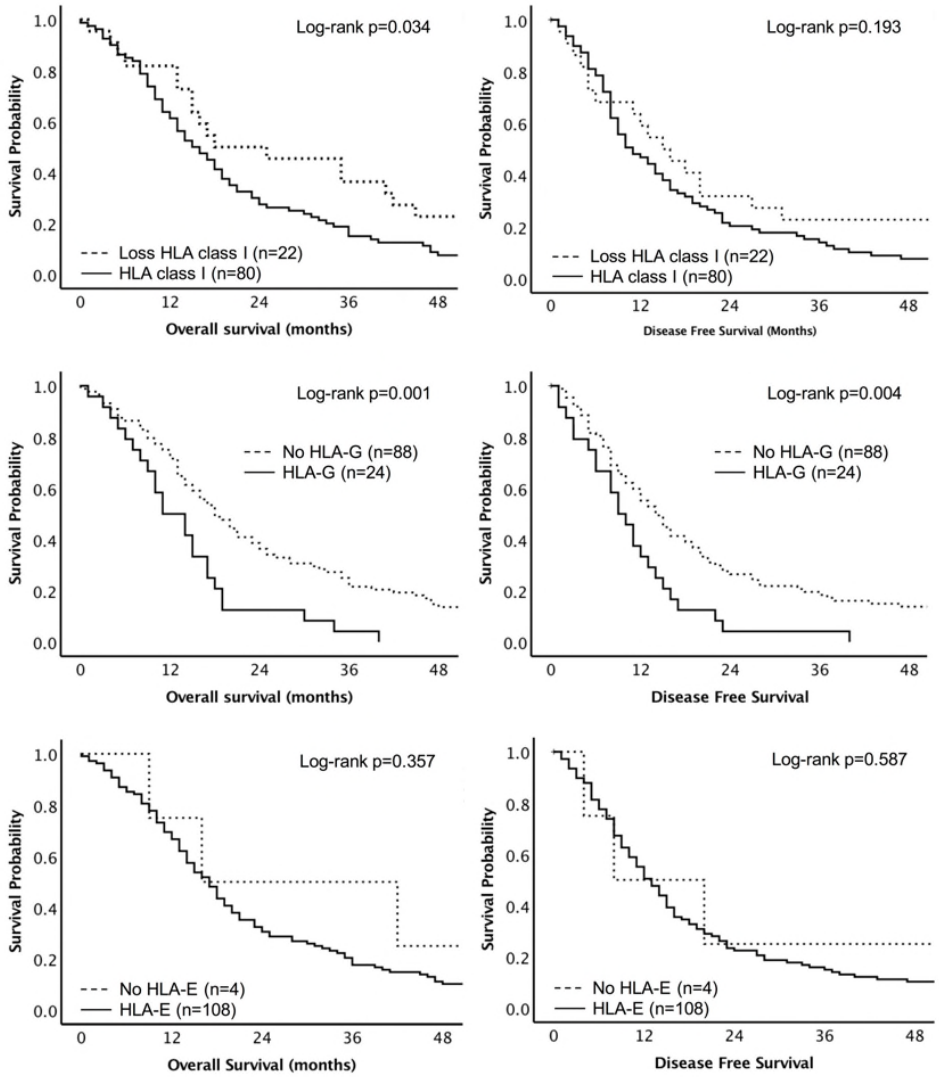

Figure 2. Kaplan-Meier survival curves for overall and disease-free survival by HLA class I, HLA-G, and HLA-E tumor expression, determined by immunohistochemical staining, in pancreatic adenocarcinoma.

Disease free survival

At time of analysis, 95% (n=123) of patients experienced tumor recurrence and the median disease free survival (DFS) in this population was 13 months. Age, gender, pT-stage disease, resection margin status, classical HLA class I (median DFS, 15 vs. 11 months; log-rank p=0.193; Fig 2), and HLA-E expression (median DFS, 8 vs. 13 months; log-rank p=0.587; Fig 2) were not significantly predictive for DFS on univariate analyses (Table 3). However, HLA-G expression (median DFS, 14 vs. 9 months; log-rank

p=0.004;Fig 2) was related with decreased DFS on both univariate and multivariate analyses (Table 3).

Table 2. Uni- and multivariate Cox proportional hazard regression analysis for overall survival.

	Univariate			Multivariate		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (≥ 65 vs. <65 years)	1.321	0.917 – 1.904	0.135			
Sex (male vs. female)	1.215	0.850 – 1.738	0.285			
pT-stage (pT2 vs. pT1)	1.300	0.823 – 2.051	0.261			
pT-stage (pT3 vs. pT1)	1.390	0.803 – 2.405	0.239			
pT-stage (pT4 vs. pT1)	0.865	0.375 – 1.995	0.734			
pN-stage (pN1 vs. pN0)	1.422	0.886 – 2.281	0.144	1.341	0.758 – 2.370	0.313
pN-stage (pN2 vs. pN0)	1.744	1.057 – 2.878	0.029	1.788	1.014 – 3.152	0.045
Margin status (R1 vs. R0)	1.480	1.013 – 2.161	0.043	1.195	0.747 – 1.912	0.458
Adjuvant therapy (no vs. yes)	1.426	0.991 – 2.054	0.056	1.943	1.258 – 3.001	0.003
HLA class I (expression vs. downregulation)	1.727	1.028 – 2.901	0.039	1.677	0.970 – 2.901	0.064
HLA-G (expression vs. loss)	2.117	1.319 – 3.398	0.002	2.113	1.263 – 3.536	0.004
HLA-E (expression vs. loss)	1.691	0.535 – 5.347	0.371			

Abbreviations: CL, Confidence Intervals; HLA, Human Leukocyte Antigen; HR, Hazard Ratio

Table 3. Uni- and multivariate Cox proportional hazard regression analysis for disease-free survival.

	Univariate			Multivariate		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (≥ 65 vs. <65 years)	1.218	0.848 – 1.749	0.286			
Sex (male vs. female)	1.165	0.815 – 1.664	0.403			
pT-stage (pT2 vs. pT1)	1.310	0.831 – 2.064	0.245			
pT-stage (pT3 vs. pT1)	1.297	0.751 – 2.243	0.351			
pT-stage (pT4 vs. pT1)	0.798	0.346 – 1.840	0.597			
pN-stage (pN1 vs. pN0)	1.365	0.853 – 2.183	0.195	1.461	0.869 – 2.458	0.153
pN-stage (pN2 vs. pN0)	1.599	0.971 – 2.633	0.065	1.702	1.003 – 2.889	0.049
Margin status (R1 vs. R0)	1.342	0.921 – 1.955	0.125			
Adjuvant therapy (no vs. yes)	1.586	1.102 – 2.282	0.013	2.056	1.361 – 3.106	0.001
HLA class I (expression vs. loss)	1.388	0.836 – 2.305	0.204			
HLA-G (expression vs. loss)	1.946	1.216 – 3.114	0.006	2.213	1.363 – 3.594	0.001
HLA-E (expression vs. loss)	1.365	0.432 – 4.313	0.596			

Abbreviations: CL, Confidence Intervals; HLA, Human Leukocyte Antigen; HR, Hazard Ratio

DISCUSSION

The plasticity of immune cancer surveillance in pancreatic cancer patients continues to be insufficiently understood, hampering endeavors to develop effective immunotherapy. A variety of immunotherapeutic agents have shown to be effective in various solid tumors, including melanoma and lung cancer[9-12]. Nonetheless, immunotherapy-based strategies have shown little success in pancreatic cancer[13, 14]. HLA expression plays a pivotal role in tumor recognition and destruction by the host immunosurveillance system, as well as in evasion of the host immune system by tumor cells. This study used a relatively large series of pancreatic adenocarcinoma to assess the survival impact of classical and non-classical HLA class I expression on the clinical

outcome for these patients. Our findings reveal that HLA-G expression was significantly associated with adverse OS and DFS, independently of classical tumor characteristics.

Previous studies investigating the survival impact of HLA-G expression in pancreatic cancer patients have shown ambiguous results. The adverse clinical prognostic value of HLA-G expression elucidated by this study, is concordant with the majority of previous studies in pancreatic cancer, as well as observations in other malignancies[21-25, 27-29]. These findings highlight the important immune privilege-inducing function of HLA-G expression on tumor cells[30]. In contrast, a recent publication describes that HLA-G expression was associated with improved cancer-specific survival [26]. Similarly, the present study also revealed an associated between high levels of FoxP3-positive Tregs and superior survival. They hypothesize that their counterintuitive findings may be explained by the host adaptive immune resistance, where immune inhibitory molecules become overexpressed on cancer cells in response to interferon gamma upregulation and tumor-infiltrating lymphocytes infiltration[26, 31-34].

Differences between studies may partly be explained by the particularities of immunohistochemistry and the ongoing controversy regarding the validity of immune-based HLA-G identification strategies[35, 36]. Sideras *et al.* (2017) used the anti-HLA-G antibody MEM-G/02, whereas our study utilized the mAbs 4H84 to detect HLA-G. MAbs 4H84 preferably recognizes unfolded HLA-G-free chains and cross-reacts with classical HLA class I molecules[35]. We previously showed that the different epitopes of HLA-G detected by 4H84, MEM-G1, and MEM-G/2 mAbs were expressed differentially in colorectal tumor tissues[37]. The latter may also apply to pancreatic cancer.

Next to the already mentioned limitations inherent to immunohistochemical analyses there are other limitations to this study that should be acknowledged. Regardless of standardization of antibodies, tissue preservation and staining protocols, the assessment of immunohistochemical-based semi-quantitative scoring is mostly subjective, due to the fact that it relies on the estimation of independent observers. The use of computer-based evaluation of immunohistochmical staining results may in future aid in overcoming these shortcomings. Furthermore, immunohistochemistry assesses the presence or absence of a moiety, but provides no information about its functional properties. Finally, there could be significant selection bias introduced in this study by non-random loss of tissue, since fibrotic pancreatic tissue is harder to manipulate.

Despite these limitations, our data suggests that HLA-G plays a critical role in the induction of immune privilege and constitutes a clinically relevant immune escape mechanisms in a subgroup of pancreatic cancer patients. The capacity of pancreatic tumor cells to evade the host immune system by expressing HLA-G has important implication for the potential efficacy of immunotherapy. The recently proposed role of HLA-G as an immune checkpoint molecule, may provide a new immunotherapeutic strategy for pancreatic cancer, but, as we show, possibly for approximately 25% of the patients[38]. Several *in vivo* studies have already demonstrated promising results for immunotherap

targeting HLA-G. Ishibashi and colleagues (2016) identified HLA-G_{26-40}, a novel peptide which induces effective antitumor CD4+ T cell response against HLA-G-positive breast cancer cells[39].

In summary, this study demonstrates that high HLA-G expression in pancreatic adenocarcinoma patients is an independent prognostic indicator of decreased OS and DFS. These findings suggest that the up-regulation of HLA-G expression in pancreatic cancer patients may enable the evasion of tumor detection and elimination by the host immune system. Consequently, HLA-G expression may be a useful therapeutic target, justifying further study for the enhancement of immunotherapy by inhibiting HLA-G in pancreatic cancer patients.

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Chapter 3

Prognostic significance of angiogenic growth factors in pancreatic cancer

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Submitted

ABSTRACT

Background: Angiogenesis plays a critical role in tumor growth and recurrence. The present study investigates the prognostic value of integrin $\alpha_v\beta_6$, vascular endothelial growth factor receptor 2 (VEGFR2), epithelial growth factor receptor (EGFR), and hepatocyte growth factor receptor (c-MET) expression in patients with resected pancreatic adenocarcinoma.

Methods: Immunohistochemistry was used to evaluate the expression of integrin $\alpha_v\beta_6$, VEGFR2, EGFR, and c-MET expression in surgical specimens from 127 patients with pancreatic adenocarcinoma. Survival analysis for overall (OS) and disease-free (DFS) survival was performed using the Kaplan-Meier method, and multivariable Cox proportional hazard models.

Results: Integrin $\alpha_v\beta_6$, VEGFR2, EGFR, and cMET expression was observed in 89%, 73%, 69%, and 87% of pancreatic cancer patients, respectively. Patients with integrin $\alpha_v\beta_6$ (median OS: 15 vs. 35 months; log-rank p=0.012), and cMET (median OS, 15 vs. 41 months; log-rank p=0.003) expression had a shorter OS. On multivariable analyses, integrin $\alpha_v\beta_6$ (HR, 1.981; p=0.037) and c-MET (HR, 1.766; p=0.051) expression remained associated with poor OS. EGFR and VEGFR2 expression were not associated with OS. In addition, c-MET expression was associated with a decrease in median DFS (12 vs. 31 months; log-rank p=0.008). c-MET expression remained predictive for unfavorable DFS on multivariable analysis (HR, 1.795; p=0.037). Integrin $\alpha_v\beta_6$, VEGFR2, and EGFR did not significantly impact DFS.

Conclusions: The results of this study suggest that expression of integrin αvβ6 and cMET expression are prognostic biomarkers in pancreatic cancer. These markers may be used to identify patients at high risk of early recurrence after pancreaticoduodenectomy. Further validation is necessary.

INTRODUCTION

Pancreatic cancer currently ranks the third leading cause of cancer-related death in the Western World and is projected to surpass colorectal cancer as the second leading cause by 2030 (1). Surgical resection remains the only chance for cure for patients with pancreatic adenocarcinoma (2). However, micro-metastasis and early distant recurrence are common, even among patients with seemingly non-metastatic upfront resectable disease at the time of surgery (3). Patients at high risk for early recurrence after pancreatectomy may benefit from neoadjuvant chemoradiation (4). Prognostic markers are pivotal to facility the selection of the patients who are likely to benefit most from an early systemic treatment strategy (5).

Angiogenesis, the recruitment of new capillaries from pre-existing blood vessels, is considered one of the hallmarks of carcinogenesis (6). Once solid tumors exceed 2-3 mm in size, angiogenesis is critical to maintain appropriate oxygen and nutritional supplementation to the core of the tumor, and allowing for further tumor growth (7). In addition, angiogenesis promotes the formation of distant metastasis by providing a pathway for tumor cells to exit the tumor and enter the circulation (8). Angiogenesis is under control of multiple molecules of which integrin $\alpha_v\beta_6$, vascular endothelial growth factor receptor 2 (VEGFR2), epithelial growth factor receptor (EGFR), and hepatocyte growth factor receptor (c-MET) are key players (9-13). Consequently, these biomarkers have been associated with survival in various gastrointestinal tumors, including pancreatic cancer (14-18).

The purpose of this study was to evaluate the prognostic value of integrin $\alpha_v\beta_6$, VEGFR2, EGFR, and c-MET expression in pancreatic cancer patients in order to identify biomarkers that might be used to identify pancreatic cancer patients at risk for early recurrence after surgery.

METHODS

Patient selection

For a consecutive series of 127 resections for pancreatic adenocarcinoma performed between June 2002 and July 2012 at the Leiden University Medical Center (LUMC) medical records and pathological specimens were revisited. Patients were excluded if they underwent neoadjuvant therapy, as this may influence the expression of molecular markers (19). Clinicopathological characteristics from these patients were retrospectively obtained from electronic hospital records. Tumor differentiation grade was determined according to the guideline of the World Health Organization, and the TNM stage was defined according to the American Joint Commission on Cancer criteria (20). All samples were nonidentifiable and used in accordance with the ethical standards of the

institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Immunohistochemistry

Tissue microarrays (TMAs) of tumor were created to conduct uniform and simultaneous immunohistochemical stainings to limit intra-assay variations. Formalin-fixed paraffin-embedded tissue blocks of the primary tumor were ovtained from the archives of the Pathology Department. A single representative block was selected for each patient based on hematoxylin-eosin-stained sections. From each donor block, triplicate 2.0-mm cores were punched from areas with clear histopathological tumor representation and transferred to a recipient TMA block using the TMA Master (3DHISTECH, Budapest, Hungary).

From each completed TMA block 5-µm sections were sliced. The sections were deparaffined in xylene and rehydrated in serially diluted alcohol solutions, followed by demineralized water according to standard protocols. Endogenous peroxidase was blocked by incubation in 0.3 % hydrogen peroxide in phosphate-buffered saline (PBS) for 20 min. For c-MET staining antigen retrieval was performed by heat induction at 95 °C using PT Link (Dako, Glostrup, Denmark) with a low-pH Envision FLEX target retrieval solution (citrate buffer pH 6.0, Dako). VEGFR2 staining required antigen retrieval with high-pH Envision FLEX target retrieval solution (Tris-EDTA pH 9.0, Dako). For staining of EGFR and integrin αvβ6, antigen retrieval was performed with 0.4 % pepsin incubation for 10 min at 37 °C. Immunohistochemical staining was performed by incubating tissue microarrays overnight with antibodies against VEGFR2 (55B11; Cell Signaling Technology, Danvers, MA, USA), c-MET (SC10; Santa Cruz Biotechnology, Santa Cruz, CA, USA), CEA (A0155; Dako, Glustrup, Denmark), EGFR (E30; Dako), and integrin αvβ6 (6.2A; Biogen Idec MA Inc., Cambridge, MA, USA) all at room temperature. All antibodies were used at predetermined optimal dilutions using proper positive and negative control tissue. Furthermore, all antibodies selected for this study were solely selective for integrin αvβ6, cMET, EGFR, and VEGFR respectively. Negative control samples were incubated with PBS instead of the primary antibodies. The sections were washed with PBS, followed by incubation with Envision anti-mouse (K4001; Dako) or Envision anti-Rabbit (K4003; Dako), where applicable, for 30 min at room temperature. After additional washing, immunohistochemical staining was visualized using 3,3-diaminobenzidine tertahydrochloride solution (Dako) for 5–10 min resulting in brown color and counterstained with hematoxylin, dehydrated, and finally mounted in pertex. All stained sections were scanned and viewed at ×40 magnification using the Philips Ultra Fast Scanner 1.6 RA (Philips, Eindhoven, Netherlands).

Immunohistochemical evaluation was performed using a four-point system for staining intensity: 0, 1, 2, and 3 (for none, light, medium, or high intense staining), as previously described. Staining was assumed positive if >10 % of the tumor cells expressed

a medium or dark staining pattern (21-25). Evaluation of the immunohistochemical staining for all molecular markers was performed blinded and independently by two observers (S.W.L.G. and H.A.J.M.P). In case of disagreement, the staining results were discussed until agreement was achieved.

Table 1. Baseline characteristic of pancreatic adenocarcinoma patients by integrin αvβ6 and vascular endothelial growth factor receptor 2 (VEGFR2) expression.

	integrin αvβ6 expression			VEGFR2 expression		
	Low expression (n=14)	High expression (n-113)	*p*	Low expression (n=33)	High expression (n=90)	*p*
Age, n (%)						
< 65 years	5 (36%)	51 (45%)	0.503	17 (52%)	37 (41%)	0.303
≥ 65 years	9 (64%)	62 (55%)		16 (48%)	53 (59%)	
Sex, n (%)						
Male	5 (36%)	56 (50%)	0.328	16 (49%)	44 (49%)	0.968
Female	9 (64%)	57 (50%)		17 (51%)	46 (51%)	
Tumor location, n (%)						
Caput	13 (93%)	106 (94%)	>0.999	31 (94%)	84 (93%)	>0.999
Other	1 (7%)	7 (6%)		2 (6%)	6 (7%)	
Tumor differentiation, n (%)						
Well differentiated	2 (20%)	11 (13%)	0.651	5 (22%)	7 (10%)	0.378
Moderately differentiated	3 (30%)	37 (45%)		9 (39%)	28 (42%)	
Poorly/undifferentiated	5 (50%)	35 (42%)		9 (39%)	32 (48%)	
Missing	4	30		10	23	
pT-stage, n (%)						
pT1	4 (29%)	25 (22%)	0.037	8 (24%)	22 (25%)	0.794
pT2	5 (36%)	60 (53%)		16 (49%)	45 (50%)	
pT3	2 (14%)	24 (21%)		6 (18%)	19 (21%)	
pT4	3 (21%)	4 (4%)		3 (9%)	4 (4%)	
pN-stage, n (%)						
pN0	6 (43%)	26 (23%)	0.142	9 (27%)	22 (25%)	0.947
pN1	3 (21%)	53 (47%)		14 (43%)	39 (43%)	
pN2	5 (36%)	34 (30%)		10 (30%)	29 (32%)	
Surgical margin status, n (%)						
R0	10 (71%)	75 (66%)	>0.999	25 (76%)	58 (64%)	0.235
R1	4 (29%)	38 (34%)		8 (24%)	32 (36%)	
Adjuvant therapy, n (%)						
No	8 (57%)	65 (58%)	0.978	20 (61%)	52 (58%)	0.778
Yes	6 (43%)	48 (42%)		13 (39%)	38 (42%)	

Statistical analysis

All statistical analyses were performed using SPSS version 23.0 software (SPSS, © IBM Corporation, Somer NY, USA). Interobserver variation of immunohistochemical results was evaluated using Cohen's kappa coefficient, and >0.8 was regarded acceptable. The Chi-square or Fisher's exact tests were used to assess association of integrin $\alpha_v\beta_6$, VEGFR2, EGFR, and cMET expression with clinical pathological characteristics. Overall survival (OS) was calculated from date of surgery until death or last follow-up. Disease free survival (DFS) was determined from date of surgery until recurrence, death of last follow-up. Survival analyses were performed using the Kaplan-Meier method and log-rank test.

In addition, multivariate analyses were performed using by the Cox proportional hazard method. Covariates assessed on univariate analysis included age, sex, pT-stage, pN-stage, and margin status, integrin αvβ6, VEGFR2, EGFR, and cMET expression. Solely covariates associated ($p<0.10$) with OS or DFS on univariate analysis were included in the multivariate model. Statistical significant was set at $p < 0.05$.

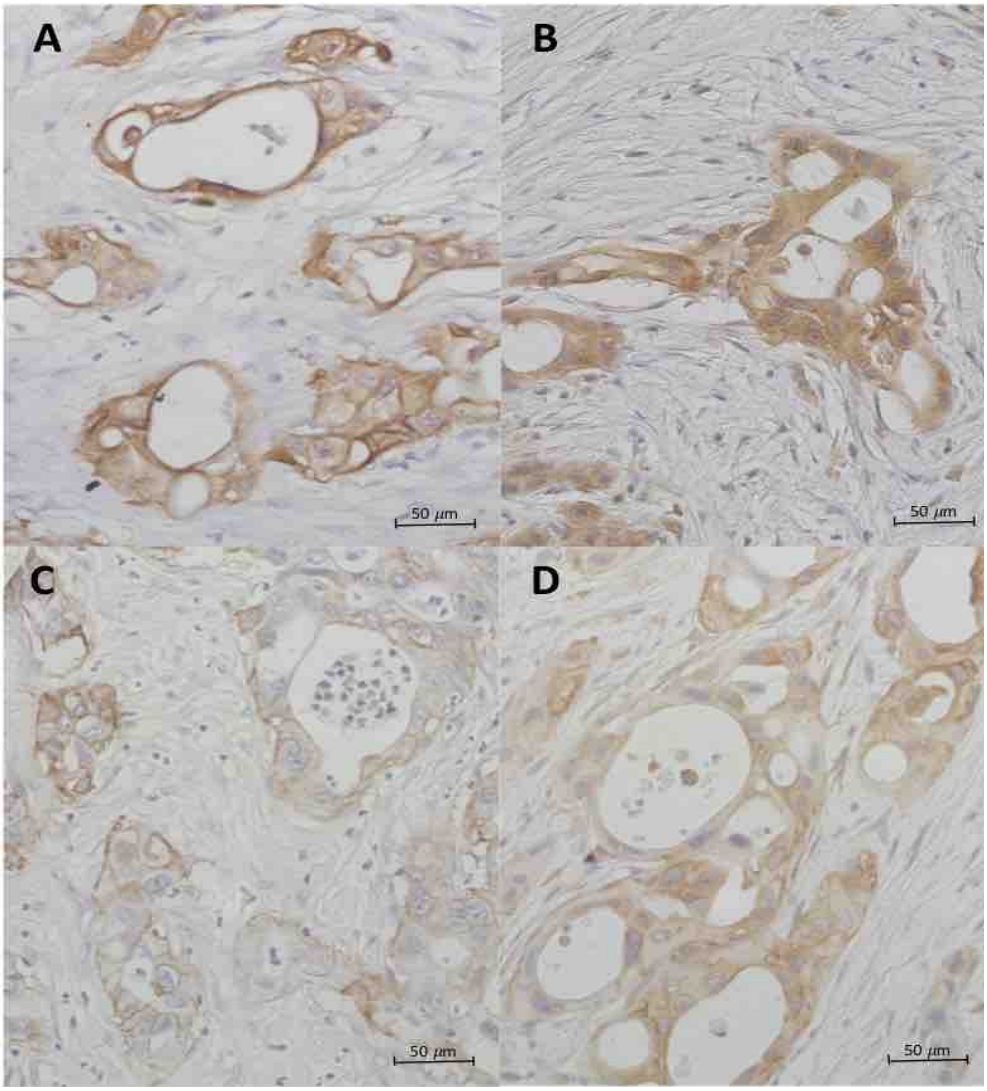

Figure 1. Representative images of predominantly cytoplasmic and membranous immunohistochemical staining for integrin αvβ6 (A), c-MET (B), EGFR (C), and VEGFR2 (D) expression in resected pancreatic adenocarcinoma.

RESULTS

Baseline characteristics

In total, 132 patients with pancreatic adenocarcinoma were identified. Baseline characteristics are summarized in Table 1. Female patients represented 52% (n=68) of the cohort, and the median patient age was 66 years (interquartile range, 60 – 72 years). Of these patients 21% (n=28) were diagnosed with stage I disease, 44% (n=58) with stage II, and 35% (n=46) with stage III. Positive resection margins were detected in 33 % (n=44) of the patients and 42% (n=55) of the patients received adjuvant therapy.

Molecular marker expression

Integrin $\alpha_v\beta_6$, VEGFR2, EGFR, and c-MET expression could be assessed in respectively 96% (n=127), 93% (n=123), 94% (n=124), and 96% (n=127) of the patient population. Missing were caused by insufficient neoplastic cellularity within the TMA tissue punches. Representative images of immunohistochemical staining for Integrin αvβ6, VEGFR2, EGFR, and c-MET expression are shown in Figure 1.

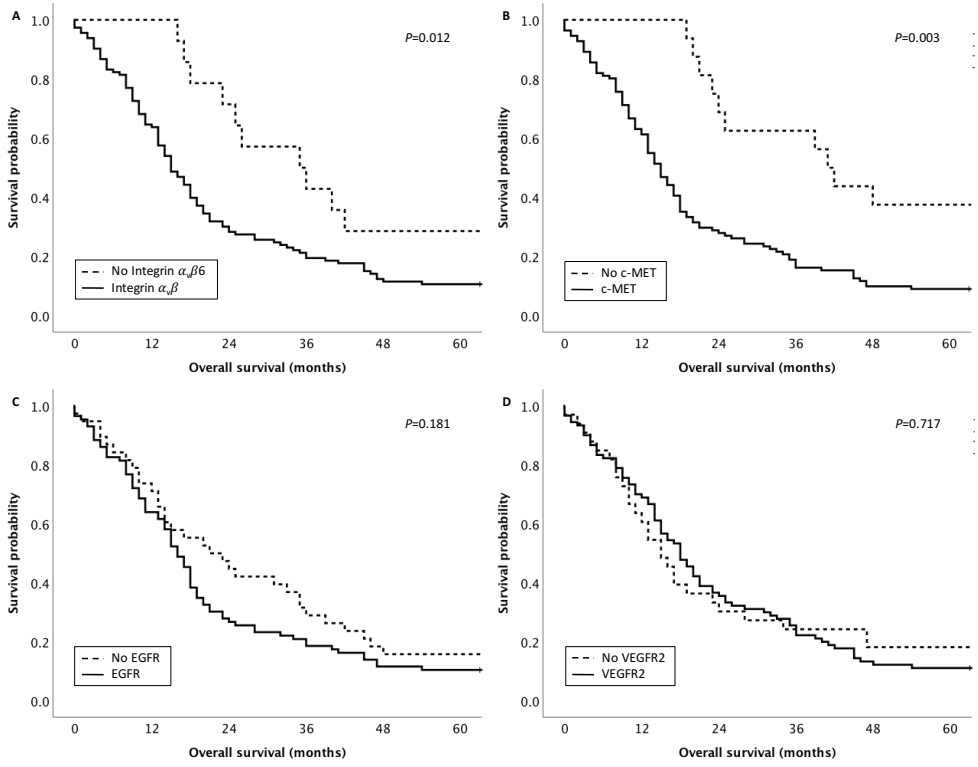

Figure 2. Kaplan-Meier survival curves for overall survival by integrin αvβ6 (A), c-MET (B), EGFR (C), and VEGFR2 (D) expression tumor expression, determined by immunohistochemical staining, in pancreatic adenocarcinoma.

The molecular markers demonstrated predominantly membranous and cytoplasmic immunoreactivity in pancreatic adenocarcinoma. Integrin $\alpha_v\beta_6$ expression was observed in 89% (n=113) of patients, VEGFR2 expression in 73% (n=90) of patients, EGFR expression 69% (n=86) of patients, and c-MET expression in 87% (n=111) of patients. Integrin $\alpha_v\beta_6$ expression was associated with higher pT-stage (p=0.037), and c-MET expression was correlated with higher pN-stage (p=0.038). No further associations between integrin $\alpha_v\beta_6$, VEGFR2, EGFR, and c-MET expression with any characteristics was found.

Table 2. Uni- and multivariate Cox proportional hazard regression analysis for overall survival.

	Univariate			Multivariate		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (≥ 65 vs. < 65 years)	1.379	0.958 – 1.984	0.083			
Sex (male vs. female)	1.243	0.870 – 1.775	0.232			
Tumor location (caput vs. other)	1.199	0.604 – 2.378	0.604			
pT-stage (pT2 vs. pT1)	1.431	0.901 – 2.274	0.129			
pT-stage (pT3 vs. pT2)	1.493	0.861 – 2.589	0.154			
pT-stage (pT4 vs. pT3)	0.979	0.423 – 2.268	0.961			
pN-stage (pN1 vs. pN0)	1.555	0.972 – 2.486	0.065	1.149	0.687 – 1.921	0.596
pN-stage (pN2 vs. pN0)	1.801	1.096 – 2.960	0.020	1.456	0.858 – 2.470	0.163
Resection margin (R1 vs. R0)	1.649	1.128 – 2.411	0.010	1.468	0.969 – 2.226	0.070
Adjuvant therapy (no vs. yes)	1.305	0.907 – 1.878	0.151			
Integrin αvβ6 (high- vs. low-expression)	2.118	1.153 – 3.890	0.016	1.981	1.041 – 3.769	0.037
VEGFR2 (high- vs. low-expression)	0.928	0.615 – 1.400	0.721			
EGFR (high- vs. low-expression)	1.307	0.876 – 1.951	0.190			
c-MET (high- vs. low-expression)	2.236	1.283 – 3.897	0.005	1.766	0.998 – 3.124	0.051

Abbreviations: c-MET, hepatocyte growth factor receptor; CI, Confidence Interval; EGFR, epithelial growth factor receptor; HR, Hazard Ratio; VEGFR2, vascular endothelial growth factor receptor 2

Overall survival

At the time of diagnosis, 92 % (n=122) of the patients had died and the median OS was 17 months. Patients with pancreatic adenocarcinoma that expressed integrin $\alpha_v\beta_6$ demonstrated a significantly shorter median OS compared to patients with no integrin $\alpha_v\beta_6$ expression (15 vs. 35 months; log-rank p=0.012; Figure 2a). Similarly, patients with high c-MET expression had significantly worse OS compared to patients with absent c-MET expression, resulting in a median OS of 15 compared to 41 months (log-rank p=0.003; Figure 2b). EGFR (median OS: 15 vs. 21 months; log-rank p=0.181; Figure 2c) and VEGFR2 (median OS: 15 vs. 18 months; log-rank p=0.717; Figure 2d) expression did not significantly impact median OS.

On multivariable analysis, integrin $\alpha_v\beta_6$ (Hazard Ratio [HR], 1.981; p=0.037) and c-MET (HR, 1.766; p=0.051) expression remained significantly predictive for unfavorable OS. However, EGFR and VEGFR2 expression did not influence OS (Table 2).

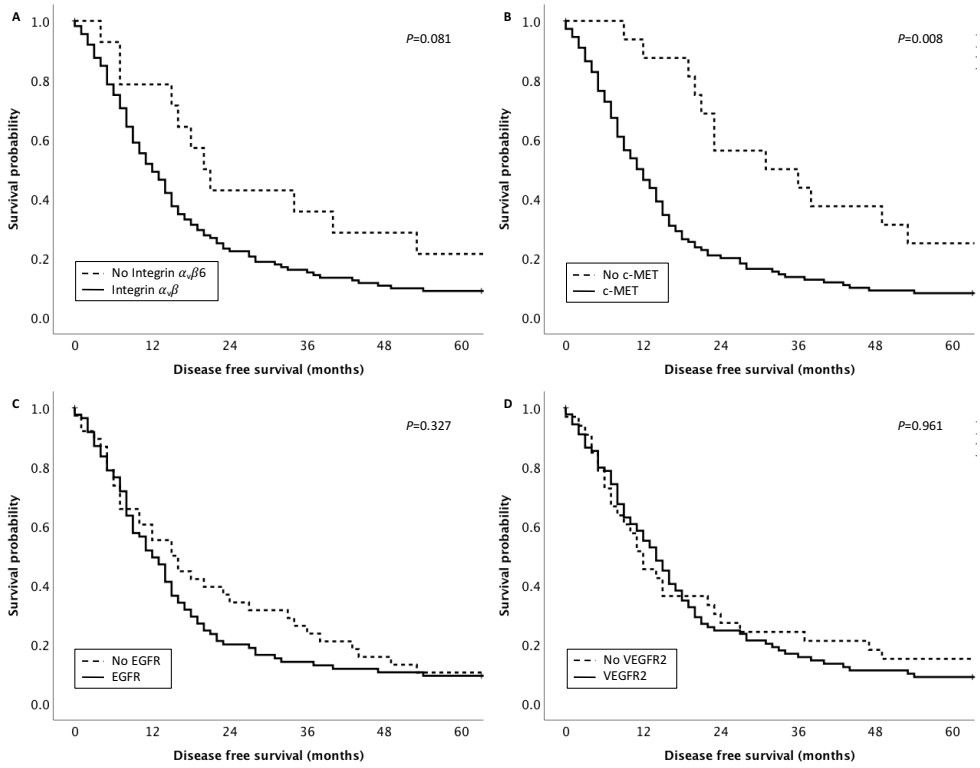

Figure 3. Kaplan-Meier survival curves for disease-free survival by integrin αvβ6 (A), c-MET (B), EGFR (C), and VEGFR2 (D) expression tumor expression, determined by immunohistochemical staining, in pancreatic adenocarcinoma.

Disease free survival

Recurrence was documented for 94% (n=124) of patients, with a median DFS of 13 months. Integrin $\alpha_v\beta_6$ expression was associated with a trend towards decreased survival (median DFS: 12 vs. 22 months; log-rank p=0.081; Figure 3a). Patients with c-MET expression had significantly worse DFS compared to patients with absent or low c-MET expression (median DFS: 12 vs. 31 months; log-rank p=0.008; Figure 3b). EGFR (median DFS: 12 vs. 15 months; log-rank p=0.327; Figure 3c) and VEGFR2 (median DFS: 12 vs. 14 months; log-rank p=0.961; Figure 3d) expression did not significantly impact DFS.

On multivariable analysis, c-MET expression remained predictive for decreased median DFS (HR, 1.795; P=0.037). However, integrin $\alpha_v\beta_6$, EGFR and VEGFR2 expression did not significantly impact survival (Table 3).

Table 3. Uni- and multivariate Cox proportional hazard regression analysis for disease-free survival.

	Univariate			Multivariate		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (≥ 65 vs. < 65 years)	1.287	0.899 – 1.845	0.169			
Sex (male vs. female)	1.173	0.822 – 1.674	0.378			
Tumor location (caput vs. other)	1.073	0.542 – 2.125	0.840			
pT-stage (pT2 vs. pT1)	1.476	0.931 – 2.340	0.098			
pT-stage (pT3 vs. pT2)	1.453	0.839 – 2.518	0.182			
pT-stage (pT4 vs. pT3)	0.920	0.398 – 2.130	0.846			
pN-stage (pN1 vs. pN0)	1.556	0.976 – 2.481	0.063	1.564	0.923 – 2.647	0.096
pN-stage (pN2 vs. pN0)	1.731	1.056 – 2.839	0.030	1.756	1.024 – 3.010	0.041
Resection margin status (R1 vs. R0)	1.466	1.006 – 2.138	0.047	1.413	0.932 – 2.142	0.103
Adjuvant therapy (no vs. yes)	1.431	0.995 – 2.056	0.053	1.940	1.302 – 2.892	0.001
Integrin αvβ6 (high- vs. low-expression)	1.636	0.927 – 2.889	0.089	1.624	0.896 – 2.944	0.110
VEGFR2 (high- vs. low-expression)	1.010	0.670 – 1.523	0.962			
EGFR (high- vs. low-expression)	1.212	0.818 – 1.795	0.337			
c-MET (high- vs. low-expression)	2.021	1.181 – 3.458	0.010	1.795	1.034 – 3.115	0.037

Abbreviations: c-MET, hepatocyte growth factor receptor; CI, Confidence Interval; EGFR, epithelial growth factor receptor; HR, Hazard Ratio; VEGFR2, vascular endothelial growth factor receptor 2

DISCUSSION

Accurate prognostic assessment of pancreatic cancer patients is essential for the appropriate selection of surgical candidates and allocation of neoadjuvant therapy. In routine, clinical practice preoperative assessment includes a multiphase computer tomography scan of the abdomen and pelvis, as well as serum levels of carbohydrate antigen 19-9 (CA 19-9) (2). However, micro-metastases often remain undetected and early metastases after upfront surgery remain common (3). In this study, patients with pancreatic adenocarcinoma expressing integrin $\alpha_v\beta_6$ had a significantly shorter OS compared to patients with low integrin $\alpha_v\beta_6$ expression (HR, 1.981; p=0.037). Similarly, c-MET upregulation was associated with unfavorable OS (HR, 1.766; p=0.051). These findings suggest that integrin $\alpha_v\beta_6$ and c-MET could potentially aid in identifying those patients at risk for early recurrence after surgery.

In line with our findings, expression of integrin $\alpha_v\beta_6$ has been associated with poor survival in variety of human cancers, including colorectal and gastric cancer, as well as in pancreatic adenocarcinoma (26-29). Upregulation of integrin $\alpha_v\beta_6$ has been recognized to play a critical role during tissue remodeling, including inflammation, wound healing, and angiogenesis (30, 31). Integrin's have not only shown promise as prognosticators, but also as pharmacological targets due to their location on the cell surface. Reader et al (2019) have demonstrated that integrin $\alpha_v\beta_6$-positive human pancreatic adenocarcinoma xenografts and transgenic mice bearing integrin $\alpha_v\beta_6$-positive pancreatic adenocarcinoma treated with integrin $\alpha_v\beta_6$ blocking antibody combined with gemcitabine significantly reduced tumor growth and increased survival (32).

Activation of c-MET by hypoxia or its ligand (hepatocyte growth factor/scatter factor) upregulates multiple neoplastic processes, including tumor invasion, migration, and

angiogenesis (33). Although, previous studies investigating the prognostic value of c-MET expression in pancreatic cancer have shown ambiguous results, the majority found an associated between c-MET expression and poor survival (18, 28, 34). A meta-analysis found that overall compared with pancreatic cancer patients showing low c-MET expression, patients with c-MET high tumors had significantly worse overall survival (HR, 1.96; $p<0.0001$) (35). Brandes et al (2015) have also shown that treatment with a c-MET inhibitor prolonged survival in an orthotropic syngeneic mouse model (36). These findings are consistent with the results of this study.

Following activation by its ligand VEGF, the tyrosine kinase receptors VEGFR2 mediates angiogenesis, potentially contributing to more aggressive tumor behavior (37). Although previous studies demonstrated an association between VEGF, VEGFR1, and VEGFR2 expression with poor survival and formation of liver metastases in pancreatic adenocarcinoma (38-41). Variability in the interpretation and comparison of immunohistochemically studies, include variation in patient selection, disparate immunohistochemistry staining evaluation criteria, and publication bias arising as a result of selective reporting of 'positive studies'(42).

EGFR is the cell surface receptor for a family of extracellular ligands, which include EGFR and TGF-α. Activation of EGFR leads to signaling cascade, which eventually promotes cell proliferation and angiogenesis (43). Previous immunohistochemisical analyses investigating the association between EGFR overexpression and overall survival demonstrated ambivalent results. However, similar to the results of the study, the majority of these studies found a negative association between EGFR overexpression and survival (42, 44-46). Likewise, prospective randomized trials investigating the value of adding erlotinib, a small molecule EGFR inhibitor, to chemotherapy or chemoradiation in patients with locally advanced pancreatic cancer have shown no difference in overall survival (47, 48).

The results of this study should be considered in light of its limitations. First, the use of tissue microarrays could have underestimated the true frequency of the molecular markers accessed in this study, especially in patients with focal expression. However, the tissue micro arrays used in this study contained relatively large punches (2 mm) and three punches for every included patient, decreasing the likelihood of underestimating the extend of focal disease. Second, all patients were treated at a single center in the Netherlands with a relatively heterogeneous patient population. Consequently, validation of these findings in an international multi-center study is pivotal. Finally, all specimens were obtained postoperatively. Clinical implementation would require sufficient tumor tissue to be acquired from preoperative biopsies, which might be challenging. Promising alternative approaches would be the use of 'liquid biopsies', such as the analyses of extracellular vesicles, and circulating tumor cells (5). However, the results of this study do not necessary translate to these emerging techniques.

Despite these limitation, we believe the results shown are an important step toward furthering our knowledge of the molecular landscape of pancreatic adenocarcinoma. The findings of this study suggest that high expression of integrin $\alpha_v\beta_6$ and c-MET was frequently observed in resected pancreatic adenocarcinoma patients and levels correlated with worse OS after pancreatic cancer surgery. These results suggest that these molecular markers may serve as predictive markers for patients who have a unfavorable prognosis after surgery and are at risk of early metastasis. Integrin $\alpha_v\beta_6$ and c-MET may not only serve as prognostic markers, but could also have additional value as therapeutic or imaging targets. Further studies validating these findings in large prospective studies are a necessary step on the path to clinical utilization.

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Chapter 4

Prognostic impact of urokinase plasminogen activator receptor expression in pancreatic cancer: malignant versus stromal cells

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Biomarker Insights 2017 June; 22(12):1-9

ABSTRACT

Background: The urokinase plasminogen activator receptor (uPAR) has been proposed as a potential prognostic factor for various malignancies. The aim of this study is to assess the prognostic value of uPAR expression in neoplastic and stromal cells of pancreatic adenocarcinoma patients.

Methods: uPAR expression was determined by immunohistochemistry in 122 pancreatic ductal adenocarcinomas. Kaplan-Meier and Cox regression analyses were used to determine the association with survival.

Results: Respectively 66%, 82% and, 62% of pancreatic cancer patients expressed uPAR in neoplastic cells, stromal, and in both combined. Multivariate analysis showed a significant inverse association between uPAR expression in both neoplastic and stromal cells and overall survival.

Conclusions: The prognostic impact of uPAR in stromal cells is substantial, but not as pronounced as that of uPAR expression in neoplastic cells. This study suggests a role for uPAR as a biomarker to single out higher risk subgroups of pancreatic cancer patients.

INTRODUCTION

Pancreatic cancer ranks the fourth leading cause of cancer-related death and is estimated to be the second leading cause of cancer death by 2020.[1,2] Complete surgical resection offers the only hope for cure; however, even after successful tumour removal, recurrence rates range from 46% to 89%.[3-8] Currently, anatomic resectability and carbohydrate antigen 19-9 (CA 19-9) serum levels are the most commonly used prognostic factors to select optimal treatment strategies for non-metastatic pancreatic cancer patients, but unfortunately with only modest impact.[9,10] Consequently, there is a necessity for novel molecular markers that are able to predict biological behaviour in order to identify patients requiring more aggressive systemic and/or surgical treatment.

Proteolysis via the plasminogen activation cascade is a crucial biological process involved in cancer cell invasion and metastasis. The urokinase plasminogen activator receptor (uPAR), a glycosyl-phosphatidylinositol-anchored membrane protein, plays a dominant role in this cascade by localizing the urokinase plasminogen activator (uPA) to the cell membrane.[11] After binding to uPAR, uPA converts the inactive zymogen plasminogen into plasmin. This active serine protease subsequently activates other proteinases, resulting in the proteolysis of basement membrane proteins and extracellular matrix.[12] Considerable evidence indicates that uPAR expression in neoplastic cells, as well as stromal cells, is correlated with shortened survival in various malignancies, including colorectal, breast, and renal carcinoma.[13-21]

In pancreatic cancer, uPAR expression has been observed in both tumour and surrounding stromal cells. However, it remains unclear which cellular uPAR localization is more immediately involved with tumour behaviour and therefore associated with patient prognosis.[15,22] In the present immunohistochemistry study, performed in a large cohort of patients with pancreatic adenocarcinoma, the expression pattern of uPAR in both tumour and stromal cells, and its clinical implications were evaluated.

METHODS

Patient selection

Retrospectively collected, formalin-fixed and paraffin-embedded tissue blocks were obtained from the archives of the Pathology Department for 137 patients with pancreatic adenocarcinoma, who underwent resection with curative intent during the period from 2001 and 2012 at the Leiden University Medical Centre, Leiden, The Netherlands. Only pancreatic adenocarcinoma were included in this study. None of the patients in this study received chemotherapy and/or radiation prior to surgery. Clinicopathological data were collected from electronic hospital records. Differentiation grade was determined according to the guideline of the World Health Organization and the TNM-stage was defined according to the American Joint Commission on Cancer criteria[23]. All samples were non-identifiable and used in accordance to the code for proper secondary use of

human tissue as prescribed by the Dutch Federation of Medical Scientific Societies. The use of archived human tissue conformed to an informed protocol that had been reviewed and approved by the institutional review board of the Leiden University Medical Centre, Leiden, The Netherlands.

Immunohistochemistry

Tissue microarrays (TMAs) of pancreatic adenocarcinoma were constructed to perform uniform and simultaneous immunohistochemical staining's to limit intra-assay variation. A single representative block was selected for each patient based on haematoxylin-eosin stained sections. From each donor block, triplicate 2.0 mm cores were punched from areas with clear histopathological tumour representation and transferred to a recipient TMA block using the TMA Master (3DHISTECH, Budapest, Hungary). From each completed TMA block, 5-µm sections were sliced. The sections were deparaffinized in xylene and rehydrated in serially diluted alcohol solutions, followed by demineralized water according to standard protocols. Endogenous peroxidase was blocked by incubation in 0.3% hydrogen peroxide in phosphate buffered saline (PBS) for 20 min. Antigen retrieval was performed by heat induction at 95°C using PT Link (Dako, Glostrup, Denmark) with a low-pH Envision FLEX target retrieval solution (citrate buffer pH 6.0, Dako). Immunohistochemical staining was performed by incubating tissue microarrays overnight with antibodies against uPAR (ATN-615, provided by Prof A.P. Mazar),[24] alpha smooth muscle actin (α-SMA) for myofibroblasts (PA5-16697; Thermo Fisher Scientific), and vimentin for mesenchymal cells (clone V9, Santa Cruz, USA), all at room temperature. All antibodies were used at predetermined optimal dilutions using proper positive and negative control tissue: ATN-615 at 1 µg/ml; PA5-16697 at 0.25 µg/ml; V9 at 2 µg/ml). Control samples were incubated with PBS instead of the primary antibodies. The sections were washed with PBS, followed by incubation with Envision anti-mouse (K4001; Dako) or Envision anti-Rabbit (K4003; Dako), where applicable, for 30 minutes at room temperature. After additional washing, immunohistochemical staining was visualized using 3,3-diaminobenzidine tetrahydrochloride solution (Dako) for 5-10 min resulting in brown colour, and counterstained with haematoxylin, dehydrated and finally mounted in pertex.

Immunohistochemistry evaluation

All stained sections were scanned and viewed at 200x magnification using the Philips Ultra Fast Scanner 1.6 EA (Philips, Eindhoven, The Netherlands). Evaluation of the immunohistochemical staining of all molecular targets was performed blinded and independently by two observers (S.G. and H.P.). In cases of discrepancy the two observers resolved the final score in accordance with a pathologist (H.M.). Immunostaining positivity was determined by a combination of staining intensity and percentage of tumour cells stained. Immunostaining intensity was scored as 0 = negative, 1 = weakly positive, 2 = moderately positive, and 3 = strongly positive. However, in this relatively small cohort the

staining intensity did not contribute substantially to the survival analyses. Therefore, in the final analysis percentages of uPAR staining in neoplastic cells were dichotomized as low (<50% moderate/strong expression) or high (≥50% moderate/strong expression)[21]. As described in a previous study, the staining results for α-SMA were scored, according to the extent of stromal positivity, as low/negative (<50% stroma positive) or high (diffuse expression throughout tumour, > 50% stroma positive).[25]

Statistical analysis

All statistical analyses were conducted using SPSS statistical software (version 23.0, IBM SPSS Inc, Chicago, USA). Baseline characteristics were reported as frequencies, and continuous data were presented as median with interquartile range [IQR] unless indicated otherwise. Comparison of the clinical and pathological characteristics of the two cohorts were made using the Chi-squared test. Fisher's exact test was used when one of the groups counted less than 5. Disease-free survival (DFS) was defined as the time from surgery to the first evidence of local or distant recurrence disease, death from any cause or lost to follow-up, whatever came first. Overall Survival (OS) was defined as the time from the date of surgery to the date of death or lost to follow-up. Kaplan-Meier estimates of the survival function, including *p*-values from the log-rank test were used to graphically compare the time-to-event outcomes based on uPAR expression and to estimate median OS and DFS. Furthermore, uni- and multivariate survival analyses were performed using the Cox proportional hazard regression model. Only variables that were significant on univariate analysis were included in multivariate analyses. Separate multivariate models were employed, one including uPAR expression in neoplastic and stromal cells as different covariates, and another incorporating uPAR expression in both neoplastic and stromal cells as one covariate. In case the proportional hazard assumption was violated the log-rank test was used and subsequently these covariates could not be included in the multivariate regression model.[26]

RESULTS

Patient and tumor characteristics

Microscopic semi-quantification of uPAR expression in neoplastic and stromal cells was successful in 89% (n=122) of pancreatic adenocarcinoma. Patient and tumor characteristics are listed in Table 1. The median age was 65 years (IQR, 60 – 72 years), 62 (51%) patients were female and 114 (93%) patients were diagnosed with pancreatic adenocarcinoma located in the head of the pancreas. Primary tumor stage was classified as pT1 in 17 (14%) patients, pT2 in 32 (26%), pT3 in 65 (53%) and pT4 in 8 (7%) patients. In addition, the majority of patients had positive nodes (n=93; 76%) and moderately

differentiated tumors (n=41; 45%). Complete surgical resection (R0) was possible in 83 (68%) cases and 61 (50%) patients underwent adjuvant chemotherapy after surgical resection.

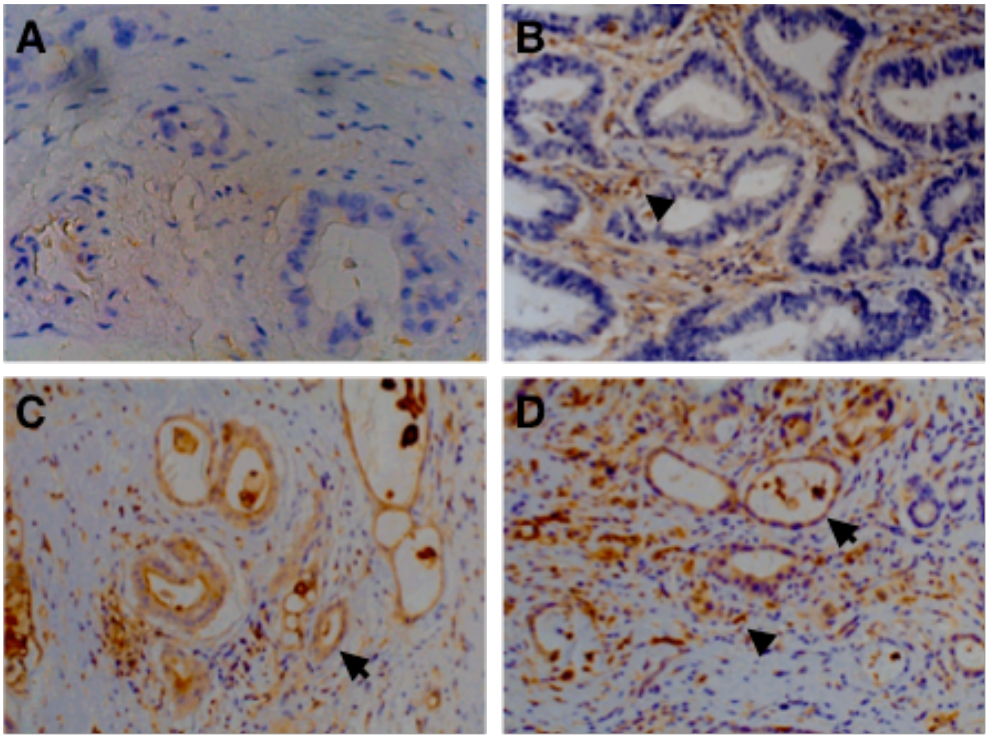

Figure 1. Representative images of pancreatic adenocarcinoma showing low urokinase plasminogen receptor (uPAR) expression in neoplastic epithelial (arrow) and stromal cells (arrow head). A) Low uPAR expression; B) uPAR expression only in stromal cells; uPAR expression in stromal and neoplastic cells (C, D) (200x magnification).

uPAR expression

In pancreatic adenocarcinoma, uPAR expression was detected in both neoplastic cells and tumour-associated stroma cells, including myofibroblasts and other mesenchymal cell, as identified by staining for α-SMA and vimentin (Figures 1 and 2). uPAR expression was elevated in neoplastic cells in 66% of the cases (n=81) and in tumour-associated cells in 82% (n=100). A significant correlation (p<0.001) was found between uPAR expression in neoplastic and tumor-associated stromal cells. 62% (n=75) of the pancreatic adenocarcinoma patients demonstrated uPAR overexpression in both neoplastic and tumor-associated tumor cells.

uPAR expression in stromal cells was significantly associated (p=0.049) with age < 65 years, whereas uPAR expression in both neoplastic and stromal cells correlated (p=0.015) with more advanced pT-stage. No association was found between baseline

clinicopathological characteristics and uPAR expression in either neoplastic and/or stromal cells (Table 1).

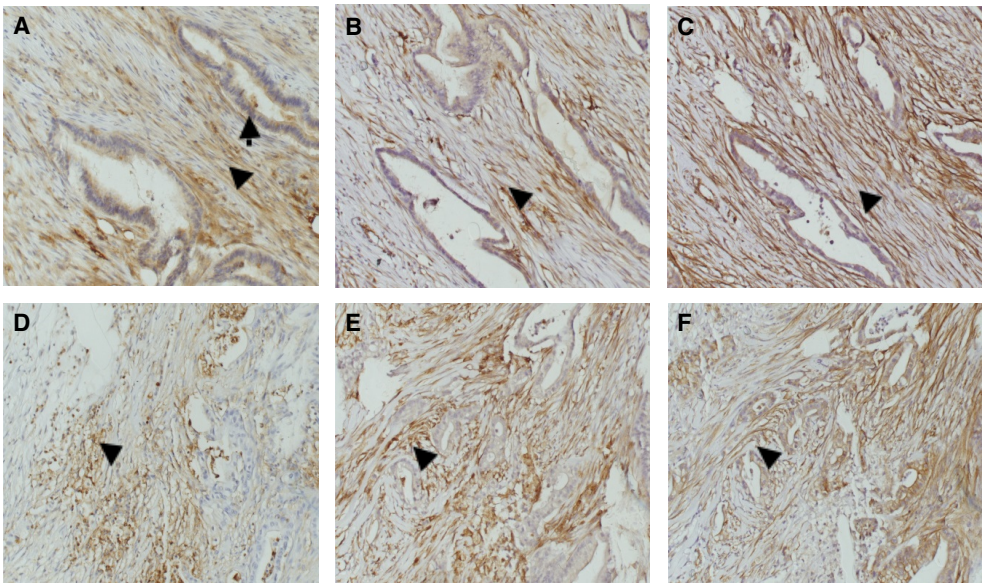

Figure 2. Representative images of immunohistochemical staining's on consecutive tissue sections demonstrating the presence of urokinase plasminogen receptor (A, D), vimentin (B, E) and alpha smooth muscle actin (C, F) in pancreatic adenocarcinoma (200x magnification). Arrows and arrow heads indicate respectively epithelial and stromal cells.

Overall survival

At time of analysis, 91% (n=111) of the study population was deceased. The median overall survival in the overall cohort was 17 months (95% CI, 15 – 19 months). Using univariate analysis, age, sex, tumour location, pT-stage, tumour differentiation, and treatment with adjuvant therapy (log-rank p=0.382) were not associated with overall survival. However, positive lymph nodes, uPAR expression in neoplastic cells (median OS, 14 vs. 23 months; Figure 2a), uPAR expression in stromal cells (median OS, 16 vs. 21 months; Figure 2c), and uPAR expression in both neoplastic and stromal cells (median OS, 13 vs. 24 months; p<0.001; Figure 2e) were significantly predictive for OS (Table 2).

In multivariate analysis, positive lymph nodes and uPAR expression in neoplastic cells were independent prognostic factors for OS, but uPAR expression in stromal cells did not keep its significance. On separate multivariate analysis, positive lymph nodes, and uPAR expression in both neoplastic and stromal cells were also significant prognostic factors for OS in pancreatic cancer (Table 2

Table 1. Characteristics of pancreatic adenocarcinoma patients subdivided by urokinase plasminogen activator receptor (uPAR) expression in neoplastic and/or stromal cells.

Characteristics	uPAR in neoplastic cells			uPAR in stromal cells		
	Low (n = 41)	High (n = 81)	*p*	Low (n = 22)	High (n = 100)	*p*
Age, n (%)						
<65 years	17 (42%)	45 (56%)	0.141	7 (32%)	55 (55%)	0.049
≥65 years	24 (58%)	36 (44%)		15 (68%)	45 (45%)	
Sex, n (%)						
Male	20 (49%)	40 (49%)	0.950	11 (50%)	49 (49%)	0.932
Female	21 (51%)	41 (51%)		11 (50%)	51 (51%)	
Tumor location, n (%)						
Head of pancreas	38 (93%)	76 (94%)	0.549	21 (96%)	93 (93%)	0.662
Other	3 (7%)	5 (6%)		1 (4%)	7 (7%)	
pT-stage, n (%)						
pT1	9 (22%)	8 (10%)	0.074	5 (23%)	12 (12%)	0.163
pT2	14 (34%)	18 (22%)		8 (36%)	24 (24%)	
pT3	16 (39%)	49 (61%)		7 (32%)	58 (58%)	
pT4	2 (5%)	6 (7%)		2 (9%)	6 (6%)	
pN-stage, n (%)						
pN0	8 (20%)	21 (26%)	0.432	4 (18%)	25 (25%)	0.496
pN1	33 (80%)	60 (74%)		18 (82%)	75 (75%)	
Tumor differentiation, n (%)*						
Well differentiated	7 (22%)	4 (7%)	0.093	3 (18%)	8 (11%)	0.426
Moderately differentiated	14 (44%)	27 (46%)		9 (53%)	32 (43%)	
Poorly differentiated	11 (34%)	28 (47%)		5 (29%)	34 (46%)	
Adjuvant therapy, n (%)						
Yes	19 (46%)	42 (52%)	0.565	11 (50%)	50 (50%)	>0.999
No	22 (54%)	39 (48%)		11 (50%)	50 (50%)	

*Tumour differentiation was only available for 75% (n=91) of the population.; significant *p*-values are bold

Table 2. Uni- and multivariate Cox proportional hazard regression analyses for the predictive value of urokinase plasminogen activator receptor (uPAR) expression on overall survival of pancreatic cancer patients.

Covariates	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Age (≥65 vs. 65 years)	1.11	0.76 – 1.60	0.598			
Sex (male vs. female)	1.38	0.95 – 2.00	0.096			
pT-stage (pT3-4 vs. pT1-2)	1.46	0.99 – 2.17	0.058			
pN-stage (pN1 vs. pN0)	1.86	1.16 – 3.00	0.011	1.96	1.21 – 3.15	0.006
Tumour differentiation* (well/moderately vs. poorly)	1.24	0.80 – 1.91	0.340			
uPAR in neoplastic cells (high vs. low)	1.93	1.28 – 2.91	0.002	1.83	1.17 – 2.85	0.008
uPAR in stromal cells (high vs. low)	1.70	1.03 – 2.81	0.036	1.31	0.76 – 2.25	0.334

*Tumour differentiation was available for 75% (n=95) of the population.

Disease-free survival

35% (n=35) of all patients reported local recurrence, 63% (n=64) distant recurrence, 42% (n=42) liver metastasis, 22% (n=22) lung metastasis, and 20% (n=20) local and distant recurrence. uPAR expression in stromal cells (p=0.018) was associated with the development of liver metastases. No correlations between uPAR expression and specific types of recurrence were found.

Multivariate analysis showed that positive lymph nodes, uPAR expression in neoplastic cells and uPAR expression in both neoplastic and stromal cells were independently associated with poor DFS (Table 3).

Table 3. Uni- and multivariate Cox proportional hazard regression analyses for the predictive value of urokinase plasminogen activator receptor (uPAR) expression on disease-free survival in pancreatic adenocarcinoma patients.

Covariates	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Age (≥65 vs. 65 years)	1.03	0.71 – 1.49	0.892			
Sex (female vs. male)	1.21	0.83 – 1.76	0.319			
pT-stage (pT3-4 vs. pT1-2)	1.01	1.00 – 1.03	0.153			
pN-stage (pN1 vs. pN0)	1.86	1.15 – 2.99	0.011	1.95	1.21 – 3.14	0.006
Tumour differentiation* (well/moderately vs. poorly)	1.16	0.75 – 1.80	0.511			
uPAR in neoplastic cells (high vs. low)	1.72	1.15 – 2.58	0.009	1.66	1.06 – 2.58	0.025
uPAR in stromal cells (high vs. low)	1.58	0.97 – 2.58	0.065	1.26	0.74 – 2.15	0.394

*Tumor differentiation was available for 75% (n=95) of the population

DISCUSSION

Outcomes after resection for pancreatic adenocarcinoma are variable and contingent on both the biology of the disease and the efficacy of the treatment. Determination of proteins or pathways that increase risk of recurrence and impair survival might be helpful to assist with the selection of pancreatic cancer patients who could benefit from (neo)adjuvant and targeted-therapies. The results of this immunohistochemical study reveal a significant inverse correlation between uPAR expression and OS and DFS of pancreatic cancer patients. The prognostic impact of uPAR in stromal cells is striking, but it is not an independent parameter, like uPAR expression in neoplastic cells.

Relatively few studies have analysed tissue expression of uPAR and its association with prognosis in pancreatic cancer[27]. In 1997 Cantero and co-workers were the first to report worse survival for patients with high uPAR positive pancreas tumours in a small cohort of 30 patients. [15]. Although they noticed uPAR staining in malignant epithelial cells and stroma cells, they did not correlate these separately with survival. More than 10 years later the level of uPAR mRNA was shown not to be correlated with prognosis in a small cohort of 25 patients, whereas in another study with 46 patients uPAR mRNA appeared to be the strongest biological prognostic marker [28,29]. The prognostic association of uPAR with pancreatic cancer was further confirmed by the measurement of high levels of soluble uPAR in urine of these patients [30]. Our data, in a relatively large cohort of pancreatic cancer patients, confirm the association between uPAR and survival. This suggests a role for uPAR as a potential independent indicator for the identification of higher risk patient subgroups, like has been found for other tumour types, including colorectal, breast, and lung cancer.[20,31,32]

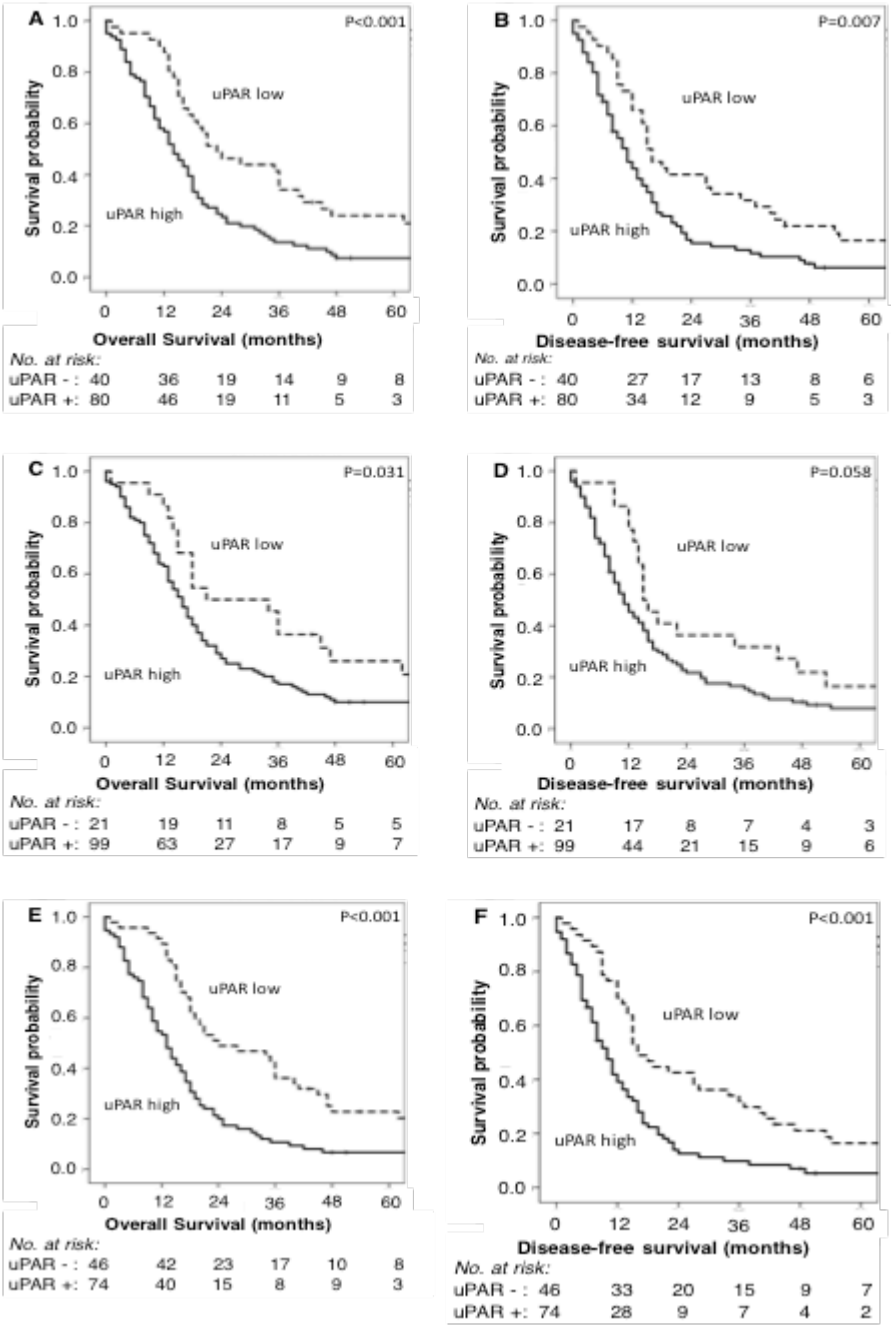

Figure 3. Kaplan-Meier curves for overall and disease-free survival for pancreatic adenocarcinoma patients after surgical treatment, stratified by the status of urokinase plasminogen receptor (uPAR) expression in neoplastic cells (A, B), uPAR expression in stromal cells (C,D), and in both neoplastic and stromal cells (E, F).

uPAR enhancement on malignant cells can partly be explained by oncogenic amplification of the PLAUR gene, as has been identified by Ströbel and co-workers in 52% of the cases in a cohort of 50 pancreatic cancer patients.[22] However, uPAR up-regulation in neoplastic cells is not dependent solely on gene amplification, as uPAR expression is also up-regulated by several oncologic pathways in which transcription factors like AP1 and PEA3/ETS are involved.[11] Furthermore, environmental factors like TNF-α and interleukins can enhance uPAR expression, which could partly explain the up-regulation in tumour stromal cells, like myofibroblasts, macrophages and endothelial cells.[33] Up-regulation of uPAR in these cells has no genetic background and is primarily a response to signals from the cancer cells. The association of uPAR up-regulation in stromal cells with survival, as found in this study, turned out not to be independent in multivariate analyses, like has been found in other tumour types.[21,34-36] However, in these other tumours the uPAR positive stromal cells were often located at the invasive front, which seems not specifically the case for uPAR expressing stromal cells in pancreatic cancer.

Just the presence of uPAR on certain cell types does not contribute to the malignancy of a tumour and could not explain a prognostic relevance. As a receptor, uPAR is strongly dependent on its interaction with other proteins for its functions[11]. The most obvious function of uPAR is the stimulation of proteolysis, which does not exist without the presence of plasminogen and uPA, and is otherwise tightly regulated by the presence of inhibitors PAI-1 and PAI-2. The chemotactic function of uPAR depends on cleavage by uPA, where again the inhibitors play a regulatory role. Also uPAR-mediated intracellular signalling relies on the binding of uPA, vimentin, and several integrins as ligands. Because uPAR itself does not contain an intracellular domain, these signals are transduced by other, 'professional' signalling proteins with transmembrane and intracellular domains like tyrosine kinase receptors, g-protein coupled receptors, and integrins.[37] All these interactions between uPAR and other proteins, plus the shedding of one of the 3 domains by uPA influence the 3-dimensional structure of uPAR. Therefore it is well established that different anti-uPAR antibodies with varying epitope specificity result in different immunohistochemical staining patterns.[38] Obviously, part of the discrepancies regarding the prognostic value of uPAR in pancreatic cancer described in the literature may be explained by the use of antibodies targeting different domains within the uPAR protein. In the present study the extensively validated antibody ATN-615 was used, which detects almost all forms of uPAR, probably explaining the abundant presence of uPAR in multiple cell types in comparison with some other studies.[24]

Another difference with previous studies is the use of a TMA, which might also be the biggest limitation of this study. Although the tumour areas were carefully selected to represent a complete overview of the tumour, the possibility of discrepant patterns of uPAR in comparison with conventional tissue sections is not ruled out. However, previous studies in breast cancer demonstrated that analysis of at least two cores on the tissue micro array is

comparable to the analysis of whole tissue sections in >95% of cases.[39] Another restriction of this study is that patients with metastatic unresectable disease at time of diagnosis could not be included, since these patients rarely have adequate tissue for detailed immunohistochemical evaluation. Considering the uPAR distribution in stage and grade, it seems not likely that including these patients with expected bad prognosis would have influenced the analysis dramatically.

Next to a possible application as prognostic marker, uPAR may also hold promise as a selective target for either tumour-specific image-guided surgery or targeted-therapy, because of its absence in normal pancreatic tissue and chronic pancreatitis.[40,41] A pre-clinical study has indeed demonstrated the ability of uPAR-targeted NIR-dye-labelled theranostic nanoparticles, to visualize residual disease in pancreatic xenografts.[42] Furthermore, uPAR-targeted magnetic iron oxide nanoparticles carrying gemcitabine were able to overcome the tumour stromal barrier and subsequently were able to enhance the efficiency of the drug. This is particularly relevant, as high resistance to therapy is a major challenge in pancreatic cancer care.[43,44]

In summary, this study demonstrates in a relatively large cohort of pancreatic adenocarcinoma patients, that uPAR expression, in particular determined in stromal cells as well as in cancerous cells, is predictive for unfavourable OS and DFS. Evaluation of uPAR expression, alone or in combination with other predictive factors, may improve the identification of patients who could benefit from more aggressive treatment. Although the combination of uPAR determination in neoplastic cells and stromal cells seemed to have the highest prognostic impact, further studies for better understanding of the mechanisms involved are still necessary.

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Chapter 5

Selecting tumor-specific molecular targets in pancreatic adenocarcinoma: paving the way for image-guided pancreatic surgery

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Molecular Imaging and Biology 2016 Dec;18(6):807-819

ABSTRACT

Background: The purpose of this study was to identify suitable molecular targets for tumor-specific imaging of pancreatic adenocarcinoma.

Methods: The expression of 8 potential imaging targets was assessed by the target selection criteria (TASC) – score and immunohistochemical analysis in normal pancreatic tissue (n=9), pancreatic (n=137) and periampullary (n=28) adenocarcinoma.

Results: Integrin $\alpha_v\beta_6$, carcinoembryonic antigen (CEA), epithelial growth factor receptor (EGFR) and urokinase plasminogen activator receptor (uPAR) showed a significantly higher (all $p<0.001$) expression in pancreatic adenocarcinoma compared to normal pancreatic tissue and were confirmed by the TASC score as promising imaging targets. Furthermore, these biomarkers were expressed in respectively 88%, 71%, 69% and 67% of the pancreatic adenocarcinoma patients.

Conclusions: The results of this study show that integrin $\alpha_v\beta_6$, CEA, EGFR, and uPAR are suitable targets for tumor-specific imaging of pancreatic adenocarcinoma.

INTRODUCTION

Pancreatic adenocarcinoma currently ranks the fourth leading cause of cancer-related death in the Western World, with a 5-year survival rate of less than 5%.[1] Radical surgical tumor resection is imperative to curative treatment of these patients as positive resection margins (defined as tumor cells present at the surface of the resection margins of the surgical specimen) are associated with a dramatic decrease in median overall survival[1-4]. Unfortunately, positive resection margins are common after pancreatic surgery and reported rates vary between 24% and 76%[5-7]. Adjuvant therapy cannot retaliate the poor survival outcome associated with residual disease[8]. The disappointing irradical resection rates after pancreatic surgery are due to our current inability to detect the true delineation of the tumor extent during surgery, which is further complicated by the intricate anatomy of the pancreas and the commonly present peritumoral inflammatory zone in pancreatic cancer. Conventional anatomic imaging modalities used for preoperative diagnosis, staging and surgical planning, include multi-phase intravenous contrast directed thin slice computed tomography, magnetic resonance imaging, endoscopic ultrasonography and endoscopic retrograde cholangiopancreatography[9-10]. However, the translation of these preoperative imaging techniques to the surgical field remains challenging and in the theater the surgical oncologist solely has to rely on vision and manual palpation to discriminate between malignant and healthy pancreatic tissue, assisted by ultrasonography and pathologic evaluation of frozen tissue sections[10].

Intraoperative tumor-specific imaging offers the opportunity to significantly improve current practice by increasing the capability to obtain negative resection margins and visualize residual disease during pancreatic surgery. This novel imaging approach uses labeled receptor ligands, nanoparticles, antibodies or antibody fragments targeting cancer-specific antigens on the tumor surface detected by positron emission tomography, single-photon emission computed tomography, ultrasonography, magnetic resonance and/or near-infrared fluorescence imaging modalities[11-13]. The feasibility of these imaging techniques has already successfully been proven in glioma and ovarian cancer surgery using respectively the fluorescent agents 5-aminolevulinic acid and folate conjugated to fluorescein isothiocyanate[11, 14]. Furthermore, the potential of image-guided surgery in pancreatic adenocarcinoma has been demonstrated by numerous preclinical studies using cancer-specific contrast-agents targeting integrin $\alpha_v\beta_6$, carcinoembryonic antigen (CEA), epithelial growth factor receptor (EGFR), human epidermal growth factor receptor (HER2), urokinase plasminogen activator receptor (uPAR), or vascular endothelial growth factor receptor 2 (VEGFR2) among others (table 1). Nevertheless, the orthotopic mouse models used in these studies are based on a small number of pancreatic adenocarcinoma cell lines originating from single patients and therefore less representative for the potential of these imaging probes in the overall population of pancreatic cancer patients. The translation from bench to bedside of this promising imaging strategy for pancreatic adenocarcinoma

currently hinges on the lack of tumor-specific and thoroughly evaluated molecular targets expressed on the general population of pancreatic adenocarcinoma patients for the further development of tumor-targeting contrast agents[15-16].

Therefore, the aim of this study was to explore the suitability of integrin $\alpha_v\beta_6$, CEA, hepatocyte growth factor receptor (cMET), EGFR, epithelial cell adhesion molecule (EpCAM), HER2, uPAR and VEGFR2 as molecular targets for tumor-targeted imaging of pancreatic adenocarcinoma patients. The primary endpoint of this study was to evaluate the ability of these markers to distinguish between normal pancreatic tissue and pancreatic and periampullary adenocarcinoma by performing immunohistochemistry on surgical specimen of these malignancies and normal pancreatic tissue obtained adjacent to the tumor. In addition, these biomarkers were judged on the Target Selection Criteria (TASC) proposed by Van Oosten et al[17].

MATERIALS AND METHODS

Patient selection

Medical records and pathology specimens of 137 patients with pancreatic ductal adenocarcinoma and 28 patients with periampullary adenocarcinoma who underwent pancreatic surgery at Leiden University Medical Center (LUMC) between June 2002 and July 2012 were retrospectively reviewed. Periampullary adenocarcinoma were included to assess the potential of tumor-specific imaging targets to visualize every pancreatic head mass, since preoperative differentiation between pancreatic, distal bile duct, ampullary, and duodenal adenocarcinoma can be challenging[18]. For the purpose of this study periampullary adenocarcinoma were defined as adenocarcinoma that invades the pancreas arising from the ampulla of Vater, duodenum or distal bile duct[19]. Patients who received any form of neoadjuvant chemotherapy and/or radiotherapy were excluded from this study, since this may influence the expression of molecular markers[20]. In addition, normal pancreatic tissue adjacent to the tumor was also obtained from 9 patients to evaluate the tumor specificity of the biomarkers. Clinicopathological data from these patients were retrospectively collected from electronic hospital records. Tumor differentiation grade was determined according to the guideline of the World Health Organization and the TNM-stage was defined according to the American Joint Commission on Cancer criteria[21]. All samples were non-identifiable and used in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

Immunohistochemistry

Tissue microarrays (TMA's) of tumor and normal tissues were constructed to perform uniform and simultaneous immunohistochemical stainings to limit intra-assay variations. Formalin-fixed paraffin-embedded tissue blocks of the primary tumor were

collected from the archives of the Pathology Department. A single representative block was selected for each patient based on hematoxylin-eosin stained sections. From each donor block, triplicate 2.0 mm cores were punched from areas with clear histopathological tumor representation and transferred to a recipient TMA block using the TMA Master (3DHISTECH, Budapest, Hungary). From each completed TMA block and normal pancreatic tissue block, 5µm sections were sliced. The sections were deparaffined in xylene and rehydrated in serially diluted alcohol solutions, followed by demineralized water according to standard protocols. Endogenous peroxidase was blocked by incubation in 0.3% hydrogen peroxide in phosphate buffered saline (PBS) for 20 min. For EpCAM, c-MET, HER2 and uPAR staining antigen retrieval was performed by heat induction at 95ºC using PT Link (Dako, Glostrup, Denmark) with a low-pH Envision FLEX target retrieval solution (citrate buffer pH 6.0, Dako). VEGFR staining required antigen retrieval with high-pH Envision FLEX target retrieval solution (Tris-EDTA pH 9.0, Dako). For staining of EGFR and integrin $\alpha_v\beta_6$, antigen retrieval was performed with 0.4% pepsin incubation for 10 minutes at 37ºC. CEA staining did not require antigen retrieval. Immunohistochemical staining was performed by incubating tissue microarrays overnight with antibodies against VEGFR2 (55B11; Cell Signaling Technology, Danvers, MA, USA), EpCAM (323A3, in-house produced hybridoma), c-MET (SC10; Santa Cruz Biotechnology, Santa Cruz, CA, USA), CEA (A0155; Dako, Glustrup, Denmark), EGFR (E30; Dako), integrin $\alpha_v\beta_6$ (6.2A; Biogen Idec MA Inc., Cambridge, MA, USA), HER2 (A0485; Dako) and uPAR (ATN-615, kindly provided by Prof A.P. Mazar, Northwestern University, Evanston, Il.) all at room temperature.[22,23] All antibodies were used at predetermined optimal dilutions using proper positive and negative control tissue. Furthermore, all antibodies selected for this study were solely selective for integrin $\alpha_v\beta_6$, CEA, cMET, EGFR, EpCAM, HER2, uPAR and VEGFR respectively, except for the CEA-antibody (A0155; Dako) that was also sensitive to CEA-like proteins (CEACAM1, CEACAM3, CEACAM4, CEACAM 6, CEACAM7, CEACAM 8) and the uPAR-antibody (ATN-615) that also recognizes the soluble form of uPAR suPAR.[22] Negative control samples were incubated with PBS instead of the primary antibodies. The sections were washed with PBS, followed by incubation with Envision anti-mouse (K4001; Dako) or Envision anti-Rabbit (K4003; Dako), where applicable, for 30 minutes at room temperature. After additional washing, immunohistochemical staining was visualized using 3,3-diaminobenzidine tertahydrochloride solution (Dako) for 5-10 min resulting in brown color and counterstained with hematoxylin, dehydrated and finally mounted in pertex. All stained sections were scanned and viewed at 40x magnification using the Philips Ultra Fast Scanner 1.6 RA (Philips, Eindhoven, Netherlands). The numerical value for overall intensity (intensity score) was based on a 4-point system: 0, 1, 2, and 3 (for none, light, medium, or high intense staining), as previously described by Choudhury et al., and staining was considered positive if > 10% of the tumor cells expressed a medium or dark

staining pattern. [23-29] Evaluation of the immunohistochemical staining of all molecular targets was performed blinded and independently by two observers (S.W.L.G. and H.A.J.M.P). In case of disagreement the stainings were discussed until agreement was reached.

Table 1a. Overview of the characteristics and preclinical experience with tumor-specific imaging of integrin $\alpha_v\beta_6$, carcinoembryonic antigen (CEA), hepatocyte growth factor receptor (cMET), epithelial growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), human epidermal growth factor receptor (HER2), urokinase plasminogen activator receptor (uPAR) and vascular endothelial growth factor receptor 2 (VEGFR2) in pancreatic adenocarcinoma animal models.

Target	Type of receptor (family)	Function
Integrin $\alpha_v\beta_6$	Transmembrane receptor (Integrin family of cell adhesion receptors)[63]	Controls extracellular matrix remodeling and provides the traction necessary for cell motility. Tumor cell migration, invasion and proliferation.[63]
CEA	Glycoprotein (Immunoglobulin superfamily)[69]	Tumor cell migration, circulation, implantation and proliferation, which is facilitated by the immunosuppressive effect of CEA.[70]
cMET	Tyrosine kinase receptor (HGFR family)[79]	Tumor cell proliferation, survival, motility and invasion. [79]
EGFR	Tyrosine kinase receptor (ErbB family)[80]	Induces tumor cell differentiation and proliferation.[81]
EpCAM	Transmembrane glycoprotein[87]	Tumor cell proliferation, migration and mitogenic signal transduction.[87]
HER2	Tyrosine kinase receptor (ErbB family)[88]	Tumor cell proliferation, survival, adhesion and migration.[88]
uPAR	GPI-anchored receptor (plasminogen activation system)[71]	Tumor cell migration, proliferation and survival.[90]
VEGFR2	Tyrosine kinases receptor (VEGFR family)[95]	Angiogenesis during tumorgenesis.[95]

Target selection criteria

The TASC–score is based on granting points for the following seven characteristics of suitable molecular targets: extracellular protein localization (receptor bound to cell surface, 5 points; in close proximity of the tumor cell, 3 points); diffuse up-regulation through tumor tissue (4 points); tumor-to-healthy cell (T/N) ratio (T/N ratio > 10, 3 points); high percentage up-regulation in patients (> 90%, 6 points; 70-90%, 5 points; 50-69%, 3 points; 10%-49%, 0 points); previous imaging success in vivo (2 points); enzymatic activity (1 point); and target-mediated internalization (1 point). All biomarkers were granted points for the seven characteristics and a total score of 18 or higher indicated that the biomarker is potentially suitable for tumor-targeted imaging in vivo.[17] Whereas, a T/N ratio could not be obtained from immunohistochemical staining we simplified the T/N ratio to a significant lower staining intensity in normal pancreatic tissue compared to

pancreatic and periampullary adenocarcinoma. For the purpose of this study, diffuse expression was defined as staining in ≥ 50% of tumor cells in the majority (>50%) of the patients; focal expression as staining in <50% of tumor cells in the majority (>50%) of the patients and negative expression as staining in 0% of the tumor cells in the majority (>50%) of the patients.

Statistical analysis

The statistical analysis was performed using SPSS version 23.0 software (SPSS, © IBM Corporation, Somer NY, USA) and GraphPad Prism 6 (Graphpad, Software, Inc, La Jolla XA, USA). Interobserver variation of immunohistochemical results was analyzed using Cohen's kappa coefficient and > 0.8 was considered as acceptable. Baseline characteristics between groups were analysed using chi-square test for categorical data. Immunohistochemistry staining intensity in normal pancreatic tissue was compared to pancreatic and periampullary adenocarcinoma using the independent student t-test. In all tests, results were considered statistically significant at the level of $p < 0.05$.

Table 1b. Overview of the characteristics and preclinical experience with tumor-specific imaging of integrin $\alpha_v\beta_6$, carcinoembryonic antigen (CEA), hepatocyte growth factor receptor (cMET), epithelial growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), human epidermal growth factor receptor (HER2), urokinase plasminogen activator receptor (uPAR) and vascular endothelial growth factor receptor 2 (VEGFR2) in pancreatic cancer animal models.

Target	Tumor-specific probe		Imaging modality	Pancreatic cancer xenograft	Ref.
Integrin $\alpha_v\beta_6$	Peptide	^{18}F-fluorobenzoic acid	PET	BxPC-3	[64-65]
	Peptide	^{99m}TC	SPECT/CT	BxPC-3	[66]
	Peptide	Phthalocyanine dye	NIRF imaging	BxPC-3	[67]
	Peptide	^{18}F-fluorobenzoate	PET	BxPC-3	[68]
CEA	scFv	800CW	NIRF imaging	BxPC-3	[71]
	MAB	IR700	NIRF imaging	BxPC-3	[72]
	MAB	AlexaFluor 488	NIRF imaging	BxPC-3	[73-77]
	scFv	I^{124}	PET/CT	BxPC-3	[78]
cMET	-	-	-	-	-
EGFR	F(ab')	^{64}Cu	PET/CT	PANC-1	[82]
	MAB	CF-750	MSOT	MiaPaCa-2	[83]
	scFv	IONP	MRI	MiaPaCa-2	[84-85]
	XIMAB	^{86}Y	PET	SHAW	[86]
EpCAM	-	-	-	-	-
HER2	MAB	^{111}In	PET	PC-Sw	[89]
uPAR	ATF-uPA	NIR-830, IONP	NIRF imaging, MRI	MiaPaCa-2	[84, 91-93]
	MAB	Cy5.5	NIRF imaging	AsPC-1	[94]
VEGFR2	MAB	Microbubbles	US	Transgenic mouse model	[96-98]

RESULTS

Patient and tumor characteristics

In total, 165 patients were included, whereof 137 and 28 with pancreatic and periampullary adenocarcinoma, respectively (Table 2). The mean age was 66 year and ranged between 38 and 84 year. Most tumors were T-stage 3 (50.9%) and poorly differentiated (44.6%). Regional lymph node involvement was found in 69.7% of patients. The majority of the patients received no adjuvant therapy after surgery.

Table 2. Baseline characteristics for the patients with pancreatic and periampullary adenocarcinoma included in this study. *p-value was obtained for patients with pancreatic adneocarcinoma compared to periampullary adenocarcinoma patients and p<0.05 was considered significant.

Characteristics	Total population (n = 165)	Pancreatic adenocarcinoma (n = 137)	Periampullary adenocarcinoma (n = 28)	p
Age, *n (%)*				
< 65 years	76 (46.1%)	66 (48.2%)	10 (35.6%)	0.228
≥ 65 years	89 (53.9%)	71 (51.8%)	18 (64.3%)	
Gender, *n (%)*				
Male	80 (48.5%)	66 (48.2%)	14 (50.0%)	0.860
Female	85 (51.5%)	71 (51.8%)	14 (50.0%)	
Tumor location, *n (%)*				
Pancreatic head	155 (93.9%)	127 (92.7%)	28 (100.0%)	-
Other	10 (6.1%)	10 (7.3%)	-	
Tumor differentiation, *n (%)*				
Well differentiated	17 (13.3%)	12 (8.8%)	5 (17.9%)	0.224
Moderately differentiated	54 (42.2%)	43 (31.4%)	11 (39.3%)	
Poorly/undifferentiated	57 (44.6%)	45 (32.8%)	12 (42.8%)	
Missing	37	37	-	
Tumor size, *n (%)*				
< 30 mm	97 (59.9%)	77 (57.5%)	20 (71.4%)	0.170
≥ 30 mm	65 (40.1%)	57 (42.5%)	8 (28.6%)	
Missing	3	3	-	
Primary tumor, *n (%)*				
pT1	31 (18.8%)	21 (15.3%)	10 (35.7%)	0.071
pT2	40 (24.2%)	36 (26.3%)	4 (14.3%)	
pT3	84 (50.9%)	72 (52.6%)	12 (42.9%)	
pT4	10 (6.1%)	8 (5.8%)	2 (7.1%)	
Regional lymph node, *n (%)*				
pN0	50 (30.3%)	34 (24.8%)	16 (57.1%)	<0.001
pN1	115 (69.7%)	103 (75.2%)	12 (42.9%)	
Surgical margin status, *n (%)*				
R0	119 (72.6%)	95 (69.3%)	24 (88.9%)	0.037
R1	45 (27.4%)	42 (30.7%)	3 (11.1%)	
Adjuvant therapy, *n (%)*				
Yes	70 (42.4%)	68 (49.6%)	2 (7.1%)	<0.001
No	95 (57.6%)	69 (50.4%)	26 (92.9%)	
Vascular invasion, *n (%)*				
Positive	48 (29.3%)	45 (32.8%)	3 (11.1%)	0.023
Negative	116 (70.7%)	92 (67.2%)	24 (88.9%)	
Perineural invasion, *n (%)*				
Positive	97 (59.1%)	87 (63.5%)	10 (37.0%)	0.011
Negative	67 (40.9%)	50 (36.5%)	17 (63.0%)	

Patients diagnosed with adenocarcinoma originating from the pancreas had, compared to patients diagnosed with periampullary adenocarcinoma, more frequently lymph node invasion (75% vs. 43%; $p < 0.001$), positive surgical margins (31% vs. 11%; $p = 0.037$), vascular invasion (33% vs. 11%; $p = 0.023$), perineural invasion (64% vs. 37%; $p = 0.011$) and received more often adjuvant therapy (50% vs. 7%; $p < 0.001$).

Table 3a. Target Selection Criteria (TASC) – score for integrin $\alpha_v\beta_6$, carcinoembryonic antigen (CEA), hepatocyte growth factor receptor (cMET), epithelial growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), human epidermal growth factor receptor (HER2), urokinase receptor (uPAR) and vascular endothelial growth factor receptor 2 (VEGFR2) in pancreatic adenocarcinoma.

Target	Extracellular localization of the protein (points awarded)	Pattern of up-regulation (points awarded)	T/N ratio (points awarded)	Percentage with positive expression (points awarded)
Integrin $\alpha_v\beta_6$	Membrane-bound (3)[99]	Diffuse (4)	Yes (3)	88% (5)
CEA	Membrane-bound (3)[101]	Diffuse (4)	Yes (3)	71.% (5)
UPAR	Membrane-bound (3)[71]	Diffuse (4)	Yes (3)	67% (3)
cMET	Membrane-bound (3)[105]	Diffuse (4)	No (0)	88% (5)
EGFR	Membrane-bound (3)[109]	Diffuse (4)	Yes (3)	69% (3)
HER2	Membrane-bound (3)[112]	Diffuse (4)	No (0)	80% (5)
VEGFR2	Membrane-bound (3)[114]	Focal (0)	No (0)	72% (5)
EpCAM	Membrane-bound (3)[118]	Focal (0)	No (0)	59% (3)

Table 3b. Target Selection Criteria (TASC) – score for integrin $\alpha_v\beta_6$, carcinoembryonic antigen (CEA), hepatocyte growth factor receptor (cMET), epithelial growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), human epidermal growth factor receptor (HER2), urokinase receptor (uPAR) and vascular endothelial growth factor receptor 2 (VEGFR2) in pancreatic adenocarcinoma.

Target	Previous imaging succes (points awarded)	Enzymatic activity (points awarded)	Internalization (points awarded)	TASC Score
Integrin $\alpha_v\beta_6$	Animal experiment (2)[68]	No (0)[100]	Yes (1)[65]	20
CEA	Animal experiment (2)[74, 77]	Unknown (0)	Yes (1)[102]	20
UPAR	Animal experiment (2)[91]	Yes (1)[103]	Yes (1)[104]	19
cMET	Animal experiment (2)[106]	Yes (1)[107]	Yes (1)[108]	18
EGFR	In patients (2)[86, 110]	Unknown (0)	Yes (1)[111]	18
HER2	Animal experiment (2)[89]	Unknown (0)	Yes (1)[113]	17
VEGFR2	Animal experiment (2)[115]	Yes (1)[116]	Yes (1)[117]	14
EpCAM	Animal experiment (2)[119]	Unknown (0)	Yes (1)[120]	11

Biomarker expression

Of the 165 pancreatic and periampullary adenocarcinoma specimens collectively present on the TMA, 159 specimens (96%) could successfully be microscopically quantified for integrin $\alpha_v\beta_6$ expression, 158 (96%) for CEA, 159 (96%) for cMET, 156 (95%) for EGFR, 151 (92%) for EpCAM, 152 (92%) for HER2, 155 (94%) for VEGFR2, and 152 (92%) for uPAR. The missing cases were due to staining artifacts, excessive necrotic tissue, or unacceptable tissue loss during the staining procedure. The molecular markers showed mainly membranous and cytoplasmic immunoreactivity in pancreatic and periampullairy adenocarcinoma cells; CEA and uPAR also showed stromal immunoreactivity (Figure 1). Diffuse membranous staining was found for integrin $\alpha_v\beta_6$, CEA, cMET, EGFR, HER2 en uPAR in pancreatic adenocarcinoma (Table 3) and integrin

$\alpha_v\beta_6$, CEA, cMET, EGFR, EpCAM, HER2 and VEGFR2 in periampullary adenocarcinoma (Table 4).

Immunohistochemistry staining, if present, in healthy pancreatic tissue was predominantly localized in the acinar cells of the pancreas. The most frequent expressed biomarkers were integrin $\alpha_v\beta_6$ and cMET that were both expressed in 88% of the pancreatic adenocarcinoma cases (Table 3). In addition, cMET was abundantly expressed in 96% of the periampullary adenocarcinoma patients (Table 4).

Table 4. Expression, as determined by immunohistochemistry, of biomarkers panels (combining the expression of two molecular markers) consisting of integrin $\alpha_v\beta_6$, carcinoembryonic antigen (CEA), epithelial growth factor receptor (EGFR) and/or urokinase receptor (uPAR) in pancreatic and periampullary adenocarcinoma**.** Overlapping expression refers to the percentage of patients that show positive expression (positive expression was defined as positive if > 10% of the tumor cells expressed a moderate or strong staining pattern) for both molecular markers in the biomarker panel. Total expression describes the frequency of patients that show positive expression (positive expression was defined as positive if > 10% of the tumor cells expressed a moderate or strong staining pattern) of one or both molecular markers in the biomarker panel and therefore could be visualized with a dual-tracer targeting both biomarkers.

Biomarker panel		**Pancreatic adenocarcinoma**		**Periampullary adencoadenocarcinoma**	
		Overlapping expression	**Total expression**	**Overlapping expression**	**Total expression**
Integrin $\alpha_v\beta_6$	CEA	64%	99%	63%	96%
Integrin $\alpha_v\beta_6$	uPAR	62%	96%	4%	73%
Integrin $\alpha_v\beta_6$	EGFR	66%	94%	44%	82%
CEA	uPAR	50%	91%	4%	88%
CEA	EGFR	52%	90%	54%	100%
uPAR	EGFR	48%	88%	60%	68%

To evaluate the ability of potential tumor-specific molecular markers to distinguish between pancreatic adenocarcinoma and healthy pancreatic tissue, the mean immunohistochemical intensity scores of the biomarkers were compared between both tissue types. In pancreatic adenocarcinoma the mean intensity score for integrin $\alpha_v\beta_6$ ($p<0.001$; $p<0.001$), CEA ($p<0.001$; $p<0.001$), EGFR ($p<0.001$; $p<0.001$) and uPAR ($p<0.001$; $p=0.056$) was significantly higher compared to normal pancreatic tissue (Figure 1). In periampullary adenocarcinoma the mean integrin $\alpha_v\beta_6$ ($p<0.001$), CEA ($p<0.001$) and VEGFR2 ($p=0.045$) staining intensity were significantly higher.

Biomarker panels

The combined expression of two biomarkers was evaluated to assess their potential as a dual-target for tumor-specific imaging (Table 5). In pancreatic adenocarcinoma integrin $\alpha_v\beta_6$ and/or CEA were expressed in 99% of the patients and 64% of the cases expressed both integrin $\alpha_v\beta_6$ and CEA, suggesting that the combination of both targets would be a promising approach for tumor-specific imaging. In periampullary adenocarcinoma the most promising combination was CEA and EGFR, whereas all cases expressed either CEA and/or EGFR. In addition, integrin $\alpha_v\beta_6$ and/or CEA were expressed in 96% of the cases.

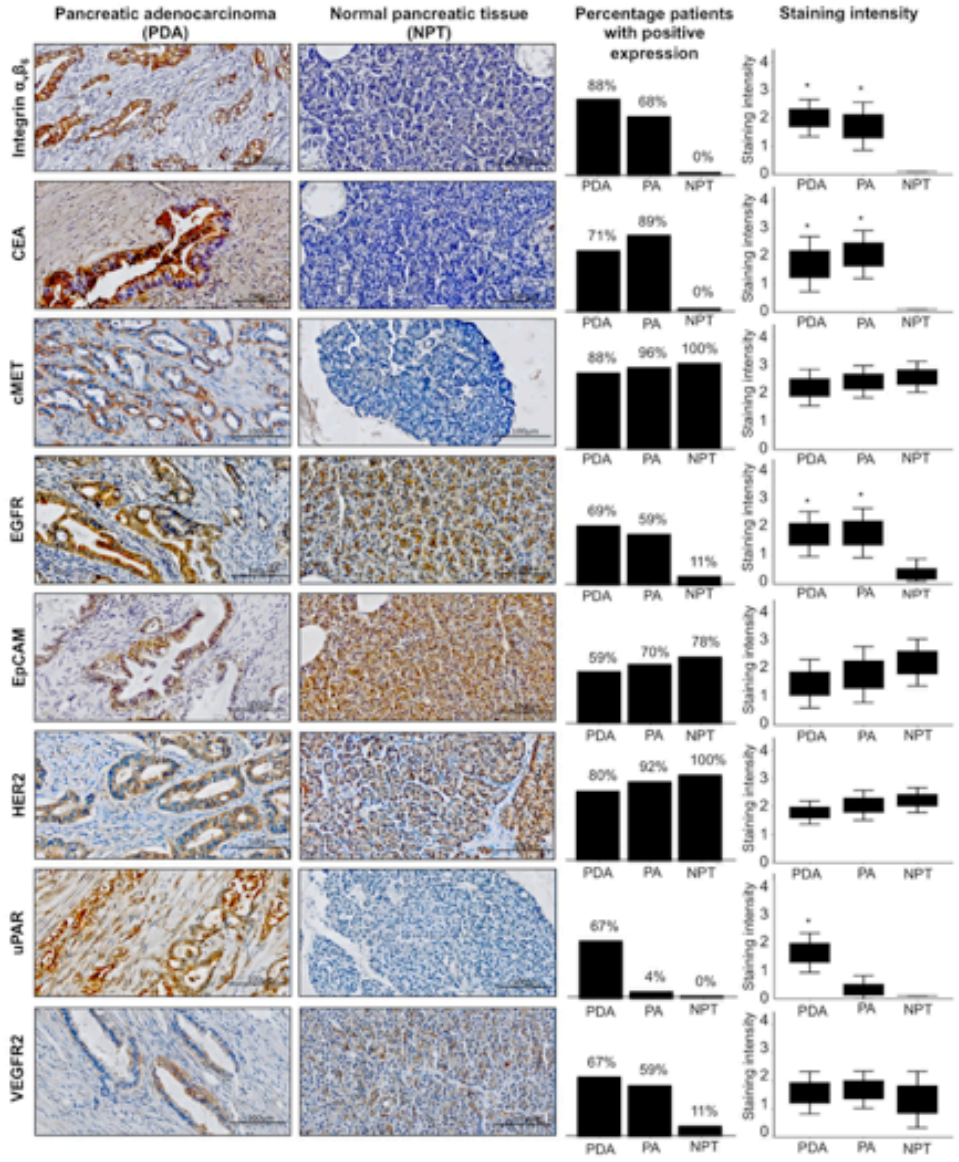

Figure 1. Representative images of moderate immunohistochemistry staining in pancreatic adenocarcinoma (*left colum*) and absent or present immunohistochemistry expression in pancreatic adenocarcinoma (*second left colum*). Followed, by barcharts (*third left colum*) displaying the percentage of PAC patients with positive staining (positive staining was defined as moderate or strong expression in >10% of tumor cells) and boxplots (*right colum*) showing the mean immunohistochemistry staining (staining intensity was classified for every patient as followed: 0 = negative, 1 = weak, 2 = moderate, and 3 = strong) in pancreatic adenocarcinoma (PDA), periampullary adenocarcinoma (PA) and normal pancreatic tissue (NPT) for integrin $\alpha_v\beta_6$, carcinoembryonic antigen (CEA), hepatocyte growth factor receptor (cMET), epithelial growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), human epithelial growth factor receptor (HER2), urokinase receptor (uPAR), and vascular endothelial growth factor receptor 2 (VEGFR2) expression. *Significant difference in staining intensity (defined as p-value of 0.05) in pancreatic or periampullary adenocarcinoma compared to normal pancreatic tissue.

Target Selection Criteria (TASC) - score

The TASC score was calculated for all molecular markers evaluated in this study (Table 3 and 4). Integrin $\alpha_v\beta_6$ (20 points), CEA (20 points), uPAR (19 points), cMET (18 points) and EGFR (18 points) were considered suitable targets for tumor-specific imaging of pancreatic adenocarcinoma according the TASC score. For tumor-specific imaging of periampullary adenocarcinoma, VEGFR2 (21 points), CEA (20 points), cMET (19 points), EGFR (18 points) and integrin $\alpha_v\beta_6$ (18 points) were categorized as potential targets by the TASC scoring system.

DISCUSSION

Tumor-specific intraoperative imaging is a rapidly emerging field that holds great promise to reduce tumor-positive resection margin rates in oncologic pancreatic surgery[30]. However, to make the transition to clinical practice, tumor-specific imaging targets and accompanying contrast-agents are prerequisite[15]. Therefore, the present study strives to provide the first steps towards clinical translation by investigating the suitability of a set of molecular markers as potential targets for tumor-specific imaging of pancreatic adenocarcinoma. The results of this study show that integrin $\alpha_v\beta_6$, CEA, EGFR and uPAR are significantly up-regulated in pancreatic adenocarcinoma compared to healthy pancreatic tissue and suggest that these biomarkers are promising targets for tumor-specific contrast agent development. By combining individual biomarkers in dual biomarker panels the coverage of patients was increased: in pancreatic adenocarcinoma, considering almost the complete population expressed either integrin $\alpha_v\beta_6$ and/or CEA. Furthermore, the TASC score confirmed the potential of integrin $\alpha_v\beta_6$, CEA, EGFR and uPAR as suitable targets for tumor-specific imaging.

Previous reports regarding the expression of integrin $\alpha_v\beta_6$ (85%-100%), cMET (82%-100%), EGFR (36%-69%), EpCAM (56%-78%), HER2 (16%-69%) and VEGFR2 (64%-93%) in pancreatic adenocarcinoma are consistent with our results[31-47]. Preceding findings demonstrate a higher expression of CEA (98%-100%) and uPAR (90%-96%) in pancreatic adenocarcinoma to our findings; however, this slight discrepancy is not likely to alter the final conclusion of this study[33, 35, 48]. Furthermore, studies of others showed analogue to our results that cMET, EpCAM and HER2 are overexpressed in healthy pancreatic tissue, which would render them less preferable as imaging targets[37-38, 40-42]. Importantly, the expression of integrin $\alpha_v\beta_6$, CEA and uPAR has been described previously in compliance with our results, as very low or undetectable in normal pancreatic tissue, which would translate to a favorable tumor-to-background ratio when used for imaging purposes[31, 35, 48-49]. EGFR and VEGFR2 were previously shown as respectively present and absent in normal pancreatic tissue, contradicting our findings[41-42, 49]. This ambiguity highlights the need to further investigate the ability of EGFR and VEGFR to distinguish between normal and malignant pancreatic tissue, especially since

fluorescence labeled contrast-agents directed at EGFR and VEGF, including bevacizumab-IRDye800CW, cetuximab-IRDye800CW and panitumab-IRDye800CW, are in various stages of clinical trials for clinical use in several other types of cancer[15].

The results of this study are posed by limitations inherent to immunohistochemical analysis, such as variation in the quality of the primary antibodies, immunohistochemical staining techniques, scoring criteria, paraffin impregnation, surgical specimen fixation delay, or diversity in the ethnic distribution of the study population[50-51]. In addition, the immunohistochemistry procedure, including tissue fixation and antigen retrieval, destroys the membrane integrity and protein conformation, which makes the protein less representative for its naïve counterpart. The antibodies used in this study were not specifically selected for the development of tumor-specific probes, since the focus of this study was to identify the most suitable targets; however, the antibodies in this study used for integrin $\alpha_v\beta_6$ (6.2A, Biogen Idec MA Inc.), CEA (A0155, Dako), EGFR (E30, Dako), EpCAM (323A3) and uPAR (ATN-615) react on the extracellular epitopes of their analogues and have been described for use on intact protein[52]. The latter could be promising for use in imaging-probes. Furthermore, the normal pancreatic tissue used in this study was obtained in proximity of the tumor for an optimal representation of the reality of image-guided surgery. Pre-malignant biological changes may already exist in this presumed normal pancreatic tissue, which could explain for the differences between our findings and the biomarker expression in normal pancreatic tissue reported in the literature. For the purpose of this study the term periampullary adenocarcinoma was used as an omnibus term for a very a heterogeneous group of adenocarcinoma that invade the head of the pancreas with distinctively different histology and expression of molecular markers as they originate from the duodenum, papilla of Vateri or the common bile duct. Hence, it is challenging or even impossible to draw conclusions that are true for the whole cohort periampullary adenocarcinoma based on our findings or represent them with a histology slide in figure 1. Moreover, this study applies a threshold of over 10% medium or dark stained tumor cells on 2 mm core TMA's to define tumor positivity. Therefore, the results of this study do not provide conclusive evidence on whether the evaluated targets could be used for tumor-specific imaging of the complete tumor and all residual disease. Nevertheless, the results of this study provide guidance on which molecular makers show the most promise for further investigation as tumor-specific imaging targets. Likewise, the reported expression of the composed biomarker panels investigated in this study indicates which biomarker combinations show complementary instead of overlapping expression in the majority of pancreatic adenocarcinoma and subsequently hold promise for future more elaborate examination. However, considering the >10% threshold these results are not decisive on whether dual-tracers directed at the inquired biomarker panels will be able to visualize the entire disease burden.

The TASC score identified cMET as a promising imaging target for pancreatic adenocarcinoma, whereas cMET did not significantly differentiate between healthy and malignant pancreatic tissue in our hands. These results suggest that the TASC score still experiences teething trouble and needs further validation and adaptation, since distinguishing between normal and malignant tissue is considered the cornerstone of surgical oncology. Various therapeutic antibodies have been investigated in preclinical models for imaging of cancer, including cetuximab, panitumumab, and bevacizumab[53-56]. Human clinical trials are underway, but none of these biologics are presently available for intraoperative imaging in humans. Use of an FDA-approved targeting molecule facilitates clinical translation, because it lowers the cost barrier to clinical practice, since revenue associated with diagnostic agents is significantly lower than for therapeutic agents[16, 57]. Therefore, for future use the TASC score should also take into consideration the availability of FDA-approved antibodies. Nevertheless, de-novo development of intraoperative diagnostics also takes place, for example, the Arg-Gly-Asp (RGD) peptide has a high affinity and selectivity for multiple integrin's, among them integrin $\alpha_v\beta_6$, and has extensively been studied for imaging objectives[58-59]. In addition, another example of de-novo developed imaging probes are autoquenched fluorescent probes, such as ProSense, that convert from a nonfluorescent to fluorescent state by proteolytic activation of lysosomal cysteine or serine proteases, hence the value of including enzymatic activity in by the TASC score[60]. Furthermore, the TASC criteria could be elaborated by adding points to the score for targets with a soluble form that can be targeted by certain antibodies, such as the ATN-615 antibody that recognizes a soluble form of uPA in addition to uPAR, which allows for antibodies to also target receptors that are already occupied by its soluble form thereby increasing its reach. Nevertheless, the TASC score is a promising tool to incorporate other favorable characteristics of potential imaging targets for pancreatic adenocarcinoma in a weighted and standardized manner in our judgment.

Despite the previously mentioned limitations this study was to the best of our knowledge the first study to assess the ability of potential targets for the image-guided surgery of pancreatic adenocarcinoma to distinguish between normal and malignant pancreatic tissue in a relatively large cohort of patients with pancreatic adenocarcinoma using the TASC score. In addition, this study was also able to investigate the expression of potential imaging targets in periampullary adenocarcinoma. The latter is of added value since the histological origin of pancreatic head masses is often unknown in wait of pancreatic surgery. Furthermore, this study was to our knowledge the first to describe the combined expression of potential imaging targets to facilitate future development of dual-labeled imaging probes; however, these dual-purpose agents present additional hurdles in development and clinical translation that are beyond the scope of this article before their potential is fully realized[16]. Moreover, aside from providing guidance for tumor surgery, molecular imaging techniques also play an increasingly important role in the preoperative staging and guidance of cancer therapy in pancreatic adenocarcinoma patients[61].

In conclusion, tumor-targeted intraoperative imaging of pancreatic adenocarcinoma has great potential to improve pancreatic surgery[12, 62]. However, the clinical implementation of this novel technique is currently halted by the lack of clinically approved tumor-specific contrast agents. Therefore, the present study sought to pave the way for future development of tumor-specific contrast-agents and consecutive image-guided resection of pancreatic adenocarcinoma, by investigating the most suitable molecular targets for tumor-specific imaging. The results of this study show that a dual-targeted tracer aimed at both integrin $\alpha_v\beta_6$ and CEA would be able to detect tumor cells in 99% of all pancreatic cancer patients.

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Part II

Multimodal treatment

Chapter 6

Neoadjuvant therapy versus upfront surgical strategies in localized pancreatic cancer: a Markov decision analysis

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EJSO 2016 Oct;42(10):1552-1560

ABSTRACT

Background: Neoadjuvant therapy is gaining acceptance as a valid treatment option for borderline resectable pancreatic cancer; however, its value for clearly resectable pancreatic cancer remains controversial. The aim of this study was to use a Markov decision analysis model, in the absence of adequately powered randomized trials, to compare the life expectancy (LE) and quality-adjusted life expectancy (QALE) of neoadjuvant therapy to conventional upfront surgical strategies in resectable pancreatic cancer patients.

Methods: A Markov decision model was created to compare two strategies: attempted pancreatic resection followed by adjuvant therapy and neoadjuvant therapy followed by restaging with, if appropriate, attempted pancreatic resection. Data obtained through a comprehensive systematic search in PUBMED of the literature from 2000-2015 were used to estimate the probabilities used in the model.

Results: Of the 786 potentially eligible studies identified, 22 studies met the inclusion criteria and were used to extract the probabilities used in the model. Base case analyses of the model showed a higher LE (32.2 vs. 26.7 months) and QALE (25.5 vs. 20.8 quality-adjusted life months) for patients in the neoadjuvant therapy arm compared to upfront surgery. Probabilistic sensitivity analyses for LE and QALE revealed that neoadjuvant therapy is favorable in 59% and 60% of the cases.

Conclusion: Although conceptual, these data suggest that neoadjuvant therapy offers substantial benefit in LE and QALE for resectable pancreatic cancer patients. These findings highlight the value of further prospective randomized trials comparing neoadjuvant therapy to conventional upfront surgical strategies.

INTRODUCTION

Pancreatic cancer represents the fourth leading cause of cancer-related death in the United States and remains an unsolved health care problem: its incidence is nearly equivalent to its annual death toll[1]. Complete surgical resection offers the only possibility for cure although the majority of operated patients develop recurrence[2]. Adjuvant therapy has been shown to improve overall survival in resected pancreatic cancer patients and is currently accepted as standard of care[3].

Unfortunately, up to 25-50% of patients who undergo pancreatic resection are unable to receive subsequent multimodal therapy as planned; postoperative complications, decreased performance status, comorbidities, or early recurrence may delay, shorten or prevent treatment delivery[4]. Neoadjuvant therapy avoids these issues and has been demonstrated to be safe and effective in subsets of other cancers[5]. Importantly, neoadjuvant therapy offers the time for patients with rapidly progressive disease who are destined to develop overt metastatic disease to "declare themselves" on re-staging scans prior to surgery and thus, avoid the morbidity and mortality of an operation. However, the latter could be interpreted by opponents of neoadjuvant therapy as loss of the "window" of resectability[6, 7] [8, 9]. Furthermore, advocates of neoadjuvant therapy have emphasized the potential added benefit of systemic therapy in an immune competent host prior to the stress imposed by major surgery[10]. To what extent the emerging data on immune checkpoint blockade and manipulation of the adoptive immune system that is seen in other solid tumors will translate to pancreatic cancer is also unknown but would argue in favor of treatment in the preoperative rather than postoperative setting[11, 12].

Neoadjuvant treatment has increasingly been accepted as a valid treatment for borderline resectable pancreatic cancer; however, the current experience with neoadjuvant therapy in evidently resectable pancreatic cancer is fairly limited[13-15]. The current study compares the life expectancy (LE) and quality-adjusted life expectancy (QALE) of resectable pancreatic cancer patients treated with neoadjuvant therapy to conventional upfront surgical strategies using a Markov decision analysis model.

METHODS

Model structure

We constructed a Markov cohort decision analysis model using TreeAge Pro 2015 (TreeAge Software Inc., Williamstown, MA) to evaluate the initial treatment options for resectable pancreatic cancer. In the model, two treatment strategies are represented: 1) attempted pancreatic resection followed by recovery and postoperative chemoradiotherapy; or 2) neoadjuvant chemotherapy or chemoradiotherapy followed by restaging and, if appropriate, attempted pancreatic resection. Patients that were not able to proceed to surgery were accounted for in the neoadjuvant therapy arm. The model did not allow

patients who received neoadjuvant therapy and successfully underwent resection to receive additional adjuvant therapy. Therefore, the neoadjuvant therapy and surgery health state included survival data from some patients that proceeded to adjuvant therapy. The Markov model included the following health states: untreated, recovering from surgery, postoperative major complications, no complication from chemotherapy or chemoradiotherapy, chemotherapy or chemoradiotherapy complication, chemotherapy or chemoradiotherapy alone, surgery alone, completed multimodal therapy and death. The cycle length in the model was three months. Patients cycled through the health states in the model, according to transition probabilities informed by the literature, until death.

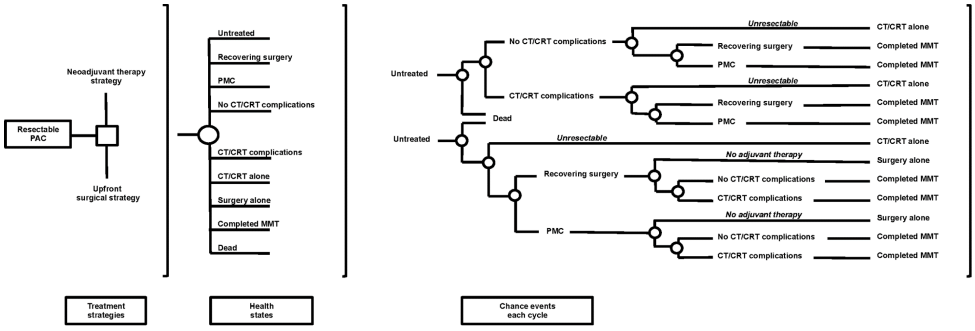

Figure 1. Schematic representation of the Markov decision analysis model. *Abbreviations:* CT, chemotherapy; CRT, chemoradiotherapy; PAC, pancreatic adenocarcinoma; MMT, multimodality therapy; PMC, postoperative major complications.

Literature search

The probabilities and survival outcomes used in the model were derived from a systematic literature search of PUBMED from January 2000 until December 2015. The search strategy included the following search terms: (("2000/01/01"[Date - Publication] : "2015/12/30"[Date - Publication])) and (“resectable”) and (“pancreas” or “pancreatic”) and (“neoplasm” or “neoplasms” or “cancer” or “cancers” or “adenocarcinoma”) and (“neoadjuvant” or “preoperative” or “adjuvant”). Inclusion or exclusion of studies was performed hierarchically based on review of the title, followed by review of the abstract, and, if warranted, review of the full text (S.W.L.de G). If the initial study was followed by a more comprehensive study or a study that included the original dataset, only the most recent and complete report or the study with the highest design in the evidence pyramid was included. These linked studies were identified based on authors, institutional affiliations, design, length of follow-up, and study populations.

The literature search was further narrowed down to English-language publications in humans age 18 years and older. This study only included publications on radiographically resectable pancreatic cancer patients with histologically or cytologically confirmed adenocarcinoma of the pancreas treated between 2000 and 2015. In this report, pancreatic cancer will refer to only pancreatic adenocarcinoma. Furthermore, studies on

intraoperative radiation, irreversible electroporation or radiotherapy without chemotherapy were excluded, as were studies on immunotherapy or vaccine therapy. All other chemotherapy or chemoradiotherapy treatment regimens were included and no specific schemata were assumed.

Utilities

Utilities are numeric estimates of preference for a given health state or outcome, reflecting the associated health-related quality of life (QOL) on a scale from 1 (corresponding to perfect health) to 0 (equivalent to dead). All utilities were based on outcomes of the European Quality of Life Survey (EQ-5D). The following utilities obtained from the literature were used in the Markov model: 0.81 for living with stable pancreatic cancer, 0.81 for undergoing CRT, 0.53 for experiencing CRT complications, 0.59 for recovering from pancreatic surgery, 0.48 for experiencing surgical complications[16-18].

Table 1. Probability distributions and parameter estimates used in the base case analyses, deterministic and probabilistic sensitivity analyses. *Abbreviations*: ADJCT, adjuvant chemotherapy; ADJCRT, adjuvant chemoradiotherapy, NACT, neoadjuvant chemotherapy; NACRT, neoadjuvant chemoradiotherapy; PMC, postoperative major complications; US, upfront surgery.

Variables	Deterministic sensitivity analysis		Probabilistic sensitivity analysis	
	Base case	Range	Distribution	Parameters
Probability NACT/NACRT complications	0.27	0.06-0.66	Beta	$\mu = 0.27$; $\sigma = 0.10$
Resectability after NACT/NACRT	0.81	0.50-0.97	Beta	$\mu = 0.81$; $\sigma = 0.03$
Surgical mortality after NACT/NACRT	0.02	0.00-0.04	Beta	$\mu = 0.02$; $\sigma = 0.01$
PMC after NACT/NACRT	0.25	0.06-0.56	Beta	$\mu = 0.25$; $\sigma = 0.05$
Probability living 3 months after NACT/NACRT alone	0.80	0.69-0.83	Beta	$\mu = 0.8034$; $\sigma = 0.1048$
Probability living 3 months after NACT/NACRT and surgery	0.93	0.90-0.95	Beta	$\mu = 0.9349$; $\sigma = 0.0476$
Resectability at US	0.92	0.65-1.00	Beta	$\mu = 0.92$; $\sigma = 0.14$
Surgical mortality during US	0.02	0.00-0.04	Beta	$\mu = 0.02$; $\sigma = 0.01$
PMC after US	0.43	0.17-0.52	Beta	$\mu = 0.43$; $\sigma = 0.06$
Probability receipt of ADJCT/ADJCRT after US	0.59	0.44-0.62	Beta	$\mu = 0.59$; $\sigma = 0.07$
Probability ADJCT/ADJCRT complications	0.54	0.31-0.68	Beta	$\mu = 0.54$; $\sigma = 0.08$
Probability living 3 months after US alone	0.86	0.85-0.87	Beta	$\mu = 0.8637$; $\sigma = 0.0725$
Probability living 3 months after US and ADJCT/ADJCRT	0.92	0.90-0.95	Beta	$\mu = 0.9200$; $\sigma = 0.0431$

Transition probabilities

To estimate pooled proportions and 95% confidence intervals for the probabilities in the model, we used random rather than fixed effect models in order to take into account the heterogeneity of the estimates[19]. Data were analyzed using a previously published Microsoft Excel spreadsheet for random effects models[20]. The probability of transitioning to the death state in any given health state of the model was calculated from the median overall survival obtained from the published literature based on the assumption that survival is an exponential function that evenly decreases over time[21]. The following

equation was used to obtain the transition probability to death after 3 months from the median survival: $S(t) = e^{-3((-\ln(0.5))/\text{median survival in months})}$ [22, 23].

Statistical analysis

The Markov decision analysis model was analyzed using TreeAge Pro 2015 (TreeAge Software Inc., Williamstown, MA). Deterministic sensitivity analyses were performed to assess the robustness of the decision analysis model for LE and QALE in response to changes in all parameters. Probabilities were varied between the highest and lowest value obtained from the published literature, while holding the other parameters fixed. In the absence of a minimal and maximal probability described in the literature, 95% confidence intervals obtained from the available data were used. In addition, two-way sensitivity analyses for LE and QALE were performed for resectability after neoadjuvant therapy and resectability during upfront surgery, since these variables play a critical role in surgical decision-making in the treatment of resectable pancreatic cancer patients. Probabilistic sensitivity analyses (PSA) using second-order Monte Carlo simulation were performed to assess the uncertainty in the LE and QALE. The PSA was performed by simultaneously drawing from beta distribution functions for each model parameter according to their means and standard deviations.

RESULTS

Literature search

Of the 786 potentially eligible studies identified, 22 studies met the inclusion criteria (supplementary figure 1 and table 1). In total, probabilities from 871 and 789 patients treated with neoadjuvant therapy (supplementary table 2) and upfront surgical strategies (supplementary table 3), respectively, were included in the Markov decision analysis model (Figure 1, schematic representation Markov model; supplementary figures 2 and 3, Markov model as used in Treeage). The weighted average transition probabilities used for the base case, deterministic and probabilistic sensitivity analysis are shown in Table 1.

Base Case Analysis

The results of the base case analysis showed that the neoadjuvant therapy strategy resulted in a substantially higher LE (32.2 vs. 26.7 months) and QALE (25.5 vs. 20.8 quality-adjusted life months) compared to the upfront surgery strategy (Table 2).

Table 2. Results of base case analyses.

Strategy	Life expectancy (In months)	Gain in life expectancy	Quality adjusted life expectancy (in months)	Gain in quality adjusted life expectancy
Neoadjuvant therapy	32.2	5.5	25.5	4.7
Upfront surgery	26.7	-	20.8	-

Deterministic sensitivity analysis

Multiple one-way sensitivity analyses were carried out for all the variables entered in the model. The optimal strategy changed at lower resectability rates after neoadjuvant therapy, lower survival rates after neoadjuvant therapy alone, lower survival rates after neoadjuvant therapy followed by surgery and higher survival rates after upfront surgery followed by adjuvant therapy than used for the base case analysis. When the rate of resectability after neoadjuvant therapy was below 0.57 and 0.55, the optimal treatment strategy changed to upfront surgery in terms of LE and QALE, respectively. Similarly, the optimal treatment strategy changed to upfront surgery when the probability of living 3 months after neoadjuvant therapy fell below 0.919 for LE (corresponding to a median survival of 24.8 months) and 0.918 for QALE (corresponding with a median survival 24.4 months). Furthermore, when the probability of living 3 months after upfront surgery followed by adjuvant therapy exceeded 0.939 (corresponding with a median survival 33.3 months) and 0.940 (corresponding to a median overall survival of 33.8) for LE and QALE, an upfront surgery approach would be preferable on one-way sensitivity analysis.

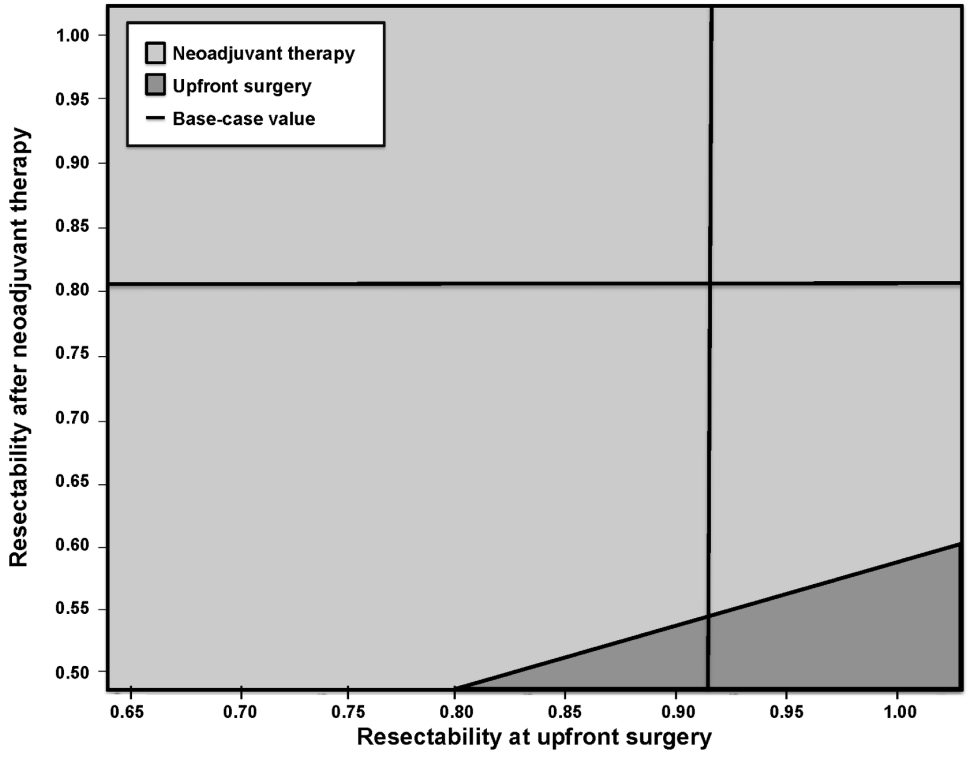

Figure 2. Two-way sensitivity analysis plotting rate of resectability after neoadjuvant therapy and rate of resectability at upfront surgery in resectable pancreatic cancer patients. Shaded regions reflect the optimal treatment strategy, in terms of life expectancy, for the corresponding values of resectability after neoadjuvant therapy and at upfront surgery.

If the probability of living after neoadjuvant therapy alone dropped below 0.698 (corresponding to a median survival of 5.8 months) the optimal treatment strategy for LE would revert to upfront surgery. The optimal treatment strategy is otherwise robust in terms of LE and QALE across the range of probabilities tested for the other probabilities used in the model.

Two-way sensitivity analyses for LE and QALE with respect to the probability of being resectable after neoadjuvant therapy and during upfront surgery are shown in Figures 2 and 3. As the rate of resectability after neoadjuvant therapy increases, the neoadjuvant therapy strategy remains optimal despite increasing probability of being resectable at upfront surgery.

Probabilistic sensitivity analysis

Incremental outcomes from the probabilistic sensitivity analysis, expressed as a difference in LE and QALE obtained between the two strategies, were obtained using a Monte Carlo simulation. The probability that the neoadjuvant therapy strategy is optimal in terms of LE and QALE is 59% and 60%, respectively.

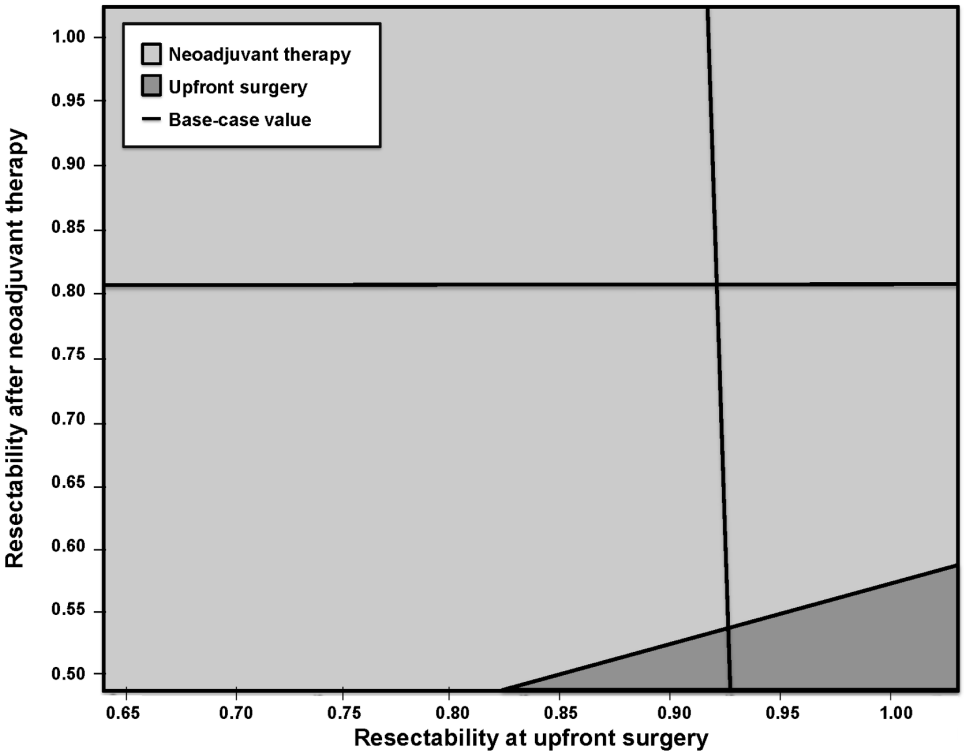

Figure 3. Two-way sensitivity analysis plotting rate of resectability after neoadjuvant therapy and rate of resectability at upfront surgery in resectable pancreatic cancer patients. Shaded regions reflect the optimal treatment strategy, in terms of quality-adjusted life expectancy, for the corresponding values of resectability after neoadjuvant therapy and at upfront surgery.

DISCUSSION

The present treatment guidelines support the use of neoadjuvant therapy for patients with borderline resectable pancreatic cancer; however, the optimal treatment strategy for patients with resectable pancreatic cancer remains controversial[24]. Therefore, in the absence of conclusive results from randomized controlled trials, the present study used an evidence-based Markov decision analysis model to compare neoadjuvant therapy to upfront surgical strategies in resectable pancreatic cancer patients. In our analysis, neoadjuvant therapy offered a 5.5 month (32.2 vs. 26.7 months) and 4.7 quality-adjusted month (25.5 vs. 20.8 quality-adjusted life months) improvement in LE and QALE, respectively, compared to a surgery-first approach in resectable pancreatic cancer patients. On probabilistic sensitivity analyses, neoadjuvant therapy resulted in a higher LE and QALE in 59% and 60% of cases, which supports the strong consideration of a neoadjuvant therapy approach in resectable pancreatic cancer patients.

Currently, upfront resectable pancreatic cancer patients rarely receive neoadjuvant therapy outside of clinical trials or a handful of unique centers. However, published data from prospective and retrospective experiences with neoadjuvant therapy have shown favorable survival durations for patients with resectable pancreatic cancer who receive neoadjuvant therapy and go on to surgical resection, with median overall survivals ranging from 26 to 45 months[25-27]. Such survival durations have not been reported, even from single institutions, with a surgery first approach with or without adjuvant therapy[28, 29]. Unfortunately, the first randomized control trial comparing neoadjuvant therapy to upfront surgery was unable to demonstrate a survival benefit for either treatment sequence due to poor accrural[30]. Given these logistical challenges for clinical trial enrollment, decision analysis may serve as an important interim source of information[31]. Previous decision analysis models comparing neoadjuvant therapy to upfront surgical strategies included both borderline resectable and clearly resectable pancreatic cancer patients, and these studies provided evidence supporting a modest benefit for neoadjuvant therapy in this population. Van Houten et al. (2012) reported a LE of 18.6 versus 17.7 months for patients treated with neoadjuvant therapy and initial surgery, respectively, whereas Sharma et al. (2015) demonstrated a slightly larger gain in LE (22 versus 20 months) and QALE (20 versus 18 quality-adjusted months) in favor of a neoadjuvant therapy[31, 32].

Markov decision analysis models are powerful analytical tools that have been widely utilized to address complex problems in medical decision-making. Using Markov modeling to compare the treatment strategies available for patients with resectable pancreatic cancer offers the opportunity to adjust for treatment delays and incorporate quality-of-life data. In concordance with our findings, previous studies using decision analysis modeling have also shown benefits in terms of overall and quality-adjusted survival in favor of neoadjuvant therapy-based management of potentially resectable

pancreatic cancer[33]. Our study was able to advance on preceding decision analysis studies by solely investigating the value of neoadjuvant therapy in resectable pancreatic cancer patients, as neoadjuvant therapy has already gained wide acceptance in the treatment of borderline resectable pancreatic cancer. Furthermore, this study improved on previous iterations by employing a robust systematic literature review with recent data as well as a comprehensive probabilistic sensitivity analysis to address the uncertainty in the Markov decision analysis model.

Decision analysis is invariably limited by the data used to inform the model, since assumptions are necessarily made in constructing the decision tree. Although probability estimates used in the model reflect the best available clinical data, the outcomes of the studies included were heterogeneous. Additionally, most articles available for this analysis are retrospective in nature and may be biased toward positive results. Different radiation doses, fields, and schedules, as well as diverse chemotherapy agents were used, which may be associated with variable response rates and complication profiles. Also, published studies often utilized different staging protocols, different definitions for radiographic resectability, and different criteria for surgical resectability. In addition, it is unknown how the more effective combination systemic therapies such as FOLFIRINOX or gemcitabine/nab-paclitaxel, which have proven effective in advanced pancreatic cancer, may impact the efficacy of neoadjuvant therapy and the interval between treatment modalities (chemotherapy, chemoradiation and surgery) [34]. Finally, some studies providing survival data for the neoadjuvant therapy and surgery arm also included patients that received adjuvant therapy; due to of lack of data on the probability of receiving adjuvant therapy, patients who received additional adjuvant therapy could not be included in the model separately.

Despite its limitations, our study is the first decision analysis model to compare neoadjuvant therapy to conventional upfront surgery in resectable pancreatic cancer patients. While a randomized controlled trial would provide conclusive evidence for the utility of neoadjuvant therapy in the care for resectable pancreatic cancer patients, we do not foresee such additional studies in the near future and decision analysis studies may serve as an interim source of information. This study accurately reflects the key tradeoffs between neoadjuvant therapy and upfront surgical strategies in patients with resectable pancreatic cancer and is populated by recent data obtained from a robust systematic literature review. Importantly, using Markov decision analysis allowed for the inclusion of patients that did not proceed to surgery in the neoadjuvant therapy arm, accounting for the probability of loss of the “window of resectability”. Furthermore, in sensitivity analysis the model responded in an intuitive manner to changes in variables within clinically plausible ranges, giving our results face validity.

In this Markov decision analysis simulation informed by the most recent data available on resectable pancreatic cancer patients, neoadjuvant therapy, as opposed to upfront surgical strategies, was the preferred treatment strategy in terms of LE (32.2 vs.

26.7 months) and QALE (25.5 vs. 20.8 quality-adjusted life months). In addition, probabilistic sensitivity analyses showed neoadjuvant therapy associated with favorable LE and QALE in 59% and 60% of cases, respectively. Although theoretical, these results suggest that neoadjuvant therapy holds promise to improve the care of resectable pancreatic cancer patients. They also lend support for further prospective randomized trials, especially in light of imminent advancements in systemic treatment.

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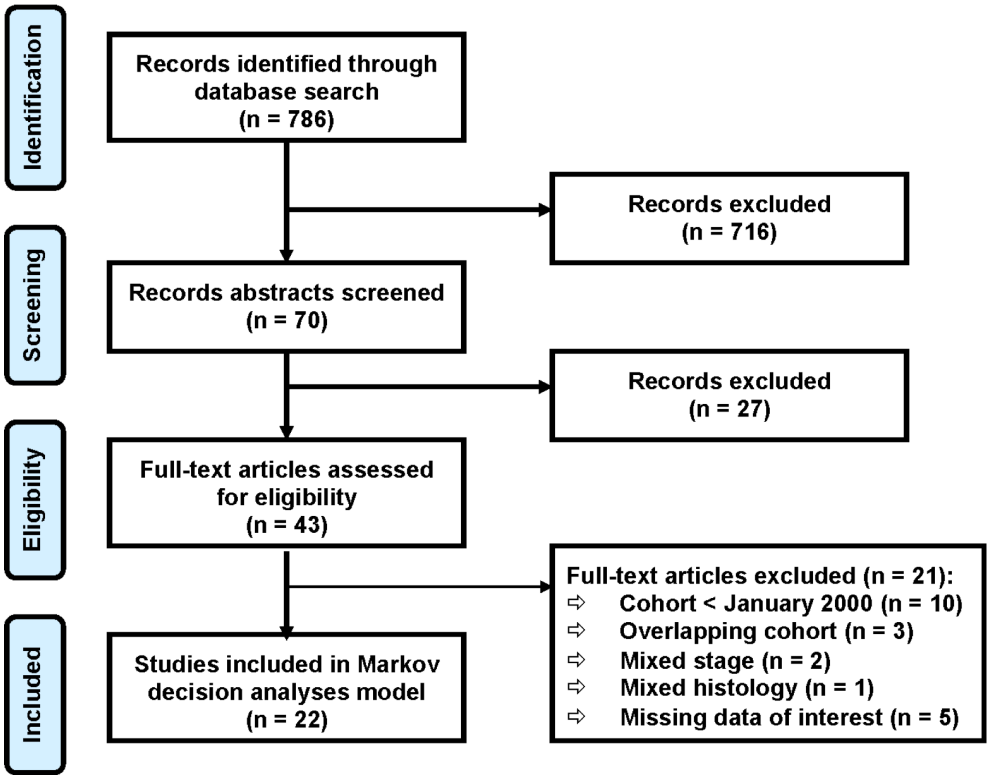
SUPPLEMENTARY FIGURES AND TABLES

Supplementary Table 1. Summary of the studies included in the Markov decision analysis model. *Abbreviations:* EBRT, external beam radiation therapy; FOLFIRINOX, 5-fluorouracil, leucovorin, ironotecan and oxaliplatin; IMRT, intensity-modulated radiation therapy; S-1, tegafur, gimeracil and oteracil.

First author, year	No. of patients	Study design	Treatment regimen
Abrams et al., 2015[46]	63	Retrospective observational	EBRT, gemcitabine and/or capecitabine
Christians et al., 2015[25]	69	Retrospective observational	EBRT, gemcitabine, capecitabine, erlotinib, cisplatin and/or FOLFIRINOX
Casadei et al., 2015[35]	38	Randomized trial	EBRT and gemcitabine
Golcher et al., 2015[30]	73	Randomized trial	EBRT, gemcitabine and/or cisplatin
Labori et al., 2015[44]	203	Retrospective observational	5-Fluorouracil and leucovorin
Kharofa et al., 2014[26]	30	Retrospective observational	IMRT, gemcitabine, cisplatin, erlotinib and/or FOLFIRINOX
Okabayashi et al., 2014[29]	189	Retrospective observational	Gemcitabine or S-1
O'Reilly et al., 2014[40]	38	Nonrandomized trial	Gemcitabine and oxaliplatin
Hong et al., 2014[36]	50	Nonrandomized trial	Proton beam therapy and capecitabine
Tzeng et al., 2014[28]	167	Retrospective observational	EBRRT, gemcitabine and 5-fluorouracil
Herman et al., 2013[47]	48	Nonrandomized trial	IMRT, erlotinib and capecitabine
Motoi et al., 2013[41]	36	Nonrandomized trial	Gemcitabine or S-1
Takahashi et al., 2013[37]	188	Nonrandomized trial	EBRT and gemcitabine
Sho et al., 2013[38]	61	Retrospective observational	EBRT and gemcitabine
Reni et al., 2012[49]	51	Randomized trial	EBRT and gemcitabine
Turrini et al., 2010[39]	34	Nonrandomized trial	EBRT and docetaxel
VanLaethem et al., 2010[50]	90	Randomized trial	EBRT and gemcitabine
Heinrich et al., 2008[42]	28	Nonrandomized trial	Gemcitabine and cisplatin
Linehan et al., 2008[48]	53	Nonrandomized trial	EBRT, 5-fluorouracil, cisplatin, interferon-α and/or gemcitabine
Varadhackary et al., 2008[7]	90	Nonrandomized trial	EBRT, gemcitabine and cisplatin
Takai et al., 2008[43]	32	Retrospective observational	EBRT, 5-fluorouracil, cisplatin and gemcitabine
Talamonti et al., 2006[27]	20	Nonrandomized trial	EBRT and gemcitabine

Supplementary Figure 1. PRISMA diagram for study selection.



Chapter 7

Neoadjuvant therapy versus upfront surgery for resected pancreatic adenocarcinoma: a nationwide propensity score matched analysis

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Surgery 2017;161(3):592-601

ABSTRACT

Background: Neoadjuvant therapy is an emerging paradigm in pancreatic cancer care; however, its role for resectable disease remains controversial in the absence of conclusive randomized controlled trials. The purpose of the present study is to assess the impact of neoadjuvant therapy on survival in resected pancreatic cancer patients by clinical stage.

Methods: A retrospective cohort study using the National Cancer Data Base from 2004 to 2012 including nonmetastatic pancreatic adenocarcinoma patients who underwent pancreatectomy and initiated chemotherapy. Propensity score matching within each stage was used to account for potential selection bias between patients undergoing neoadjuvant therapy and upfront surgery. Overall survival was compared by the Kaplan-Meier method.

Results: In the study, 1,541 and 7,159 patients received neoadjuvant therapy followed by surgery and upfront surgery succeeded by adjuvant therapy, respectively. In clinical stage III pancreatic cancer
(n = 486), neoadjuvant therapy was associated with significant survival benefit after matching (median survival 22.9 vs 17.3 months; log-rank P < .0001) compared with conventional upfront surgery followed by adjuvant therapy; however, no survival difference was found between the 2 treatment sequences in patients with clinical stage I (n = 3,149; median survival, 26.2 vs 25.7 months; P = .4418) and II (n = 5,065; median survival, 23.5 vs 23.0 months; P = .7751) disease after matching.

Conclusion: The survival impact of neoadjuvant therapy is stage-dependent. Neoadjuvant therapy does not disadvantage survival compared with conventional upfront surgery followed by adjuvant therapy in any stage, and is associated with a significant survival advantage in stage III pancreatic cancer.

INTRODUCTION

Pancreatic adenocarcinoma ranks the fourth leading cause of cancer-related death in the Western World.[1] Complete surgical resection provides the only hope for cure; however, recurrence rates after surgery range from 46% to 89%.[2, 3] These high recurrence rates provide evidence for likely unrecognized micro-metastatic disease at diagnosis.[4, 5] Therefore, there is widespread consensus that multimodality therapy is superior to surgery alone. However, the optimal treatment sequence of multimodality therapy remains an ongoing debate [6, 7]

Adjuvant therapy is currently the standard of care throughout the United States.[8] Nevertheless, 25%-48% of upfront resected patients fail to complete adjuvant therapy due to surgical complications and disease progression.[9-11] Neoadjuvant therapy circumvents these impediments and likely increases the chance of receiving all components of recommended care.[12] In addition, neoadjuvant therapy provides early treatment of systemic disease.[13] Furthermore, neoadjuvant therapy can be a tool for optimal patient selection, protecting those with rapidly progressive disease from the morbidity and mortality of surgery.[14]

In the absence of conclusive randomized trials, the potential benefit of neoadjuvant therapy has never been substantiated; however, it is likely stage-dependent.[15] The current study is a propensity score matched analysis of a contemporary nationwide cohort, comparing the clinical outcomes of neoadjuvant therapy vs upfront surgery for pancreatic cancer by stage.

METHODS

Cohort assembly

The National Cancer Data Base (NCDB) from 2004 to 2012 was queried for patients with histologically confirmed pancreatic adenocarcinoma defined according to the third edition International Classification of Disease for Oncology (ICD-O-3) codes for morphology (8140-and 8500) and topography (C25.0, C25.1, C25.2, C25.3, C25.7, C25.8, C25.9). The NCDB is a collaborative initiative of the American Cancer Society and the American College of Surgeons that captures over 70% of all newly diagnosed cancer patients in the United States. Data are obtained from more than 1500 Commission on Cancer approved cancer centers, including academic and community cancer programs.[16] Each participating center annually reports de-identified information according to a standardized set of data elements.

Exclusion criteria

Patients who were not reported as having received systemic treatment, radiotherapy and/or surgery in the NCDB (n = 67,546), had metastatic disease (clinical

stage IV; n = 85,653), were missing clinical stage (n = 36,251), were diagnosed after 2012 and had missing/unknown vital status (n = 24,828), were not treated at the reporting hospital (n = 25,867), had a history of other malignancies (n = 35,550), or received hormone therapy (n = 553), immunotherapy (n = 920) or intraoperative chemotherapy and/or radiation (n = 107) were excluded. Patients were also excluded if any of the following essential variables was listed as missing or unknown: treatment sequence (n = 3,081), race (n = 419), type of treatment center (n = 2,850), surgical margins (n = 738), insurance status (n = 1,028) or tumor differentiation (n = 1,931).

Definition of variables

Patients who underwent surgery of the primary site were identified according to their Facility Oncology Registry Data Standards (FORDS) surgery codes (25, 30, 35, 36, 37, 40, 60, 70, 80 and 90). All patients included in the study received chemotherapy and surgery. Patients were categorized into two exposure groups based on the timing of chemotherapy relative to the pancreatic resection: neoadjuvant therapy and upfront surgery. Neoadjuvant therapy was defined as chemotherapy with or without radiation before surgery independent of any treatment succeeding surgery. Upfront surgery was defined as surgery followed by chemotherapy with or without radiation without any treatment prior to surgery. Neoadjuvant and upfront surgery were categorized via the variables for sequencing of treatment when available in the NCDB; otherwise, we used the date of chemotherapy and surgery to determine the sequence, with neoadjuvant and adjuvant therapy defined as treatment preceding and following surgery, respectively.

Age was categorized as < 65 years or ≥ 65 years; race as white and non-white; patient insurance status as private/managed care and other; tumor location as head/neck (ICD-O-3 topography code C25.0 and C25.7) and other (ICD-O-3 topography codes C25.1, C25.2, C25.3, C25.8 and C25.9); treatment center as academic and non-academic institution and tumor differentiation as well/moderately differentiated and poor/undifferentiated. Clinical stage was defined according to the 7th edition of the American Joint Committee on Cancer (AJCC) cancer staging manual. If clinical tumor stage was missing, the individual clinical T, N and M stages were combined according to AJCC staging guidelines into a group stage; survival was cross-checked with the original staged cohort. Patient comorbidity was approximated using the Charlson comorbidity index modified by Deyo, which is comprised of up to six pre-existing comorbidities (not including cancer); this was grouped into no comorbidities and any comorbidities.[17, 18]

Statistical analysis

Only patients who received multimodal treatment were included in the final analysis. Patient characteristics were compared using Chi-square tests for categorical variables. The primary outcome of interest was overall survival, defined as the time

between diagnosis and death. Patients who were still alive at the time of analysis were censored at the time they were last known to be alive.

Within each stage, propensity score matching was used to reduce selection bias between the allocation of neoadjuvant therapy and upfront surgery. Propensity score models were built predicting the odds of assignment to upfront surgery. All available potential confounders were included, including variables with even a weak effect on outcomes.[19, 20] Propensity score models were adjusted for: age, sex, race, comorbidities, insurance status, type of treatment center, tumor location and tumor differentiation. Nearest-neighbor matching without replacement was performed with a caliper width equal to 0.2 of the standard deviation of the estimated probability of receiving upfront surgery and adjuvant therapy, eliminating 99% of the selection bias.[21] The c-statistic for the propensity score models for stage I, II, and III patients were 0.578, 0.618, and 0.647, correspondingly. In addition, the caliper width ranges for the difference between case and control propensity score for stage I, II, and III patients were (-0.0055436 ~ 0.0055436), (-0.012301 ~ 0.012301), and (-0.0268583 ~ 0.0268583), respectively. Survival analyses following propensity score matching were performed using the Kaplan-Meier method. Survival analysis was restricted to the first 30 months from diagnosis (with patients surviving beyond that time point censored at 30 months), taking into account the limited follow-up thereafter. Sensitivity analysis was performed on the unrestricted cohort. All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). P-values less than 0.05 were considered statistically significant. For the purpose of this study, the terms pancreatic adenocarcinoma and pancreatic cancer are used interchangeably.

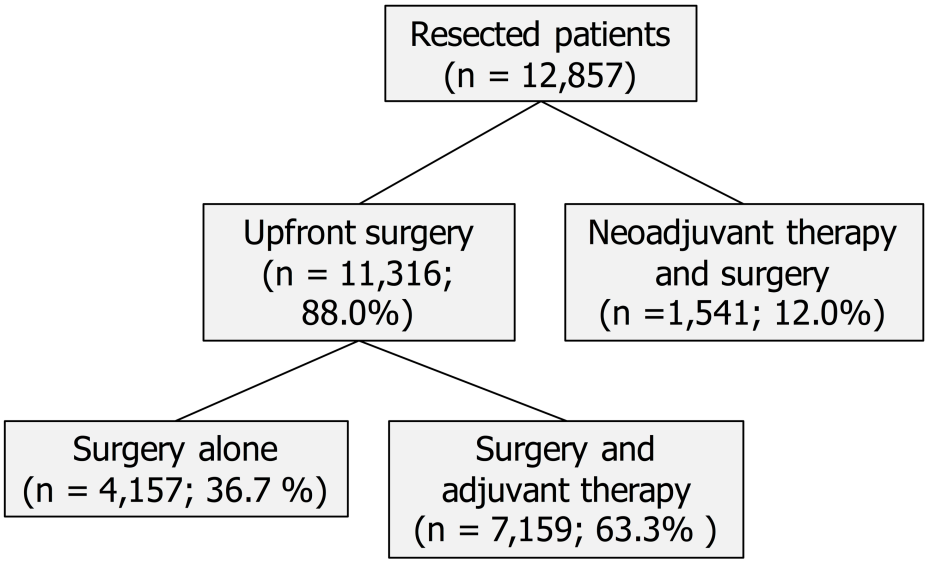

Figure 1. Flow diagram of patients with resected non-metastatic pancreatic adenocarcinoma by treatment approach.

RESULTS

Baseline characteristics

12,857 patients met our inclusion criteria. The majority of patient received upfront surgery (88.0%; n = 11,316), and 1,541 (12.0%) patients received neoadjuvant therapy followed by surgery. Only 63.3% (n = 7,159) of the patients were resected upfront underwent adjuvant therapy, as shown in Figure 1. In the cohort of patients that received multimodal treatment, the majority of patients were under 65 years at diagnosis (n = 4,384; 50.4%), male (n = 4,531; 52.1%), white (n = 7,275; 83.6%), treated in academic treatment centers (n = 5,443; 62.6%) and privately insured (n = 3,971; 45.6%). There were 3,149 (36.2%) patients with clinical stage I disease, 5,065 (58.2%) patients with clinical stage II disease, and 486 (5.6%) patients with clinical stage III pancreatic cancer.

Table 1. Baseline characteristics and surgical outcomes for unmatched and matched patients receiving neoadjuvant therapy followed by surgical resection vs upfront surgery followed by adjuvant therapy for resected clinical stage I pancreatic adenocarcinoma.

Characteristics	Initial population (n = 3,149)			Matched population (n = 662)		
	Neoadjuvant therapy (n = 332)	**Upfront surgery (n = 2,817)**	**p**	**Neoadjuvant therapy (n = 331)**	**Upfront surgery (n = 331)**	**p**
Sex, n (%)						
Male	171 (51.5%)	1,396 (49.6%)	0.517	170 (51.4%)	163 (49.2%)	0.586
Female	161 (48.5%)	1,419 (50.4%)		161 (48.6%)	168 (50.8%)	
Age, n (%)						
< 65 years	173 (52.1%)	1,309 (46.5%)	0.052	172 (52.0%)	172 (52.0%)	> 0.999
≥ 65 years	159 (47.9%)	1,508 (53.5%)		159 (48.0%)	159 (48.0%)	
Race, n (%)						
White	267 (80.4%)	2,356 (83.6%)	0.138	267 (80.7%)	271 (81.9%)	0.690
Non-white	65 (19.6%)	461 (16.4%)		64 (19.3%)	60 (18.1%)	
Charlson score, n (%)						
0	214 (64.5%)	1,859 (66.0%)	0.577	214 (64.7%)	214 (64.7%)	>0.999
≥ 1	118 (35.5%)	958 (34.0%)		117 (35.3%)	117 (35.3%)	
Insurance, n (%)						
Private	169 (50.9%)	1,196 (42.5%)	0.003	168 (50.8%)	162 (48.9%)	0.641
Not private	163 (49.1%)	1,621 (57.5%)		163 (49.2%)	169 (51.5%)	
Type of center, n (%)						
Academic	223 (67.2%)	1,680 (59.6%)	0.008	222 (67.1%)	219 (66.2%)	0.805
Non-academic	109 (32.8%)	1,137 (40.4%)		109 (32.9%)	112 (33.8%)	
Location, n (%)						
Head/neck	261 (78.6%)	2,077 (73.7%)	0.054	260 (78.6%)	263 (79.5%)	0.775
Other	71 (21.4%)	740 (26.3%)		71 (21.4%)	68 (20.5%)	
Tumor differentiation, n (%)						
Well	38 (11.5%)	257 (9.1%)	0.219	37 (11.2%)	41 (12.4%)	0.440
Moderately	186 (56.0%)	1,536 (54.5%)		186 (56.2%)	197 (59.5%)	
Poorly/ undifferentiated	108 (32.5%)	1,024 (36.4%)		108 (32.6%)	93 (28.1%)	

Patients with clinical stage I pancreatic cancer receiving neoadjuvant therapy followed by surgery were noted to be significantly younger at diagnosis ($p = 0.052$), more likely to have private insurance ($p = 0.003$) and more frequently treated at academic institutions ($p = 0.008$). Clinical stage II patients who received neoadjuvant therapy tended to be younger ($p = 0.017$), more often privately insured ($p = 0.002$) and were more likely treated in academic institutions ($p < 0.001$). In patients with clinical stage III disease, receipt of neoadjuvant therapy was associated with treatment at an academic institution ($p < 0.001$).

Table 2. Baseline characteristics and surgical outcomes for unmatched and matched patients receiving neoadjuvant therapy followed by surgical resection vs upfront surgery followed by adjuvant therapy for resected clinical stage II pancreatic adenocarcinoma.

Characteristics	Initial population (n = 5,065)			Matched population (n = 1,862)		
	Neoadjuvant therapy (n = 931)	Upfront surgery (n = 4,134)	p	Neoadjuvant therapy (n = 931)	Upfront surgery (n = 931)	p
Sex, n (%)						
Male	484 (52.0%)	2,222 (53.7%)	0.330	484 (52.0%)	508 (54.6%)	0.265
Female	447 (48.0%)	1,912 (46.3%)		447 (48.0%)	423 (45.4%)	
Age at diagnosis, n (%)						
< 65 years	512 (55.0%)	2,095 (50.7%)	0.017	512 (55.0%)	529 (56.8%)	0.428
≥ 65 years	419 (45.0%)	2,039 (49.3%)		419 (45.0%)	402 (43.2%)	
Race, n (%)						
White	791 (85.0%)	3,451 (83.5%)	0.268	791 (85.0%)	771 (82.8%)	0.207
Non-white	140 (15.0%)	683 (16.5%)		140 (15.0%)	160 (17.2%)	
Charlson score, n (%)						
0	626 (67.2%)	2,848 (68.9%)	0.326	626 (67.2%)	639 (68.6%)	0.519
≥ 1	305 (32.8%)	1,286 (31.1%)		305 (32.8%)	292 (31.4%)	
Insurance status, n (%)						
Private/managed care	483 (51.9%)	1,864 (45.1%)	0.0002	483 (51.9%)	480 (51.6%)	0.889
Not private	448 (48.1%)	2,270 (54.9%)		448 (48.1%)	451 (48.4%)	
Type of center, n (%)						
Academic	712 (76.5%)	2,493 (60.3%)	<0.001	712 (76.5%)	707 (75.9%)	0.786
Non-academic	219 (23.5%)	1,641 (39.7%)		219 (23.5%)	224 (24.1%)	
Tumor location, n (%)						
Head/neck	774 (83.1%)	3,181 (77.0%)	<0.001	774 (83.1%)	793 (85.2%)	0.228
Other	157 (16.9%)	953 (23.0%)		157 (16.9%)	138 (14.8%)	
Tumor differentiation, n (%)						
Well differentiated	98 (10.5%)	355 (8.6%)	0.020	98 (10.5%)	99 (10.6%)	0.642
Moderately differentiated	504 (54.1%)	2,137 (51.7%)		504 (54.1%)	522 (56.1%)	
Poorly/undifferentiated	329 (35.3%)	1,642 (39.7%)		329 (35.3%)	310 (33.3%)	

In the unmatched stage I, II and III groups that received neoadjuvant therapy, 239 (72.0%), 669 (71.9%) and 232 (83.5%) patients received neoadjuvant radiation in addition to neoadjuvant chemotherapy, respectively. In the upfront surgery group 1457 (51.7%) stage I, 2254 (54.5%) stage II and 122 (58.7%) stage III patients received adjuvant radiation in addition to adjuvant chemotherapy.

The demographic, tumor and treatment characteristics of each cohort before and after matching are summarized by clinical stage in Tables 1, 2 and 3. There were no

significant differences between patients receiving neoadjuvant treatment and upfront surgery after matching. After matching, 105 (31.6%), 274 (29.4%) and 69 (24.8%) patients with stage I, II and III disease in the neoadjuvant therapy group, respectively, also received adjuvant therapy after surgery.

Table 3. Baseline characteristics and surgical outcomes for unmatched and matched patients receiving neoadjuvant therapy followed by surgical resection vs upfront surgery followed by adjuvant therapy for resected clinical stage III pancreatic adenocarcinoma.

Characteristics	Initial population (n =486)			Matched population (n = 358)		
	Neoadjuvant therapy (n = 278)	Upfront surgery (n = 208)	p	Neoadjuvant therapy (n = 179)	Upfront surgery (n = 179)	p
Sex, n (%)						
Male	136 (48.9%)	120 (57.7%)	0.055	98 (54.8%)	98 (54.8%)	>0.999
Female	132 (51.1%)	88 (42.3%)		81 (45.2%)	81 (45.2%)	
Age at diagnosis, n (%)						
< 65 years	175 (63.0%)	120 (57.7%)	0.240	104 (58.1%)	105 (58.7%)	0.915
≥ 65 years	103 (37.0%)	88 (42.3%)		75 (41.9%)	74 (41.3%)	
Race, n (%)						
White	234 (84.2%)	176 (84.6%)	0.894	154 (86.0%)	150 (83.8%)	0.555
Non-white	44 (15.8%)	32 (15.4%)		25 (14.0%)	29 (16.2%)	
Charlson score, n (%)						
0	199 (71.6%)	147 (70.7%)	0.827	128 (71.5%)	127 (71.0%)	0.907
≥ 1	79 (28.4%)	61 (29.3%)		51 (28.5%)	52 (29.0%)	
Insurance status, n (%)						
Private/managed care	158 (56.8%)	101 (48.6%)	0.070	95 (53.1%)	92 (51.4%)	0.751
Not private	120 (43.2%)	107 (51.4%)		84 (46.9%)	87 (48.6%)	
Type of center, n (%)						
Academic	216 (77.7%)	119 (57.2%)	<0.001	118 (65.9%)	118 (65.9%)	> 0.999
Non-academic	62 (22.3%)	89 (42.8%)		61 (34.1%)	61 (34.1%)	
Tumor location, n (%)						
Head/neck	186 (66.9%)	133 (63.9%)	0.496	118 (65.9%)	116 (64.8%)	0.824
Other	92 (33.1%)	75 (36.1%)		61 (34.1%)	63 (35.2%)	
Tumor differentiation, n (%)						
Well differentiated	39 (14%)	15 (7.2%)	0.055	14 (7.8%)	15 (8.4%)	0.942
Moderately differentiated	140 (50.4%)	109 (52.5%)		93 (51.0%)	95 (53.1%)	
Poorly/undifferentiated	99 (35.6%)	84 (40.4%)		72 (40.2%)	69 (38.6%)	

Survival analysis

At the time of statistical analysis, 34% of all stage I pancreatic cancer patients who completed multimodality therapy were still alive. Patients with stage I disease treated with neoadjuvant therapy and surgery had a similar overall survival compared with upfront surgery followed by adjuvant therapy in both unmatched (median survival, 26.2 vs 24.5 months; p = 0.3421; Figure 2a) and matched (median survival, 26.2 vs 25.7 months; p = 0.4418; Figure 2b) non-metastatic pancreatic adenocarcinoma patients.

Of the patients with stage II pancreatic adenocarcinoma 25.9% (n = 1,314) were still alive at time of survival analysis. Unmatched (median survival, 23.5 vs 20.9 months; log-rank p = 0.063; Figure 2c) and matched (median survival, 23.5 vs 23.0 months; p = 0.7751; Figure 2d) patients with clinical stage II disease who received neoadjuvant therapy

and surgery had similar survival to those who were resected upfront followed by adjuvant therapy.

For clinical stage III patients, 24.1% (n = 117) were still alive at survival analysis. Overall survival was significantly improved for the neoadjuvant therapy group for both unmatched (median survival, 23.5 vs 15.5; log-rank $p < 0.001$; Figure 2e) and matched patients (median survival, 22.9 vs 17.3 months; log-rank $p < 0.001$; Figure 2f) compared to those receiving conventional upfront surgery followed by adjuvant therapy.

The conclusions of the previous survival analyses were robust on sensitivity analyses in an unrestricted cohort (without censoring at 30 months).

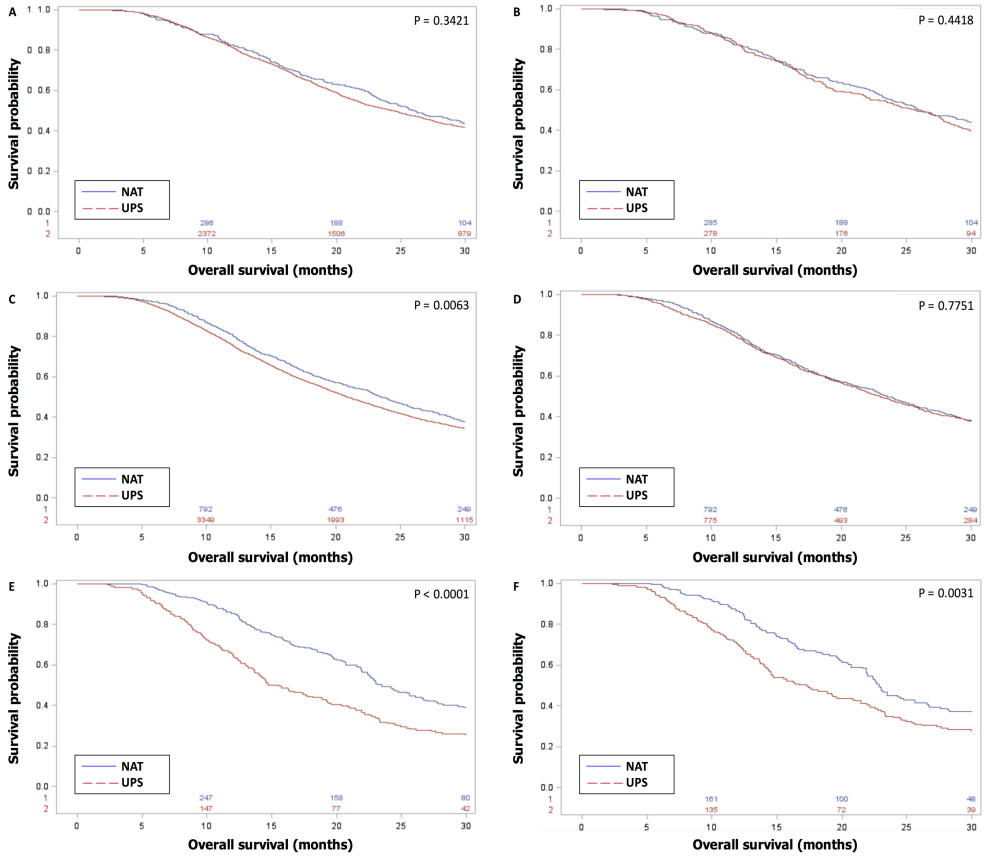

Figure 2. Kaplan-Meier survival curves comparing neoadjuvant therapy followed by surgery (NAT) to surgery succeeded by adjuvant therapy (UPS) for A+B) Clinical Stage I, C+D) Clinical Stage II and E+F) Clinical Stage III patients. Unmatched data is presented on the left and matched data on the right.

DISCUSSION

Multimodality therapy is crucial to the curative treatment of pancreatic cancer; however, the optimal treatment sequence of surgery and chemotherapy remains controversial. In this national study investigating the impact of neoadjuvant therapy vs. upfront surgery for pancreatic cancer, we examined overall survival in both an unmatched and a matched cohort. Before matching, neoadjuvant therapy appeared to be associated with a survival benefit compared to conventional upfront surgery and adjuvant therapy in stage III patients. This survival benefit of neoadjuvant therapy in stage III patients persisted after matching. No survival benefit was found in stage I and stage II disease either before or after matching. Therefore, after adjusting for the selection bias inherent in treatment allocation, neoadjuvant therapy provided a statistically significant and clinically relevant benefit only to stage III patients in this retrospective study.

The current NCCN Clinical Practice Guidelines in Oncology support the use of neoadjuvant therapy in borderline resectable and locally advanced disease, while adjuvant therapy continues to be the standard of care in resectable pancreatic cancer.[8] Irrefutable evidence for either treatment strategy has yet to be discovered, as the first worldwide randomized controlled trial in resectable patients remained inconclusive.[22] Single-center studies (many with small sample sizes) have reported favorable survival outcomes for neoadjuvant therapy, with median survivals ranging from 15 to 45 months.[5, 6, 11, 12, 23-36] In comparison, comparable, well-known adjuvant therapy trials demonstrate median survivals between 20 and 22 months.[5, 6, 11, 12, 23-36] Nevertheless, the generalizability of these single-institution experiences is limited.

More representative nationwide studies have produced widely contrasting results. Using the California Cancer Surveillance Program, Artinyan et al. found that patients with pathological stage I and II pancreatic adenocarcinoma showed survival improvements attributable to neoadjuvant therapy (median survival, 34 vs 19 months; $p = 0.003$).[37] Another study by Dimou et al. demonstrated similar results (median survival of 24 vs 22 months; $p = 0.01$) in pathological stage I and II patients identified from the NCDB (2004-2011).[38] In contrast, retrospective analyses employing data from the Surveillance, Epidemiology, and End Results Program (SEER) have identified no difference in median overall survival between the two approaches, with median survival ranging from 17 to 19 months after upfront surgery and from 20 to 23 months after neoadjuvant therapy.[39-41]

In contrast to previous findings in nationwide population-based studies, this study showed that in stage I and II disease, the treatment sequence has no effect on overall survival.[37, 38] These conflicting results may be explained by residual selection bias in previous studies, as patients who receive neoadjuvant therapy likely differ from the patients who were resected upfront. The current study used propensity score matching to balance potential confounders that could be associated with both treatment allocation and survival. Consequently, the results of this study may represent a more accurate estimation of the

impact of neoadjuvant therapy. Additionally, our analysis was based on clinical stage instead of pathological stage, accounting for potential bias due to down-staging after neoadjuvant therapy.[42] Our findings are supported by a previous study by Stessin et al. using SEER data; however, the SEER registry was not able to identify the receipt of chemotherapy, which is vital to understanding the role of neoadjuvant treatment.[39] The studies in SEER also analyzed an earlier cohort, whereas novel chemotherapeutic agents and radiation regimen have increased the efficacy of neoadjuvant therapy over time.[43, 44]

There are limitations inherent to the use of administrative database registries and the NCDB in particular. Although the NCDB has extensive external validity, as it encompasses 70% of all newly diagnosed pancreatic cancer cases nationwide, it fails to report important variables. These variables include disease-free survival, specific type of chemotherapeutic regimen, completion of chemotherapy or radiation, portal vein involvement, perineural invasion and surgical morbidity. In addition, CA 19-9 values were not coded for a substantial proportion of the study cohort. Furthermore, tumor size was not coded uniformly by the NCDB, since tumor size was recorded from the pathology report, if it was available, when the patients received no radiation or systemic treatment prior to surgery; however, if the patient received no radiation or systemic treatment prior to surgery; however, if the patient received no radiation or systemic therapy (chemotherapy, hormone therapy, immunotherapy) or radiation therapy, the largest tumor size was reported whether prior to or following treatment. Consequently, these variables could not be included in the propensity score models. However, both elevated CA 19-9 levels and large tumor size have been associated with both worse prognosis and allocation to neoadjuvant therapy.[45, 46] Not correcting for these variables in our propensity score models may underestimate the positive survival impact of neoadjuvant therapy, resulting in a type II error. The NCDB also lacks data on intention-to-treat, restricting our analysis to patients that received multimodality therapy. Consequently, this study was not able to take into consideration the number of patients that failed to proceed to surgery after neoadjuvant therapy or those patients that were resected upfront and failed to receive adjuvant therapy. However, previous studies have reported that neoadjuvant therapy significantly increases the likelihood of receiving all components of recommended therapy.[12] Therefore, despite our findings, neoadjuvant therapy could still improve survival in early-stage disease by increasing the likelihood of multimodality therapy completion. The lack of intention to treat may also have resulted in patients receiving palliative chemotherapy for early recurrence to be included in the upfront surgery arm.

Retrospective, nonrandomized database studies, by their very essence, are plagued by selection bias – the patients that received neoadjuvant therapy in this study may have differed from those resected upfront. In an effort to eliminate selection bias, we performed a propensity score matched analysis. Although propensity score matching is an excellent method to diminish selection bias, it is restricted to known available covariates and cannot

account for confounding by unknown variables. Furthermore, clinical decision-making is often based on resectability at diagnosis, dividing non-metastatic patients into clearly resectable, borderline resectable and locally advanced. In the absence of data on resectability, subset analysis was performed by clinical AJCC tumor stage, which does not completely correspond with anatomical resectability. Stage III tumors involve the celiac axis or the superior mesenteric artery.[47] Consequently, patients with clinical stage III disease in this analysis may include both borderline resectable and unresectable locally advanced patients. Birkenbach et al. (2012) demonstrated that in 73% of selected initially unresectable stage III patients, pathology revealed stage II lesions.[48] These results suggest that our stage III patients may include a substantial portion of borderline resectable patients. This study shows that initially unresectable stage III patients who can be converted to resectable patients benefit from neoadjuvant therapy. However, it remains uncertain how to interpret these results for stage III patients who appear fully resectable without vascular involvement. In addition, the utilization of neoadjuvant therapy increased substantially over time compared to conventional adjuvant therapy.[38] Therefore, the neoadjuvant therapy group may have received more advanced chemotherapeutic agents and radiation regimens. Propensity score matching inevitably reduces sample size, as not all patients can be matched, although significance was not lost after matching among Stage III patients. The primary survival analyses in this study were restricted to the first 30 months after diagnosis, although the reported survival effects seen in some previous neoadjuvant therapy studies have attenuated at 5 years.[37, 49] However, sensitivity analysis for stage III patients showed a persistent robust survival benefit in an unrestricted cohort. Finally, this study did not take into account the possible receipt of adjuvant therapy after receipt of neoadjuvant therapy followed by surgery, but rates are reported.

Despite the previously described limitations, the current study adds novel insights. To the best of our knowledge, this is the first stage-specific analysis of a nationwide contemporary cohort. The results of this study confirm that the benefit of neoadjuvant therapy is likely stage-dependent. This study employed all the available statistical protections against bias. Propensity score matching was used, eliminating 99% of the selection bias.[21] Survival was calculated from date of diagnosis and included a primary analysis within a restricted survival cohort (to account for limited follow-up thereafter) as well as confirmation in an unrestricted sensitivity analysis. Clinical stage was used instead of pathological stage to account for pathological down-staging. Importantly, in contrast with studies performed in SEER, this study was able to account for the use of chemotherapy. Finally, the use of a current nationwide study population improves the generalizability of these results.

This propensity score matched stage-specific analysis of a large contemporary nationwide hospital-based cancer registry confirms that neoadjuvant therapy should be offered to patients with clinical stage III disease, in concordance with current guidelines. This study found no evidence for a benefit to neoadjuvant treatment over conventional

upfront surgery followed by adjuvant therapy in stage I and II patients. However, the potential survival gain caused by maximizing the likelihood of receiving all components of recommended care was not taken into consideration.[11, 12]

This study suggests that neoadjuvant therapy has a stage-dependent impact on survival but does not significantly disadvantage survival in any stage of the disease. This acknowledges neoadjuvant therapy as an emerging paradigm in pancreatic cancer care and provides evidence to justify future randomized controlled trials.

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Chapter 8

Is neoadjuvant therapy sufficient in resected pancreatic cancer patients? A national study

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Journal of Gastrointestinal Surgery 2018 Feb; 22(2):214-225

ABSTRACT

Background: Despite the increasing use of neoadjuvant treatment, the question of whether preoperatively treated, successfully resected patients should receive additional postoperative adjuvant treatment remains unanswered. We evaluate the impact of adjuvant therapy following neoadjuvant treatment and pancreatectomy in pancreatic cancer patients in a large national study.

Methods: We used the National Cancer Data Base between 2006-2013 to identify resected, non-metastatic pancreatic adenocarcinoma patients who received neoadjuvant chemo(radio)therapy followed by pancreatectomy. Kaplan-Meier and multivariate Cox proportional hazard regression analyses were performed to compare survival between groups.

Results: In total, 1,357 patients were identified. 38.6% (n=524) of patients were treated with postoperative therapy. There was no difference in unadjusted median overall survival between patients who did and did not receive postoperative therapy (median survival, 27.5 months vs. 27.1 months, log-rank p=0.5409). Postoperative therapy was not significantly associated with favorable prognosis in patients with positive resection margins (log-rank p=0.6452) or positive lymph nodes (log-rank p=0.6252). On multivariate analysis, receipt of postoperative therapy was not predictive of survival (HR: 0.972; 95% CI, 0.848-1.115; p=0.6876).

Conclusions: Our results using national data suggest that after receipt of neoadjuvant therapy and pancreatectomy, additional postoperative therapy may not provide additional survival benefit. These data warrant further prospective data collection and consideration for clinical trials.

INTRODUCTION

Pancreatic cancer is the third leading cause of adult cancer deaths in the United States, with an estimated 5-year survival of less than 8% for all patients and 20-25% among resected patients.[1, 2] Complete surgical resection provides the only hope for long-term survival; however, even after surgery the majority will develop disease recurrence.[3] The frequent early systemic failure and disappointing overall survival observed for patients with localized pancreatic adenocarcinoma emphasize the importance of adjuvant systemic chemotherapy, which is now the standard of care for upfront resected patients.[4-6] Newer drug combinations and multimodality regimens will likely continue to appreciably extend survival.[7-9]

Unfortunately, 25-48% of upfront resected patients fail to initiate adjuvant therapy due to major postoperative complications and early cancer progression.[10-12] Neoadjuvant therapy overcomes these barriers and increases the proportion of patients receiving all components of recommended care.[12, 13] In addition, patients with unfavorable tumor biology who develop disease progression during neoadjuvant therapy can be spared the morbidity of a surgery.[14] Furthermore, neoadjuvant therapy may increase R0-resection rates and potentially cause tumor down-staging.[13, 15] Neoadjuvant therapy is currently recommended for patients with borderline resectable disease and its use appears to be increasing over time.[16, 17]

The most recent American Society of Clinical Oncology (ASCO) clinical practice guidelines for pancreatic cancer state that all patients who underwent resection of their tumors and did not receive preoperative therapy should be offered 6 months of adjuvant chemotherapy in the absence of medical or surgical contraindications.[9, 16] However, despite increasing use of neoadjuvant treatment, the question of whether preoperatively treated, successfully resected patients should receive additional postoperative adjuvant treatment remains unanswered.

A recent prospective single-institution study demonstrated that postoperative chemotherapy after neoadjuvant therapy in patients with low lymph node radio is associated with improved oncologic outcomes.[18] Therefore, this study evaluated the impact of adjuvant therapy followed by neoadjuvant treatment and resection for pancreatic adenocarcinoma in a large national study.

METHODS

Data Source

The National Cancer Data Base (NCDB) is jointly maintained by the American College of Surgeons' Commission on Cancer (CoC) and the American Cancer Society (ACS). The NCDB is a nationwide oncology outcomes database for more than 1500 CoC-accredited cancer programs in the United States and estimated to capture approximately

70% of all newly diagnosed cancer cases.[19] Furthermore, the NCDB requires centers to maintain a 90% follow-up rate for patients diagnosed within 5 years to remain accredited.[20] All data within the NCDB are de-identified of patient and hospital specific factors and in compliance with the Health Insurance Portability and Accountability Act (HIPAA).

Cohort assembly

The NCDB was queried for patients that received neoadjuvant chemotherapy or chemoradiotherapy followed by pancreatic resection for non-metastatic pancreatic adenocarcinoma between 2006 and 2013. Patients with pancreatic adenocarcinoma were identified using the International Classification of Disease for Oncology, 3rd edition (ICD-O-3) morphology (8140, 8500) and topography (C25.0, C25.1, C25.2, C25.3, C25.7, C25.8, C25.9) codes. The cohort was further restricted to patients who underwent operative procedures of the primary site. These patients were identified using the following Registry Operations and Data Standards and Facility Oncology Registry Data Standards (FORDS) procedure codes (25, 30, 35, 36, 37, 40, 60, 70, and 80) for pancreatectomy.[21] Neoadjuvant therapy was defined as systemic therapy with or without radiation before surgery. Postoperative therapy was classified as patients who received systemic therapy and/or radiotherapy after surgery. Patients who received neoadjuvant therapy with or without postoperative therapy were identified via the variables for treatment sequence when available in the NCDB.

Exclusion criteria

Patients with pathologic metastatic disease as specified by the 7th edition American Joint Commission on Cancer (AJCC) staging were excluded.[22] Patients were also excluded if they had no surgical procedure of the primary site or were diagnosed at autopsy (n=373), did not receive treatment at the reporting facility (n=213), received hormone therapy or immunotherapy (n=111), had other malignancies (n=918), died within 6 months from surgery of the primary site (defined by subtracting the time from the date of diagnosis to surgery from the time from date of diagnosis to last contact/death, n=1,568) or received palliative care (n=98). In addition, patients were also excluded if any of the following essential variables were listed as missing or unknown: surgery of the primary site (n=73), surgical margins status (n=114), receipt of hormone or immunotherapy (n=165), age, sex, receipt of palliative care, comorbidities, insurance status or race (n=104), treatment facility (n=37), tumor location, pathological primary tumor stage (n=304), pathological lymph node stage or tumor differentiation (n=585), vital status, and pathological distant metastasis (n=24). In addition, patient that solely received postoperative radiotherapy (n=89) were excluded, as well as patients with positive aspiration of lymph node(s), no nodes examined, no regional lymph nodes removed, unknown number of positive lymph nodes or lymph nodes examined (n=29), and patients that received intraoperative radiotherapy or unknown sequence of radiotherapy and surgery (n=13).

Definition of variables

Age at diagnosis was dichotomized in < 65 years and ≥ 65 years. Race was grouped as white and non-white. Insurance status was examined as private and other insurance. Tumor location was categorized as head/neck (ICD-O-3 topography code C25.0 and C25.7) and other (ICD-O-3 topography codes C25.1, C25.2, C25.3, C25.8 and C25.9); and tumor differentiation as well/moderately differentiated and poorly differentiated/undifferentiated. Treatment center was divided into academic and non-academic institution, including Community Cancer Program, integrated Network Cancer Program, and other or unknown type of cancer treatment program. Patient comorbidity was approximated using the Charlson comorbidity index modified by Deyo, which is comprised of up to six pre-existing comorbidities (not including cancer); this was grouped into no comorbidities (Charlson score of 0) and any comorbidities (Charlson score of ≥ 1).[23, 24] Pathologic T- and N- stages were defined according to the 7th edition of the AJCC cancer staging manual.[22] Lymph node status was designated according to the pathologic N-stage. Lymph node ratio was defined as the ratio of the number of positive lymph nodes to the total number of lymph nodes removed and examined by the pathologist. Based on previous findings by Roland et al (2015) we categorized all patients into lymph node ratio < 0.15 group and ≥ 0.15.[18] Patients with negative lymph nodes were assigned to the < 0.15 group. Using the final pathologic report, resection margin status was graded as negative (no residual tumor) or positive (microscopic residual tumor, macroscopic residual tumor, or residual tumor not otherwise specified).

Statistical analysis

Patient characteristics were compared using Chi-square tests for categorical variables. A multivariate logistic regression model was used to assess predictors for receipt of adjuvant therapy after neoadjuvant therapy followed by surgery, which was adjusted for the following covariates: age, sex, race, facility type, insurance status, comorbidities, T-stage, N-stage, tumor differentiation, and margin status.

Overall survival (OS) was defined as the time from diagnosis to death and patients who were still alive at the time of analysis were censored at the time they were last known to be alive. Survival analyses were performed using the Kaplan-Meier method with log-rank tests and a multivariate stratified Cox proportional hazards model including the following covariates: postoperative therapy, age, sex, race, insurance, comorbidities, tumor location, T-stage, N-stage, tumor differentiation, and margin status, and was stratified by facility type.

Separate subset analyses were performed in patients with: 1) negative resection margins, 2) positive resection margins, 3) negative lymph nodes, 4) positive lymph nodes, 5) lymph node ratio < 0.15, and 6) lymph node ratio ≥ 0.15. Subset analysis in patients with both positive margins and positive lymph nodes was not performed, due to a lack of power to detect a statistically significant difference.

Sensitivity analyses were performed investigating the individual impact of postoperative chemotherapy and postoperative chemoradiotherapy. All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). P-values less than 0.05 were considered statistically significant.

Table 1. Clinical and pathologic features of non-metastatic pancreatic adenocarcinoma patients who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection.

Variables	Total (n=1,357)	No postoperative therapy (n=833)	Postoperative therapy (n=524)	p
Age, n (%)				
< 65 years	771 (56.8%)	461 (55.3%)	310 (59.2%)	0.1668
≥ 65 years	586 (43.2%)	372 (44.7%)	214 (40.8%)	
Sex, n (%)				
Female	659 (48.6%)	409 (49.1%)	250 (47.7%)	0.6180
Male	698 (51.4%)	424 (50.9%)	274 (52.3%)	
Race, n (%)				
White	1,139 (83.9%)	678 (81.4%)	461 (88.0%)	0.0013
Non-white	218 (16.1%)	155 (18.6%)	63 (12.0%)	
Charlson score, n (%)				
0	907 (66.8%)	548 (65.8%)	359 (68.5%)	0.2992
≥1	450 (33.2%)	285 (34.2%)	165 (31.5%)	
Insurance status, n (%)				
Private	736 (54.2%)	432 (51.9%)	304 (58.0%)	0.0267
Other	621 (45.8%)	401 (48.1%)	220 (42.0%)	
Type of treatment center, n (%)				
Academic	959 (70.7%)	579 (69.5%)	380 (72.5%)	0.2355
Non-academic	398 (29.3%)	254 (30.5%)	144 (27.5%)	
Tumor location, n (%)				
Head	1,072 (79.0%)	651 (78.2%)	421 (80.3%)	0.3344
Other	285 (21.0%)	182 (21.8%)	103 (19.7%)	
Tumor differentiation, n (%)				
Well/moderately differentiated	924 (68.1%)	579 (69.5%)	345 (65.8%)	0.1581
Poorly differentiated	433 (31.9%)	254 (30.5%)	179 (34.2%)	
pT-stage, n (%)				
pT1-2	364 (26.8%)	241 (28.9%)	123 (23.5%)	0.0271
pT3-4	993 (73.2%)	592 (71.1%)	401 (76.5%)	
pN-stage, n (%)				
pN0	670 (49.4%)	466 (55.9%)	204 (38.9%)	<0.0001
pN1	687 (50.6%)	367 (44.1%)	320 (61.1%)	
Resection margins status, n (%)				
Negative margins	1,123 (82.8%)	706 (84.8%)	417 (79.6%)	0.0140
Positive margins	234 (17.2%)	127 (15.2%)	107 (20.4%)	

RESULTS

Baseline characteristics

In total, 1,357 patients who received neoadjuvant chemotherapy and/or radiotherapy were identified. 38.6% (n=524) of patients were treated with postoperative therapy. Baseline characteristics are summarized in Table 1. The majority of patients were

younger than 65 years (n=771; 56.8%), white (n=1,139; 83.9%), had no comorbidities (n=907, 66.8%), treated at academic center (n=959; 70.7%), privately insured (n=736; 54.2%), diagnosed with a proximal tumor (n=1,072; 79.0%), pathological peripancreatic invasion (n=993; 73.2%), positive lymph nodes (n=687; 50.6%), and positive resection margins (n=1,123; 82.8%). The median number of examined lymph nodes was 16 (interquartile range [IQR], 10-24 nodes) and median number of involved lymph nodes was 1 (IQR, 0-2 nodes).

Table 2. Clinical and pathologic features of non-metastatic pancreatic adenocarcinoma patients who underwent neoadjuvant chemotherapy or chemoradiotherapy stratified by receipt of postoperative therapy and margin status.

Variables	R0-resection (n=1,123)		p	R1-resection (n=234)		p
	No postoperative therapy (n=706)	Postoperative therapy (n=417)		No postoperative therapy (n=127)	Postoperative therapy (n=107)	
Age, n (%)						
< 65 years	391 (55.4%)	242 (58.0%)	0.3867	70 (55.1%)	68 (63.6%)	0.1914
≥ 65 years	315 (44.6%)	175 (42.0%)		57 (44.9%)	39 (36.4%)	
Sex, n (%)						
Female	351 (n=49.7%)	205 (49.2%)	0.8571	58 (45.7%)	45 (42.1%)	0.5791
Male	355 (n=50.3%)	212 (50.8%)		69 (54.3%)	62 (57.9%)	
Race, n (%)						
White	578 (81.9%)	369 (88.5%)	0.0032	100 (78.7%)	92 (86.0%)	0.1505
Non-white	128 (18.1%)	48 (11.5%)		27 (21.3%)	15 (14.0%)	
Charlson score, n (%)						
0	473 (67.0%)	292 (70.0%)	0.2930	75 (59.1%)	67 (62.6%)	0.5784
≥1	233 (33.0%)	125 (30.0%)		52 (40.9%)	40 (37.4%)	
Insurance status, n (%)						
Private	365 (51.7%)	240 (57.6%)	0.0572	67 (52.8%)	64 (59.8%)	0.2786
Other	341 (48.3%)	177 (42.4%)		60 (47.2%)	43 (40.2%)	
Type of center, n (%)						
Academic	499 (70.7%)	304 (72.9%)	0.4255	80 (63.0%)	76 (71.0%)	0.1939
Non-academic	207 (29.3%)	113 (27.1%)		47 (37.0%)	31 (29.0%)	
Tumor location, n (%)						
Head	548 (77.6%)	335 (80.3%)	0.2835	103 (81.1%)	86 (80.4%)	0.8880
Other	158 (22.4%)	82 (19.7%)		24 (18.9%)	21 (19.6%)	
Tumor differentiation, n (%)						
Well/moderately	490 (69.4%)	284 (68.1%)	0.6494	89 (70.1%)	61 (57.0%)	0.0379
Poorly	216 (30.6%)	133 (31.9%)		38 (29.9%)	46 (43.0%)	
pT-stage, n (%)						
pT1-2	223 (31.6%)	111 (26.6%)	0.0785	18 (14.2%)	12 (11.2%)	0.5001
pT3-4	483 (68.4%)	306 (73.4%)		109 (85.8%)	95 (88.8%)	
pN-stage, n (%)						
pN0	415 (58.8%)	175 (42.0%)	<0.0001	51 (40.2%)	29 (27.1%)	0.0360
pN1	291 (41.2%)	242 (58.0%)		76 (59.8%)	78 (72.9%)	

Predictors postoperative therapy

On multivariate analysis, white race (vs. non-white race: odds ratio (OR), 1.577; 95% CI, 1.141-2.179; p=0.0058) and positive regional lymph nodes (vs. negative lymph nodes: OR, 1.896; 95% CI, 1.500-2.396; p<0.0001) were predictive for receipt of

postoperative therapy after neoadjuvant therapy followed by surgery. However, male sex (vs. female sex: OR, 0.995; CI 95%, 0.794-1.247; p=0.9658), age < 65 years (vs. age ≥ 65 years: OR, 1.041; 95% CI, 0.771-1.405; p=0.7954), no comorbidities (vs. comorbidities: OR, 1.104; 95% CI, 0.869-1.404; p=0.4179), non-academic treatment center (vs. academic treatment center: OR, 0.867; 95% CI, 0.677-1.111; p=0.2593), private insurance (vs. non-private insurance: OR, 1.249; 95% CI, 0.926-1.686; p=0.1451), poor tumor differentiation (vs. well/moderate tumor differentiation: OR, 1.120; 95% CI, 0.882-1.423; p=0.3537), pathological stage T3-4 (vs. stage T1-2: OR, 1.023; 95% CI, 0.782-1.339; p=0.8667), tumor location in the head of the pancreas (vs. other tumor locations: OR, 1.076; 95% CI, 0.814-1.421; p=0.6064), and positive resection margins (vs. negative resection margins: OR 1.294; 95% CI, 0.963-1.740; p=0.0872 were not significant predictors.

Survival analysis

Among all patients undergoing neoadjuvant therapy and resection, median overall survival was similar (log-rank p=0.5409; Fig. 1) for patients who received postoperative chemotherapy (median OS, 27.5 months; 95% CI, 25.3-29.9 months) and who did not receive any additional treatment (median OS, 27.1 months; 95% CI, 25.6-29.5 months).

After controlling for differences in patient and tumor characteristics, the long-term hazard of death remained comparable among patients who did versus who did not receive postoperative adjuvant therapy (Hazard Ratio (HR), 0.972; 95% CI, 0.848-1.115; p=0.6876). In contrast, poor/undifferentiated tumors (vs. well/moderate differentiated tumors: HR, 1.229; 95% CI, 1.069-1.413; p=0.0039), positive lymph nodes (vs. negative lymph nodes: HR, 1.334; 95% CI, 1.160-1.534, p<0.0001) and positive resection margins (vs. negative resection margins: HR, 1.514; 95% CI, 1.283-1.787; p<0.0001) were significantly associated with decreased long-term survival outcomes. Age < 65 years (vs. ≥ 65 years: HR, 1.102; 95% CI, 0.928-1.309; p=0.2684), male sex (vs. female sex: HR, 1.004; 95% CI, 0.879-1.148, p=0.9494), white race (vs. non-white race: HR, 1.056; 95% CI, 0.880-1.268; p=0.5586), no comorbidities (vs. any comorbidities: HR, 0.953; 95% CI, 0.830-1.094; p=0.4947), tumor located in the head of the pancreas (vs. other tumor location: HR, 1.017; 95% CI, 0.863-1.200; p=0.8369), private insurance (vs. other/non-private insurance: HR, 0.930; 95% CI, 0.783-1.105; p=0.4092), non=academic treatment facility (vs. academic treatment facility: HR, 1.026; 95% CI, 0.889-1.185; p=0.7240) and pathological stage T3-4 (vs. stage T1-2: HR, 1.106; 95% CI, 0.942-1.297; p=0.2187) did not significantly affect survival on multivariate survival in patients who received neoadjuvant therapy and resection for pancreatic adenocarcinoma.

Subset analyses

On final pathologic assessment, resection margins were positive in 17.2% (n=234) of patients (Table 2). Postoperative therapy did not significantly affect survival in patients with positive margins (median OS, 20.1 vs. 21.9 months; log-rank p=0.6452; Fig. 2a), as

well as in patients with negative margins (median OS, 29.2 vs. 29.1 months; log-rank p=0.9292; Fig 2b).

Overall 687 and 670 patients were diagnosed with respectively positive and negative lymph nodes. Baseline characteristics are summarized in table 3. Postoperative therapy was not associated with survival in patients with positive lymph nodes (median OS, 25.0 vs. 25.3 months; log-rank p=0.6252; Fig. 3a) or negative nodes (median OS, 32.8 vs. 29.7 months; log-rank p=0.3655; Fig 3b).

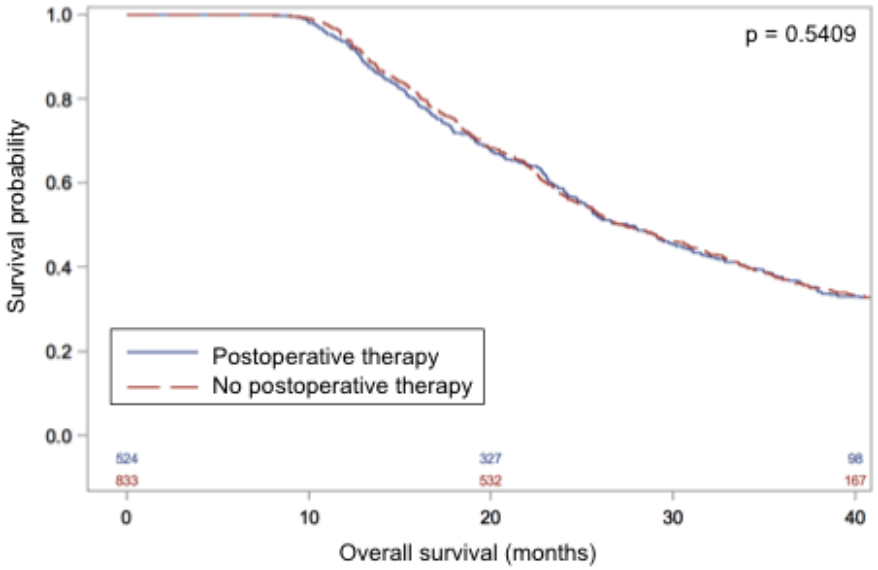

Figure 1. Kaplan-Meier curve for non-metastatic pancreatic adenocarcinoma patients who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection stratified by receipt of postoperative therapy.

There were 1,057 patients in the lymph node ratio < 0.15 group, and 300 patients in the lymph node ratio ≥ 0.15 group. Postoperative therapy did not confer a survival benefit among patients with lymph nodes ratio <0.15 (median OS, 30.6 vs. 29.1 months; log-rank p=0.8860; Fig 4a). Similar results were observed for patient with a lymph node ratio ≥ 0.15 (median OS, 22.8 vs. 22.2 months; log-rank p=0.8807; Fig 4b).

Sensitivity analyses

A total of 381 patients received postoperative chemotherapy whereas 143 patients received postoperative chemoradiotherapy. There was no significant difference (log-rank p=0.5237; Fig 5.) in survival among patients treated with postoperative chemotherapy (median OS, 28.0 months), postoperative chemoradiotherapy (median OS, 24.8 months), or no postoperative therapy (median OS, 27.1 months).

Table 3. Clinical and pathologic features of non-metastatic pancreatic adenocarcinoma patients who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection stratified by receipt of postoperative therapy and resection margin status.

Variables	Negative lymph nodes (n=670)			Positive lymph nodes (n=687)		
	No postoperative therapy (n=466)	Postoperative therapy (n=204)	p-value	No postoperative therapy (n=367)	Postoperative therapy (n=320)	p-value
Age, n (%)						
< 65 years	274 (58.8%)	122 (59.8%)	0.8075	187 (51.0%)	188 (58.8%)	0.0406
≥ 65 years	192 (41.2%)	82 (40.2%)		180 (49.0%)	132 (41.2%)	
Sex, n (%)						
Female	229 (49.1%)	94 (46.1%)	0.4653	180 (49.0%)	156 (48.8%)	0.9382
Male	237 (50.9%)	110 (53.9%)		187 (51.0%)	164 (51.2%)	
Race, n (%)						
White	372 (79.8%)	173 (84.8%)	0.1282	306 (83.4%)	288 (90.0%)	0.0114
Non-white	94 (20.2%)	31 (15.2%)		61 (16.6%)	32 (10.0%)	
Charlson score, n (%)						
0	304 (65.2%)	144 (70.6%)	0.1756	244 (66.5%)	215 (67.2%)	0.8454
≥1	162 (34.8%)	60 (29.4%)		123 (33.5%)	105 (32.8%)	
Insurance status, n (%)						
Private	254 (54.5%)	120 (58.8%)	0.3004	178 (48.5%)	184 (57.5%)	0.0184
Other	212 (45.5%)	84 (41.2%)		189 (51.5%)	136 (42.5%)	
Type of center, n (%)						
Academic	313 (67.2%)	151 (74.0%)	0.0769	266 (72.5%)	229 (71.6%)	0.7893
Non-academic	153 (32.8%)	53 (26.0%)		101 (27.5%)	91 (28.4%)	
Tumor location, n (%)						
Head	355 (76.2%)	160 (78.4%)	0.5249	296 (80.7%)	261 (81.6%)	0.7617
Other	111 (23.8%)	44 (21.6%)		71 (19.4%)	59 (18.4%)	
Tumor differentiation, n (%)						
Well/moderately	331 (71.0%)	143 (70.1%)	0.8072	248 (67.6%)	202 (63.1%)	0.2210
Poorly	135 (29.0%)	61 (29.9%)		119 (32.4%)	118 (36.9%)	
pT-stage, n (%)						
pT1-2	185 (39.7%)	72 (35.3%)	0.2805	56 (15.3%)	51 (15.9%)	0.8067
pT3-4	281 (60.3%)	132 (64.7%)		311 (84.7%)	269 (84.1%)	
Margins status, n (%)						
Negative margins	415 (89.1%)	175 (85.8%)	0.2295	291 (9.3%)	242 (75.6%)	0.2503
Positive margins	51 (10.9%)	29 (14.2%)		76 (90.7%)	78 (24.4%)	

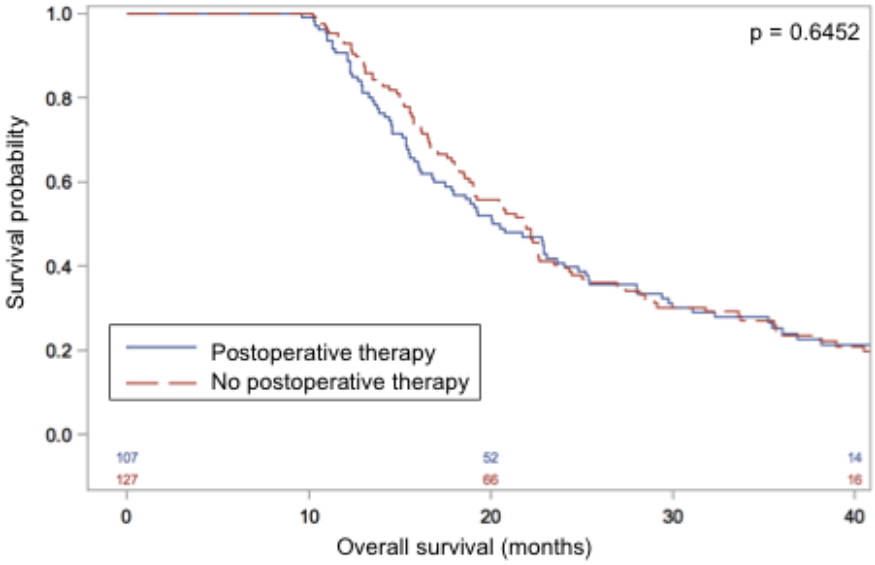

Figure 2a. Kaplan-Meier curve for non-metastatic pancreatic adenocarcinoma patients who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection with positive resection margins stratified by receipt of postoperative therapy.

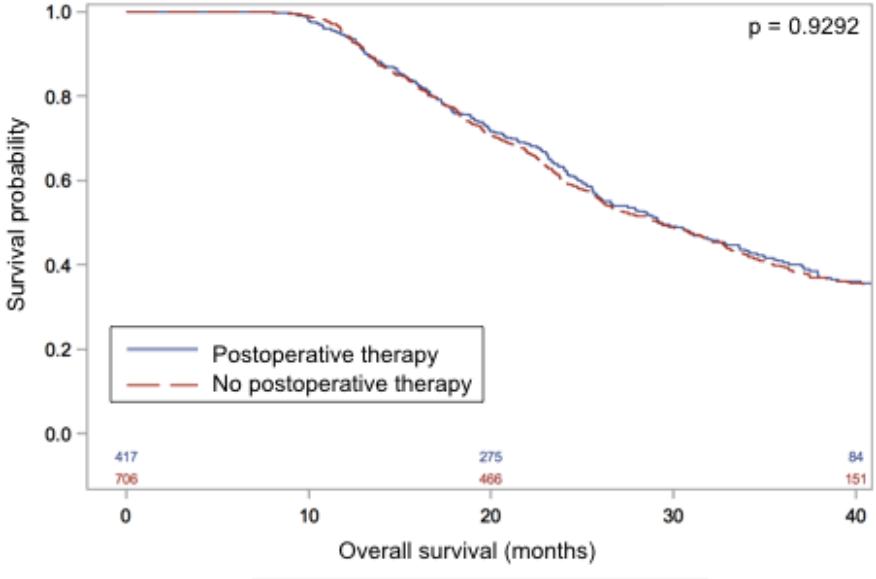

Figure 2b. Kaplan-Meier curve for non-metastatic pancreatic adenocarcinoma patients who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection with negative resection margins stratified by receipt of postoperative therapy.

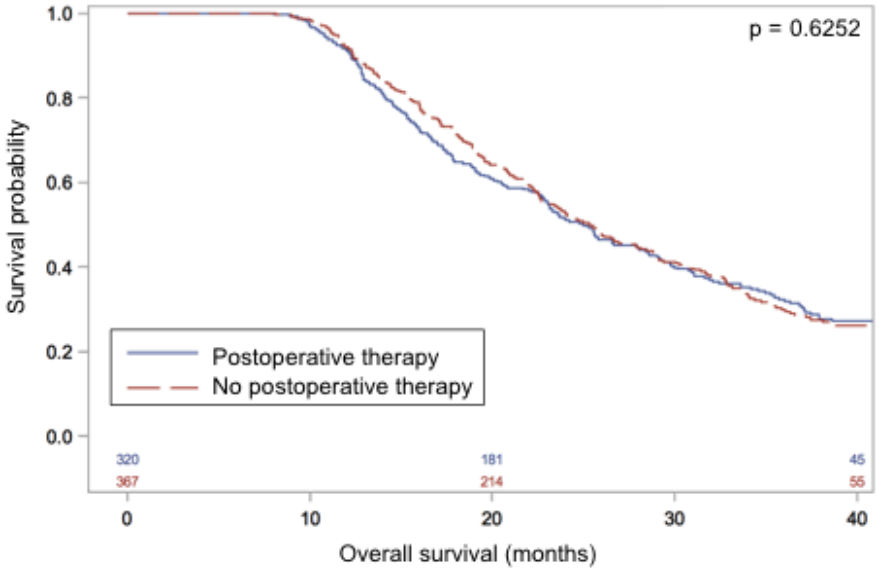

Figure 3a. Kaplan-Meier curve for non-metastatic pancreatic adenocarcinoma patients with pN1 who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection stratified by receipt of postoperative therapy.

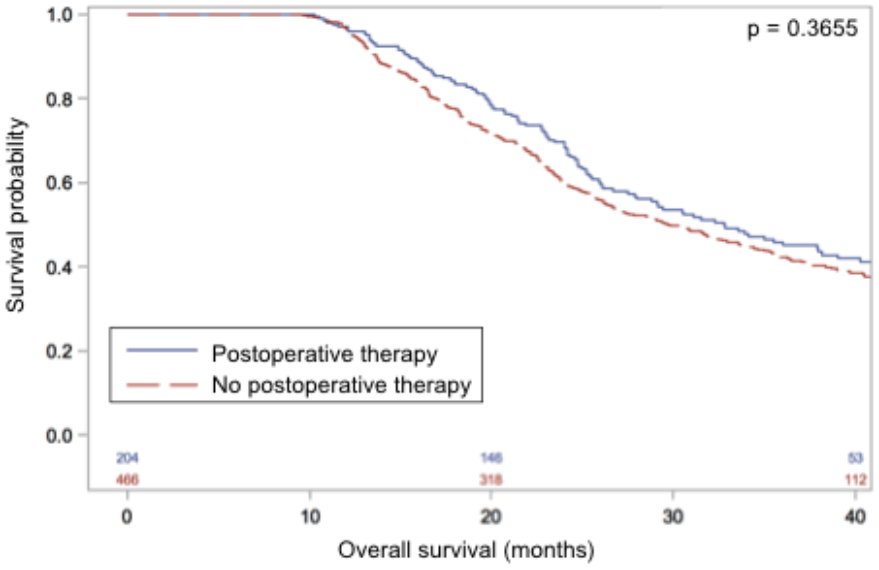

Figure 3b. Kaplan-Meier curve for non-metastatic pancreatic adenocarcinoma patients with pN0 who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection stratified by receipt of postoperative therapy.

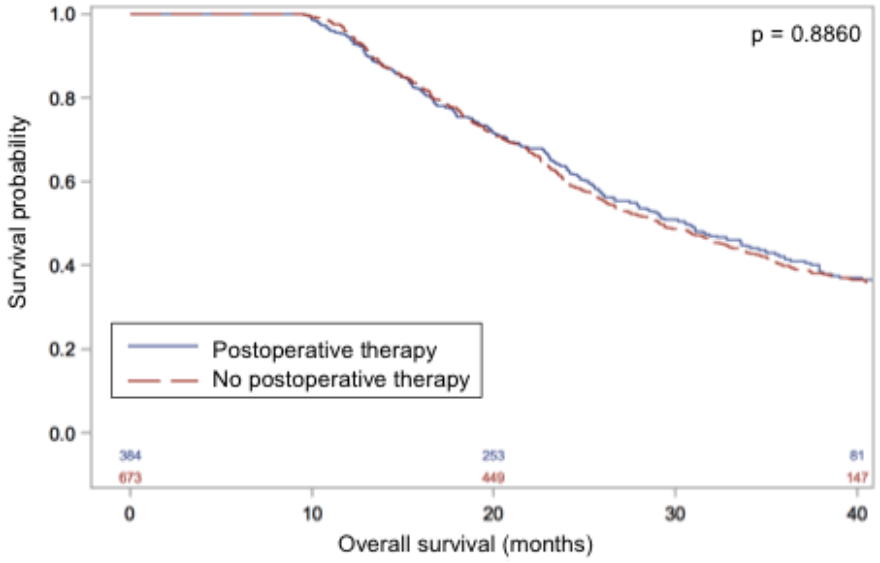

Figure 4a. Kaplan-Meier curve for non-metastatic pancreatic adenocarcinoma patients with lymph nodes ratio < 0.15 who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection stratified by receipt of postoperative therapy.

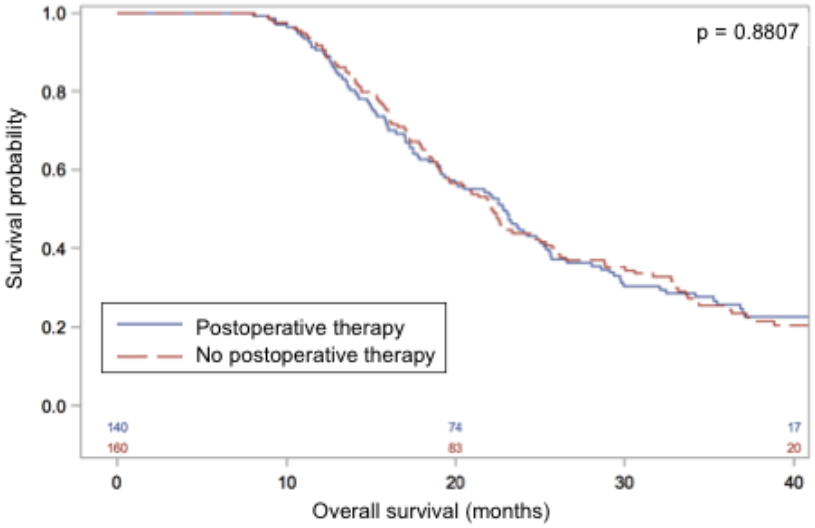

Figure 4b. Kaplan-Meier curve for non-metastatic pancreatic adenocarcinoma patients with lymph nodes ratio ≥ 0.15 who underwent neoadjuvant chemotherapy or chemoradiotherapy followed by surgical resection stratified by receipt of postoperative therapy

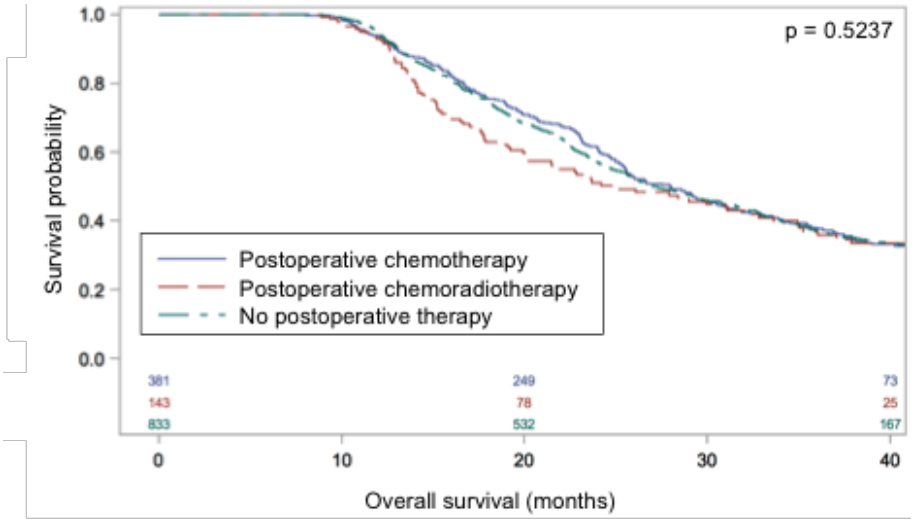

Figure 5. Kaplan-Meier curve comparing receipt of postoperative chemotherapy, postoperative chemoradiotherapy, and no postoperative therapy for non-metastatic pancreatic adenocarcinoma patients who underwent neoadjuvant therapy followed by surgical resection.

DISCUSSION

Postoperative chemotherapy is the standard of care for upfront resected chemo-naive pancreatic cancer patients, based on the favorable outcomes of various well-known randomized controlled trials.[4, 5, 9] However, the role of postoperative chemotherapy among patients who received neoadjuvant therapy followed by surgery remains unclear. To the best of our knowledge the current study is the first to investigate the survival impact of postoperative therapy after neoadjuvant therapy followed by surgery using national data. Our results demonstrate that survival for patients undergoing neoadjuvant treatment followed by pancreatectomy is impacted by additional postoperative therapy (median OS, 27.1 vs. 27.5 months; log-rank p=0.5409). Postoperative therapy was also not significantly associated with more favorable prognosis after neoadjuvant therapy and surgery among patients with positive resection margins (log-rank p=0.6452, negative resection margins (log-rank p=0.9292), positive (log-rank p=0.6252) or negative (log-rank p=0.3655) lymph nodes. These data collectively highlight that after receipt of neoadjuvant chemo(radio)therapy and pancreatectomy, additional postoperative therapy may not be required. On multivariate survival analysis, after adjustment for critical confounders, no benefit of additional post-operative therapy was found (HR: 0.972; 95% CI, 0.848-1.115; p=0.6876).

Interest for neoadjuvant therapy as a new treatment paradigm for pancreatic cancer is growing from the emerging conviction that pancreatic cancer is a systemic disease at the time of discovery. Neoadjuvant therapy provides early treatment for microscopic metastatic

disease, aids in the identification of patients who are unlikely to benefit from pancreatectomy, decreases treatment delay caused by postoperative recovery and complications, and increases the potential for negative resection margins.[15, 13] Current National Comprehensive Cancer Network (NCCN) guidelines support the use of neoadjuvant therapy for patients with borderline resectable pancreatic cancer and several centers of excellence have expanded the use of neoadjuvant therapy by including patients who are anatomically resectable.[17, 12, 25] The first worldwide randomized trial for neoadjuvant chemoradiotherapy versus upfront surgery in patients with pancreatic cancer was terminated early due to slow recruitment and failure to demonstrate a significant difference between groups; however, non-randomized single-institution studies have reported prolonged survival in patients undergoing neoadjuvant therapy, with median overall survival ranging from 21 to 45 months.[26-29] In addition, previous national studies investigating the survival impact of neoadjuvant therapy found median overall survival rates of 23-26 months depending on the tumor stage.[29, 30] This study demonstrate more favorable survival compared to previous nationwide appraisals, which is likely caused by the exclusion of patients who died within 6 months from date of surgery in our study.

Postoperative chemotherapy is currently widely recommended for resected pancreatic cancer patients, on the basis of the European Study Group for Pancreatic Cancer 1 (ESPAC-1), the Charite Onkologie 001 (CONKO-001), and the Japan Adjuvant Study Group of Pancreatic Cancer (JASPAC) 01 trials, all demonstrating a survival benefit using fluorouracil or gemcitabine monotherapy.[4, 5, 31] The results of the recently published ESPAC-4 study, a multicenter, international, open-label randomized controlled trial indicated that multi-agent chemotherapy further increased overall survival compared with monotherapy after resection for pancreatic cancer in an adjuvant setting.[8] However, the role of postoperative therapy after neoadjuvant therapy followed by pancreatectomy remains unclear. Roland and colleagues performed a retrospective single-institution study in 263 patients who underwent resection following neoadjuvant therapy and found that the addition of postoperative chemotherapy was associated with improved survival (median OS, 32.8 vs. 72.1 months; p=0.007) outcomes only in patients who had a low lymph node ratio (<0.15).[18] Patients with a persistently high lymph node ratio (≥0.15) following neoadjuvant therapy reported poor survival (median OS, 22.3 vs. 18.2; p=0.79), despite additional postoperative therapy.[18]

Several studies described that the benefit of radiotherapy may be limited to patients with lymph node-positive disease.[32, 33] In contrast, it is more likely that patients who had persistently elevated lymph node ratio after neoadjuvant therapy in the study by Roland et al. represented a cohort of patients with aggressive tumor biology who did not respond to chemotherapy.[32, 33, 18] However, in our study postoperative therapy was not associated with a survival benefit for patients with either positive lymph nodes, negative lymph nodes, lymph node ratio < 0.15 or lymph node ratio ≥0.15. These findings may in part be explained by the stringent control for immortality bias by excluding patients that

died within 6 months after diagnosis in our study, as patients who were able to receive postoperative may have had more favorable tumor biology and less major surgical complications, which is why they were able to receive additional postoperative therapy. Discordant with previous reports that postoperative chemoradiotherapy after upfront surgery is associated with improved survival, our results demonstrate no significant difference between patients that received postoperative chemoradiotherpy and postoperative chemotherapy or no postoperative therapy et al.[34, 35] The latter may be caused by the fact that, for a variety of reasons, patients with resected adenocarcinoma of the pancreas who subsequently receive adjuvant radiotherapy may have different patient characteristics from those who do not.[6, 36]

Several limitations should be considered when interpreting the data. Due to the constraints of the NCDB, relevant covariates, such as specific type of chemotherapeutic regimen, number of chemotherapy cycles, anatomical tumor resectabiliy, and vascular invasion, were not available, as well as certain important disease-specific outcomes, surgical morbidity, and quality of life indicators. In addition, CA 19-9 values were not coded for a substantial proportion of the study cohort.[37] In addition, the specific clinical rationale for not receiving postoperative therapy after neoadjuvant therapy and surgery cannot be accurately obtained from the NCDB. Similar to upfront resected patients, the most common reasons for not receiving postoperative therapy after neoadjuvant therapy and surgery were likely postoperative complications, cancer progression and/or poor patient performance status.[38, 39] Patients may also not have received postoperative therapy due to the absence of conclusive guidelines before the introduction of the latest ASCO recommendations.[9] They may have selected patients at high risk for recurrence who would benefit most from addition postoperative therapy.[32] In accordance, our results demonstrate that there is a significant association between resection margins and/or lymph nodes and receipt of postoperative therapy. Subsequently, patients who received postoperative therapy may have important difference from those who do not. This type of selection bias was controlled for to a considerable extent through the use of multivariate Cox proportional hazard and logistic regression analyses models. However, given the retrospective non-randomized nature of the study, unmeasured confounding and selection bias may have influenced the results. Furthermore, there may be significant biologic heterogeneity between groups that is not accounted for by this study.

Despite the previously describes limitations, the present study represents, to the best of our knowledge, the first report investigating the survival impact of postoperative therapy after neoadjuvant therapy followed by resection for pancreatic adenocarcinoma. The current study also constitutes the largest cohort to date to examine this unanswered clinical dilemma. Guidelines are present as testimony to the absence of scientific evidence, with both the NCCN and ASCO clinical practice guidelines leaving consideration of additional postoperative therapy after neoadjuvant therapy and surgery at the discretion of the physician.[17] Furthermore, we performed rigorous subset analyses in patients with

negative lymph nodes, as well as patients with positive lymph node, negative resection margins, or positive resection margins. In addition, this study improved on previous iterations by performing a multivariate Cox proportional hazard analysis controlling for multiple known potential confounders.

In conclusion, following neoadjuvant therapy and definitive resection of pancreatic adenocarcinoma, 38.6% of patients received additional postoperative therapy. After adjusting for various clinical and pathologic factors in the no postoperative versus postoperative therapy cohorts using a multivariate Cox proportional hazard model, there were no difference in survival among patients who did versus who did not receive postoperative therapy. Even in subgroups with less favorable prognosis, postoperative therapy was not significantly associated with superior outcomes after neoadjuvant therapy and surgery, which indicates that additional postoperative therapy may not be obligatory after neoadjuvant therapy. These data warrant further prospective data collection and consideration for clinical trials, especially in light of the significant survival benefit of multi-agent chemotherapy in an adjuvant setting.[8] Furthermore, added traction may be gained by the identification of biomarkers to guide therapeutic response for pancreatic cancer.

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Chapter 9

Neoadjuvant therapy affects margins and margins affect all: perioperative and survival outcomes in resected pancreatic cancer

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HPB 2018 Jun; 20(6):573-581

ABSTRACT

Background: Resection margin status is an important prognostic factor in pancreatic cancer; however, the impact of positive resection margins in those who received neoadjuvant therapy remains unclear. The current study investigates the prognostic impact of resection margin status after neoadjuvant therapy and pancreaticoduodenectomy for patients with pancreatic adenocarcinoma.

Methods: Patients who underwent pancreaticoduodenectomy for pancreatic adenocarcinoma between 2006 and 2013 were identified from the National Cancer Database. Multivariable logistic regression analysis was utilized to examine the predictive value of neoadjuvant therapy for resection margin status. Long-term outcomes were compared using a Cox proportional hazards model.

Results: 7 917 patients were identified in total: 1 077 (13.6%) and 6 840 (86.4%) patients received neoadjuvant therapy and upfront surgery, respectively. Upfront surgery was independently predictive of a positive margin (25.7% vs. 17.7%; OR, 1.54) compared to neoadjuvant therapy. After receipt of neoadjuvant therapy, positive margins (median overall survival, 18.5 vs. 25.9 months; HR, 1.58) remained significantly associated with poor survival on multivariable analysis.

Discussion: While neoadjuvant therapy is associated with decreased R1/R2-resection rates after pancreaticoduodenectomy, the poor prognostic impact of positive margins is not abrogated by neoadjuvant therapy, stressing the need for complete tumor clearance and postoperative treatment even after neoadjuvant therapy.

INTRODUCTION

Pancreatic adenocarcinoma currently ranks as the third leading cause of cancer-related death and is estimated to become the second most common cause by 2030 (1). Despite advances in operative techniques, postoperative care, and therapeutic agents, improving the prognosis of pancreatic cancer patients remains a formidable challenge (2). Complete surgical resection offers the only hope for meaningful survival, yet 50%-86% of patients develop local recurrence following a presumed curative resection (3-6). The high frequency of local recurrence points to the necessity of multimodal therapy (7, 8). Adjuvant therapy represents the standard of care throughout the United States (9). While the use of neoadjuvant therapy is recommended in patients with borderline resectable disease and increasingly applied for resectable pancreatic adenocarcinoma, there remains a lack of conclusive results from randomized controlled trials (10, 11).

Over the past decades, pancreatic surgeons have been pushing the boundaries of surgical resection in an effort to attain negative margins. Nonetheless, data from high-volume academic centers show R0-resection rates of only 70% to 76% after upfront surgery (12-14). Previous studies have suggested that neoadjuvant chemoradiation may potentially downstage pancreatic tumors to attain locoregional control and subsequently reducing positive margin rates (15, 16). However, controversy continues to exist as to whether neoadjuvant therapy has the ability to abrogate the negative survival impact of positive margins after pancreatic cancer surgery (17).

As neoadjuvant therapy becomes a more widespread treatment strategy for pancreatic adenocarcinoma, a better understanding of its potential significance for surgical margin clearance is pivotal (11). Therefore, the aim of this study was to assess the impact of neoadjuvant therapy on resection margin status, and the prognostic impact of incomplete margin clearance after neoadjuvant therapy in resected stage I-III pancreatic adenocarcinoma patients using national cancer registry data.

METHODS

Data source

The National Cancer Database is a nationwide hospital-based cancer registry, founded as a joint initiative of the Commission on Cancer of the American College of Surgeons and the American Cancer Society. The National Cancer Database comprises more than 30 million patient records collected by over 1,500 Commission on Cancer accredited facilities across the United States (US) (18). Furthermore, the National Cancer Database requires centers to maintain a 90% follow-up rate for patients diagnosed within 5 years to remain accredited (19).

Selection criteria

Using the National Cancer Database, patients diagnosed with pancreatic adenocarcinoma between 2006 and 2013 were identified according to the third edition of the International Classification of Disease for Oncology (ICD-O-3) morphology (8140 and 8500) and topography codes (C25.0, C25.1, C25.2, C25.3, C25.7, C25.8, and C25.9). Patients were excluded if they did not receive pancreaticoduodenectomy (n=195,674) based on the following Facility Oncology Registry Data Standards (FORDS) site-specific procedure coding: 35, 36, 37, and 70.

The cohort was further limited by sequentially excluding patients diagnosed with clinical M1 disease (n=748), that did not receive any treatment at the reporting facility (n=1,072), reported other malignancies or received hormone therapy (n=95), immunotherapy (n=339), and/or intraoperative chemotherapy and radiation (n=45). Furthermore, patients were excluded if one of the following variables was unknown or missing: type of surgery, other malignancies (n=5 815), receipt of hormone therapy (n=1 492), receipt of immunotherapy (n=25), sequence of radiation and surgery (n= 302), sequence of systemic treatment and surgery or receipt of intraoperative systemic therapy (n=3 730), surgical margin status (n=426), length of inpatient stay (n=2 117), vital status (n=2 421), 90-day mortality (n=149), age, sex, race (n=151), comorbidities, insurance status (n=184), type of treatment facility (n=2 003), clinical stage (n=4 488), tumor differentiation (n=837), lymph node status (n=110), and treatment sequence (n=350).

Predictive variables

Systemic therapy categories were defined using the National Cancer Database PUF's variables for systemic and surgical therapy sequencing. Patients who received any neoadjuvant systemic therapy were grouped together regardless of receipt of adjuvant systemic therapy. Age was dichotomized into <65 years and ≥ 65 years. Race was grouped as white and other. Comorbid conditions were analyzed using the Deyo modified Charlson comorbidity index and divided into Charlson-Deyo scores of 0 and ≥ 1 (20). Insurance status was dichotomized into private and other. Type of treatment facility was divided in academic and non-academic center. Clinical stage was defined in compliance with the $6^{th}/7^{th}$ edition staging system proposed by the American Joint Committee on Cancer (AJCC). Whenever clinical tumor stage was missing, the individual clinical T, N and M stages were combined according to AJCC staging guidelines into a group stage (21). Tumor differentiation was categorized into well, moderately, and poorly or undifferentiated tumors. Receipt of chemotherapy included both single- and multi-agent chemotherapy. Radiotherapy was classified as beam radiation, radioactive implants, and/or radioisotopes. Neoadjuvant therapy was defined as neoadjuvant chemotherapy with or without radiation either before or after surgery. Upfront surgery was defined as either no chemotherapy or chemotherapy administered only after surgical resection, independent of receipt of radiation.

Using the final pathologic report, resection margin status was graded as “negative” (no residual tumor) or “positive” (microscopic residual tumor, macroscopic residual tumor, or residual tumor not otherwise specified). Prolonged hospital stay was defined as over 14 days of hospital admission after date of surgery. 90-day mortality was defined from date of most definitive surgery. Overall survival was calculated as the number of months between the date of diagnosis and the date on which the patient was last contacted or died.

Statistical analysis

Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Baseline characteristics were reported as frequencies and distributions. Categorical variables were compared using the chi-square test. The predictors of positive resection margins, prolonged hospital stay, and 90-day mortality were identified using multivariable logistic regression models, including receipt of neoadjuvant therapy, gender, age at diagnosis, race, comorbidities, insurance status, type of treatment center, clinical stage, lymph node status, and tumor differentiation. Since the objective of these multivariable regression models is assessing the association between neoadjuvant therapy and positive resection margins, prolonged hospital stay, and 90-day mortality, these models take into account most confounders and effect modifiers to reduce potential bias at the cost of the discriminative ability of the overall model. This study did not use automated variable selection or resampling (22-24). The results of the logistic regression models were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). The c-statistics for the multivariable models were as follows: 0.608 for positive resection margins, 0.576 for prolonged hospital stay, and 0.650 for 90-day mortality.

Survival analysis in all resected pancreatic cancer patients was performed by treatment sequence and by resection margins status using the Kaplan-Meier method. In addition, the prognostic impact of resection margins was assessed separately after neoadjuvant therapy, upfront surgery with or without adjuvant therapy, and upfront surgery succeed by adjuvant chemoradiotherapy using a multivariable Cox proportional hazard model. For patients that received neoadjuvant therapy the model adjusted for: insurance status, type of treatment center, clinical stage and lymph node status, stratified by age group and tumor differentiation grade. For patients that received upfront surgery with or without adjuvant therapy the model adjusted for clinical stage and tumor differentiation, stratified by age group, type of treatment center, insurance status, lymph node status, and receipt of adjuvant therapy. For patients that received upfront surgery succeeded by adjuvant chemoradiotherapy the model adjusted for age group, insurance status, type of treatment center, lymph node status, and clinical stage, stratified by tumor differentiation.

Sensitivity analyses were carried out to assess the robustness of our findings. Multivariable regression analyses predicting positive resection margins (c-statistics, 0.605), prolonged hospital stay (c-statistics, 0.571), and 90-day mortality (c-statistics, 0.645) were

performed in patients that underwent neoadjuvant chemoradiotherapy followed by surgery and upfront surgery with or without adjuvant therapy. In addition, multivariable Cox proportional hazard survival analysis adjusted for insurance status, type of treatment center, clinical stage, lymph node status, and margin status, stratified by age and tumor differentiation was performed after excluding patients with macroscopically residual tumor documented. Furthermore, after exclusion of patients treated at non-academic centers, additional multivariable regression analyses for positive resection margins (c-statistics, 0.599), prolonged hospital stay (c-statistics, 0.580), and 90-day mortality (c-statistics, 0.639) were performed. Kaplan-Meier survival analysis was also executed in the afore mentioned subgroup, as the proportional hazard assumption did not hold for margin status in this subgroup. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

7 917 patients were identified. In the entire cohort, 1 077 (13.6%) patients received neoadjuvant therapy and 6 840 (86.4%) patients were resected upfront. The majority of patients were over 65 years old (n=4 427; 55.9%), male (n=4 027; 50.9%), white (n=6 601; 83.4%), had no comorbidities (n=5 265; 66.5%), had non-private insurance (n=4,654; 58.8%), were treated at academic centers (n=5 401; 68.2%), had clinical stage II disease (n=4 576; 57.8%), had positive lymph nodes (n=5 470; 69.1%), and moderately differentiated tumors (n=4 126; 52.1%).

Baseline characteristics are summarized in Table 1. Neoadjuvant therapy was significantly associated with age group, insurance, treatment center, clinical stage, lymph nodes, tumor differentiation, and resection margin status (all $p<0.0001$).

Surgical outcomes

17.7% (n=191) and 25.8% (n=1 761) of patients had positive resection margins after neoadjuvant therapy and upfront surgery, respectively. On multivariable analysis, the probability of positive resection margins, male sex, non-white race, non-academic treatment center, upfront surgery, poorly differentiated tumor grade, negative lymph nodes, stage II disease, and stage III disease were predictive for positive resection margins (Table 2). However, age, comorbidities, and insurance were not correlated with resection margin status (Table 2).

17.6% (n=190) and 21.7% (n=1 481) of patients experiences prolonged hospital stay after surgery following neoadjuvant therapy and upfront surgery, respectively. On multivariable analysis, the probability of experiencing prolonged hospital stay, age ≥ 65 years at diagnosis, non-white race, any comorbidities, non-private insurance, non-academic treatment center, positive resection margins, upfront surgery, and negative regional lymph

nodes were significant predictors of prolonged hospital stay on multivariable analysis (Table 2). However, sex, tumor differentiation, and clinical stage were not significantly associated with prolonged hospital stay (Table 2).

Table 1. Characteristics and surgical outcomes of resected stage I-III pancreatic adenocarcinoma patients by treatment sequence.

Characteristics	No neoadjuvant therapy (n=6 840)	Neoadjuvant therapy (n=1 077)	p
Sex, n (%)			
Male	3 464 (50.6%)	563 (52.3%)	0.3195
Female	3 376 (49.4%)	514 (47.7%)	
Age at diagnosis, n (%)			
< 65 years	2 886 (42.2%)	604 (56.1%)	<0.0001
≥ 65 years	3 954 (57.8%)	473 (43.9%)	
Race, n (%)			
White	5 702 (83.4%)	899 (83.5%)	0.9282
Non-white	1 138 (16.6%)	178 (16.5%)	
Comorbidities, n (%)			
No comorbidities	4 529 (66.2%)	736 (68.3%)	0.1697
Comorbidities	2 311 (33.8%)	341 (31.7%)	
Insurance, n (%)			
Private	2 693 (39.4%)	570 (52.9%)	<0.0001
Not private	4 147 (60.6%)	507 (47.1%)	
Treatment center, n (%)			
Academic	4 532 (66.3%)	869 (80.7%)	<0.0001
Non-academic	2 308 (33.7%)	208 (19.3%)	
Clinical stage, n (%)			
Stage I	2 778 (40.6%)	246 (22.8%)	<0.0001
Stage II	3 905 (57.1%)	671 (62.3%)	
Stage III	157 (2.3%)	160 (14.9%)	
Lymph node status, n (%)			
Negative nodes	1 913 (28.0%)	534 (49.6%)	<0.0001
Positive nodes	4 927 (72.0%)	543 (50.4%)	
Tumor differentiation, n (%)			
Well	574 (8.4%)	135 (12.5%)	<0.0001
Moderate	3 553 (51.9%)	573 (53.2%)	
Poor/undifferentiated	2 713 (39.7%)	369 (34.3%)	
Margin status, n (%)			
Negative	5 079 (74.3%)	886 (82.3%)	<0.0001
Positive	1 761 (25.7%)	191 (17.7%)	
Prolonged inpatient stay, n (%)			
No	5 359 (78.4%)	887 (82.4%)	0.0027
Yes	1 481 (21.6%)	190 (17.6%)	
90-day mortality, n (%)			
No	6 333 (92.6%)	1 016 (94.3%)	0.0388
Yes	507 (7.4%)	61 (5.7%)	

Table 2. Multivariable logistic regression analyses predicting positive resection margins (R1/R2), prolonged hospital stay, and 90-day mortality in resected pancreatic adenocarcinoma patients.

	Positive resection margins		Prolonged hospital stay		90-day mortality	
	Odd ratio (95% CI)	p	Odd ratio (95% CI)	p	Odd ratio (95% CI)	p
Sex						
Female	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Male	1.14 (1.03-1.26)	0.0155	0.98 (0.88-1.09)	0.6816	1.02 (0.86-1.21)	0.8335
Age at diagnosis						
< 65 years	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
≥ 65 years	1.00 (0.88-1.14)	0.9858	1.21 (1.05-1.39)	0.0069	1.80 (1.42-2.28)	<0.0001
Race						
White	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Non-white	1.15 (1.00-1.32)	0.0448	1.24 (1.08-1.43)	0.0027	1.10 (0.87-1.38)	0.4186
Comorbidities						
No comorbidities	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Comorbidities	1.05 (0.94-1.17)	0.3726	1.13 (1.01-1.27)	0.0325	1.25 (1.04-1.49)	0.0157
Insurance						
Private	1.000 (Ref)		1.00 (Ref)		1.00 (Ref)	
Not private	1.06 (0.93-1.21)	0.4122	1.19 (1.03-1.37)	0.0171	1.58 (1.24-2.01)	0.0002
Treatment center						
Academic	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Non-academic	1.34 (1.20-1.50)	<.0001	1.20 (1.07-1.35)	0.0020	1.31 (1.10-1.57)	0.0029
Clinical stage						
Stage I	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Stage II	1.19 (1.07-1.34)	0.0018	0.97 (0.86-1.09)	0.6027	1.16 (0.97-1.40)	0.1132
Stage III	2.44 (1.88-3.17)	<.0001	1.18 (0.89-1.57)	0.2601	1.54 (1.00-2.38)	0.0498
Lymph node status						
Positive nodes	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Negative nodes	0.53 (0.47-0.60)	<.0001	1.21 (1.08-1.37)	0.0015	1.07 (0.88-1.29)	0.5188
Differentiation						
Well	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Moderate	1.20 (0.98-1.46)	0.0793	1.00 (0.83-1.22)	0.9684	1.06 (0.77-1.47)	0.7269
Poor/undifferentiated	1.25 (1.02-1.54)	0.0293	1.00 (0.82-1.22)	0.9791	1.12 (0.80-1.56)	0.5170
Treatment						
Neoadjuvant therapy	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Upfront surgery	1.54 (1.29-1.83)	<.0001	1.25 (1.05-1.49)	0.0130	1.15 (0.86-1.54)	0.3472
Margin status						
Negative	-		1.00 (Ref)		1.00 (Ref)	
Positive	-	-	1.21 (1.07-1.37)	0.0032	1.62 (1.34-1.95)	<0.0001

The 90-day mortality rate was 5.7% (n=61) after neoadjuvant therapy and 7.4% (n=507) after upfront surgery. On multivariable analysis modeling the probability of dying within 90 days of surgery, age ≥ 65 years at diagnosis, any comorbidities, non-private insurance, non-academic treatment center, and positive resection margins were associated with 90-day mortality (Table 2). However, sex, race, treatment sequence, tumor differentiation, lymph node status, and tumor stage were not associated with 90-day mortality (Table 2).

Survival

Kaplan-Meier survival curves for resected pancreatic cancer patients by multimodality treatment sequence and resection margin status are shown in Figure 1. After

neoadjuvant therapy, patients with negative resection margins (median overall survival, 25.9 months; 95% CI, 24.7-27.6 months) demonstrated a 7.4 month longer median overall survival compared to patients with positive resection margins (median overall survival, 18.5 months; 95% CI, 15.7-20.8 months). On multivariable survival analysis, positive resection margins, positive lymph node status, and non-academic treatment center were predictive for poor overall survival (Table 3). Insurance status and clinical tumor stage were not significantly associated with overall survival (Table 3).

In patients that received upfront surgery, negative resection margins (median overall survival, 20.8 months; 95% CI, 20.2-21.4 month) showed improved survival compared to patients with positive margins (median overall survival, 14.7 months; 95% CI, 13.8-15.4 months; p<0.0001). After adjustment for patient characteristics, positive resection margins, clinical stage II/III disease, and tumor differentiation were significantly associated with decreased survival (Table 3).

Table 3. Multivariate Cox models in stage I-III pancreatic adenocarcinoma that underwent neoadjuvant therapy and surgery, upfront surgery with or without adjuvant therapy, or upfront surgery followed by adjuvant chemoradiotherapy.

	Neoadjuvant therapy		**Upfront surgery**		**Upfront surgery and adjuvant chemoradiotherapy**	
	Hazard ratio (95% CI)	**p**	**Hazard ratio (95% CI)**	**p**	**Hazard ratio (95% CI)**	**p**
Age at diagnosis						
< 65 years	-		-		1.00 (Ref)	
≥ 65 years	-	-	-	-	1.14 (1.00-1.29)	0.0455
Insurance						
Private	1.00 (Ref)		-		1.00 (Ref)	
Not private	1.14 (0.94-1.37)	0.1752	-	-	1.13 (0.99-1.28)	0.0655
Treatment center						
Academic	1.00 (Ref)		-		1.00 (Ref)	
Non-academic	1.29 (1.08-1.55)	0.0047	-	-	1.18 (1.07-1.31)	0.0009
Clinical stage						
Stage I	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Stage II	1.19 (0.99-1.43)	0.0683	1.08 (1.02-1.14)	0.0109	1.11 (1.00-1.23)	0.0451
Stage III	1.19 (0.93-1.52)	0.1587	1.39 (1.16-1.67)	0.0003	1.51 (1.10-2.08)	0.0107
Lymph node status						
Negative nodes	1.00 (Ref)		-		1.00 (Ref)	
Positive nodes	1.23 (1.06-1.42)	0.0063	-	-	1.46 (1.30-1.65)	<0.0001
Differentiation						
Well	-		1.00 (Ref)		-	
Moderate	-	-	1.29 (1.16-1.44)	<0.0001	-	-
Poor/undifferentiated	-	-	1.65 (1.48-1.85)	<0.0001	-	-
Margin status						
Negative	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Positive	1.58 (1.32-1.89)	<0.0001	1.48 (1.39-1.57)	<0.0001	1.40 (1.27-1.55)	<0.0001

Table 4. Multivariate Cox model or stage I-III pancreatic adenocarcinoma patients that underwent R0/R1-resection.

Characteristics	Hazard ratio (95% CI)	p-value
Insurance		
Private	1.00 (Ref)	
Not private	1.14 (0.94-1.37)	0.1821
Treatment center		
Academic	1.00 (Ref)	
Non-academic	1.29 (1.08-1.54)	0.0050
Clinical stage		
Stage I	1.00 (Ref)	
Stage II	1.19 (0.99-1.43)	0.0656
Stage III	1.19 (0.93-1.52)	0.1636
Lymph node status		
Negative nodes	1.00 (Ref)	
Positive nodes	1.22 (1.05-1.41)	0.0092
Margin status, n (%)		
Negative	1.00 (Ref)	
Positive	1.59 (1.33-1.91)	<0.0001

After upfront surgery, 31.9% (n=2 177) of patients did not proceed to receive any adjuvant treatment, 32.6% (n= 2 222) received adjuvant chemoradiotherapy, 34.7% (n=2 367) received adjuvant chemotherapy, and 0.8% (n=54) received adjuvant radiotherapy. For 20 patients, it was unknown whether they received any and/or what type of adjuvant therapy. In patients that received upfront surgery followed by adjuvant chemoradiotherapy, negative resection margins were significantly (log-rank p<0.0001) associated with survival benefit compared to positive resection margin, resulting in a median overall survival of 24.2 months (95% CI, 22.9-25.6 months) versus 19.0 months (95% CI, 17.4-20.5 months). On multivariable analysis, negative margins remained associated with favorable survival in patients that received upfront surgery followed by adjuvant chemoradiotherapy (Table 3).

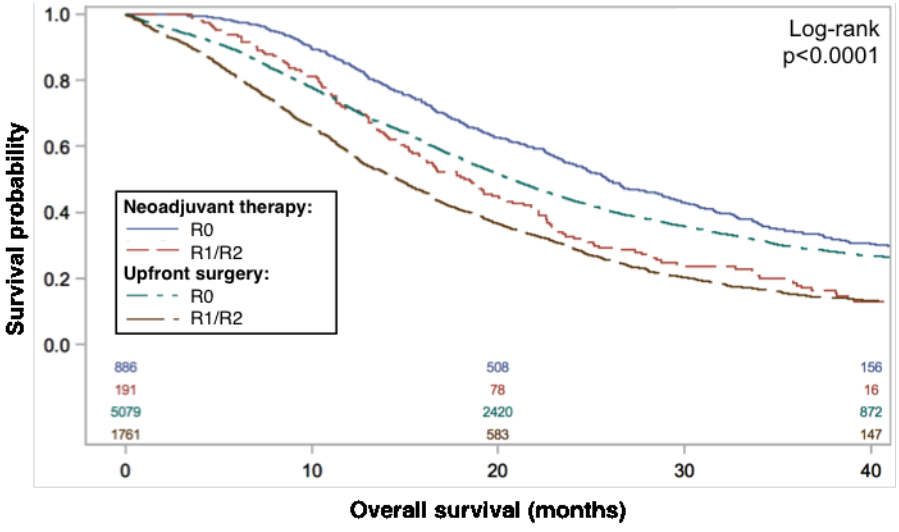

Figure 1. Kaplan-Meier survival curves for pancreatic adenocarcinoma patients by treatment sequence and resection margin.

Sensitivity analysis

Sensitivity analysis was performed in patients that received neoadjuvant chemoradiotherapy succeeded by surgery (n=617) or upfront surgery (n=6 840). 17.0% (n=105) and 25.8% (n=1,761) of patients demonstrated positive margins after neoadjuvant chemoradiotherapy and upfront surgery, respectively. After adjustment for differences in patient characteristics and tumor factors, upfront surgery remained predictive for positive resection margins (Supplementary Table 1.) 20.8% (n=128) of patients experienced prolonged hospital stay after neoadjuvant chemoradiotherapy and 21.7% (n=1,481) after upfront surgery. 6.5% (n=40) of patients that received neoadjuvant chemoradiotherapy and 7.4% (n=507) of patients that received upfront died within 90 days of primary surgery. Multivariable logistic regression demonstrated that treatment sequence did not significantly influence 90-day mortality (Supplementary Table 1.).

In patients treated at academic centers (n=5 367), 16.4% (n=142) and 23.5% (n=1 058) demonstrated positive margins after neoadjuvant therapy and upfront surgery, respectively. On multivariable analysis, upfront surgery significantly increased the likelihood of positive resection margins (Supplementary Table 2). Prolonged hospital stay occurred in 18.0% (n=156) of the patients that received neoadjuvant therapy and 20.4% (n=916) of upfront resected patients. Treatments sequence did not significantly impact hospital stay on multivariable analysis (Supplementary Table 2). After adjusting for difference in patient and tumor characteristics, the 90-day mortality rate was 5.4% (n=47) for the neoadjuvant therapy and 6.7% (n=300) upfront surgery group (Supplementary Table 2).

In patients that received neoadjuvant therapy followed by surgery without macroscopically residual disease reported, positive resection margin status remained associated with decreased overall survival, as well as positive lymph nodes, and non-academic treatment center (Table 4). Insurance status and clinical tumor stage did not significantly impact overall survival (Table 4).

DISCUSSION

This population-level analysis demonstrates that neoadjuvant therapy significantly decreases the likelihood of positive resection margins after pancreaticoduodenectomy. These data provide evidence that a neoadjuvant treatment approach may allow the surgeon to more easily attain a complete resection. Nonetheless, although previously suggested otherwise, positive resection margins remain associated with poor prognosis (median overall survival, 18.5 vs. 25.9 months; HR, 1.58) after neoadjuvant therapy (17). These findings suggest that, despite neoadjuvant therapy improving the probability of complete tumor clearance, negative resection margins continue to be critical to long-term overall survival, and should remain the goal of curative resections. Moreover, these findings

highlight the potential for additional postoperative therapy after neoadjuvant therapy and pancreatectomy.

Previous studies have reported varying R0 resection rates, ranging from 15% to 94%(25, 26). This substantial inconsistency in R0 resection rates can partly be explained by intercontinental variability in the definition of R0, which is a 0 mm tumor distance from the resection margin in the US, and ≥ 1 mm across Europe and Australia (27-29). In addition, standardized examination has been demonstrated to significantly decrease R0-resection rates (26). Therefore, the definition of margin involvement wielded in the US and lack of standardization may account for the relatively low R0 resection rates found by this nationwide review in both arms. However, a meta-analysis by Andriulli et al. (2012) demonstrated a pooled proportion R0 resection rate of 89% (95% CI, 83%-94%) after neoadjuvant therapy, which is comparable to our findings (30). Furthermore, similar to our study, a meta-analysis by Laurence et al. (2011) demonstrated that patients receiving neoadjuvant chemoradiotherapy were significant less likely to have positive margins (31). Furthermore, this study also revealed that positive margins were associated with increased 90-day mortality, which suggests that positive resection margins are more common in patients that underwent an anatomically challenging resection.

Positive resection margins have been shown to impact outcomes for pancreatic adenocarcinoma, with prior experiences describing a median survival of 10-15 months for patients with positive margins compared to 16-23 months for patients with negative margins after upfront surgery with or without postoperative therapy (32-37). Raut and colleagues (2007) found no statistically significant difference in survival or recurrence based on resection margin status after the use of neoadjuvant therapy followed by pancreatic surgery with or without postoperative therapy (17). In their study patients who underwent an R1 resection had a median overall survival of 21.5 months compared with 27.8 months in patients who underwent an R0 resection (17). Although, the present study was not able to confirm the previously described potential mitigating effect of neoadjuvant therapy on the unfavorable impact of incomplete tumor clearance, it demonstrated a similar survival advantage for patients with negative resection margins (17).

This study is limited by its retrospective nature and inherent selection bias. In addition, the National Cancer Database has several limitations, including the potential for coding errors, missing data, and the absence of several critical outcomes and variables, including local recurrence rates, specific chemotherapeutic agents, the precise location of margin involvement, vascular invasion, surgeon experience, standardized pathology assessment, and use of frozen section analysis (28, 38, 39). Pursuit of negative margins after positive intraoperative frozen-section analysis has been shown to be associated with worse survival than negative margins on initial intraoperative frozen sections (40, 41). In addition, comparability of studies on resection margin status is often plagued by frequent underreporting of microscopic margin involvement due to inconsistent pathologic review

practices (42). Neoadjuvant therapy has also shown to alter the consistency of the pancreas, which may impact pathologic evaluation of tumor cells at the circumferential margin (43). Furthermore, it should be acknowledged that this study did not take into account intention to treat, and does not account for patients who progressed on chemotherapy and were thus never resected. Patients who undergo resection following neoadjuvant therapy may be considered as a distinct subset of patients with better tumor biology (15). Therefore, the favorable impact of neoadjuvant therapy on R0 resection rates and R0 resection on survival demonstrated by this study suggests, but does not prove that neoadjuvant therapy increases overall survival compared to upfront surgery. Moreover, the c-statistics for the multivariable regression models predicting positive margins, prolonged hospital stay, and 90-day mortality suggest that residual confounding exists; however, this study is limited by the covariates provided by the NCDB (44).

Despite these limitations, this study is, to the best of our knowledge, the first to investigate the survival impact of positive resection margins after neoadjuvant therapy in pancreatic cancer patients on a population-level. Previous studies have suggested that worse outcomes after R1 resection are associated with advanced disease stage, which is technically more challenging to resect (33). Therefore, we used a stratified multivariable Cox proportional hazard survival analysis to partially control for these potential confounders. In addition, a sensitivity analysis excluding patients with macroscopically residual disease was performed. Furthermore, neoadjuvant therapy is more often administrated to patients with more advanced disease and in academic centers (45). To control for selection bias multivariable logistic regression analyses were performed to investigate the potential predictive value of neoadjuvant therapy for positive resection margins, prolonged hospital stay, and 90-day mortality. This study was not able to distinguish pancreatic adenocarcinoma patients with initially resectable and non-resectable (borderline/locally advanced/unresectable) disease; however, a previous meta-analysis revealed that R0 resection rates were comparable between these groups (46).

In conclusion, the findings of this population-level analysis emphasize the ability of neoadjuvant therapy to decrease margin positivity rates after pancreaticoduodenectomy, even after controlling for critical confounders. Nevertheless, the poor prognostic impact of incomplete tumor clearance was not abrogated by neoadjuvant therapy, and resection margin status remains a critical prognosticator after neoadjuvant therapy. Therefore, complete margin clearance should continue to be the central aim of pancreatic cancer surgery, even in light of the current increase in the use and efficacy of neoadjuvant therapy (11). New innovative surgical techniques, methods of intraoperative margin assessment, preoperative imaging for patient selection, and improved neoadjuvant chemotherapy are needed to further decrease the rates of incomplete tumor clearance.

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Chapter 10

Reappraisal of the American Joint Commission on Cancer (8th Edition) Changes in Patients with Pancreatic Adenocarcinoma who Underwent Neoadjuvant Therapy

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Submitted

ABSTRACT

Purpose: This study aimed to compare and validate the seventh and eighth edition American Joint Commission on Cancer (AJCC) staging criteria in pancreatic cancer patient who underwent neoadjuvant therapy followed by pancreaticoduodenectomy.

Methods: The National Cancer Data Base was queried for pancreatic adenocarcinoma patients who underwent neoadjuvant therapy followed by pancreaticoduodenectomy. Patients were staged according to the seventh and eighth edition AJCC staging criteria. Prognostic performance was assessed using multivariable Cox proportional hazards analyses and concordance statistics.

Results: A total of 2,019 patients were identified. The median survival was 24.2 months. The seventh edition staging significantly discriminate survival between stage IIB vs. IIA (HR, 1.143; p=0.0299), but not between stage IB vs. IA (HR, 1.189; p=0.2278), stage IIA vs. IB (HR, 1.134; p=0.2532), and stage III vs. IIB (HR, 0.955; p=0.7560). The eighth edition staging was able to distinguish survival between stage III vs. IIB (HR, 1.173; p=0.0278), but not between stage IA vs. IB (HR, 1.138; p=0.2458), stage IIA vs. IB (HR, 1.063; p=0.5759), and stage IIB vs. IIA (HR, 1.072; p=0.5146). A simplified eighth edition staging significantly distinguished survival for all stages (II vs. I: HR, 1.157; p=0.0168; III vs. II: HR, 1.187; p=0.0142). The C-statistics for the group staging improved from 0.54 for the seventh to 0.56 for the eighth edition.

Conclusions: Neoadjuvant therapy is known to induce fibrosis in pancreatic adenocarcinoma, hampering accuracy and granularity of pathologic assessment. Therefore, a simplified eighth edition staging system might be more clinically applicable after neoadjuvant therapy.

INTRODUCTION

Pancreatic adenocarcinoma ranks the third leading cause of cancer-related death in the United States and is expected to become the second leading cause by 2030.[1-3] To date, surgical resection remains at the nexus of pancreatic cancer care, providing the sole – albeit rare – prospect of cure, and the best chance at prolonged survival.[4] Neoadjuvant therapy is a relatively recent treatment strategy, which is gaining traction with the increasing availability of more effective chemotherapeutic agents, such as FOLFIRINOX and gemcitabine/nab-paclitaxel.[5-7] The advantages of neoadjuvant therapy, include early treatment of micro-metastatic disease, optimized selection of surgical candidates, and increased likelihood of negative resection margins, and multimodality therapy completion.[8]

In pancreatic adenocarcinoma patients, neoadjuvant therapy frequently induces pronounced fibrosis involving both the tumor and the adjacent non-neoplastic parenchyma, which may hamper the accurate pathological staging of the surgical specimen.[9] The American Joint Committee on Cancer (AJCC) has recently introduces the eighth edition staging manual for pancreatic cancer.[10,11] To reduce the substantial inter-observer bias reported for preceding iterations of the AJCC pancreatic cancer staging system, the eighth edition has introduced a size-based staging paradigm.[10-17] Furthermore, lymph node staging was adjusted to take into account the number of positive lymph nodes.[10,11] Previous studies in upfront resected pancreatic cancer patients have shown that the eighth edition TNM staging may be able to more equally distribute patients among stages and increase prognostic accuracy.[11,18] However, data regarding the performance of the eighth edition staging system in patients who underwent neoadjuvant therapy remain scarce.[9]

A single-institution study at a high-volume tertiary center has suggested that the new AJCC eighth edition ypT stage grouping performs better than the AJCC seventh edition ypT stage in patients who underwent neoadjuvant therapy.[9] The present study aims to compare and validate the seventh and eighth edition AJCC staging criteria in pancreatic cancer patient who underwent neoadjuvant therapy followed by pancreaticoduodenectomy using a large nationwide dataset.

METHODS

Data Source

Patient data were obtained from the National Cancer Data Base (NCDB), a joint program of the Commission on Cancer of the American College of Surgeons and the American Cancer Society. This database contains more than 30 million records of individual cancer cases collected from over 1,500 Commission on Cancer approved facilities across the United States.[19] Enhancing this data beyond mere numbers, the Commission on Cancer requires centers to maintain a 90% follow-up rate for patients diagnosed within 5 years to remain accredited, improving long-term survival analsyses.[19]

All NCDB data are de-identified, and hence this study was deemed exempt from institutional review board review.

Inclusion/Exclusion Criteria

Patients with pancreatic adenocarcinoma who underwent neoadjuvant chemotherapy or chemoradiation followed by pancreaticoduodenectomy between 2006 to 2015 were identified using Facility Oncology Registry Data Standard (FORDS) procedure (35, 36, 37, 70), International Classification of Disease for Oncology, 3rd edition (ICD-O-3), morphology (8140 and 8500) and topography (C25.0, C25.1, C25.2, C25.3, C25.7, C25.9, and C25.9) codes, as well as the NCDB systemic treatment and surgical procedure sequence variable.[20] Patients who did not undergo surgery at the reporting facility (n=166), had malignancies other than pancreatic cancer (n=862), clinical or pathologic metastatic disease (n=139), or pathologic tumor stage p0, pIS, or pX (n=591) were excluded, as well as patients with missing for any of the following variables: age, sex, race, tumor size, comorbidities, insurance status, facility type, number of positive lymph nodes, number of lymph nodes examined, resection margin status, tumor grade, pathologic primary tumor stage, pathologic lymph node stage, radiation and surgery treatment sequence, and vital status (n=1,529).

Covariates

Patient demographics included: age (<65 years, ≥ 65 years), gender (male, female), race (white, black, other), insurance (private insurance, Medicare, Medicaid/uninsured/other Govermental), treatment facility (community, academic center, Integral cancer network), tumor differentiation (well/moderately differentiated, poorly/undifferentiated), type of neoadjuvant therapy (chemotherapy, chemoradiation), and receipt of any adjuvant therapy. Type of neoadjuvant therapy and receipt of any adjuvant therapy were identified based on the surgery and systemic therapy treatment sequence variable, as well as the surgery and radiation therapy treatment sequence variable provided by the NCDB. Comorbidities were reported as previously described by Charlson/Deyo based on ten comorbidities.[21] For the purpose of this study patients were grouped as no comorbidities, 1 comorbidity, and more than 1 comorbidity. Hospital volume was calculated based on the number of pancreaticoduodenectomies per year, and dichotomized in low- and high volume centers based on the median hospital volume (23 cases per year). Resection margin status was recorded as it appeared in the pathology report, and dichotomized as negative (all margins are grossly and microscopically negative) or positive (microscopic residual tumor, margin involvement is indicated, but not otherwise specified). The total number of pathologically assessed lymph nodes was recorded from the final pathology report for each surgical specimen.

Patients were staged according to the seventh and eighth edition AJCC staging manuals, as shown in Supplementary Table 1.[22,23] For the purpose of this study solely

pathology stage was assessed. The T-, and overall stage according to the seventh edition staging criteria were readily provided by the NCDB.[12] For both staging criteria N0 was defined as all nodes examined are negative. The seventh edition stage N1 included patients with any or an unspecified number of positive regional lymph nodes. The eighth edition staging variables were created based on tumor size and number of positive lymph nodes reported by the NCDB. Stage T1 was coded in the eighth edition as tumor size ≥ 0.01 cm ≤ 2.0 cm. The eighth edition stage T4 was substituted by the seventh edition stage T4, as they are defined the same. The eighth edition stage N2 was defined as 4-89 nodes. For both staging criteria, the M0 group was defined as absence of pathological distant metastases. Previous studies have suggested that in patients who underwent neoadjuvant therapy a simplified group stage may be more appropriate, grouping stage as I (IA and IB), II (IIA and IIB), and III.[24]

Statistical analysis

All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). Descriptive statistics were summarized with frequencies and percentages for categorical variables and as medians with interquartile range (IQR) for continuous variables. Categorical and continuous variables were compared using the Chi-square test and Kruskal-Wallis test, respectively. The primary outcome was overall survival, which was calculated from the date of diagnosis to the patient's death or last known follow-up. Survival curves and median survival estimated were obtained using the Kaplan-Meier method and compared using the log-rank test. Univariate Cox proportional hazard analyses for seventh and eighth edition group stage, simplified group stage, T- and N-stage, stratified for resection margin status and tumor differentiation, were performed. Chatterjee et al (2017) identified resection margin status and tumor differentiation as the most important prognosticators in pancreatic cancer patients who received neoadjuvant therapy.[9]

Prognostic accuracy for overall survival of the seventh and eighth edition of the TNM staging system was assessed using concordance statistics (Uno C statistics), the traditional receiver operating characteristics (ROC) curve, and the time-dependent area under the curve (AUC).[18,25,26] The Uno C statistic is comparable with a routinely used C statistic, but it accounts for a covariate- dependent censoring distribution; in addition, 95% CIs were calculated based on 100 perturbation samples.[25] The time- dependent AUC can be appreciated as the predictive accuracy over time, as derived from each ROC curve.[26]

RESULTS

Patient population

In total, the study cohort comprised of 2,019 patients. Baseline characteristics are shown in table 1. The median age was 64 (IQR, 57 – 70) years, and 1,058 patients (52.4%) were male. The median tumor size was 30 (IQR, 25 – 40) mm and the median number of

lymph nodes retrieved was 17 (IQR, 11 -24) nodes. A total of 381 patients (18.9%) had positive resection margins and 959 patients (47.5%) underwent neoadjuvant chemoradiation.

Table 1. Clinicopathologic features of patients with pancreatic adenocarcinoma who underwent neoadjuvant therapy and pancreaticoduodenectomy.

Characteristics	Total (n=2,019)
Age at diagnosis, n (%)	
≤ 65 years	1,186 (58.7%)
> 65 years	833 (41.3%)
Sex, n (%)	
Male	1,058 (52.4%)
Female	961 (47.6%)
Race, n (%)	
White	1,671 (82.8%)
Black	211 (10.4%)
Other	137 (6.8%)
Charles/Deyo score, n (%)	
0	1,344 (66.6%)
1	536 (26.5%)
≥2	139 (6.9%)
Tumor differentiation, n (%)	
Well differentiated	235 (11.6%)
Moderately differentiated	1,139 (56.4%)
Poor/undifferentiated	645 (32.0%)
Insurance status, n (%)	
Private insurance	988 (48.9%)
Medicare	881 (43.7%)
Medicaid/uninsured	150 (7.4%)
Facility type, n (%)	
Community	375 (18.6%)
Academic	1,405 (69.6%)
Integrated Network Cancer Network	239 (11.8%)
Hospital volume, n (%)	
Low	990 (49.0%)
High	1,029 (51.0%)
No. of lymph nodes resected, n (%)	
< 15 nodes	788 (39.0%)
≥ 15 nodes	1,231 (61.0%)
Resection margin status, n (%)	
Negative	1,638 (81.1%)
Positive	381 (18.9%)
Neoadjuvant therapy, n (%)	
Chemotherapy	1,060 (52.5%)
Chemoradiation	959 (47.5%)
Adjuvant therapy, n (%)	
No	1,208 (59.8%)
Yes	811 (40.2%)

Via the seventh edition AJCC TNM staging system, stage IA was found in 165 patients (8.2%), stage IB in 157 patients (7.8%), stage IIA in 593 patients (29.4%), stage

IIB in 1,039 patients (51.5%), and stage III in 65 patients (3.2%), and via the eighth edition, stage IA was found in 163 patients (8.1%), stage IB in 597 patients (29.6%), stage IIA in 155 patients (7.7%), stage IIB in 692 patients (34.3%), and stage III in 412 patients (20.4%) (Figure 1). Using the eighth-edition classification, 967 patients (47.9%) migrated to a different stage, of who 496 patients (24.6%) were assigned to a lower stage and 471 patients (23.3%) to a higher stage.

Table 2. Univariable cox regression analysis of the overall survival effect by tumor stage according to the seventh and eighth edition AJCC staging criteria.

	Seventh edition		Eighth edition	
	Hazard Ratio (95% CI)*	***P***	**Hazard Ratio (95 CI)***	***P***
Group stage				
Stage IB (vs. IA)	1.189 (0.897 – 1.576)	0.2278	1.138 (0.915 – 1.417)	0.2458
Stage IIA (vs. IB)	1.134 (0.914 – 1.406)	0.2532	1.063 (0.857 – 1.319)	0.5759
Stage IIB (vs. IIA)	1.143 (1.013 – 1.290)	0.0299	1.072 (0.868 – 1.325)	0.5146
Stage III (vs. IIB)	0.955 (0.716 – 1.275)	0.7560	1.173 (1.018 – 1.352)	0.0278
Simplified group stage				
Stage II (vs. I)	1.339 (1.150 – 1.560)	0.0002	1.157 (1.027 – 1.304)	0.0168
Stage III (vs. II)	0.998 (0.750 – 1.329)	0.9910	1.187 (1.035 – 1.362)	0.0142
Primary Tumor Stage				
ypT2 (vs. ypT1)	1.275 (1.015 – 1.601)	0.0370	1.160 (0.993 – 1.355)	0.0621
ypT3 (vs. ypT2)	1.088 (0.931 – 1.271)	0.2906	1.039 (0.906 – 1.191)	0.5868
ypT4 (vs. ypT3)	1.002 (0.752 – 1.334)	0.9912	0.996 (0.727 – 1.336)	0.9253
Lymph Node Stage				
ypN1 (vs. ypN0)	1.204 (1.083 – 1.339)	0.0006	1.134 (1.009 – 1.276)	0.0356
ypN2 (vs. ypN1)	-		1.202 (1.037 – 1.392)	0.0144

Abbreviations: CI, Confidence Interval
*Stratified by resection margin status and tumor differentiation

Via the seventh edition staging system, 220 patients (10.9%) were stage ypT1, 266 patients (13.2%) were stage ypT2, 1,468 patients (72.7%) were stage ypT3, and 65 (3.2%) were stage ypT4 (Figure 1). According to the eight edition AJCC staging, 300 patients (14.9%) were stage ypT1, 1,272 patients (63.0%) were stage ypT2, 382 patients (18.9%) were stage ypT3, and 65 patients (3.2%) were stage ypT4 (Figure 1). Overall, 33.0% of ypT1 according to the eighth edition corresponded with the seventh edition ypT1 stage, 16.0% of eighth edition ypT2 stage corresponded with the seventh edition ypT2 stage, and 80.9% of eighth edition ypT3 stage corresponded with the seventh edition ypT3 (Supplementary Table 2).

Via the seventh edition staging system, 945 patients (46.8%) were staged as ypN0, and 1,074 patients (53.2%) as ypN1 (Figure 1). In agreement with the eighth edition, 945 patients (46.8%) were staged as ypN0, 716 patients (35.5%) were staged as ypN1, and 358 patients (17.7%) were staged as ypN2 (Figure 1).

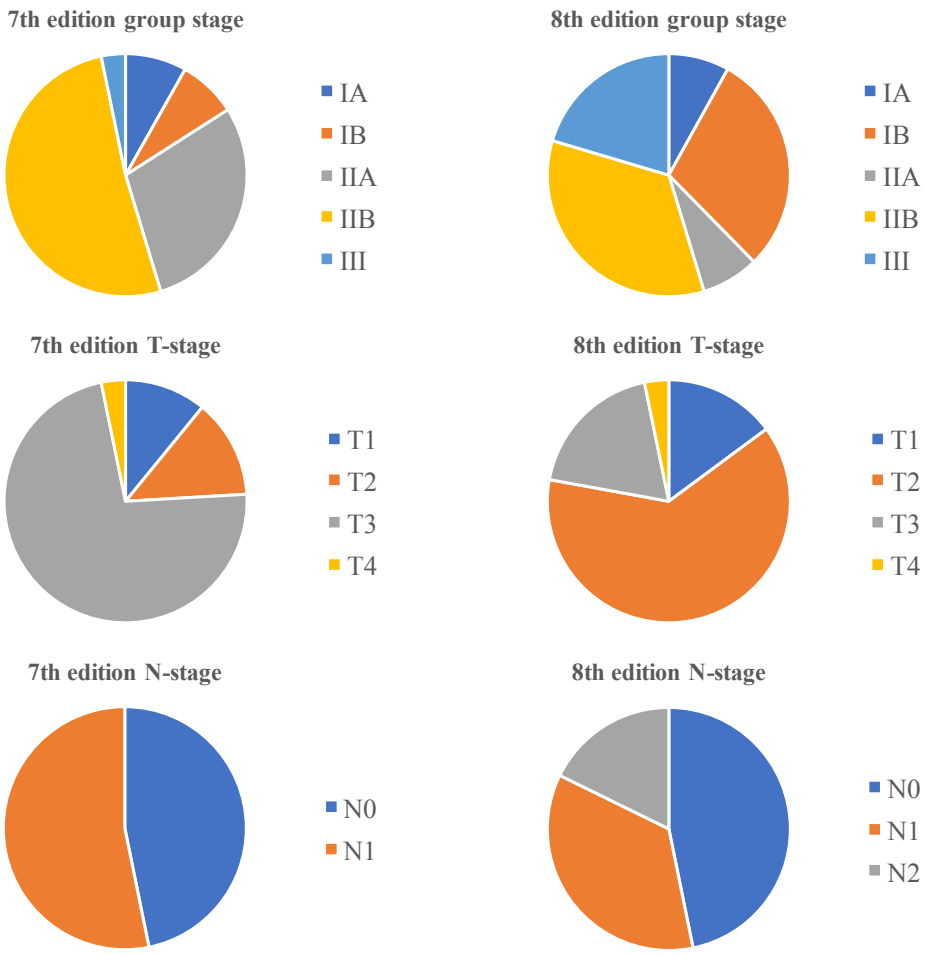

Figure 1. Distribution of TNM classification in the seventh and eighth edition AJCC staging system.

Survival by TNM Stage

At the time of last follow-up, 597 patients (29.6%) were alive, median overall survival for the entire cohort was 24.2 months, and the 5-year survival rate was 17.3%. Median overall survival changed from 34.5 months for patients in stage IA, 27.0 months for patients in stage IB, 25.0 months for patients in stage IIA, 22.1 months for patients in stage IIB, 22.8 months for those in stage III (Figure 2a; log-rank $p<0.0001$) under seventh-edition classification to 30.9 months for patients in stage IA, 25.7 months for those in stage IB, 24.6 months for those in stage IIA, 23.0 months for those in stage IIB, and 21.1 months for those in stage III (Figure 2b; log-rank $p<0.0001$) under eighth-edition classifications. On multivariable Cox proportional hazard analyses, the seventh edition AJCC staging was able to significantly distinguish survival between stage IIB vs. IIA (HR, 1.143; $p=0.0299$), but not for any of the successive stages (Table 2). The eighth edition TNM stage was able to

discriminate survival between stage III vs. IIB (HR, 1.173; p=0.0278), but not between the other subsequent stages (Table 2).

Via the seventh edition staging, the median survival was 30.9 months for the simplified stage I (IA + IB), 23.4 months for stage II (IIA + IIB), and 22.8 months for stage III (Figure 3a; p<0.0001). For the eighth edition simplified group stage, patients with stage I (IA + IB) disease demonstrated a median survival of 26.8 months, patients with stage II (IIA + IIB) had a median survival of 23.3 months, and patients with stage III disease a median survival of 21.1 months (Figure 3b; p<0.0001). The simplified version of seventh edition group stage was able to distinguish survival between stage I vs. II (HR, 1.339; p=0.0002). However, not between stage II vs. III (HR, 0.998; p=0.9910; Table 2). The simplified eighth edition group stage was able to discriminate survival between stage I vs. II (HR, 1.157; p=0.0168) and stage II vs. III (HR, 1.187; p=0.0142; Table 2).

For the seventh edition TNM stage, median survival was 33.7 months for patients with stage ypT1, 25.9 months for stage ypT2, 23.3 months for stage ypT3, and 22.8 months for stage ypT4 (Figure 4a). Via the eighth edition, the median survival was 27.3 months for stage ypT1, 24.1 months for stage ypT2, 22.8 months for stage ypT3, and 22.8 months for stage ypT4 (Figure 4b). On multivariable Cox proportional hazard analyses, the seventh edition was able to distinguish survival between stage ypT2 vs. ypT1 (HR, 1.275; p=0.0370), but not between ypT3 vs. ypT2 (HR, 1.088; p=0.2906) and ypT4 vs. ypT3 (HR, 1.002; p=0.9912; Table 2). The eighth edition distinguished survival between stage ypT2 vs. ypT1 (HR, 1.160; p=0.0621), but not between ypT3 vs. ypT2 (HR, 1.039; p=0.5868), and ypT4 vs. ypT3 (HR, 0.996; p=0.9253; Table 2).

The median survival for patients with negative and positive lymph nodes was 26.1 and 22.5 months, respectively (Figure 5a). The new classification of the ypN-stage in the eighth edition was highly discriminative, with median survival of 26.1 months for stage ypN0, 23.1 months for stage ypN1, and 20.9 months for stage ypN2 (Figure 5b). Adjusted for other covariates, multivariable analysis of the seventh (ypN1 vs. ypN0: HR, 1.204; p=0.0006) and eighth (ypN1 vs. ypN0: HR, 1.134; p=0.0356; ypN2 vs. ypN1: HR, 1.202; p=0.0144) edition demonstrated a significant survival difference between all lymph node stage (Table 2).

Prognostic Accuracy

When assessing prognostic accuracy on survival, the Uno C statistics was 0.54 for the seventh edition group stage and 0.55 for the eighth edition of the TNM group stage. The Uno C statistics was 0.53 for the seventh edition simplified group stage and 0.54 for the eighth edition simplified group stage. Furthermore, the Uno C statistics was 0.53 for the seventh edition T-stage and dropped to 0.52 for the eighth edition T-stage. In addition, the Uno C statistic was 0.53 for the seventh edition N-stage and 0.54 for the eighth edition N-stage.

The ROC curve at 5-year survival demonstrated a time-dependent area under the curve (AUC) of 0.57 with the seventh edition group stage compared to 0.57 with the eighth edition group stage. For the seventh edition simplified group stage the AUC was 0.54 and 0.56 for the eighth edition simplified group stage. The AUC was 0.54 and 0.54 for respectively the seventh and eighth T-stage. For the seventh edition N-stage the AUC was 0.55 and 0.56 for the eighth edition N-stage.

DISCUSSION

Neoadjuvant therapy for pancreatic adenocarcinoma is on the rise.[5] To the best of our knowledge, this study is the first to validate the eighth edition AJCC staging system in a nationwide cohort of pancreatic cancer patients who underwent neoadjuvant therapy. This study demonstrated that neither the seventh nor the eighth edition staging system was able to significantly distinguish survival between all subsequent group stages. However, a simplified group stage, collapsing stage IA with IB and stage IIA with IIB, was able to distinguished survival for all stages defined according to the eighth edition staging system (II vs. I: HR, 1.157; p=0.0168; III vs. II: HR, 1.187; p=0.0142). The simplified seventh edition group stage was only able to distinguish survival between stage II vs. I (HR, 1.339; p=0.0002), but not between stage III vs. II (HR, 0.998; p=0.9910). The Uno C-statistics for the modified group stage improved from 0.54 for the seventh to 0.56 for the eighth edition. In conclusion, a novel simplified staging system might be more practicable for pancreatic cancer patients who received neoadjuvant therapy.

Previous studies validating the value of the recently introduced eighth edition AJCC staging in patients who underwent upfront surgery for pancreatic adenocarcinoma have shown improved prognostic accuracy with the eighth edition staging system.[11,18] The Uno C-statistics for both the seventh and eighth edition staging systems reported by these studies were slightly higher than found in our study, indicating that the prognostic accuracy of pathologic staging decreases after neoadjuvant therapy.[11,18] These findings may be ascribed to the impeding effect of neoadjuvant therapy on pathologic examination. After neoadjuvant therapy the tumor and the adjacent non-neoplastic pancreatic parenchyma become fibrotic, which makes it challenging to delineate the boundaries of the tumor. In addition, pancreatic adenocarcinomas often have a heterogeneous response to neoadjuvant therapy, resulting in islands and nests of surviving tumor, with stretched of tumor-free fibrotic tumor bed in between, further complicating pathologic examination.[9]

A single-center study performed at the MD Anderson Cancer Center in 398 patients with pancreatic ductal adenocarcinoma who underwent neoadjuvant therapy followed by pancreaticoduodenectomy demonstrated that the eighth edition ypT stage better stratified survival than the seventh edition ypT AJCC stage.

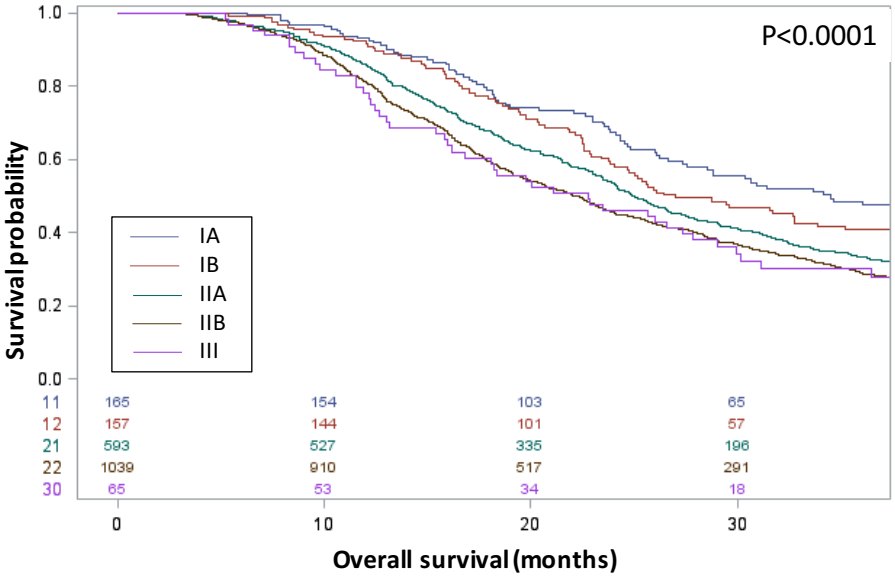

Figure 2a. Kaplan-Meier survival curves for pancreatic cancer patients staged according to the seventh edition AJCC group stage.

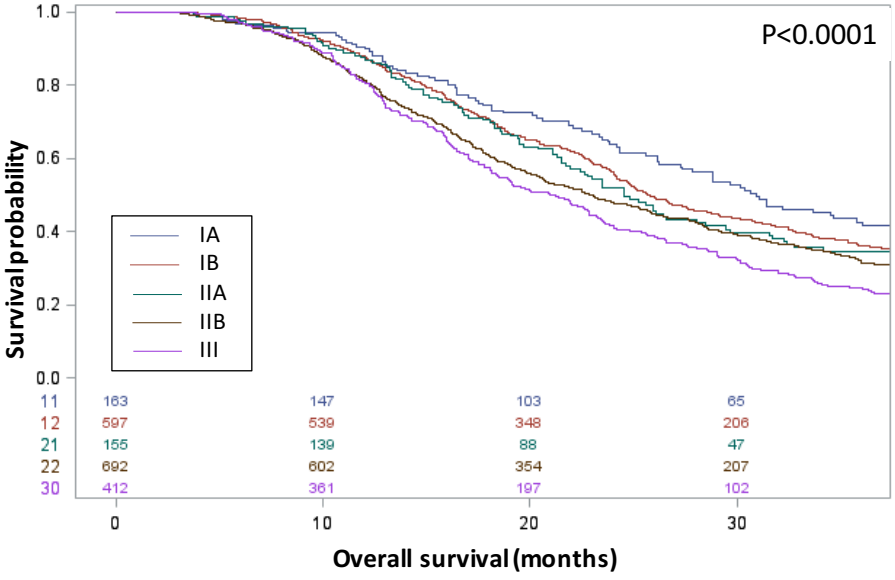

Figure 2b. Kaplan-Meier survival curves for pancreatic cancer patients staged according to the eighth edition AJCC group stage.

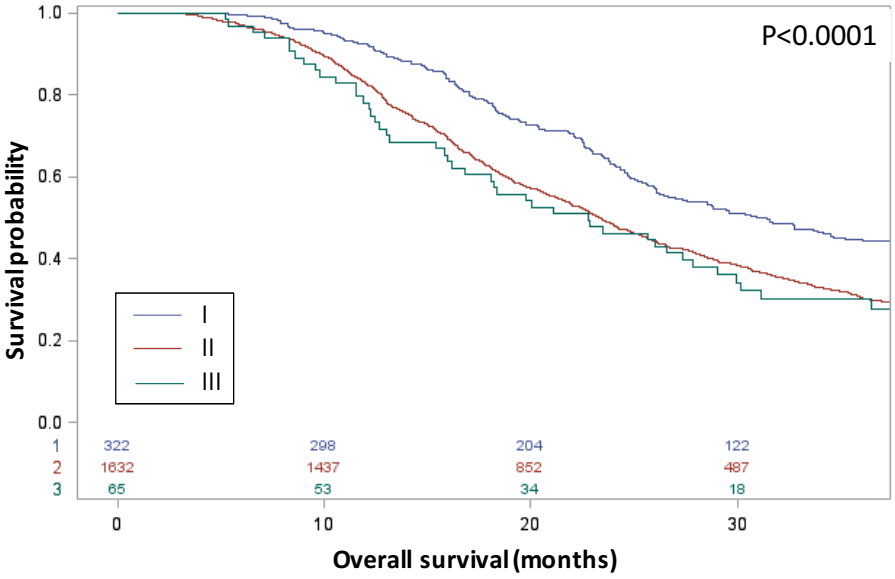

Figure 3a. Kaplan-Meier curves for pancreatic cancer patients staged according to the seventh edition AJCC modified group stage.

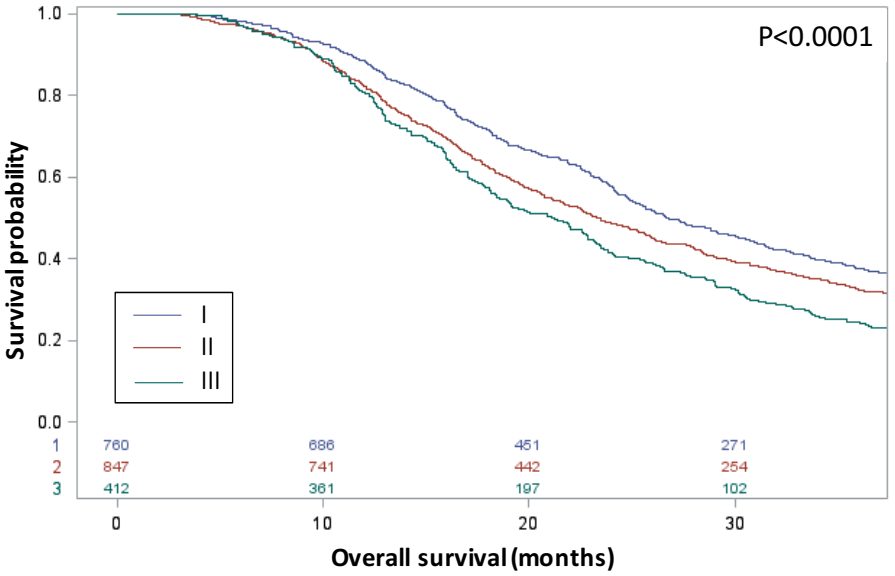

Figure 3b. Kaplan-Meier curves for pancreatic cancer patients staged according to the eighth edition AJCC modified group stage.

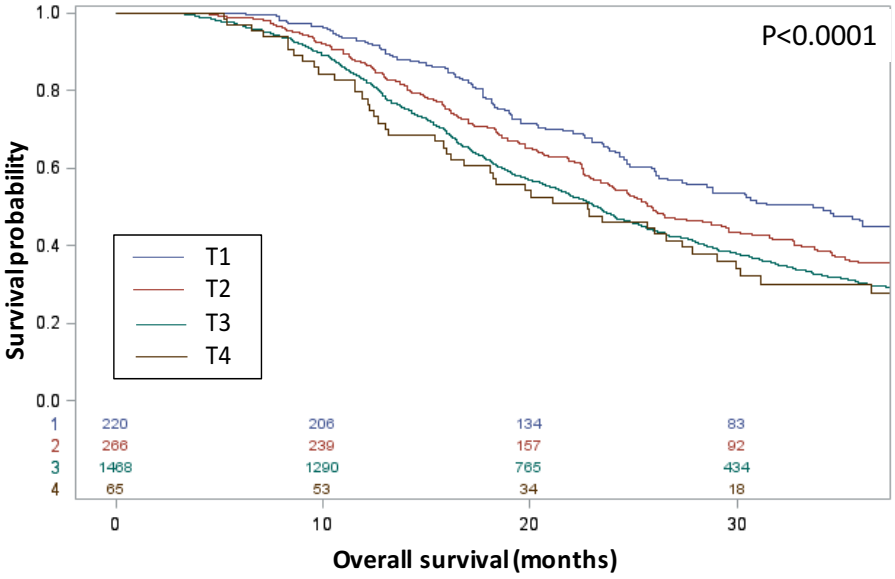

Figure 4a. Kaplan-Meier curves for pancreatic cancer patients staged according to the seventh edition AJCC T-stage.

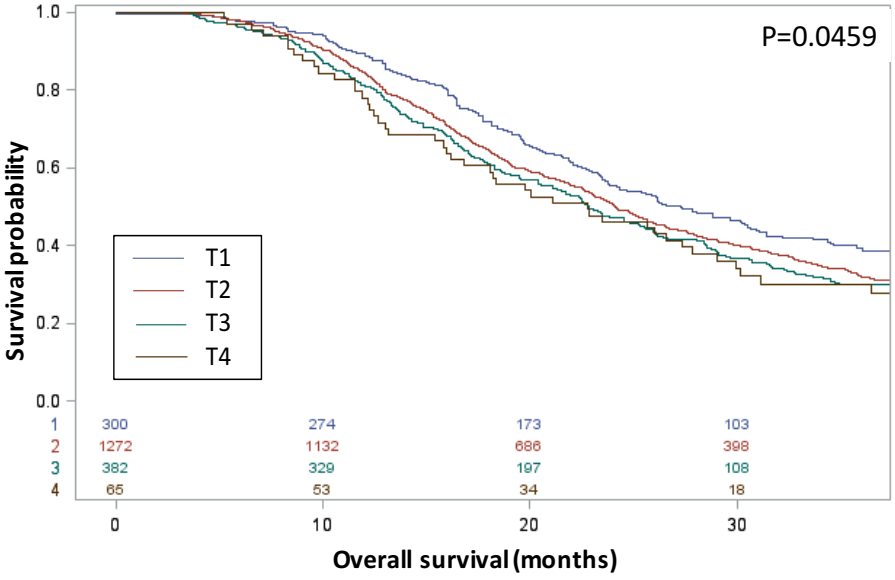

Figure 4b. Kaplan-Meier curves for pancreatic cancer patients staged according to the eighth edition AJCC T-stage.

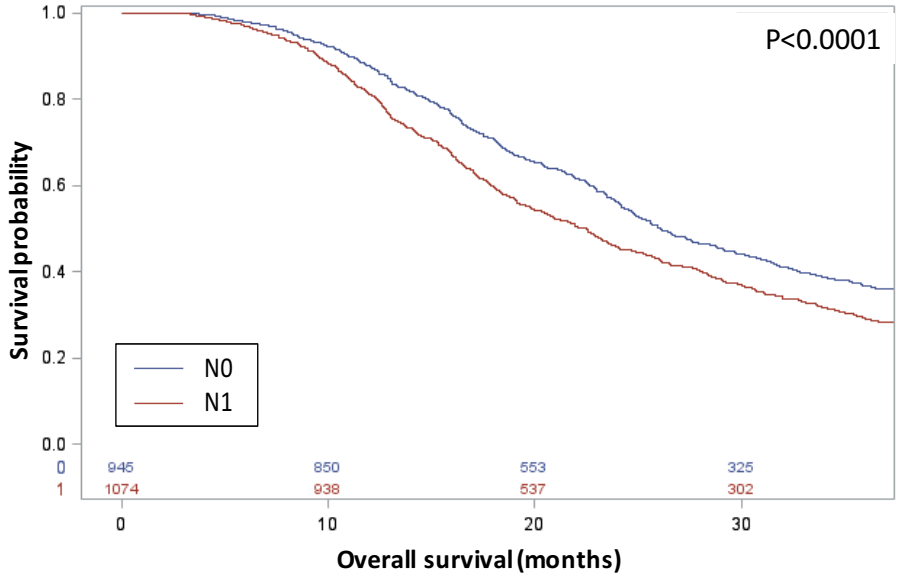

Figure 5a. Kaplan-Meier curves for pancreatic cancer patients staged according to the seventh edition AJCC N-stage.

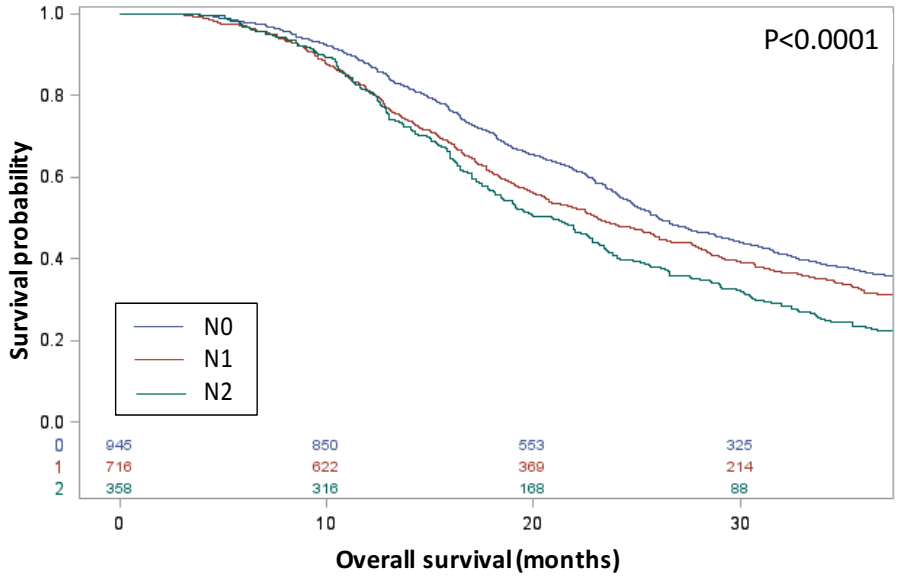

Figure 5b. Kaplan-Meier curves for pancreatic cancer patients staged according to the eighth edition AJCC N-stage.

In absence of pancreatic cancer specific guidelines, their study applied the standardized post-neoadjvuant therapy pathologic evaluation guidelines recommended for breast cancer by the international working group. Despite the stringent pathologic examination, they were not able to find a significant survival difference between the eighth edition ypT2 vs. ypT1 and ypT1 vs. ypT3 stage, similar to our results. Their study did not assess grouped stage.[9] These findings confirm that even when using a standardized pathology approach, assessment of tumor size after neoadjuvant therapy remains challenging.

The results of this study should be interpreted with respect to its limitations. First, the NCDB is subject to coding errors and does not report on various covariates, including the specific neoadjuvant chemotherapeutic regimen used, postoperative carbohydrate antigen 19-9 (CA 19-9) lab values, and the presence of perineural and/or vascular invasion.[27,28] The second limitation is the lack of standardization of the pathological examination guidelines, in particular for the pathologic assessment of pancreatic cancer specimen after neoadjuvant therapy, resulting in considerable variability in tumor size and lymph node yield. This variability may further comprise the relationship between pathology stage and clinical outcomes after pancreatic cancer surgery.[18] Finally, the NCDB does only collect data from commission on cancer approved hospitals. Although these centers capture 74% of all newly diagnosed pancreatic cancer cases in the United States, these findings may not be entirely generalizable to all centers.[29]

Despite these limitations, the present is to the best of our knowledge the first to validate the prognostic accuracy of the new eighth edition AJCC staging in pancreatic adenocarcinoma patients who underwent neoadjuvant therapy at a nationwide level. As neoadjuvant therapy is becoming more common, these findings will become more important over time. In addition, the present study possesses adequate statistical power to warrant its negative findings. Furthermore, the results of this study reflect the value of the eighth edition staging system beyond the high-volume specialized pancreatic cancer clinics were this staging system originated.[9,11]

In conclusion, the results of this study suggest that neither the seventh nor the eighth edition AJCC staging criteria are able to significantly discriminate survival between subsequent tumor stages. Neoadjuvant therapy has been well known to induce fibrosis in pancreatic adenocarcinoma and its surrounding non-neoplastic pancreatic parenchyma. These changes hamper the accuracy and granularity of the pathologic assessment of the tumor extend and therefore the prognostic value of the primary tumor stage. Although evidence-based consensus guidelines regarding the pathologic examination of post-neoadjuvant therapy surgical pancreatic cancer specimen are pivotal, previous work suggests this may not be sufficient to give the eighth edition staging system clinical relevance in patients who underwent neoadjuvant therapy.[9] In this study, a simplified eighth edition group stage , collapsing stage IA with IB, and IIA with IIB, was able to significantly discriminate survival between all subsequent stages. This simplified group stage may

provide a more practicable clinically relevant alternative to the original eighth edition group stage.

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SUPPLEMENTARY TABLES

Supplementary table 1. Pancreatic cancer staging according to the seventh and eighth edition AJCC staging criteria.

	Seventh edition			Eighth edition		
Primary Tumor (T)						
ypT1	Tumor limited to the pancreas, ≤ 2 cm in greatest dimension			Maximum tumor diameter ≤ 2 cm		
ypT2	Tumor limited to the pancreas, > 2 cm in greatest dimension			Maximum tumor diameter > 2 ≤ 4 cm		
ypT3	Tumor extend beyond the pancreatic without involvement of the celiac axis or the superior mesenteric artery			Maximum tumor diameter > 4 cm		
ypT4	Tumor involves the celiac axis or the superior mesenteric artery			Tumor involves the celiac axis or the superior mesenteric artery		
Regional Lymph Nodes (N)						
ypN0	No regional lymph node metastasis			No regional lymph node metastasis		
ypN1	Regional lymph node metastasis			Metastasis in 1-3 regional lymph nodes		
ypN2	-			Metastasis in ≥ 4 regional lymph nodes		
Distant Metastases (M)						
ypM0	No distant metastases			No distant metastases		
ypM1	Distant metastases			Distant metastases		
Overall stage						
Stage IA	ypT1	ypN0	ypM0	ypT1	ypN0	ypM0
Stage IB	ypT2	ypN0	ypM0	ypT2	ypN0	ypM0
Stage IIA	ypT3	ypN0	ypM0	ypT3	ypN0	ypM0
Stage IIB	ypT1-T3	ypN1	ypM0	ypT1-T3	ypN1	ypM0
Stage III	ypT4	ypN0-N1	ypM0	ypT4	ypN0-N1	ypM0
	-	-	-	ypT1-T4	ypN2	ypM0

Supplementary Table 2. Correlation between primary tumor stage by the American Journal Committee of Cancer (AJCC) 8th Edition and clinicopathologic factors in pancreatic cancer patients who underwent neoadjuvant therapy and resection.

Characteristics	**Primary Tumor Stage, Eighth Edition**				
	T1 (n=300)	**T2 (n=1,272)**	**T3 (n=382)**	**T4 (n=65)**	***p***
Primary Tumor Stage, Seventh Edition, n (%)					
T1	99 (33.0%)	98 (7.7%)	23 (6.0%)	0 (0.0%)	<0.0001
T2	12 (4.0%)	204 (16.0%)	50 (13.1%)	0 (0.0%)	
T3	189 (63.0%)	970 (76.3%)	309 (80.9%)	0 (0.0%)	
T4	0 (0.0%)	0 (0.0%)	0 (0.0%)	65 (100.0%)	

Supplementary table 3. 5-year survival for the seventh and eighth edition AJCC staging.

	Seventh edition	**Eighth edition**
Group stage		
Stage IA	26.4%	23.2%
Stage IB	21.2%	21.0%
Stage IIA	18.7%	15.0%
Stage IIB	14.9%	16.0%
Stage III	11.1%	12.5%
Simplified group stage		
Stage I	23.7%	21.5%
Stage II	16.3%	15.7%
Stage III	11.1%	12.5%
Primary Tumor Stage		
T1	24.2%	20.6%
T2	17.2%	17.5%
T3	16.6%	15.1%
T4	11.1%	11.1%
Lymph Node Stage		
N0	19.9%	19.9%
N1	15.0%	15.9%
N2	-	13.5%

Chapter 11

A tale of two cities: reconsidering adjuvant radiation in pancreatic cancer care

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Journal of Gastrointestinal Surgery 2016 Jan;20(1):85-92

ABSTRACT

Background: Adjuvant chemotherapy plays a critical role in the treatment of resected pancreatic cancer patients. However, the role of adjuvant radiation remains controversial. This study compares survival between resected pancreatic cancer patients who received adjuvant radiation and no adjuvant radiation.

Methods: Medical records of patients with pancreatic ductal adenocarcinoma who underwent surgical resection from January 2003 through 2013 at medical centres in Boston and Leiden were retrospectively reviewed. Propensity score matching was used to correct for potential selection bias in the allocation of adjuvant chemoradiation versus chemotherapy alone.

Results: 350 total patients were identified, of whom 138 (39.4%) received adjuvant radiation. On pathological staging 245 (70.0%) had positive lymph nodes, and these patients gained a significant survival benefit from adjuvant radiation (HR 0.74; 95% CI 0.56 – 0.99) in the complete cohort. After propensity score matching, adjuvant radiation lost its prognostic significance in the complete cohort. However, after matching, patients who survived longer than 12 months and had positive lymph nodes (n=108) demonstrated a significant (log-rank p = 0.04) survival benefit from adjuvant radiation.

Discussion: This study, while nonrandomized, suggests that adjuvant radiation may be associated with a survival benefit for resected pancreatic cancer patients in specific situations.

INTRODUCTION

Pancreatic head adenocarcinoma has a poor prognosis, with a 5-year survival rate of less than 5%. For 2014, 46,420 new cases were predicted in the United States, a number almost equivalent to the annual pancreatic cancer mortality rate [1]. Surgical resection offers the only hope for cure, but even if negative resection margins can be obtained, recurrence rates remain high; 46% - 99% of resected pancreatic cancer patients develop recurrence as late as 7 years after pancreatectomy [2-4]. These facts illustrate that pancreatic adenocarcinoma should be considered a systemic disease at any stage and emphasize the need for adjuvant therapy [5].

However, the role of adjuvant radiation in resected pancreatic cancer patients remains controversial. Multiple randomized trials have been performed with contradictory results. In 1985, the Gastrointestinal Tumor Study Group (GITSG) provided the first evidence for the survival benefit of adjuvant radiation therapy compared to observation alone for patients with resected pancreatic adenocarcinoma [6]. It was then followed by multiple trials from large cooperative groups in the United States and Europe utilizing adjuvant radiation with varying results: the European Organization for Research and Treatment of Cancer (EORTC) and European Study Group for Pancreatic Cancer (ESPAC-1). In addition, several nonrandomized trials as well as retrospective single institution and large database reports in the US have suggested a survival benefit for adjuvant radiation for patients treated with adjuvant radiation [6-10].

The purpose of this study was to explore the value of adjuvant radiation in two pancreatic centres of excellence in Europe and the United States.

METHODS

Data collection

The medical records of patients undergoing pancreatectomy from January 2002 through December 2012 at a medical center in Boston, Massachusetts, USA and a medical center in Leiden, The Netherlands~~,~~ were retrospectively reviewed. For the purpose of this study, patients with a tissue diagnosis other than pancreatic adenocarcinoma were excluded, as were those with incomplete follow-up data or those treated with neoadjuvant therapy. In this paper, pancreatic cancer and pancreatic adenocarcinoma will be used interchangeably.

Patients were categorized by pathologic stage according to the American Joint Committee on Cancer (AJCC) 7th edition staging definitions for adenocarcinoma of the pancreas. Postoperative complications were defined according to guidelines of the International Study Group of Pancreatic Surgery. Standardized histological evaluation of the surgical specimens was performed as described by the American Joint Committee on Cancer. Final margins were recorded as negative (R0) or microscopically positive (R1) for tumor. There were no R2 resections for this cohort. A margin was designated positive if

tumor cells were present at the inked resection margin, whether retroperitoneal, pancreatic or biliary. All chemotherapy administrated in this study consisted of systemic chemotherapy and receipt of radiation also included concurrent chemosensitizing doses of chemotherapy.

Statistical analysis

SAS 9.3 software (SAS Institute, Cary, NC) was used for all statistical analyses. Clinical and pathological characteristics between both treatment groups were comparing using χ^2 and Fischer's exact tests. The primary endpoint was overall survival, which was calculated from date of surgery until death with censoring at date of last contact for living patients. Kaplan-Meier methods and log-rank tests were used to assess differences between survival curves. In addition, survival analyses were performed using univariate and multivariate Cox proportional hazard models. As part of the Cox proportional hazard model building technique, each variable was evaluated using LLS plots: Log (-log(survival probability)) against log of time to determine if the hazards were proportional. If the proportional hazard assumption was violated, the variable was withheld from the univariate and multivariate Cox proportional hazard analyses and p-values of the log-rank test were reported. Statistical significance was set at $p<0.05$.

In order to reduce residual confounding by indication between patients receiving chemotherapy versus those receiving both chemotherapy and radiotherapy, we performed propensity score matching for patients receiving adjuvant chemoradiotherapy with patients that received chemotherapy alone. We performed 1:1 nearest-neighbor matching without replacement with caliper width equal to 0.2 of the pooled standard deviation of the logit of the propensity score, thus eliminating 99% of the bias [11].

This study was approved by the Dana Farber / Harvard Cancer Center, the Beth Israel Deaconess Medical Center and the Leiden University Medical Center Institutional Review Boards. Study data were collected and managed using the REDCap (Research Electronic Data Capture) electronic data capture tools hosted at BIDMC. REDCap is a secure, web-based application designed to support data capture for research studies that provides an intuitive interface for validated data entry, audit trails for tracking data manipulation and export procedures, automated export procedures for seamless data downloads to common statistical packages, and procedures for importing data from external sources [12].

RESULTS

Characteristics of initial cohort

The study population was comprised of 350 patients who underwent surgical resection for a pancreatic adenocarcinoma, of whom 209 (59.7%) were patients at a medical center in Boston and 141 (40.3%) patients at a medical center in Leiden, respectively.

Baseline demographics and clinical characteristic are shown in Table 1. The median age of the patients was 66 years (range 36 – 86 years); 44.6% of patients were male. The median tumor size was 3.0 cm ranging from 0.9 to 8.0 cm. Pathologic staging was T3 for 240 cases (68.6%) and N1 for 245 cases (70.0%). An R0 surgical resection was achieved in 207 patients (59.1%); there were no R2 resections. 135 (38.6%) patients received adjuvant chemotherapy with radiation, 105 (30.0%) received adjuvant chemotherapy without radiation, 107 (30.6%) received no treatment, and 3 (0.9%) received radiation alone

The distribution of baseline characteristics was similar in the radiation and non-radiation groups, except for tumor differentiation and T stage. In the group that received adjuvant radiation, tumor differentiation ($p = 0.002$) and pT-stage ($p<0.001$) were significantly more common.

Table 1. Baseline characteristics of the initial cohort by receipt of radiation therapy. EBRT, external beam radiation therapy.

	Total population (n = 350)	**Received EBRT (n = 138)**	**No EBRT (n = 212)**	**p**
Age, n (%)				
< 65 years	154 (44.0%)	66 (47.8%)	88 (41.5%)	0.245
≥ 65 years	156 (56.0%)	72 (52.2%)	124 (58.5%)	
Sex, n (%)				
Male	156 (44.6%)	59 (42.7%)	97 (45.8%)	0.581
Female	194 (55.4%)	79 (57.3%)	115 (54.2%)	
Tumor differentiation, n (%)				
Well differentiated	64 (18.3%)	32 (23.2%)	32 (15.1%)	0.002
Moderately differentiated	190 (54.3%)	82 (59.4%)	108 (50.9%)	
Poorly/undifferentiated	96 (27.4%)	24 (17.4%)	72 (34.0%)	
AJCC T stage, n (%)				
T1	32 (9.1%)	5 (3.6%)	27 (12.7%)	< 0.001
T2	65 (18.6%)	18 (13.0%)	47 (22.2%)	
T3	240 (68.6%)	112 (81.2%)	128 (60.4%)	
T4	13 (3.7%)	3 (2.2%)	10 (4.7%)	
AJCC N stage, n (%)				
N0	105 (30.0%)	37 (26.8%)	68 (32.1%)	0.294
N1	245 (70.0%)	101 (73.2%)	144 (67.9%)	
Perineural invasion**, n (%)**				
No	45 (14.4%)	17 (12.8%)	29 (12.0%)	0.477
Yes	267 (85.6%)	116 (87.2%)	151 (70.5%)	
Vascular invasion, n (%)				
No	149 (54.4%)	57 (49.6%)	92 (57.9%)	0.174
Yes	125 (45.6%)	58 (50.4%)	67 (42.1%)	
Margin status, n (%)				
R0-resection	207 (59.1%)	70 (50.7%)	137 (64.6%)	0.009
R1-resection	143 (40.9%)	68 (49.3%)	75 (35.4%)	

Survival outcomes of initial cohort

With a median overall survival of 19.5 months (95% CI 17.5 – 21.8 months) for all 350 patients, there were 273 deaths reported in the study cohort (78%). The median overall survival for patients who received adjuvant radiation was 21.8 months (95% CI 18.7 – 26.1 months) compared to 18.4 months (95% CI 15.9 – 20.5 months) in patients who did not receive adjuvant radiation. The 2-year survival rates were 30.7% and 41.3% for the

patients who received no adjuvant radiation and adjuvant radiation, respectively. There were 4 deaths (1.1%) occurring within 30 days of surgical resection of the pancreatic mass.

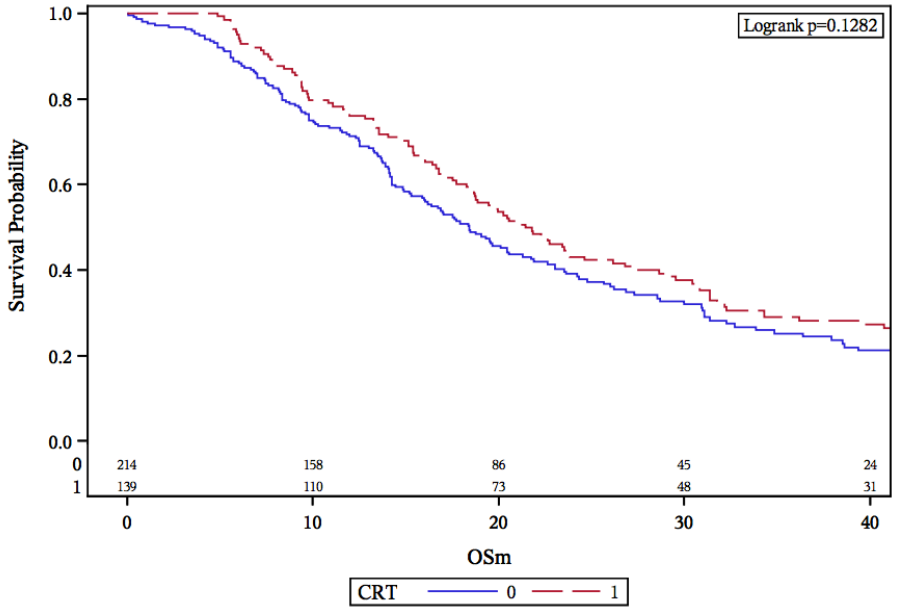

Figure 1. Kaplan-Meier curves for adjuvant radiation and no adjuvant radiation in the initial cohort of pancreatic cancer patients.

On survival analyses, well differentiation tumors (log-rank p = 0.001) and lymph node positive (log-rank p =0.004) were predictive for survival benefit. However, sex (log-rank p = 0.51), age (log-rank p = 0.17), pT-stage (log-rank p = 0.06), resection margin status (log – rank p = 0.08) and receipt of adjuvant radiation (HR 0.82; 95% CI 0.64-1.05; p = 0.11) did not significantly influence survival in resected pancreatic cancer patients. Figure 1 shows the survival of resected pancreatic cancer stratified for receipt of adjuvant radiation therapy.

Impact of lymph node status

After patients were stratified for lymph node invasion (n = 245), adjuvant radiation was significantly protective (HR 0.74; 95% CI 0.56 – 0.99; p = 0.04) in patients with only resected pancreatic cancer with lymph node invasion, as shown in Figure 2. However, sex (log-rank p = 0.87), age (log-rank p = 0.24), pT-stage (log-rank p = 0.39), tumor differentiation (log-rank p = 0.13) and resection margin status (log-rank p = 0.49) did not influence survival in these patients.

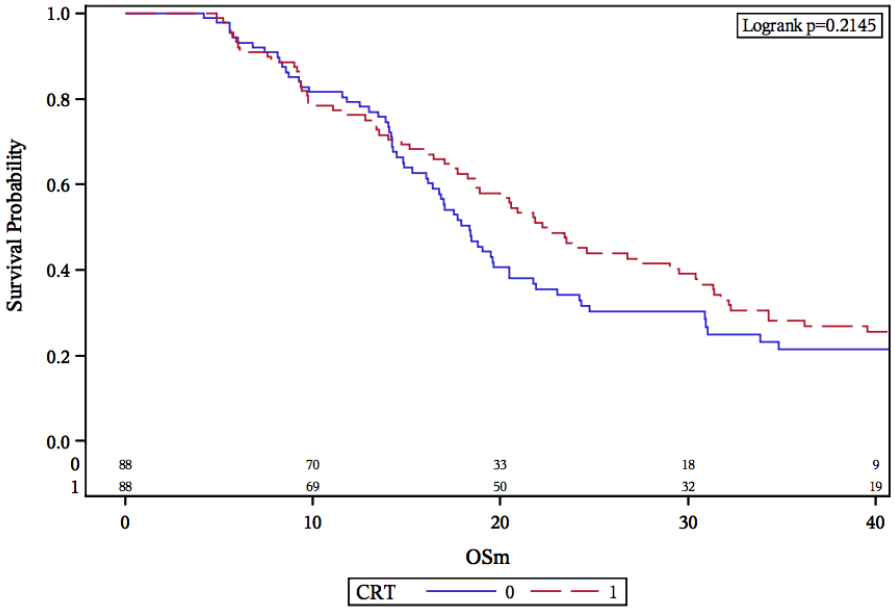

Figure 2. Kaplan-Meier curves for adjuvant radiation and no adjuvant radiation in the initial cohort of resected pancreatic cancer patients with positive lymph nodes.

Propensity score matched cohort

After matching, the cohort consisted of 88 patients treated with adjuvant chemotherapy and 88 patients treated with adjuvant chemoradiotherapy. All baseline clinical and pathological characteristics were equally distributed over both groups and shown in Table 2.

The median survival in the complete matched population was 19.6 months (range 17.5 – 22.3 months), and 35 (19.9%) patients survived to the end of follow-up. The median survival and 2-year survival for resected pancreatic adenocarcinoma patients treated with only adjuvant chemotherapy were 17.9 months (95% CI 16.0 – 19.6 months) and 70.4%, compared to 22.3 months (95% CI 18.7 – 29.5 months) and 56.8% in the group that received adjuvant chemoradiation (Figure 3).

After propensity score matching, sex (log-rank p = 0.76), age (log-rank p = 0.14) pT-stage (log-rank p = 0.11), pN-stage (log-rank p = 0.26), tumor differentiation (HR 1.80; 95% CI 0.95 – 3.44; p = 0.07), resection margin status (log-rank p = 0.73) and adjuvant treatment (log-rank p = 0.16; figure 3) did not influence survival in resected pancreatic cancer patients.

One year Survivors

In total, 135 patients (76.7%) in the matched cohort survived longer than 12 months. In this subgroup, sex (log-rank p = 0.81), age (Log-Rank p = 0.32), pT-stage

(log-rank p = 0.33), tumor differentiation (log-rank p = 0.16) and margin status (log-rank p = 0.76) were not prognostic for overall survival. However, lymph node invasion (HR 1.69; 95% CI 1.00 – 2.85; p = 0.05) was predictive for a poor prognosis and a trend was observed towards survival benefit for patients that received adjuvant chemoradiation compared to chemotherapy alone (log-rank p = 0.06).

In patients that survived over one year with positive lymph nodes (n =108), receipt of adjuvant chemoradiation resulted in significant (log-rank p = 0.04) survival benefit, with a median overall survival of 29.0 months (95% CI 22.3 – 32.3 months) compared to 18.8 months (95% CI 17.0 – 24.2 months) for adjuvant chemoradiation and chemotherapy alone respectively (Figure 5). Sex (log-rank p = 0.59), age (log-rank p = 0.11), pT-stage (log-rank p = 0.56), tumor differentiation (log-rank p = 0.45) and resection margin status (log-rank p = 0.40) had no predictive value for overall survival in this patient population.

Table 2. Baseline characteristics of the population resected pancreatic cancer patients after propensity score matching stratified for adjuvant treatment.

	No radiation (n = 88)	**Received radiation (n = 88)**	**p-value**
Age, n (%)			
< 65 years	43 (48.9%)	40 (45.5%)	0.65
≥ 65 years	45 (51.1%)	48 (54.5%)	
Sex, n (%)			
Male	39 (44.3%)	39 (44.3%)	>0.99
Female	49 (55.7%)	49 (55.7%)	
Tumor differentiation, n (%)			
Well differentiated	9 (10.2%)	9 (10.2%)	0.005
Moderately differentiated	49 (55.7%)	67 (76.1%)	
Poorly differentiated	30 (34.1%)	12 (13.6%)	
AJCC T stage, n (%)			
T1	8 (9.1%)	5 (5.7%)	0.005
T2	8 (9.1%)	12 (13.6%)	
T3	67 (76.1%)	68 (77.3%)	
T4	5 (5.7%)	3 (3.4%)	
AJCC N stage, n (%)			
N0	21 (23.9%)	17 (19.3%)	0.464
N1	67 (76.1%)	71 (80.7%)	
Perineural invasion, n (%)			
No	13 (14.8%)	11 (12.9%)	0.438
Yes	62 (70.5%)	74 (87.1%)	
Vascular invasion, n (%)			
No	36 (54.6%)	40 (54.5%)	0.95
Yes	30 (45.4%)	34 (45.9%)	
Margin status, n (%)			
R0-resection	54 (61.4%)	53 (60.2%)	0.877
R1-resection	34 (38.6%)	35 (39.8%)	

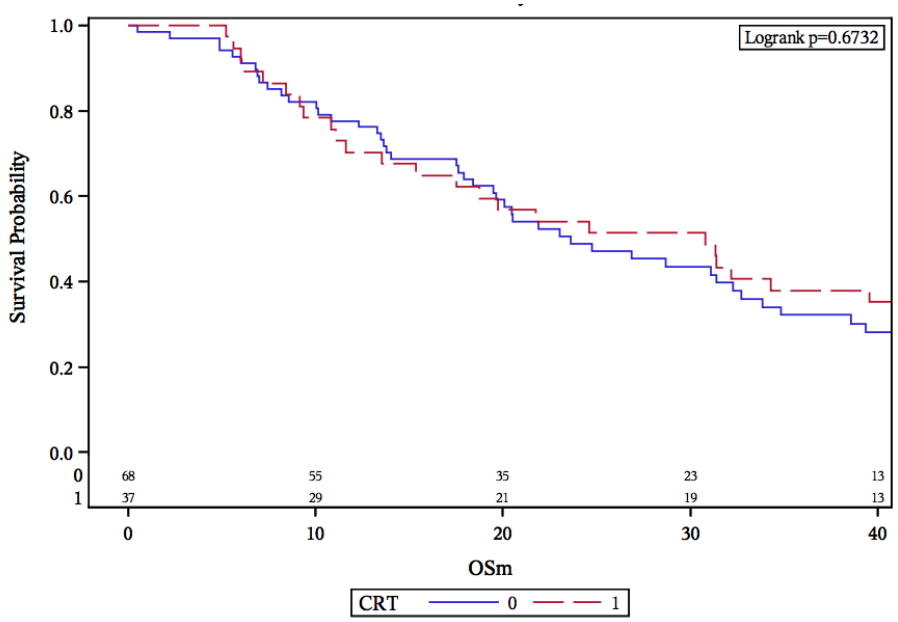

Figure 3. Kaplan-Meier curves for adjuvant chemoradiation and adjuvant chemotherapy alone in the propensity score matched cohort of resected pancreatic cancer patients.

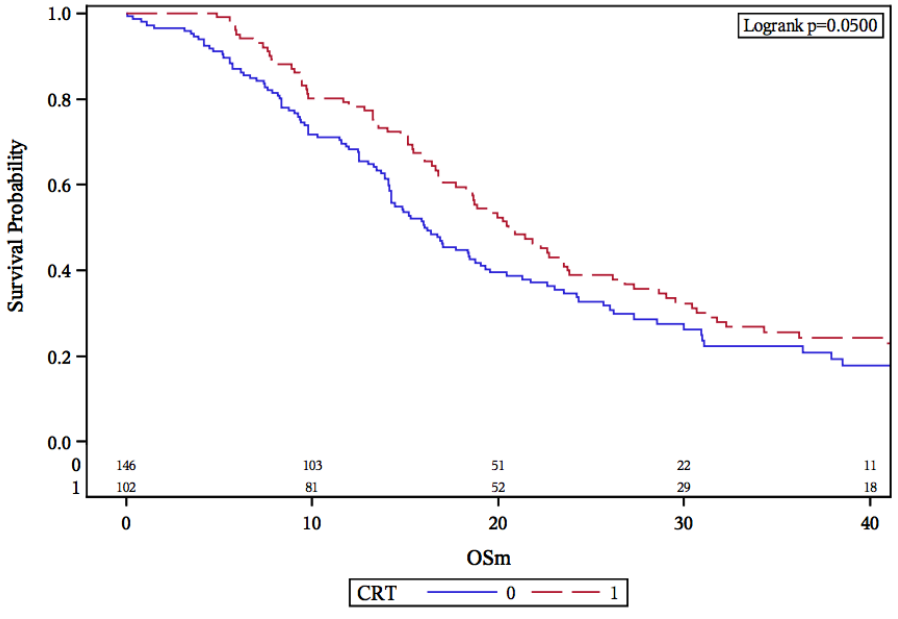

Figure 4. Kaplan-Meier curves for adjuvant chemoradiation and no adjuvant chemotherapy alone in the propensity score matched cohort of resected pancreatic cancer patients that lived over twelve months.

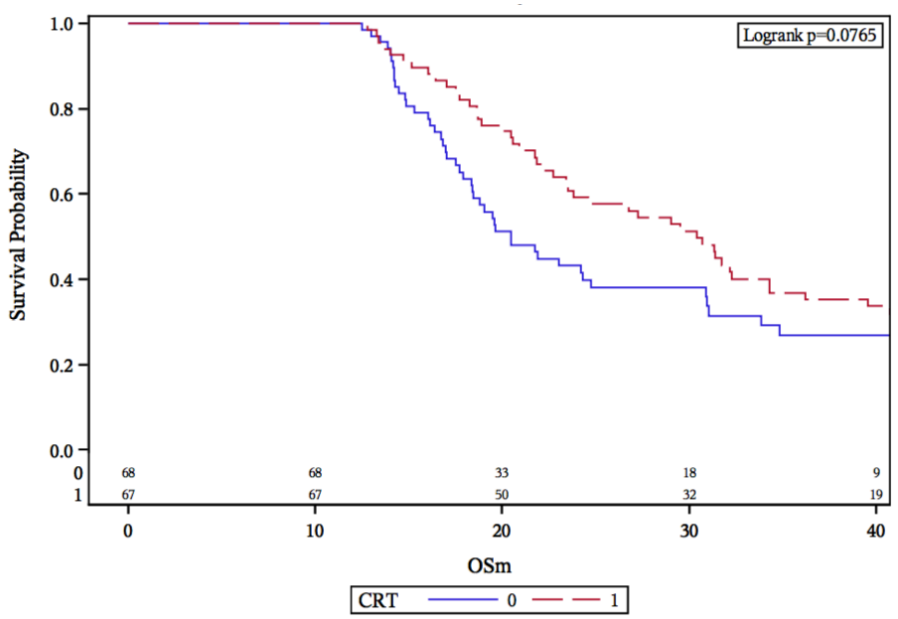

Figure 5. Kaplan-Meier curves for adjuvant chemoradiation and no adjuvant chemotherapy in the propensity score matched cohort of resected pancreatic cancer patients that lived over twelve months with positive lymph nodes.

DISCUSSION

Adjuvant therapy has been proven to prolong survival for resectable pancreatic adenocarcinoma patients. However, the role of adjuvant radiation, although standard of care in many centers of excellence throughout the United States, remains controversial. In this study, we used data from two pancreatic cancer centers with different approaches to adjuvant therapy to explore the survival benefit of adjuvant radiation for resected pancreatic cancer patients. Our study suggests there might be a survival benefit for resected pancreatic cancer patients treated with adjuvant chemoradiation compared to chemotherapy alone. However, the survival benefit appeared to be limited to patients with positive lymph nodes and patients who survived over one year.

The first trial to indicate survival benefit for adjuvant radiation in pancreatic cancer patients was the Gastrointestinal Tumor Study Group (GITSG) trial that randomized patients to either adjuvant radiation or observation alone. This study reported an overall median survival of 21 months for patients in the adjuvant radiation group versus 14 months for the observation alone group [6]. The subsequent randomized controlled trial conducted by the European Organization for Research and Treatment of Cancer (EORTC) showed a slight, non-significant survival benefit of 24.5 months overall survival for patients treated with adjuvant radiotherapy versus 19 months in the observation only arm [8]. However, both randomized controlled trials compared adjuvant radiation to observation alone an more

recently published phase III trials on adjuvant therapy clearly demonstrate improved survival for patients treated with adjuvant chemotherapy [10, 13]. These results established a controversy around the addition of radiation to adjuvant chemotherapy in resected pancreatic cancer patients. To date, the European Study Group for Pancreatic Cancer (ESPAC)-1 trial is still the only formal randomized controlled trial addressing the value of adjuvant chemoradiation compared to chemotherapy alone. The ESPAC-1 trial results contradicted both the GITSG and EORTC trial conclusions; it showed no short-term survival benefit for adjuvant radiation and a negative effect on long-term survival [9, 14]. However, the ESPAC-1 trial has extensive technical and methodological limitations [15, 16]. In contrast, subsequent non-randomized studies showed potential therapeutic benefit for patients treated with adjuvant radiation [7, 17, 18]. In addition, multiple studies have shown promising results with chemoradiation in the neoadjuvant setting [2, 19-21].

The results of our study, although non-randomized tentative suggest a survival benefit for patients with node-positive resected pancreatic adenocarcinoma that received chemoradiation over patients who received chemotherapy alone, if they survived over one year[22]. However, a major problem inherent to exploring the additional value of radiation to chemotherapy in a non-randomized setting is that radiation is typically administrated following chemotherapy, as is the case in this study. Therefore, any survival advantage attributed to radiation in this study could easily be due to the fact that patients who received radiation simply lived long enough to receive it. This fundamental problem can't completely be addressed with any other method than a randomized controlled trial. However, in the absence of such a trial we attempted to minimize the selection bias between the two treatment approaches in this study by performing propensity score matching and subset analyses in patients who lived over a year, which is long-enough to receive radiation. After propensity matching and restriction to patients who survived long enough to receive radiation, the remaining cohort may be underpowered, which makes it less likely to detect significant differences between both groups. Nevertheless, this study was still able to detect significant survival benefit for patients who received chemoradiation compared to patient who received chemotherapy alone. In addition, the Boston medical center is a high-volume referral center; its patients may not reflect the typical American pancreatic cancer population in contrast to the Leiden medical center which is located in the Netherlands, where all pancreatic cancer patients receive care at high-volume centers, which could add additional heterogeneity.

Despite its limitations, this study indicates the potential survival benefit of chemoradiation for resected pancreatic cancer patients and emphasizes the need for further randomized prospective exploration of this evident caveat in the evidence-based care for pancreatic cancer patients. In addition, it is the first study incorporating populations from both Europe and the USA to explore the radiation question making gradual steps toward a more global comprehension of the treatment of pancreatic cancer [6, 8, 9].

CONCLUSION

The results of this study, although non-randomized and slightly underpowered, still suggest a significant survival benefit for lymph node positive pancreatic adenocarcinoma patients receiving adjuvant chemoradiation to chemotherapy alone. This reinforces the need for additional research on the value of adjuvant radiation to answer this critical question in a well-powered and randomized setting.

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Chapter 12

Stereotactic body radiotherapy for unresected pancreatic cancer: a nationwide review

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Cancer 2017;123(21):4158-4167

ABSTRACT

Background: Over the past decade, stereotactic body radiotherapy (SBRT) has emerged as a novel therapeutic option in pancreatic cancer care. We sought to evaluate the survival impact of SBRT in unresected pancreatic cancer patients.

Methods: The National Cancer Database was queried for unresected patients who received chemotherapy for non-metastatic pancreatic adenocarcinoma between 2004 and 2012. Four treatment groups were identified: chemotherapy alone, or chemotherapy combined with one of the following: external beam radiotherapy (EBRT), intensity-modulated radiotherapy (IMRT), or SBRT. Propensity score models predicting the odds of receiving SBRT were created to control for potential selection bias, and patients were matched on propensity score.

Results: A total of 14,331 patients met the inclusion criteria. Chemotherapy alone was delivered to 5,464 (38.1%) patients. 6,418 (44.8%), 322 (2.3%) and 2,127 (14.8%) patients received chemotherapy along with EBRT, IMRT, or SBRT, respectively. The median survival before matching was 9.9, 10.9 12.0, and 13.9 months for patients treated with chemotherapy, EBRT, IMRT, and SBRT, respectively. In separate matched analyses, SBRT remained superior to chemotherapy alone (log-rank $p<0.0001$) and EBRT (log-rank $p=0.0180$). After matching, survival did not differ between patients receiving IMRT and SBRT (log-rank $p=0.0492$).

Conclusions: SBRT is associated with a significantly better outcome than chemotherapy alone or in conjunction with traditional EBRT. These results emphasize that SBRT is a promising treatment approach for unresected pancreatic cancer patients.

INTRODUCTION

Pancreatic cancer ranks the third leading cause of cancer death and is approximated to become the second leading cause by 2020[1, 2]. Despite continued advancements in treatment modalities, its 5-year survival rate remains less than 8%[1]. Complete surgical tumor resection provides the only hope for long-term survival. Unfortunately, 80-85% of patients are diagnosed with unresectable disease, due to distant metastases or locally advanced tumors.[3] Even technically resectable patients often do not receive surgery due to poor performance status, lack of access to care, or patient preference[3, 4]. Chemotherapy, often combined with traditional fractionated external beam radiotherapy (EBRT), is considered the standard of care for unresectable pancreatic cancer patients throughout the United States[5]. However, the role of radiation constitutes an area of controversy as trials demonstrate ambiguous results[6, 7].

Over the last decade, stereotactic body radiotherapy (SBRT) has been progressively used for the treatment of inoperable pancreatic cancer. This novel approach permits the precise application of high dose radiation in one to five fractions to a limited target volume. The accuracy and swift dose fall-off of SBRT reduce the dose to adjacent healthy tissue and subsequently minimize toxicity[8]. In addition, considering the high rates of distant failure in localized disease, fewer intrusions in the delivery of chemotherapy may enhance survival. Furthermore, the majority of emerging data on SBRT demonstrate promising rates of local control, ranging from 49-100%[8-10].

The current study investigates the survival impact of chemotherapy plus SBRT compared to chemotherapy alone and chemotherapy combined with EBRT or intensity-modulated radiotherapy (IMRT) using population data.

METHODS

Database

The National Cancer Database (NCDB) is a collective venture of the American College of Surgeons and the American Cancer Society. This nationwide database comprises oncologic outcomes for more than 1500 Commission on Cancer accredited programs. Accordingly, the NCDB captures roughly 70% of all newly diagnosed cases of cancer in the United States. Furthermore, the NCDB requires centers to maintain a 90% follow-up rate for patients diagnosed within 5 years to remain accredited[11].

Patient selection

This retrospective study queried the NCDB for patients with pancreatic adenocarcinoma (n=204,387) diagnosed between 2004 and 2012. Pancreatic adenocarcinoma was defined based on ICD-O-3 (International Classification of Disease for Oncology) morphology codes for adenocarcinoma (8140 and 8500), in combination with

the following ICD-O-3 topography codes: C25.0, C25.1, C25.2, C25.3, C25.7, C25.8, and C25.9.[12] For the purpose of this study, the terms “pancreatic cancer” and “pancreatic adenocarcinoma” are used interchangeably.

Patients with metastatic disease at diagnosis (n=85,653) or surgery of the primary site (n=42,794) were excluded. Patients who underwent resection were identified according to the Facility Oncology Registry Data Standards (FORDS) surgery codes: 25, 30, 35, 36, 37, 40, 60, 70, 80, and 90. The cohort was further restricted by sequentially excluding patients that were not treated at the reporting facility (n=13,659), were diagnosed with other malignancies (n=11,501) or did not receive chemotherapy (n=22,417), treatment including electrons and/or neutrons (n=54), proton therapy (n=43), or radioisotopes (n=11), or started treatment more than 90 days after diagnosis (n=1,222). In addition, patients that died or were last contacted within 3 months after diagnosis (n=2,421) were excluded to correct for potential immortality bias[13].

Furthermore, patients with at least one of the following missing or unknown variables were excluded sequentially: overall survival (n=3,049), age, sex, race (n=259), comorbidities, other malignancies or insurance status (n=750), facility type or treated at integrated network cancer programs (n=1,513), tumor location, clinical stage (n=3,061), surgery of the primary site (n=47), receipt of chemotherapy (n=787), receipt of radiation (n=152), or number of days from diagnosis to treatment greater than 90 days (n=663).

Construction of variables

Age at diagnosis was dichotomized into those < 65 and ≥ 65 years. Race was collapsed to white and non-white. Comorbidities are reported in the NCDB based on the Charlson/Deyo scoring system, and were divided into zero versus any comorbid conditions recorded[14, 15]. Insurance status was categorized into private insurance and other type of insurance or no insurance. Treatment centers were divided into academic and non-academic. Tumor location was dichotomized based on ICD-O-3 topography codes for the site of origin into head/neck (C25.0, and C25.7) vs. other (C25.1, C25.2, C25.3, C25.8, and C25.9). Clinical tumor stage was defined according to the 7th edition of the American Journal Committee on Cancer (AJCC) staging manual; if the group stage was not available the definitive stage was computed by combining clinical primary tumor (cT), regional lymph node status (cN) and the presence or absence of distant metastasis (cM)[5, 16, 17].

All patients included in this study received chemotherapy. Receipt of chemotherapy was defined as reported by the NCDB. Radiotherapy was coded according the Commission on Cancer facility oncology registry data standard.[18] In addition, for the purpose of this study radiotherapy was categorized as no radiotherapy administrated, EBRT (external beam not otherwise specified, orthovoltage, Cobalt-60 or Cesium-137, photons alone or mixed energies, or conformal or 3-D therapy), IMRT, and SBRT (SBRT not otherwise specified, linac radiosurgery, or gamma knife).[19] EBRT refers to all patients that reported EBRT as first course of treatment typically in the radiation oncologist’s summary

letter in the medical record; IMRT and SBRT were defined as external beam techniques that were clearly defined in the patient record. In cases where multiple radiotherapy modalities were employed, radiation treatment was categorized in compliance with the dominant modality stated in the medical record. Radiotherapy dose was defined as radiotherapy delivered to the tumor during treatment. For estimation of the median radiotherapy dose the cohort was capped at 100.1 Gy, as higher values (e.g. 504.0 Gy) were judged clinically impossible. Radiotherapy fractions were recorded as the number of sessions received to the total volume.

Statistical analysis

Baseline characteristics were reported as frequencies, and continuous data were presented as median with interquartile range [IQR] unless indicated otherwise. Frequencies between two treatment groups were compared using the chi-square test. Three separate cohorts and propensity score models were created for the odds of receiving SBRT as compared with chemotherapy alone, EBRT, or IMRT, respectively. Models were adjusted for: age, sex, race, comorbidity, insurance, type of treatment center, tumor location and clinical stage. We performed 1:1 matching without replacement for the two compared treatments' propensity scores difference with caliper width equal to 0.2 of the standard deviation of the estimated probability of receiving treatment (relative to SBRT), eliminating 99% of the bias[20]. Three separate cohorts and propensity score models were created for the odds of receiving SBRT as compared to chemotherapy alone, EBRT, or IMRT, with respectively the following c-statistics: 0.636 (calipers, -0.005053 ~ 0.005053), 0.678 (calipers, -0.006073 ~ 0.006073), and 0.672 (calipers, -0.014145~ 0.014145).

Survival was calculated from the date of diagnosis to the date of last follow-up or death. Survival analyses were performed using the Kaplan-Meier method and tested using log-rank statistics stratified by treatment received. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC) and a 0.05 level of significance.

RESULTS

Patient characteristics

A total of 14,331 patients met the inclusion criteria. Chemotherapy alone was delivered to 5,464 (38.1%) patients. 6,418 (44.8%), 322 (2.3%) and 2,127 (14.8%) patients received chemotherapy combined with EBRT, SBRT, and IMRT, respectively. In the whole cohort, the majority of patients were: 65 years of age or older at diagnosis (n=7,901; 55.1%), male (n=7,104; 49.6%), white (n=11,398; 79.5%), had no comorbidities (n=10,251; 71.5%), treated in academic centers (n=7,290; n=50.9%), not privately insured (n=8,727; 60.9%), had proximal pancreatic tumors (n=9,665; 67.4%), and clinical stage III disease (n=7,480; 52.2%

Patients that received SBRT were more often white (p=0.0191) and treated in academic centers (p<0.0001) compared to patients that received chemotherapy alone. In addition, SBRT was associated with age over 65 years, compared with EBRT(p=0.0002) and IMRT (p=0.0002), and treatment at an academic facility relative to EBRT(p<0.0001) and IMRT (p<0.0001). However, after matching covariates were equally distributed, as shown in Tables 1-3.

Table 1. Baseline characteristics for unmatched and matched unresected pancreatic adenocarcinoma patients treated with stereotactic body radiation therapy (SBRT) versus chemotherapy alone.

Characteristics	Initial cohort (n=5,786)		P	Matched cohort (n=644)		P
	Chemotherapy (n=5,464)	SBRT (n=322)		Chemotherapy (n=322)	SBRT (n=322)	
Sex, n (%)						
Male	2,610 (47.8%)	167 (51.9%)	0.153	164 (50.9%)	167 (51.9%)	0.813
Female	2,854 (52.2%)	155 (48.1%)		158 (49.1%)	155 (48.1%)	
Age, n (%)						
< 65 years	2,264 (41.4%)	118 (36.7%)	0.090	115 (35.7%)	118 (36.7%)	0.806
≥ 65 years	3,200 (58.6%)	204 (63.4%)		207 (64.3%)	204 (63.3%)	
Race, n (%)						
White	4,262 (78.0%)	269 (83.5%)	0.019	269 (83.5%)	269 (83.5%)	>0.999
Non-white	1,202 (22.0%)	53 (16.5%)		53 (16.5%)	53 (16.5%)	
Comorbidities, n (%)						
No comorbidities	3,837 (70.2%)	239 (74.2%)	0.126	247 (76.7%)	239 (74.2%)	0.464
Any comorbidities	1,627 (29.8%)	83 (25.8%)		75 (23.3%)	83 (25.8%)	
Insurance, n (%)						
Private	1,991 (36.4%)	127 (39.4%)	0.277	124 (38.5%)	127 (39.4%)	0.809
Other	3,473 (63.6%)	195 (60.6%)		198 (61.5%)	195 (60.6%)	
Facility type, n (%)						
Academic	3,010 (55.1%)	237 (73.6%)	<0.001	233 (72.4%)	237 (73.6%)	0.723
Community	2,454 (44.9%)	85 (26.4%)		89 (27.6%)	85 (26.4%)	
Location, n (%)						
Pancreas head	3,552 (65.0%)	224 (69.6%)	0.095	223 (69.3%)	224 (69.6%)	0.932
Pancreas other	1,912 (35.0%)	98 (30.4%)		99 (30.7%)	98 (30.4%)	
Clinical stage, n (%)						
Stage I	652 (11.9%)	28 (8.7%)	0.215	25 (7.8%)	28 (8.7%)	0.285
Stage II	2,108 (38.6%)	129 (40.1%)		112 (34.8%)	129 (40.1%)	
Stage III	2,704 (49.5%)	165 (51.2%)		185 (57.4%)	165 (51.2%)	

Treatment characteristics

In the overall population, 7,989 (55.8%) patients received single-agent chemotherapy, 5,534 (38.6%) patients received multi-agent chemotherapy, and for 808 (5.6%) patients the type and number of chemotherapeutic agents was not recorded. Patients that received chemotherapy plus any additional radiotherapy were more likely to be treated with a single agent (59.8%) compared to those that received chemotherapy alone (49.2%); however, patients were more likely to be treatment with a multi-agent regimen if they received chemotherapy alone (44.8%) versus chemotherapy plus additional radiation (34.8%) (p<0.0001).” However, 5.6% (n=808) of the total population did not have a documented number and/or type of chemotherapeutic regimen.

5,969 (93.0%), 295 (91.6%), and 2,090 (98.3%) patients in the EBRT, SBRT and IMRT cohorts reported radiation dose, respectively. In the overall cohort, the median radiation dose of EBRT was 45.0 Gy (IQR, 45.0-50.4 Gy), for SBRT was 30.0 Gy (IQR, 24.0 - 36.0 Gy), and for IMRT was 50.4 Gy (IQR, 45.0 - 52.5 Gy).

The number of fractions administered was available for 5,755 (89.7%), 297 (92.2%) and 2,033 (95.6%) patients treated with EBRT, SBRT and IMRT, correspondingly. 37.8% reported 0 fractions and were excluded as they had no clinically possible radiation dose documented. Patients in the EBRT cohort received a median number of 28 fractions (IQR, 25 - 29 fractions), whereas patients treated with SBRT and IMRT were treated with a median number of 3 (IQR, 3 - 5) and 28 (IQR, 25 - 30) fractions, respectively.

Table 2. Baseline characteristics for unmatched and matched unresected pancreatic adenocarcinoma patients treated with stereotactic body radiation therapy (SBRT) versus conventional external beam radiation (EBRT).

Characteristics	Initial cohort (n=6,740)		P	Matched cohort (n=644)		P
	EBRT (n=6,418)	SBRT (n=322)		EBRT (n=322)	SBRT (n=322)	
Sex, n (%)						
Male	3,249 (50.6%)	167 (51.9%)	0.664	183 (56.8%)	167 (51.9%)	0.206
Female	3,169 (49.4%)	155 (48.1%)		139 (43.2%)	155 (48.1%)	
Age, n (%)						
< 65 years	3,029 (47.2%)	118 (36.7%)	0.002	113 (35.1%)	118 (36.7%)	0.681
≥ 65 years	3,389 (52.8%)	204 (63.3%)		209 (64.9%)	204 (63.3%)	
Race, n (%)						
White	5,153 (80.3%)	269 (83.5%)	0.151	268 (83.2%)	269 (83.5%)	0.916
Non-white	1,265 (19.7%)	53 (16.5%)		54 (16.8%)	53 (16.5%)	
Comorbidities, n (%)						
No	4,646 (72.4%)	239 (74.2%)	0.472	251 (78.0%)	239 (74.2%)	0.268
Any	1,772 (27.6%)	83 (25.8%)		71 (22.0%)	83 (25.8%)	
Insurance, n (%)						
Private	2,651 (41.3%)	127 (39.4%)	0.507	127 (39.4%)	127 (39.4%)	>0.999
Other	3,767 (58.7%)	195 (60.6%)		195 (60.6%)	195 (60.6%)	
Facility type, n (%)						
Academic	3,009 (46.9%)	237 (73.6%)	<0.001	233 (72.4%)	237 (73.6%)	0.723
Community	3,409 (53.1%)	85 (26.4%)		89 (27.6%)	85 (26.4%)	
Location, n (%)						
Pancreas head	4,421 (68.9%)	224 (69.6%)	0.797	226 (70.2%)	224 (69.6%)	0.864
Pancreas other	1,997 (31.1%)	98 (30.4%)		96 (29.8%)	98 (30.4%)	
Clinical stage, n (%)						
Stage I	640 (10.0%)	28 (8.7%)	0.236	33 (10.3%)	28 (8.7%)	0.592
Stage II	2,279 (35.5%)	129 (40.1%)		136 (42.2%)	129 (40.1%)	
Stage III	3,499 (54.5%)	165 (51.2%)		153 (47.5%)	165 (51.2%)	

Table 3. Baseline characteristics for unmatched and matched unresected pancreatic adenocarcinoma patients treated with stereotactic body radiation therapy (SBRT) versus intensity-modulated radiation therapy (IMRT).

Characteristics	Initial cohort (n=2,449)		P-value	Matched cohort (n=644)		P-value
	IMRT (n=2,127)	SBRT (n=322)		IMRT (n=322)	SBRT (n=322)	
Sex, n (%)						
Male	1,078 (50.7%)	167 (51.9%)	0.693	165 (51.2%)	167 (51.9%)	0.875
Female	1,049 (49.3%)	155 (48.1%)		157 (48.8%)	155 (48.1%)	
Age, n (%)						
< 65 years	1,019 (47.9%)	118 (36.7%)	0.002	127 (39.4%)	118 (36.7%)	0.465
≥ 65 years	1,108 (52.1%)	204 (63.3)		195 (60.6%)	204 (63.3%)	
Race, n (%)						
White	1,714 (80.6%)	269 (83.5%)	0.208	279 (86.7%)	269 (83.5%)	0.269
Non-white	413 (19.4%)	53 (16.5%)		43 (13.3%)	53 (16.5%)	
Comorbidities, n (%)						
No comorbidities	1,529 (71.9%)	239 (74.2%)	0.383	240 (74.5%)	239 (74.2%)	0.928
Any comorbidities	598 (28.1%)	83 (25.8%)		82 (25.5%)	83 (25.8%)	
Insurance, n (%)						
Private	835 (39.3%)	127 (39.4%)	0.950	128 (39.8%)	127 (39.4%)	0.936
Other	1,292 (60.7%)	195 (60.6%)		194 (60.2%)	195 (60.6%)	
Facility type, n (%)						
Academic	1,034 (48.6%)	237 (73.6%)	<0.001	238 (73.9%)	237 (73.6%)	0.929
Community	1,093 (51.4%)	85 (26.4%)		84 (26.1%)	85 (26.4%)	
Tumor location, n (%)						
Pancreas head	1,468 (69.0%)	224 (69.6%)	0.843	222 (68.9%)	224 (69.6%)	0.864
Pancreas other	659 (31.0%)	98 (30.4%)		100 (31.1%)	98 (30.4%)	
Clinical stage, n (%)						
Stage I	228 (10.7%)	28 (8.7%)	0.396	26 (8.1%)	28 (8.7%)	0.686
Stage II	787 (37.0%)	129 (40.1%)		120 (37.3%)	129 (40.1%)	
Stage III	1,112 (52.3%)	165 (51.2%)		176 (54.6%)	165 (51.2%)	

Survival analyses

In the unmatched cohort, the median survival was 10.8 for the overall cohort, and 9.9, 10.9 13.9, and 12.0 months for patients treated with chemotherapy alone, EBRT, SBRT, and IMRT, respectively. There was a significant difference in survival between treatment groups in the unmatched cohort (log-rank $p<0.0001$; Fig. 1).

After matching, patients who received SBRT had significantly improved median survival compared with patients who received chemotherapy alone (13.9 vs. 10.2 months; log-rank $p<0.0001$; Fig 2b.). Furthermore, the median survival improved significantly after SBRT compared to EBRT (13.9 vs. 11.6 months; log-rank $p=0.0180$; Fig2d.). However, SBRT did not significantly advance survival over IMRT after matching (13.9 vs. 12.2 months; log-rank $p=0.0492$; Fig. 2f).

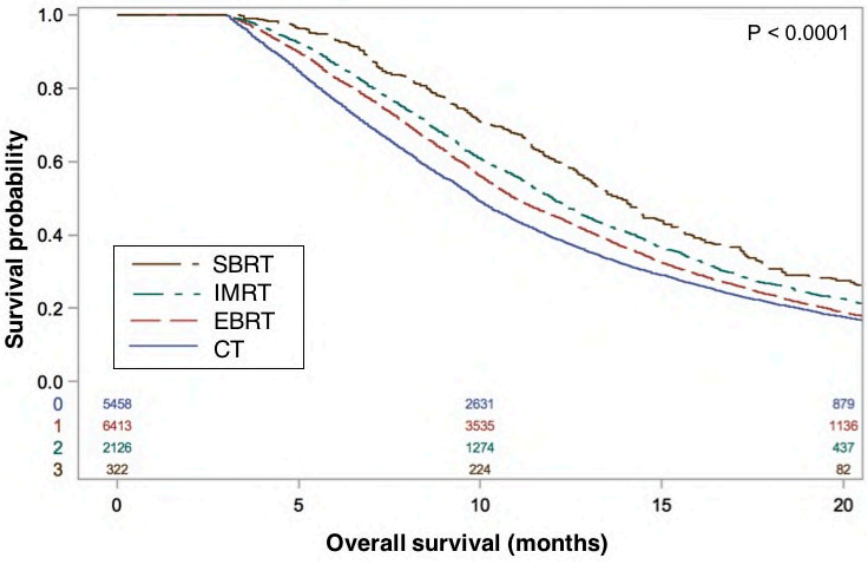

Figure 1. Kaplan-Meier curves of overall survival for patients with unresected pancreatic adenocarcinoma patients treated with chemotherapy alone, external beam radiation therapy (EBRT), stereotactic body radiation therapy (SBRT) or intensity-modulated radiation therapy (IMRT).

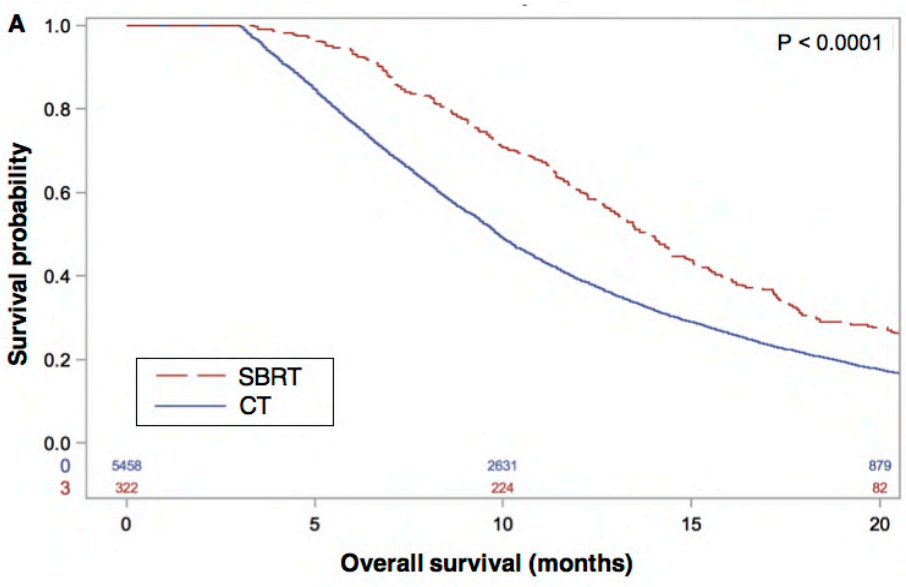

Figure 2a. Kaplan-Meier survival curves comparing stereotactic body radiation therapy (SBRT) to chemotherapy alone.

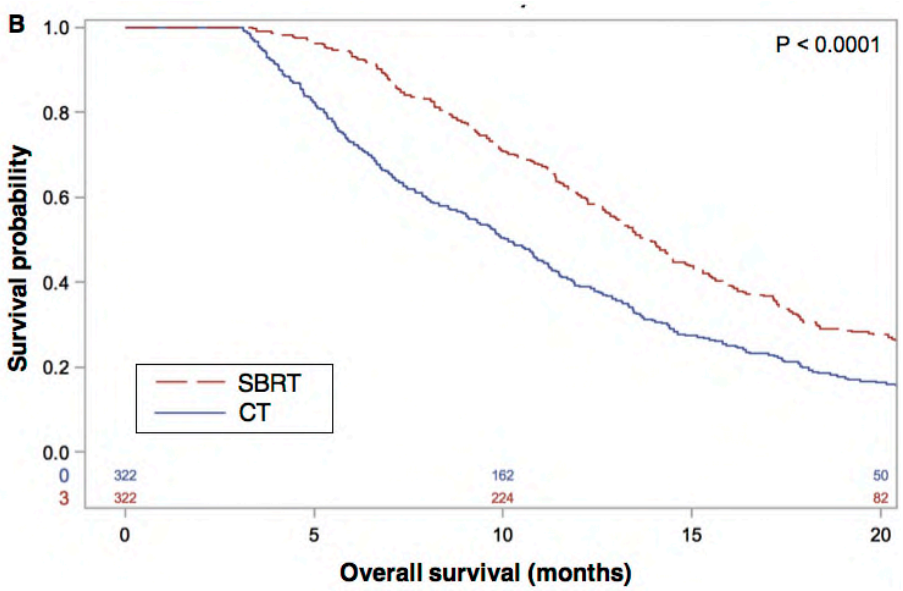

Figure 2b. Kaplan-Meier survival curves comparing stereotactic body radiation therapy (SBRT) to chemotherapy alone after matching.

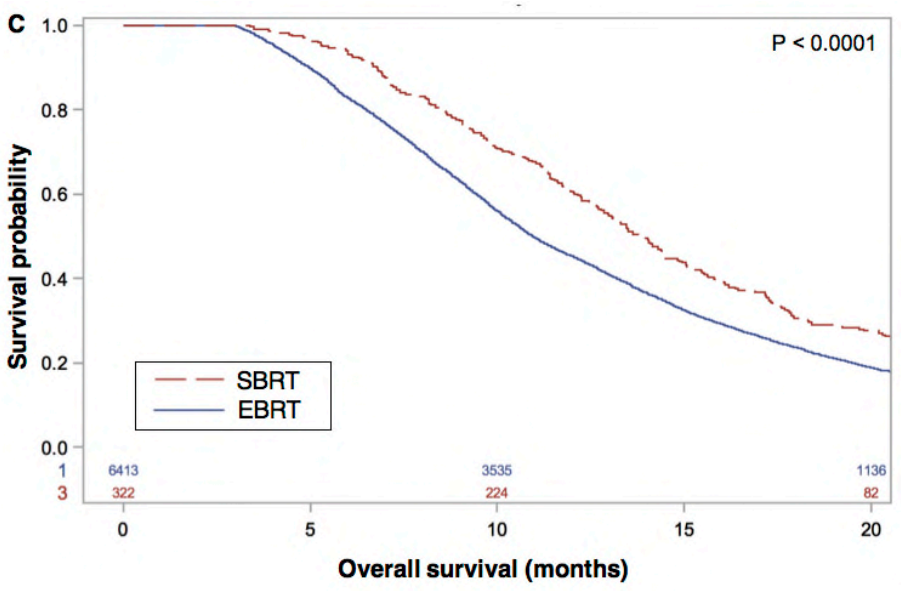

Figure 2c. Kaplan-Meier survival curves comparing stereotactic body radiation therapy (SBRT) to external beam radiation therapy (EBRT).

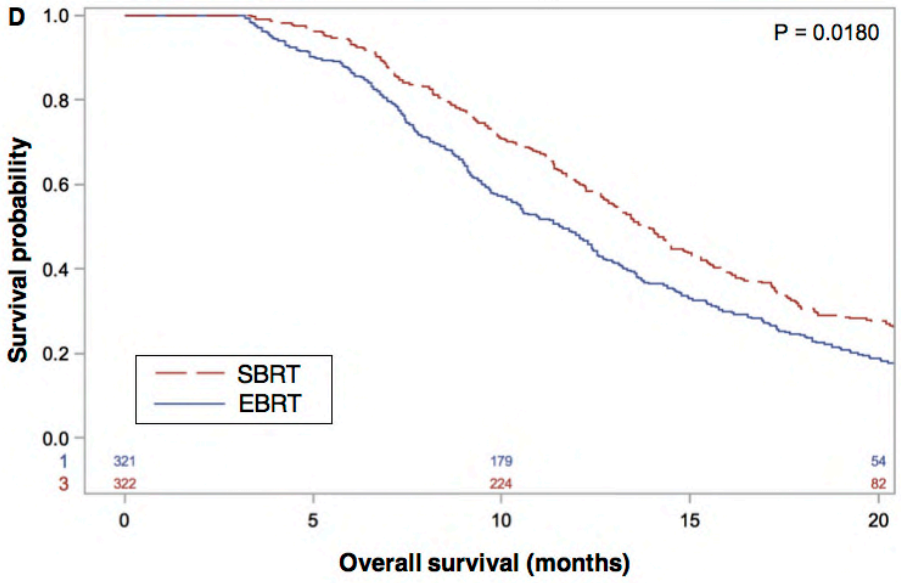

Figure 2d. Kaplan-Meier survival curves comparing stereotactic body radiation therapy (SBRT) to external beam radiation therapy (EBRT) after matching.

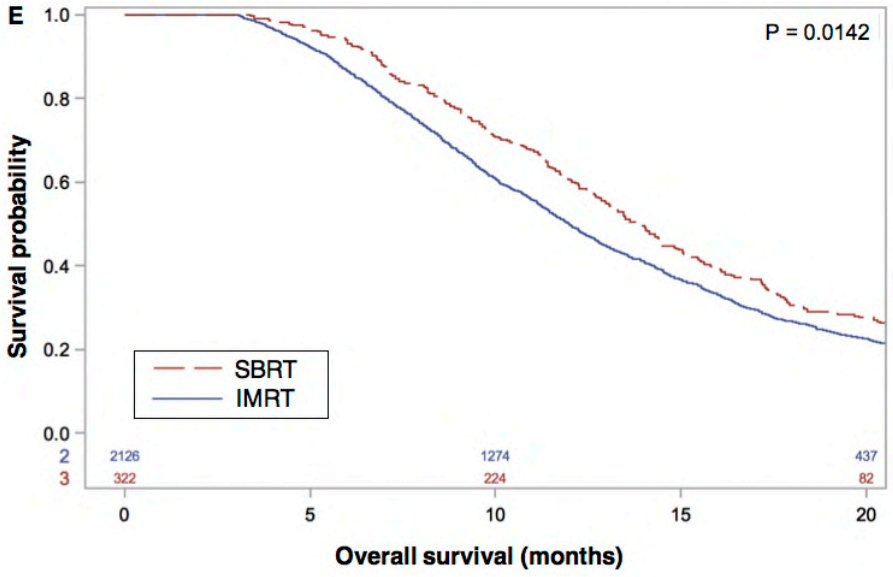

Figure 2e. Kaplan-Meier survival curves comparing stereotactic body radiation therapy (SBRT) to intensity-modulated radiation therapy (IMRT).

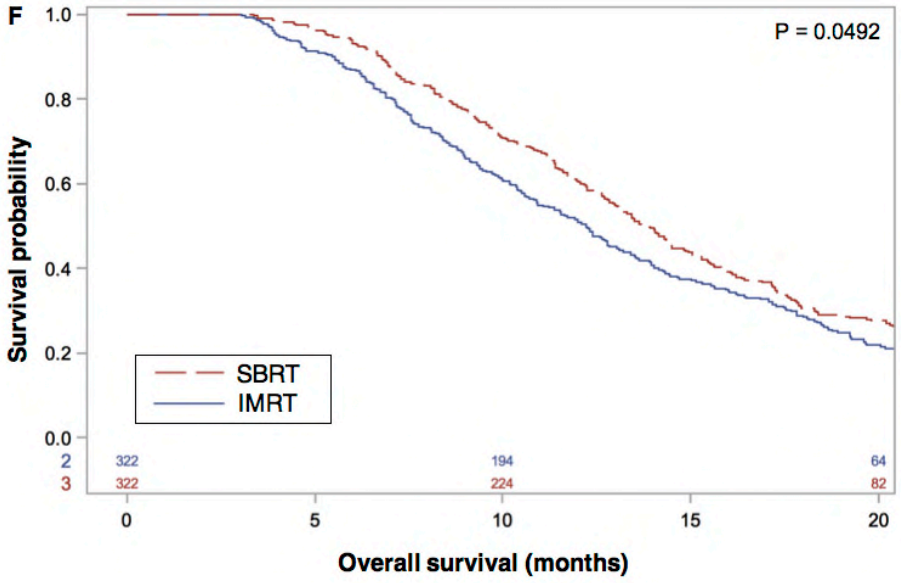

Figure 2f. Kaplan-Meier survival curves comparing stereotactic body radiation therapy (SBRT) to intensity-modulated radiation therapy (IMRT) after matching.

DISCUSSION

This population-level analysis examined survival outcomes for unresected pancreatic cancer patients after SBRT compared to chemotherapy alone, EBRT, and IMRT. This study reveals a promising median survival of 13.9 months after SBRT. In addition, our analyses suggest a meaningful survival gain for SBRT over chemotherapy alone (median survival, 13.9 vs. 10.2 months; log-rank p<0.0001) or chemotherapy plus EBRT (median survival, 13.9 vs. 11.6 months; log-rank p=0.0180) after matching. After adjustment, patients that received SBRT and IMRT demonstrated similar survival outcomes (median survival, 13.9 vs. 12.2 months; log-rank p=0.0492).

The ideal treatment approach for unresectable pancreatic cancer remains a matter of debate. Chemotherapy is essential in the treatment of locally advanced patients[21]. However, in the last two decades the prognosis for unresected patients, as reported in six randomized trials, has only modestly improved. In these trials, the median survival for patients who received chemotherapy alone or combined with EBRT ranged from 7 to 13 months [6, 7, 22]. Additionally, multiple meta-analyses comparing chemotherapy to chemoradiotherapy have failed to demonstrate a significant survival difference between the two approaches[22, 23]. Nevertheless, despite the fact that randomized trials demonstrate ambiguous results, chemotherapy and concurrent EBRT are frequently employed in clinical

practice. Unfortunately, approximately 50% of patients experience local progression after conventional fractionated EBRT[24]. Autopsy studies highlight the importance of improved local control, as 30% of locally advanced patients die from local disease burden[25].

In the last decade, the disappointing results of conventional radiotherapy has led to the investigation of novel radiation modalities for pancreatic cancer. SBRT is a type of EBRT that enables precise delivery of high dose radiation over a short time period. In addition, this novel therapeutic option may offer a high probability of local control[9, 10, 26, 27]. In 2004, Koong et al. pioneered SBRT for pancreatic cancer, demonstrating a median survival of 8 months and a 100% local control rate[26]. Since then, various phase 2 trials have confirmed SBRT as a safe therapeutic option for patients with unresectable pancreatic cancer[10, 28, 29]. Furthermore, local control rates were improved in multiple subsequent studies, with reported local control rates of 40-100%[9, 10, 26, 27]. The published data regarding SBRT in locally advanced disease also describes median survival ranging from 5 to 20 months[10, 30-33]. These studies administrated SBRT in various treatment regimens, with a high dose of 15-50 Gy in 1-5 fractions[8, 10, 27, 33-36].

In addition to SBRT, other new radiation modalities have been applied to locally advanced pancreatic cancer, including IMRT. This approach is expected to provide more conformal dose delivery in comparison to conventional 3D-conformal radiotherapy, consequently permitting higher dose radiation and reducing treatment-related toxicity[37]. Median survival times in unresected pancreatic cancer patients receiving IMRT range from 12 to 14 months[38-41]. Lin et al. compared SBRT to IMRT for locally advanced patients and showed an advantage for SBRT in achieving local control.[32] Nevertheless, no difference in overall survival or radiation toxicity was observed between the two radiotherapy techniques[32].

Our unadjusted results confirm previous trials demonstrating a slightly better survival after conventional EBRT (median survival, 11.1 months) compared to chemotherapy alone (median survival, 10.1 months)[6, 42, 43]. Recently, multi-agent chemotherapeutic regimens, such gemcitabine/nab-paclitaxel and fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRINOX) have demonstrated improved survival compared to gemcitabine alone[44, 45]. In this study, while the majority of patients received single-agent chemotherapy (55.8%), amongst those patients who received chemotherapy alone, the administration of multi-agent chemotherapy was much more common (44.8%) than amongst patients who received additional radiation (34.8%) ($p<0.0001$). The higher rates of multi-agent utilization amongst patients who received chemotherapy alone may improve the survival outcomes such that the difference between groups who were treated with addition radiotherapy is underestimated. With the increased utilization of newer multi-agent regimens, a survival gain for all treatment groups may be observed in the future. In addition, we were not able to distinguish between patients with resectable, borderline resectable, and locally advanced disease at presentation. However, no significant differences in the clinical stage were noted between treatment groups before and after

matching. This cohort may also include patients that developed metastatic disease on neoadjuvant therapy, as intention-to-treat was unknown. This study also corroborates previous findings regarding IMRT, as we found no difference in overall survival between the two approaches after matching. However, previous studies have reported more favorable local control rates after SBRT compared to IMRT[32].

The current study improves on previous iterations by comparing SBRT to chemotherapy alone, conventionally fractionated treatment, and IMRT in unresected patients. This study uses population-wide data and employs matching to adjust for potential confounders. The median survival times for chemotherapy alone, EBRT, IMRT, and SBRT are comparable with historic series[8, 10, 30-33, 35, 40]. Moreover, the findings regarding the dose and number of fractions for EBRT, IMRT and SBRT correspond with the appropriate guidelines. To control for immortality bias the cohort was restricted to patients that lived 3 months beyond diagnosis[13]. Furthermore, to ensure curative intent, all patients with metastatic disease were excluded. These results highlight the generalizability of this work.

This study is based on data from a nationwide registry, which may be limited by coding errors. Several important prognostic variables were not reported by the NCDB or missing for a majority of patients, including Karnofsky performance status and CA 19-9 levels[46, 47]. Comparative effectiveness studies are often hampered by selection bias, due to systematic differences in the characteristics across study arms. To control for the impact of these potentially confounding factors, this study utilized matching to balance patient and tumor characteristics. However, unknown confounders may have influenced treatment allocation for which the propensity score matching process could not completely account. In addition, the NCDB provides no data on key oncologic outcomes, including progression-free survival, treatment-related toxicity, and quality of life. Furthermore, this study did not take into account specific SBRT regimens or the impact of volumetric-modulated arc therapy.

Widespread implementation of SBRT remains yet to be achieved. Consequently, these findings are limited to early adopters. Furthermore, Commission on Cancer approved hospitals are larger, more frequently accredited by major oversight agencies, and exhibit a higher degree of oncology-related specialization[48]. Hence, this study may also not be entirely representative of all hospitals in the United States. In addition, the median biologically equivalent radiation dose in the SBRT group was lower compared to the EBRT and IMRT groups. Consequently, this study may underestimate the survival benefit of SBRT. However, dose-escalation of SBRT for pancreatic cancer is limited by nearby critical structures, especially the duodenum, and surpassing the commonly used dose of 50.4 Gy considerably may therefore be undesirable[49, 50].

Despite its limitations, the current study employed a nationwide cohort to investigate the survival impact of SBRT compared to both traditional and novel therapeutic approaches for unresected patients using propensity-score matched models. The NCDB allows for analysis of quality-assured data of a large cohort of patients from different

institutions. In addition, the use of the NCDB improves on other nationwide datasets by including radiation dose, fractionations, and treatment modality. Consequently, the results of this study likely provide an appropriate reflection of the comparative effectiveness of this novel treatment approach.

In summary, this population-level propensity score matched appraisal demonstrates that SBRT is associated with substantial survival benefit compared to chemotherapy alone and EBRT. In the retrospective analysis, the use of SBRT resulted in a 3-month survival gain over traditional approaches. In addition, our results show a trend towards a survival advantage (median survival, 13.9 vs. 12.8 months) for SBRT over IMRT after matching. Previous studies have demonstrated that SBRT is an attractive therapeutic option due to its short duration, safety, and high rate of local control[8, 10, 27, 33, 34, 51-53]. Our data, in conjunction with the published literature, support the expanded implementation of SBRT for unresected pancreatic cancer patients and highlight the need for future trials.

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Chapter 13

Big Data vs. Clinical Trials in HPB Surgery

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J Gastrointest Surg. 2020 May;24(5):1127-1137

ABSTRACT

Randomized controlled clinical trials (RCTs) are at the heart of ‘evidence-based’ medicine. However, in surgical practice RCTs remain uncommon. Conducting well-designed RCTs for surgical procedures is often challenged by inadequate recruitment accrual, blinding, or standardization of the surgical procedure, as well as lack of funding and evolution of the treatment strategy during the many years over which such trials are conducted. In addition, most clinical trials are performed in academic high-volume centers in highly selected patients, which may not necessary reflect a ‘real-world’ practice setting. Over the past decades, surgical outcomes research using nationwide administrative and registry databases has become increasingly common. Large databases provide easy and inexpensive access to data on a large and diverse patient population at a variety of treatment centers. Furthermore, large database studies provide the opportunity to answer questions that would be impossible or very arduous to answer using RCTs, including questions regarding health policy efficacy, trends in surgical practice, access to health care, impact of hospital volume, and adherence to practice guidelines, as well as research questions regarding rare disease, infrequent surgical outcomes, and specific subpopulation. Prospective data registries may also allow for quality benchmarking and auditing. This review outlines the role, advantages and limitations of RCTs and large database studies in answering important research questions in surgery.

INTRODUCTION

The term evidence-based medicine (EBM) was first coined in 1992 by Gordan Guyatt in an article in JAMA, and quickly became the sine qua non of medical practice.[1, 2] However, the struggle to balance the uncontrolled experience of physicians with observations obtained by rigorous empirical evaluation of the effect of health interventions goes back to the time of Hippocrates.[3] The core epistemology of EBM is that scientific evidence should guide clinical decision-making, and the extent to which we believe and implement that evidence is determined by a credible process.[3] EBM views randomized controlled trials (RCTs) and meta-analyses of those trials as the 'gold standard' for evidence-based practice.[3]

Surgical interventions remain less likely to be investigated using full-scale RCTs than medical therapies, and the number of surgical RCTs has decreased over the past decades, especially in the United States.[4, 5] Furthermore, the quality of many surgical RCTs that are published are lacking.[6, 7] Conducting RCTs in surgery differs substantially from medicine, as surgical procedures are more difficult to standardize than pharmacologic or radiotherapeutics interventions. Every surgery is unique, since it is highly dependent of the skill and learning curve of the surgeon, as well as the patient's anatomic variation and habitus.[8-10] In addition, surgical RCTs are often challenged by inadequate patient accrual, blinding, and funding.[8]

Although RCTs have long been presumed to be the ideal source for evidence regarding treatment effects, there is growing interest in other methods of obtaining evidence for decisive action, giving rise to new research methods, such as the use of large database studies, to leverage the strengths and overcome the limitations of RCTs.[11] The purpose of this review it to reflect on the role, advantages, and limitations of RCT and large database studies in surgical science.

ADVANTAGES OF SURGICAL TRIALS

The main reason why RCTs are considered more rigorous than other methods is that randomization of study subjects not only controls for measurable confounders between two treatment groups, but also balances unmeasured confounders.[12, 13] In addition, although often challenging in surgical research, RCTs allow for blinding of participants, physicians, outcomes assessors, and data analysts to reduce detection and performance bias.[14] Therefore, well-designed and conducted RCTs are able to identify causal relationships and establish definitively which treatment methods is superior.[11] Previously, many procedures were introduced into surgical practice solely based on observational data until a randomized trial disproved their efficacy. Surgical procedures such as the extra cranial intracranial bypass operation for stroke prevention were common practice for at least 15 years before and an RCT demonstrated that it actually increased the likelihood of developing a

stroke.[15, 16] In similar fashion, the unmerited use of internal mammary ligation for ischemic heart disease was discredited.[15, 17]

LIMITATION OF SURGICAL TRIALS

Poor accrual

There are several barriers to conducting surgical RCTs. It is often difficult to accrue a sufficient number of patients for RCTs in a timely and cost-effective manner. Unless this procedure or disease process is a relatively common one, it can take months to years to collect sufficient patients in a single-institutions to power a study adequately. While multi-institutional studies can recruit patients more quickly, such studies are resource intensive and demand complicated coordination to assure consistent protocol application.[18] In addition, the lack of clinical equipoise can be another obstacle to randomization and trial inclusion. Surgeons are often convinced that what they do is the best for their patients, when other ways to achieve comparable or even superior results might actually exist. In particular, surgeons pioneering a new technique are often 'true-believers', and less likely to participate in a RCT. Furthermore, surgeons are frequently not reimbursed, or only partially reimbursed, for performing additional therapeutic or diagnostic interventions, which makes participation in a clinical trial less attractive.[8] Another problem applies to the competitive culture in which surgeons' work. Many surgeons may not enroll patients in trials where the patients are assigned to a non-operative study arm, because of competition among surgeons to recruit patients and out of fear to losing a source of referrals.[19]

As a result of poor accrual, a third of RCTs remains unpublished, and 20% of trials are discontinued at 5 years.[8, 7, 20] In addition, a third of published surgical trials are underpowered to demonstrate clinically significant differences. Unfortunately, negative findings in underpowered trials are often interpreted as showing the equivalence of the treatment arms with no discussion of the issue of being underpowered, resulting in a type II error.[7].[21] This may lead clinicians to accept new treatments that have not been validated.[21]

Generalizability

RCTs often have strong internal validity, but sometimes lack external validity; generalizations of findings outside the study population may be invalid.[11] Although RCTs exist on a continuum, with a progression from efficacy to effectiveness studies. The primary goal of efficacy trials is to determine whether an intervention produced the expected result under ideal circumstances. Whereas effectiveness trials (also known as pragmatic trials) are designed to inform general guidelines, clinical or policy decisions by measuring the degree of real world effectiveness.[22] Nonetheless, in reality the majority of RCTs are optimized to determine efficacy and may not necessary adequately inform practice.[23] The treatment setting and the patients included in most RCTs do not reflect 'real' world population. Previous studies have shown that only approximately 2% to 3% of all

patients with cancer ever enroll in a trial.[24, 25] Traveling to and receiving care at tertiary medical centers where a trial involving complex operations are often conducted requires considerable financial resources and often takes patients away from their family and support system. Consequently, racial and ethnic minorities, elderly and women are less likely to enroll in RCTs. In addition, patients enrolled in trials are often healthier, more compliant and of a higher socioeconomic status.[24, 26, 19] Furthermore, these types of studies are often performed in highly controlled conditions, with strict in- and exclusion criteria.[18] Limiting the generalizability of the findings in RCTs to 'real' world practice.

Resource intensive

RCTs are resource intensive with regards to costs and time.[11] The lion's share of funding to carry out RCTs comes from two main sources: industry and the federal government. While financial support is often readily available for pharmaceutical trials, there are fewer industry sponsors of surgical research.[19] The majority of industry research payments towards surgeons are related to novel pharmaceuticals, with the most funding being procured from Novartis, Amgen, and Merck.[27] Previous studies have also shown that surgical grant proposals are less likely to be funded by the National Institute of Health (NIH) and carry significantly smaller awards compared to nonsurgical proposals.[28] The latter has been partly explained by the low percentage of surgeon-scientists participation in the reviewing of NIH grant proposals. High costs may sometimes result in RCT designs with in adequate sample size.

In addition, RCTs often take years to plan, implement, and analyze reducing the ability of RCTs to keep pace with clinical innovations; new products and standards of care are often developed before earlier studies complete evaluation.[11] This makes trial outcomes at risk of becoming obsolete before they get published. Consequently, some interventions have been widely adopted without rigorous evaluation.[29] The increasingly high costs and time constraints of RCTs can also lead to reliance on surrogate markers that may not correlate well with the outcome of interest and create additional bias.[11]

Bias

Many surgeons believe that every RCT is bias-free. This belief is not, in fact, reflected in reality, as poorly designed and conducted RCTs provide distorted, confounded results that are not beneficial for improving current surgical practice.[19] A common problem with conducting surgical RCT is adequate blinding, due to the frequent lack of placebo controls (surgical placebo, sham surgery). A surgical placebo represents a simulated operation in which the skin incisions are done without actually performing the operation. In most cases, both the patient and surgeon are able to determine which procedure was done, potentially leading to post-randomization bias.[18] Another frequent criticism of surgical RCTs, particularly when evaluation a new innovation against a standard intervention, is that the comparison may be inherently 'unfair' due to an imbalance in expertise.[29]

Table 1. Overview characteristics of the Medicare Claims Data, Healthcare Cost and Utilization Project National Inpatient Sample (NIS), National Surgical Quality Improvement Program (NSQIP), National Cancer Database (NCDB), and the Surveillance, Epidemiology, and End Results (SEER) database.

Database	Population	Advantages	Limitations
Medicare Claims Data[36]	- 70% of adults aged 65 years and older - People who qualify for Social Security Administration disability benefits.	- The data sets available from the Centers for Medicare and Medicaid Services (CMS) are suitable for linkage to several existing data sets (eg, other CMS data, SEER, Medicaid). - Data can be tracked longitudinally across episodes of care, making this a uniquely positioned dataset to study long-term outcomes in surgical patients.	- Only includes diagnosis documented via international Classification of Disease, Ninth version (ICD-9) or ICD-10 codes. - No physiological or biochemical patient information, such as vital signs, laboratory test results, and pathology results. - Lack of data on uncovered outpatient services and managed care information.
NCDB[38]	- 70% of all newly diagnosed cancer cases in the United States. - Not population based, only patients treated at commission on cancer approved centers.	-Strengths of the NCDB are in examining treatment patterns and trends over time across the United States.	- Only reports treatment that was used in the 6 months after diagnosis. - Surgical procedures reported will only include the most definitive intervention. - The readmission variable only captures readmission to the same hospital within 30 days of discharge (reporting bias).
NSQIP[44]	- Random sampling of one out of eight cases performed at the ± 700 hospitals participating in NSQIP. - Does not represent a valid nationally representative sample. - Excludes trauma and transplant cases.	- Provides data on a broad range of 30-day outcomes, including mortality, readmission, and length of stay, as well as timing of postoperative discharge complications. - Provides the ability to account for preoperative comorbidity, as well as complications that occur in the perioperative period. - Targeted NSQIP for hepatectomy and pancreatectomy.	- Does not contain hospital or clinical identifiers. - No data on type of insurance, type of treatment facility, and surgeon or hospital volume. - Follow-up limited to 30 days.
NIS[39]	- 20% representative sampling of all inpatient hospital encounters in the US - Designed to be representative for health care use overall	- Ideal for researching national prevalence/incidence, changes over time, and associations between diagnosis, procedures, and outcomes.	- Lack of longitudinal data. - Only diagnosis identified by ICD-9 ICD-10 codes. - Systematic undercoding of certain low-cost diagnostic procedures can lead to inaccurate estimations of procedure us - Redesign of NIS in 2012
SEER[37]	- Cancer cases only - Population-based - Captures 28% of the US population	- Includes a high proportion of racial/ethnic minorities, foreign-born individuals, and those with income below the poverty line. - Longitudinal trends in cancer incidence, prevalence, treatment, and survival can be analyzed starting from 1974 to the present. - Longitudinal studies on specific subpopulations and rare or indolent cancer types.	- Comparative effectiveness analyses are limited by lack of information on comorbidities, surgical approach (minimally invasive vs. open), systemic treatment (chemotherapy, hormonal therapy, or immunotherapy), radiation dose, and recurrence. - HER2 status is coded inconsistently and should not be used in analysis.[84]

ADVANTAGES OF BIG DATA

The first large database study in the Unites States is the Framingham Heart Study, which was initiated in 1948 to study risk factors for cardiovascular disease.[30] Data from this large longitudinal database have resulted in 3,819 publications to data.[31] In recent decades, the use of big data for research studies has increased significantly (Figure 1), and currently

nine of ten research papers published in clinical specialty journal describe observational research.[32, 33] This development has been fueled in large part by the Health Information Technology for Economic and Health Act of 2009 which helped fund the adoption of electronic health records which in turn facilitated the creation of large clinical databases.[34] The first surgery specific database was the Veterans Affairs National Quality Improvement Program (VA NSQIP), which was created in response to concerns regarding high mortality rates in the VA system and ultimately gave rise to the American College of Surgeons (ACS) NSQIP in 2004.[35] Other frequently used databases, include the National Cancer Database (NCDB), the Surveillance, Epidemiology, and End Results (SEER) database, Healthcare Costs and Utilization Project (HCUP) National Inpatient Samples, and Medicare Claims Data.[36-39]

Outcomes research is in general defined as any study of the end results of health services, including mortality, physiological functional measures, definable clinical events, and patient satisfaction.[40] For the purpose of this study we use a more narrow definition of outcomes research, only referring to outcomes research that is observational in nature and performed using multi-institutional pro- or retrospectively collected datasets.

The use of large database studies for surgical research has several advantages. First, large databases are easy and cheap (sometimes free) to obtain, and most of the time the data can be analyzed using ubiquitous statistical programs.[12] Second, in contrast to RCTs, large database studies often provide sufficient power to detect a significant difference. This may be of particular importance, considering the relative rarity of certain diseases treated by Hepato-Pancreato-Biliary (HPB) surgeons, such as hilar cholangiocarcinoma and pancreatic neuroendocrine tumors. In addition, the large sample sizes of nationwide databases als0 provides the opportunity to answer a wide variety of research questions with sufficient statistical power, as well as study rare diseases, infrequent postoperative outcomes, and subsets of patients that benefit the most from a specific procedure.[24, 26, 19] Third, RCTs often adhere to strict inclusion and exclusion criteria, and are often performed at high-volume academic centers in highly selected patients, which limits the generalizability of its findings. Large database studies include a wide variety of patients and treatment centers, and allow for the investigation of 'real' world practice patterns, treatment efficacy and outcomes.[12]

Finally, outcomes research enables researchers to answer relevant questions that cannot be answered through a randomized clinical trial, because the latter would require prohibitively complex, costly, or even ethically unacceptable practices.[19, 41] Large database studies have shown to be ideal to investigate the impact of hospital volume, access to health care, geographic variations in care, risk stratification protocols, trends in practice patterns, adherence to practice guideline, effectiveness of novel treatment strategy, and also to evaluate health care policy effectiveness.[34, 42] Furthermore, large data registries are a useful tool for quality benchmarking. A major strength of the ACS NSQIP it not only that it provides granular risk-adjusted, and case-mix-adjusted surgical outcomes data, but also that

it allows participating hospital to benchmark their performance to an estimate average of all hospitals providing data to NSQIP, which has resulted in significantly reduced morbidity and mortality in these centers.[43, 44] This is similar to large nationwide audit programs common in Europe.[45-47] In addition, some of the most important published surgical research is based on retrospective studies reported in high impact-factor journal, often because randomizing patients between two very diverse treatment arms is impossible, although this limitation is not always explicitly stated as the reason for not undertaking a prospective RCT.[48-51] Much can be learned from the use of prospective registries for the introduction of innovative procedures in other aspects of surgery, such as the introduction of associating liver partition and portal vein ligation (the ALPSS procedure), the modified 2-stage hepatectomy procedure for liver tumors – for which the registry demonstrated increased perioperative morbidity in older patients compared to conventional liver resection procedures.[52]

LIMITATION OF BIG DATA

Statistical versus clinical significant

The large sample size available in administrative data sets have the potential to reveal statistical significance even when very small absolute differences exist. Although the conventional threshold for statistical significance of $P < .05$ is widely used, one should keep in mind that this threshold is arbitrary.[53] Previous studies have shown that when the total sample size of two groups combined exceeds 250,000, the p-value will meet traditional significance levels (ie, a of 0.05) without substantial differences in outcomes.[54] Disproportionate focus on a P value of less than .05 can exaggerate the importance of statistically significant, but clinically meaningless results. Similarly, this approach can cast aside potentially meaningful information obtained simply because the P value exceeds an arbitrary threshold. This practice carries a higher risk of type I error, concluding that a treatment is effective or a difference exists between two groups when in reality the treatment is not effective or no difference exists.[12]

In particular, any large database analysis that does not begin with an a-priori hypothesis is susceptible to ‘‘data mining’’ or ‘‘data dredging’’—a non-hypothesis-driven quest for a statistically significant result.[53] In addition, the difference in the effect estimate should be reported as a patient-centered, clinically meaningful, and interpretable difference in addition to the statistical result.[53, 55] The use of confidence intervals (CIs) should help distinguish between clinical significance and statistical significance. CIs are in general more informative when comparing two treatment groups because they are generated around the absolute or relative difference between those populations.[54] When reporting the results of observational studies, authors should also consider following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

Table 2. Checklist for good conduct large database research.

□	Identify a hypothesis driven research question
□	Choose the appropriate data set to address the question of interest (see Table 1).[38]
□	A flow diagram should be included that shows the number of patients included and excluded, along with reasons for exclusion, documenting a stepwise derivation of the final sample.[85-87]
□	All predictor and outcomes variables should be defined a priori.[87]
□	A justification should be provided regarding categorizing continuous variables.[87]
□	Check or variables of interest have changed over time.[38]
□	Shifts in cancer stage classifications over time should be accounted for[37]
□	Define study population according to Current Procedural Terminology (CPT) codes and then validate this population using the International Classification of Disease Ninth Revision (ICD-9) and ICD-10 diagnostic codes.[44]
□	Account for differences between the International Classification of Disease, Ninth Edition (ICD-9) and ICD-10 systems.[39]
□	When the study spans many years, determine whether the period qualitatively changes the study results, if present, consider a stratified analysis.[38]
□	Variables with less than 50% of data available for analysis should be discarded.[38]
□	In case, over 30% of patients has missing variables, multiple imputation should be used to control for missing variables. In addition, the cause of 'missingness' should be investigated and described.[61, 85]
□	In case of health policy evaluation, difference-in-difference assessments should be performed to controlled for any unrelated changed over time.[36]
□	For multivariable analyses, variable selection should be based on prior evidence and biological/clinical plausibility, not necessary any variable that is statistically significant.[86, 87]
□	In case multivariable models include variables based on statistical significant criteria, model performance statistics and whether multicollinearity and effect modification were assessed should be specified.[87]
□	For logistic multivariable regression analysis, coefficients should be interpreted using odds ratios, while linear and Poisson models should incorporate effect size.[86]
□	In case of rare events of interest (less than 10-15 events per variable in the model), the propensity score method should be used instead of a multivariable analysis.[53]
□	In case the covariates of two groups under investigation are not sufficiently overlapping, the propensity score method should be used instead of a multivariable analysis.[53]
□	Check for immortal time bias, especially in studies investigating the efficacy of (neo)adjuvant therapy or transplantation, perform a landmark analysis or extended Cox model.[80]
□	Perform extensive sensitivity analyses to evaluate and address confounding and selection bias[38]
□	Emphasize practical clinical findings instead of incidental statistically significant results.[39, 86]
	A power calculation should be included when dealing with small subgroups or rare disease; the findings for a subgroup or rare disease may be susceptible to bias if the sample size is small.[86]
	In case, the database included facility identifiers, hierarchical analyses should be used including the identifier as the random effect in the model to account for the correlated patient outcomes, as patients are nested within facilities.[87, 38, 88]
□	Avoid use of language implying causal inference in reporting results from observational studies; Instead, these studies are best suited for hypothesis generation.[39]
□	Ensure that your article has a clear take-home message that addresses how your research advances current knowledge and has important policy or clinical implications.[85]

Coding errors

Although databases have the ability to investigate a wide range of surgical hypotheses, the information included in different national databases is highly variable and, as such, the questions that can be answered and the conclusions that can be reached are restricted by the extent of the available data (Table 1).[42] There are two types of databases: administrative and registry databases (Figure 2). Administrative databases are generally assembled from billing information and were not created for clinical research. These databases obtain their information typically from two sources: requests to insurers for healthcare payments and claims for clinical services. In general, reimbursed procedures are more often coded accurately, while the coding of comorbidities and complications (other

than death) may be less dependable. Over-coding has been reported as a potential source of distortion of administrative data. For instance, if hospitals are reimbursed based on the complexity of the patient's disease, there may be a propensity to over-code primary and secondary diagnoses, a phenomenon called diagnosis-related group (DRG) creep.[56] Miscoding constitutes an innate limitation that must be carefully considered when interpreting the results of studies based on administrative data.[19]

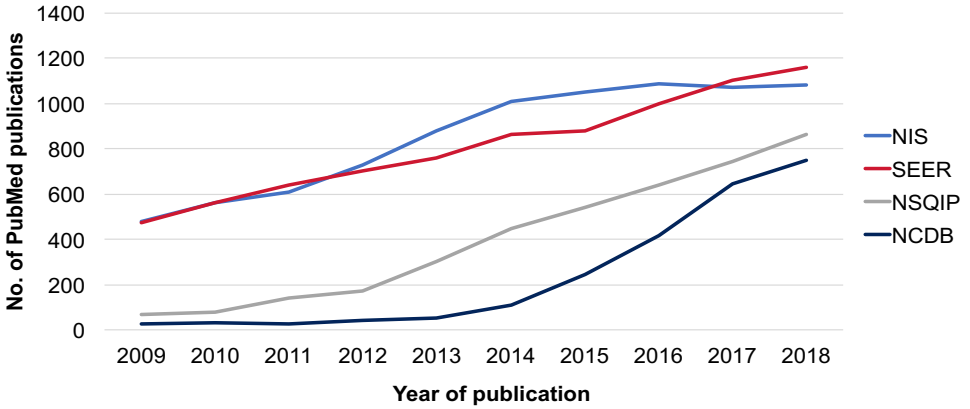

Figure 1. Trends in the number of PubMed publication of manuscripts using data obtaines the Nationwide Inpatient Sample (NIS), Surveillance, Epidemiology, and End Results (SEER), National Surgical Quality Improvement Program (NSQIP), and National Cancer Database (NCDB).

Clinical or registry databases, on the other hand, are composed of a given patient population with a priori defined patient information. These databases were created to record and track information, allowing for the investigation of specific clinical questions.[57] Compared with administrative data, registry data are widely considered to have a greater degree of accuracy, mainly due to (I) a greater level of clinical training within the staff abstracting data and (II) a rigorous set of clinical criteria used in interpreting clinical phenomena. When registry data are considered a gold standard, these comparisons find administrative data to have rates of false positives ranging from 48% to 84% and false negatives ranging from <1% to 5%.[58]

A common limitation of both administrative and registry databases is that they are dependent on International Statistical Classification of Disease, Ninth edition (ICD-9) or ICD-10, and Current Procedural Terminology (CPT) coding to isolate comorbidities, diagnoses, procedures, and complications. These codes were not originally created for research purposes and their use may only be valid for certain diagnoses, procedures, or complications.[59, 60] Variations in coding may be caused by clerical errors or different interpretations of discharge summaries, operative records, or other healthcare documentation. Diagnostic accuracy may also differ between type of treatment facilities;

challenging diagnoses that require extensive work-up by an experienced physician may be more accurate coming from a tertiary referral center than a community hospital.[54, 42]

Missing data

Missing data are common in clinical research, particularly for variables requiring complex, time-sensitive, resource-intensive, or longitudinal data collection methods.[61] Some variables are missing at random, which does not necessarily presume patients with missing values are similar to those with complete data, but instead presumes that observed values can be used to "explain" which values are missing and assist predicting what the missing values would be. There are various methods of dealing with missing variables. Often patients with missing variables are omitted from an analysis, which is known as complete case analysis and is the default methods used by most statistical software. The primary limitation of complete case analysis is reduced sample size, resulting in reduced study power. In addition, unless variables are missing completely at random (very unlikely), estimates using observed case analysis will be biased and the direction of the bias unpredictable. In general, multiple imputation is the best approach for modeling the effect of missing data in studies.[61] Multiple imputation uses the available data to predict plausible values for missing data through the use of regression models. Missing data are then replaced with predicted, or imputed, values. By using multiple imputed data sets, the subsequent analyses appropriately consider both the uncertainty of the observed values and the uncertainty of the imputed values, thereby resulting in more valid inferences.[61]

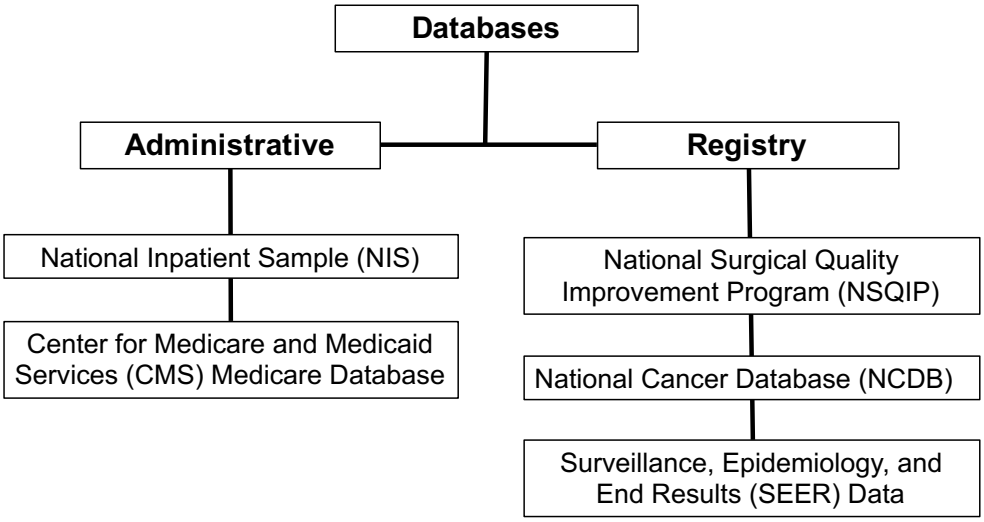

Figure 2. Administrative and registry databases commonly used for surgical outcomes research in Hepato-Pancreato-Biliary surgery.

Variables that are missing not at random are the most troublesome and occur when missing values are dependent on unobserved or unknown factors. When variables are missing not at random, statistical adjustment for missing information is effectively impossible. Because an investigator usually cannot establish the actual mechanism for why the date is missing, statistical analyses usually continue assuming the data are missing at random.[61]

Studies using administrative data should report the extent of missing data, use proper methods to account for missing data in analyses, and describe their potential impact on inferences and conclusions. The proportion of missing data for the variables and outcomes of interest should be clearly discussed in the resulting manuscript. When there is a large proportion of missing data (>30%), the author should investigate and describe the pattern of 'missingness' in the data, and there should be consideration for using techniques such as multiple imputation.[61]

Bias and confounding

Large database studies are a valuable tool for surgical outcomes research. However, a constant challenge in observational designs is to rule out bias.[62] Bias is the systematic deviation of study results or inferences from the truth. Because bias can lead to erroneous conclusions, its minimization is pivotal to all good research.[63] The two most common types of bias are selection bias and confounding. Selection bias arises when certain types of patients are more or less likely to receive treatment owing to possible confounding by indication.[64] Interestingly, a common and often intractable form of confounding results from good medical practice: overall healthier patients tend to undergo more aggressive treatments, which improves survival seen with these more aggressive treatments, but may actually be more of a sign of the patients' overall health at diagnosis rather than the treatment itself.[65] Another common, but often unrecognized, type of selection bias is immortal time bias, also known as guarantee-time bias or survivorship bias, which occurs when a time-dependent exposure (such as initiation of a medical treatment) is not included appropriately in an analysis of a survival outcome. It is termed immortal time bias, because patients must survive sufficiently long enough to receive treatment; hence, they are immortal by definition before exposure.[66] The latter places a disproportionate number of the early deaths in the control group, lowers its survival rate and artificially makes the treatment group seem better in comparison.[67] In a systematic review, over 40% of studies with a survival end point and time-varying treatment were susceptible to immortal time bias.[63]

Confounding stems from measured or unmeasured factors that affect the outcome of interest and are unevenly distributed among study arms. A variable may introduce confounding only if it manifests three characteristics. First, it must be a risk factor for the outcome of interest. Second, it must be associated with the exposure of primary interest. Finally, it must not be affected by the exposure or the outcome of interest.[12] For example, when extended lymphadenectomy is more commonly performed at high-volume treatment

centers, studies may demonstrate that patients undergoing extended lymphadenectomy have better long-term outcomes compared to patients who did not undergo extended lymphadenectomy. However, the improved survival is actually caused by receiving better care at a high-volume center, which is in this case the confounder.

Statistical consideration

There are several approaches to dealing with potential selection bias and confounding. However, multivariate regression is the most often used technique to adjust for the presence of confounding variables. When using a multivariable model, the theoretical rationale of the model should be reported. The type of model (eg, logistic, linear, Poisson) and the assumptions on which it is based should be clearly states (eg, the model assumed linearity or normality of the distribution of the data). The authors should demonstrate that model's assumptions were not violated (eg, the hazard are proportional), thereby confirming the validity of the model. In addition, it should be clearly stated why certain predictor variables and which variables were chosen for the model. Ideally, a model its predictors will not be selected based on statistical significance. Rather, the predictor variables should be chosen based on background literature and/or biological and clinical plausibility. If selection is performed purely based on statistical significance, the model should be presented as hypothesis-generating, rather than conclusive.[68, 53] Furthermore, for every covariate included in the model there should be at least 10 to 15 participants with the outcome of interest.[53]

Propensity score methodology can be especially useful when a treatment is common but the outcome of interest is rare, a situation in which multivariate regression analysis is particularly troublesome.[69] In addition, propensity score methods should be preferred over multivariable regression strategies when the distribution of the covariates of the two treatment groups under investigation do not overlap sufficiently. [70] With propensity scores, patient and provider characteristics are used to calculate the probability that a patient will receive the intervention of interest.[71, 72] There are 4 general ways these propensity scores can be further used. The most common is propensity score matching, which involves assembling 2 groups of study participants, one group that received the treatment of interest and the other that did not, while matching individuals with similar or identical propensity scores.[73, 74] Other methods include stratification on the propensity score, covariate adjustment using the propensity score, and inverse probability of treatment weighting using the propensity score. [74, 75] In general, propensity score matching minimizes bias to a greater extent than propensity score stratification.[74] Previous studies have demonstrated that propensity score methods eliminate approximately 90% of the bias.[76-78] In addition, a review of treatment effects of published surgical studies, results by RCT and non-RCT studies were found to be very similar when non-RCT data were analyzed after matching by use of propensity analysis.[79]

Immortal time bias cannot be controlled for using multivariable models or propensity score methods. The common techniques to control or remove immortal time bias are conditional landmark analysis, time-dependent Cox regression model, and inverse probability weighting.[80] Condition landmark analyses are most frequently used: a fixed time point after the initiation of follow-up is chosen as a landmark for conducting the analysis.[81] Treatment status (exposure) is determined at the landmark, with patients having the event of interest or censored before the landmark excluded from the analysis. Patients who initiate treatment after the landmark are included in the no-exposure group.

It is critical to remember, however, that propensity score techniques, or any of the other statistical methods, can only reliably account for measured determinants of treatment selection, but not for unknown confounding. Nonetheless, as pointed out by Birkmeyer and colleagues, if the differences are large even after adjusting for putative confounding factors, it can be presumed that they cannot be explained solely by residual confounding.[82, 19] In addition, even more important than post hoc adjustment—because it is never perfect—is the thorough investigation and description of the potential impact of both overt and hidden biases in the manuscript of a study. [12] Furthermore, extensive sensitivity analyses should be performed to test the robustness of outcomes for confounders and missing data.[12]

CONCLUSIONS

RCTs in surgery rightfully constitute the most reliable scientific approach to comparative effectiveness studies and should be conducted when feasible. However, considering the rarity of certain surgical conditions, lack of funding, and time constrains, not every research question can be answered by a RCT. Furthermore, RCTS are limited by strict inclusion criteria and exclusion criteria, as well as their highly selected patient population. This raised concerns regarding the translation of results obtain from RCTs into everyday practice. Large database studies are able to provide a ‘real’ world perspective on surgical practice, and could aid in conducting well-designed RCTs by provided pretrial data to enable power calculations, to clarify the definition and indication of the intervention, as well as to develop quality measures. Furthermore, they could provide external validation of RCTs results after completion. There even have been initiatives to integrated the strengths of large database studies and RCTs by performing for example registry-based pragmatic RCTs.[83] In addition, large database studies are able to reflect on research questions regarding the efficacy of health policy, access to health care, trends and geographic variation in practice patterns, as well as the treatment of rare disease or patients subgroups, which would be impossible or very strenuous by using RCTs. Moreover, large nationwide datasets, such as NSQIP, provide the tremendous opportunity to benchmark surgical outcomes and subsequently improve quality of care. On the other hand, it has been well established that large database studies are prone to bias. Therefore, comprehensive understanding of the limitation of these studies, well-thought study-designs and rigorous

statistical analyses are pivotal to conducting worthwhile large database studies.

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Chapter 14

Summary and future perspectives

SUMMARY

Despite progress in our knowledge of the molecular aspects of pancreatic cancer, survival has improved minimally over the past decades.[1, 2] Even among patients with radiographically resectable disease postoperative recurrence rates are high.[3] More effective systemic therapies, and clinically relevant biomarkers to guide decision making are essential to improve survival.

The first part of this thesis focused on molecular biomarkers that have potential to identify resectable pancreatic cancer with poor postoperative survival outcomes. The second part investigates the value of neoadjuvant therapy compared to conventional upfront surgical strategies in patients with potentially resectable pancreatic cancer.

MOLECULAR BIOMARKERS

In **Chapter 2,** the prognostic value of classical (-A, -B, -C), and non-classical (-E, -G) HLA class I expression in pancreatic cancer patients was investigates using immunohistochemistry. Classical HLA class I, HLA-G and HLA-E expression was observed in respectively 78%, 21%, and 96% on tumor cells of the pancreatic adenocarcinomas. On multivariate analysis, HLA-G expression was significantly associated with decreased overall survival (median overall survival, 11 vs. 18 months; HR, 1.863; 95% CI, 1.124 – 3.090; P=0.016). Multivariate analyses did not identify classical HLA class I and HLA-E expression as independent predictive factors for overall survival. These findings provide further evidence for the immunogenic character of pancreatic cancer and the potential of HLA-G as a tumor marker for patients with unfavorable survival..

In **Chapter 3**, the clinical significance of the integrin $\alpha_v\beta_6$, VEGFR2, EGFR, and c-MET expression in patients with resected pancreatic adenocarcinoma was assessed using immunohistochemistry. Integrin $\alpha_v\beta_6$, VEGFR2, EGFR, and cMET expression was observed in 89%, 73%, 69%, and 87% of pancreatic cancer patients, respectively. Patients with integrin $\alpha_v\beta_6$ (median OS: 15 vs. 35 months; log-rank p=0.012), and cMET (median OS, 15 vs. 41 months; log-rank p=0.003) expression had a shorter overall survival. On multivariable analyses, integrin $\alpha_v\beta_6$ (HR, 1.981; p=0.037) and c-MET (HR, 1.766; p=0.051) expression remained associated with poor overall survival. EGFR and VEGFR2 expression were not associated with overall survival. These results suggest that integrin αvβ6 and cMET expression may be used to identify patients at high risk of early recurrence after pancreaticoduodenectomy for pancreatic cancer.

In **Chapter 4**, the prognostic value of uPAR expression in neoplastic and stromal cells of pancreatic adenocarcinoma patients was evaluated using immunohistochemistry. Respectively 66%, 82% and, 62% of pancreatic cancer patients expressed uPAR in neoplastic cells, stromal, and in both combined. uPAR expression in neoplastic cells (median OS, 14 vs. 23 months; p<0.001), uPAR expression in stromal cells (median OS, 16

vs. 21 months; p=0.031), and uPAR expression in both neoplastic and stromal cells (median OS, 13 vs. 24 months; $p<0.001$) were significantly predictive for overall survival. Multivariate analysis showed a significant inverse association between uPAR expression in neoplastic cells (HR, 1.72; p=0.009), as well as uPAR expression in both neoplastic and stromal cells (HR, 2.00; p=0.001), and overall survival. These results suggest a role for uPAR as a biomarker to single out higher risk subgroups of pancreatic cancer patients.

In **Chapter 5**, suitable molecular targets for tumor-specific imaging of pancreatic adenocarcinoma were identified. The expression of 8 potential imaging targets was assessed by the target selection criteria (TASC) – score and immunohistochemical analysis in normal pancreatic tissue (n=9), pancreatic (n=137) and periampullary (n=28) adenocarcinoma. Integrin $\alpha_v\beta_6$, CEA, EGFR, and uPAR showed a significantly higher (all $p<0.001$) expression in pancreatic adenocarcinoma compared to normal pancreatic tissue and were confirmed by the TASC score as promising imaging targets. The results of this study show that integrin $\alpha_v\beta_6$, CEA, EGFR, and uPAR are suitable targets for tumor-specific imaging of pancreatic adenocarcinoma.

NEOADJUVANT THERAPY

In **Chapter 6**, a Markov decision analysis model was used to compare the life expectancy (LE) and quality-adjusted life expectancy (QALE) of neoadjuvant therapy to conventional upfront surgical strategies in resectable pancreatic cancer patients. Data obtained through a comprehensive systematic search in PUBMED of the literature from 2000-2015 were used to estimate the probabilities used in the model. Of the 786 potentially eligible studies identified, 22 studies met the inclusion criteria and were used to extract the probabilities used in the model. Base case analyses of the model showed a higher LE (32.2 vs. 26.7 months) and QALE (25.5 vs. 20.8 quality-adjusted life months) for patients in the neoadjuvant therapy arm compared to upfront surgery. Although conceptual, these data suggest that neoadjuvant therapy offers substantial benefit in LE and QALE for resectable pancreatic cancer patients.

In **Chapter 7**, the impact of neoadjuvant therapy on survival in resected pancreatic cancer patients was assessed by clinical stage using a nationwide cohort. Propensity score matching within each stage was used to account for potential selection bias between patients undergoing neoadjuvant therapy and upfront surgery. In clinical stage III pancreatic cancer (n = 486), neoadjuvant therapy was associated with significant survival benefit after matching (median survival 22.9 vs 17.3 months; log-rank $p < 0.0001$) compared to upfront surgery followed by adjuvant therapy; nevertheless, no survival difference was found between the two treatment sequences in patients with clinical stage I (n = 3,149; median survival, 26.2 vs 25.7 months; p = 0.4418) and II (n = 5,065; median survival, 23.5 vs 23.0 months; p = 0.7751) disease. These results suggest that the survival impact of neoadjuvant therapy is stage-dependent.

In **Chapter 8**, the survival impact of adjuvant therapy following neoadjuvant treatment and pancreatectomy in pancreatic cancer patients was evaluated in a large national cohort. In total, 1,357 patients were identified. 38.6% (n=524) of patients were treated with postoperative therapy. There was no difference in unadjusted median overall survival between patients who did and did not receive postoperative therapy (median survival, 27.5 months vs. 27.1 months, log-rank p=0.5409). On multivariate analysis, receipt of postoperative therapy was not predictive of survival (HR: 0.972; 95% CI, 0.848-1.115; p=0.6876). The findings of this study suggest that after receipt of neoadjuvant therapy and pancreatectomy, additional postoperative therapy may not provide additional survival benefit.

In **Chapter 9**, the prognostic impact of resection margin status after neoadjuvant therapy and pancreaticoduodenectomy for patients with pancreatic adenocarcinoma was investigated. 7,917 patients were identified in total: 1,077 (13.6%) and 6,840 (86.4%) patients received neoadjuvant therapy and upfront surgery, respectively. Upfront surgery was independently predictive of a positive margin (25.7% vs. 17.7%; OR, 1.54) compared to neoadjuvant therapy. After receipt of neoadjuvant therapy, positive margins (median overall survival, 18.5 vs. 25.9 months; HR, 1.58) remained significantly associated with poor survival on multivariable analysis. These findings show that while neoadjuvant therapy is associated with decreased R1/R2-resection rates after pancreaticoduodenectomy, the poor prognostic impact of positive margins is not abrogated by neoadjuvant therapy, stressing the need for complete tumor clearance and postoperative treatment even after neoadjuvant therapy.

In **Chapter 10**, seventh and eight edition American Joint Commission on Cancer (AJCC) staging for pancreatic cancer were validated in patients who underwent neoadjuvant therapy followed by pancreaticoduodenectomy. The eighth edition staging was able to distinguish survival between stage III vs. IIB (HR, 1.173; p=0.0278), but not between stage IA vs. IB (HR, 1.138; p=0.2458), stage IIA vs. IB (HR, 1.063; p=0.5759), and stage IIB vs. IIA (HR, 1.072; p=0.5146). A simplified eighth edition staging significantly distinguished survival for all stages (II vs. I: HR, 1.157; p=0.0168; III vs. II: HR, 1.187; p=0.0142). The C-statistics for the group staging improved from 0.54 for the seventh to 0.56 for the eighth edition. The results of this study suggest that a simplified eighth edition staging system might be more clinically applicable after neoadjuvant therapy.

In **Chapter 11**, the role of adjuvant chemoradiation compared to adjuvant chemotherapy in patients with resected pancreatic adenocarcinoma was evaluated using an international cohort of patients who underwent surgery in Boston or Leiden. Propensity score matching was used to correct for potential selection bias in the allocation of adjuvant chemoradiation versus chemotherapy alone. In total, 350 patients were identified, of whom 138 (39.4%) received adjuvant chemoradiation. After propensity score matching, adjuvant chemoradiation did not significantly impact survival. However, after matching patients

who survived longer than 12 months and had positive lymph nodes (n=108) demonstrated a significant (log0tank p=0.04) survival benefit from adjuvant chemoradiation. This study, while nonrandomized, suggest that adjuvant radiation may be associated with a survival benefit for resected pancreatic cancer patients in specific situations.

In **Chapter 12**, the survival impact of SBRT was evaluated in unresected pancreatic cancer patients. Four treatment groups were identified: chemotherapy alone, or chemotherapy combined with one of the following: EBRT, IMRT, or SBRT. Propensity score models predicting the odds of receiving SBRT were created to control for potential selection bias, and patients were matched on propensity score. A total of 14,331 patients met the inclusion criteria. The median survival before matching was 9.9, 10.9 12.0, and 13.9 months for patients treated with chemotherapy, EBRT, IMRT, and SBRT, respectively. In separate matched analyses, SBRT remained superior to chemotherapy alone (log-rank $p<0.0001$) and EBRT (log-rank $p=0.0180$). After matching, survival did not differ between patients receiving IMRT and SBRT (log-rank $p=0.0492$). These findings suggest that SBRT is associated with a significantly better outcome than chemotherapy alone or in conjunction with traditional EBRT. These results emphasize that SBRT is a promising treatment approach and may also have promise in a neoadjuvant setting.

In **Chapter 13**, the role, advantages and limitations of randomized controlled trials (RCTs) and large database studies in answering important research questions in surgery was discussed. RCTs are at the heart of 'evidence-based' medicine. However, in surgical practice RCTs remain uncommon. At present, RCTs investigating the value of neoadjuvant therapy compared to upfront surgery in pancreatic cancer have been scarce. Surgical outcomes research using nationwide administrative and registry databases has become increasingly common and may be able to fill part of gap. Large databases provide easy and inexpensive access to data on a large and diverse patient population at a variety of treatment centers. Furthermore, large database studies provide the opportunity to answer questions that would be impossible or very arduous to answer using RCTs, including questions regarding adherence to practice guidelines. Prospective data registries may also allow for quality benchmarking and auditing.

GENERAL CONCLUSION

Pancreatic cancer carries a dismal prognosis.[4] Surgical resection represents the only hope for long-term survival, but even after resection with curative intent relapse is common, often due to undetected micro-metastatic disease at diagnosis.[3, 5] Improved stratification of risk for recurrence in patients with radiographically resectable pancreatic cancer is critical, as these patients are likely to benefit from an early systemic treatment approach, instead of upfront surgery. Although validation in larger multi-center cohorts is essential before clinical implementation, the results of the studies included in this thesis suggest that HLA-G, integrin $\alpha v\beta 6$, cMET, and uPAR expression represent promising

molecular biomarkers to preoperatively identify patients with rapidly progressing disease that may benefit from neoadjuvant therapy.

The high recurrence rates after pancreatic cancer surgery have led to widespread consensus regarding the superiority of multimodal therapy over surgery alone.[5] However, the optimal treatment sequence of multimodal therapy remains an ongoing debate. Neoadjuvant therapy is gaining more wide spread acceptance for pancreatic cancer, but RCTs have not yet demonstrated conclusive results.[6, 7] While we are waiting the outcomes of currently ongoing trials, the results of the present thesis suggest that neoadjuvant chemoradiation has the potential to significantly increase survival in well selected patients with locally advanced, borderline resectable, or even upfront resectable pancreatic cancer. The rise of FOLFIRINOX, gemcitabine/nab-paclitaxel, and novel radiation delivery strategies, such as SBRT, will likely even further increase the survival benefit of neoadjuvant chemoradiation.[8-11] Future randomized controlled multi-center international trails are necessary to investigate the optimal neoadjuvant treatment strategy.

FUTURE PERSPECTIVES

Over the past decade, our understanding of tumor biology has grown exponentially, which has led to a vast expansion in attempts to measure aberrant pathways at the molecular level.[12] However, the route from bench to bedside has proven to be arduous and a large gap exists between multiple initial reports or promising biomarkers and eventual full clinical implementation and validation.[12] One of the main challenges in biomarker development is the collection of tumor tissue of sufficient quality for analysis.[13, 14] Early diagnosis of pancreatic cancer is usually performed with ultrasound guided fine-needle aspiration, which provides a limited number of cells for cytological analysis, not always allowing complete molecular profilling.[13, 14] This process is further complicated by the large amount of tumor stroma in pancreatic cancer.[15] Recent studies have explored non-invasive approaches, also known as liquid biopsies, that can be used for molecular profiling, including circulating tumor cells, circulating-free DNA or RNA, exosomen and secretomes.[16, 17] These strategies hold great promise for precision medicine in pancreatic cancer, but are still in the early developmental stages and reliability still needs to be proven.[16, 17] In addition, many previous biomarker discovery and validation studies have been hampered by the lack of statistical power, as pancreatic cancer is a relatively rare and heterogeneous disease.[1] However, the recent increase in the number of large biobank initiatives with uniform collection of biomaterials and associated clinical data are likely going to bring more opportunities for large validation studies, bringing us closer to clinical implementation of novel biomarkers.[18, 19] Finally, it is unlikely that just one molecular biomarker will be sufficient to identify potentially resectable pancreatic cancer patients with more aggressive underlying tumor biology. Instead, it is more likely that tumor marker

panels are necessary to guide clinical decision making and more personalized pancreatic cancer care.[20]

Re-staging

The increasing use of neoadjuvant therapy for potentially resectable pancreatic cancer provides use with novel challenges. Previous studies haves suggested that current radiologic imaging modalities can no longer predict resectability after neoadjuvant chemoradiation, due to the induction of fibrosis.[21, 22] Therefore, improved imaging strategies and re-staging treatment algorithms for patients who underwent neoadjuvant chemoradiation are pivotal. Serum carbohydrate antigen (CA 19-9) levels have been recommended as part of the workup for pancreatic cancer who underwent neoadjuvant chemoradiation.[5] The utilization of CA 19-9 as a treatment response biomarker during neoadjuvant therapy has particular appeal, as changes in CA 19-9 may correlate with otherwise unmeasurable changes in disease response. Approximately, 90% of patients demonstrates a decline in CA 19-9 with neoadjuvant therapy, but normalization of preoperative CA 19-9 occurred much less frequently (39%). Normalization of CA 19-9 with treatment was associated with improved survival.[23] Interestingly, the magnitude of change in CA 19-9 with neoadjuvant therapy seems to be not associated with improved survival.[24] Although the positive predictive value of CA 19-9 for resectability has shown to be high, its clinical utility is compromised by a low negative predictive value.[25, 26] Therefore, several other biomarkers, including carcinoembryonic antigen (CEA), are under investigation to improve prediction of unresectable disease after neoadjuvant therapy.[27] In addition, circulating tumors cells dynamics have shown to reflect progression of disease and response to treatment, providing important information on clinical outcomes, not available by current tumor markers and imaging.[17]

Staging laparoscopy

Not only the assessment local tumor expansion is challenging in patients who underwent neoadjuvant chemoradiation, but also the presence of metastatic disease. Approximately 20-40% of patients with ‘resectable’ pancreatic adenocarcinoma by imaging criteria after neoadjuvant chemoradiation was found to have occult metastatic disease on exploration.[28, 29] Staging laparoscopy ensures that patients do not have metastatic disease before proceeding to a more invasive laparotomy. The argument against staging laparoscopy used to be that patients would need a prophylactic bypass of the tumor with either a gastrojejunostomy, bilioenterostomy, or both, to prevent presumed future obstruction.[30] In the era of metal biliary stents, it is unclear whether patients benefit from a surgical biliary bypass. However, various studies have shown that staging laparoscopy offers patients found to have metastatic disease a shorter operation, a shorter length of stay, a quicker initiation of palliative chemotherapy, and a longer survival.[31]

Radical resection

In line with the paradigm of surgical oncology, radical resection has been widely established as essential to long-term survival in pancreatic cancer patients. However, in light of the increasing use of neoadjuvant chemoradiation for pancreatic cancer patients, some studies have deemed positive resection margins acceptable after neoadjuvant chemoradiation.[32] Considerable international variation exists in what constitutes a microscopically negative margin, which refers to the absence of tumor cells at the inked resection margin (margin clearance > 0 cm) according to the College of American Pathologists, but many European centers define a margin-negative resection as no tumor cells within 1 mm of the resection margin, according to the UK Royal College of Pathologists.[33, 34] In addition, neoadjuvant therapy has shown to alter the consistency of the pancreas, which may impact pathologic evaluation of tumor cells at the circumferential margin.[22] Despite considerable variability and challenges in pathologic examination, the results of this thesis suggest that poor prognostic impact of positive margins is not abrogate by neoadjuvant therapy, stressing the need for surgeons to still pursue complete tumor clearance even after neoadjuvant therapy.[35] New innovative surgical techniques, methods of intraoperative margin assessment, preoperative imaging for patient selection, and improved neoadjuvant chemotherapy are needed to further decrease the rates of incomplete tumor clearance.

Intraoperative assessment

Intraoperative assessment of vascular involvement may also be more challenging after neoadjuvant chemoradiation, especially considering the unreliability of preoperative imaging in this situation, and the lack of historically accepted criteria for resection on imaging.[21, 22] Involved arterial structures or narrowing of venous structures should be approached via serial frozen-section biopsies before attempted resection. If biopsies are positive, resection should be abandoned because R1 and R2 resection is associated with poor overall survival.[36, 37] In addition, molecular intraoperative imaging, used in combination with tumor-specific imaging agents, may improve intraoperative visualization between tumors cells and surrounding benign tissue.[38, 39] Previous studies have suggested that intergrin αVβ6, CEA, epithelial growth factor receptor, and urokinase plasminogen activator receptor would be suitable targets for tumor-specific imaging of pancreatic adenocarcinoma.[40] Furthermore, intraoperative ultrasound has shown some potential to aid the surgical team in assessing respectability, in particular extend vascular invasion, in real time during surgery.[41]

Personalized medicine

Toxicity remains a major concern with the use of combined chemotherapy modalities in pancreatic cancer.[8, 9] Severe (grade 3-4) quite frequently occur and raise questions of patient selection, asking for dose-reduction of dose-modification.[42] This is

especially important in the neoadjuvant setting, as reduced performance status may significantly delay surgical resection, or even worse, may lead to closure of the window to resectability completely.[43] Proper patient selection is crucial to identify the patients that are most likely to benefit from aggressive chemotherapy approaches, and also separate them, who will likely have only benefit due to increased rates of severe side effects.[44] Some studies have identified pretreatment thrombocytosis, low body mass index, and female sex as predictors of early toxicity for patients receiving FOLFIRINOX.[44, 45] Others have suggested that absence of class III β-tubulin expression in specimens obtained by EUS-FNA may be a favorable predictive marker of response to gemcitabine and nab-paclitaxel.[46] However, at present no prospective validated models are available to guide decision making for upfront patient identification.

Radiation therapy

Few issues in oncology have been more divisive in pancreatic cancer care than the role of radiation therapy.[47] Although the value of radiation in the adjuvant setting remains unclear, with widely varying practice patterns and gross international differences, most guidelines currently endorse the use of radiation the neoadjuvant setting.[48, 49] With the intent of increasing efficacy, different modes of delivery, fractionation schedules and/or increasing radiation doses have been tested in combination with chemotherapy.[50] The possibility of combining ablative radiation doses to the tumor and minimizing interruption of systemic therapy has made SBRT an attractive option as neoadjuvant therapy in pancreatic cancer patients. Chuong et al. reported a resection rate of 56% in borderline resectable pancreatic cancer patients who underwent neoadjuvant SBRT after induction therapy with gemcitabine, docetaxel, and oxaliplatin.[51] In addition, a series performed at the John Hopkins Hospital demonstrates that neoadjuvant SBRT does not negatively impact postoperative complications.[52] Trials future investigating the role of SBRT combined with FOLFIRINOX in the neoadjuvant setting are currently ongoing.[10]

Prehabilitation

In patients receiving neoadjuvant therapy, the hiatus between the time of cancer diagnosis and a scheduled operation provides a unique opportunity for patient optimization, both physically and mentally.[53] The implementation of upfront, preoperative habilitation ("prehabilitation"), as opposed to postoperative habilitation (rehabilitation), provides a unique opportunity to optimize outcomes, while ensuring that patients receive necessary conditioning that may otherwise be significantly delayed by postoperative complications. With the implementation of a goal-directed prehabilitation program, perioperative complication rates may decrease and cancer-specific outcomes could potentially be improved. The necessary components of a prehabilitation program would ideally include emotional support, physical therapy, and nutritional optimization. Patients should be enrolled in such a program as soon as the diagnosis is established by the multidisciplinary

team, and continued through the time of their operation.[54] Previous studies have suggested that introduction of prehabilitation prevents nutritional deterioration, improves physical fitness before surgery, and shortened the postoperative hospital stay for patients undergoing hepato-pancreato-biliary surgery.[55]

Centralization

Pancreatic resections are technically complex procedures that historically have been associated with extremely high rates of post-operative morbidity and mortality – to the point where the value of performing the operation at all was questioned openly in medical journals.[56, 57] During the 1960s and 1970s, pancreatic resection, most commonly a pancreaticoduodenectomy, for carcinoma was associated with a perioperative mortality rate exceeding 20% and a considerably higher morbidity rate.[58-60] Patient outcomes have improved over time, with pancreatectomies currently being performed routinely at centers of excellence with operative mortality rate of less than 2% and acceptable rate of morbidity.[56, 61-63] Numerous studies have addressed factors contributing to improved patients outcomes. For pancreatectomy, as for many complex surgical procedures, operative mortality rates are substantially lower at hospitals that perform them more frequently.[64-66] As a result, concentrating pancreatic surgery in high-volume hospitals is advocated by many.[67-69] For example, the American Leapfrog Group, a large business coalition, has been among the strongest proponent of volume-based referral, and uses a variety of incentives to encourage its employees and enrollees to receive care at hospitals exceeding minimum volume standards of 20 pancreatectomies per year.[70] Similarly, in the Netherlands, centralization initiative came to bear based on mutual agreement between surgeon, and proved effective in improving patients outcomes.[71] Although minimum volume standards are not in place, larger countries, including the United States and France, have also seen a shift to high-volume hospitals.[66, 72] These findings suggest that more widespread centralization of pancreatic cancer care would substantial benefit patients survival. However, centralization also raises questions of increased travel burden and decreased spatial access to surgery, with vulnerable patients (e.g., elderly, racial minorities, and uninsured) being the most sensitive to these changes.[66, 73]

Quality cancer care

In past decade, overall patient safety and quality of cancer care has risen to attention. Considering the central role of surgery in the treatment of pancreatic cancer, both surgeon and institutional volume are critical to providing high quality pancreatic cancer care, but are far from the only measures of quality.[74] Especially with the increasing popularity of neoadjuvant therapy, care processes, including diagnostic procedures, multidisciplinary decision making and combined modality treatment are becoming more and more complex, demand more specific knowledge, expertise, and infrastructure in institutions providing cancer care.[75] Similar to other gastrointestinal malignancies,

high-quality pancreatic cancer care can only be provided in specialized pancreatic cancer care centers, which have both a core multidisciplinary team and an extended team of allied professionals, which are subject to quality and audit procedures.[76] Unfortunately, such centers of excellence are far from universal in both Europe and the United States. To improve comprehensive pancreatic cancer care, it is essential that Essential Requirements for Quality Cancer Care guidelines are established for pancreatic cancer, similar to previous iteration for colorectal, esophageal, and gastric cancer.[76, 77] The guidelines should strongly encourage the initiation of dedicated comprehensive pancreatic cancer care centers, with cancer care pathway, timeline of care, minimum case volume requirements, and multidisciplinary teamworking among care and extended groups of professionals (e.g., gastroenterology, pathology, radiology, radiotherapy, medical oncology, interventional radiology, and nursing). In addition, the same approach to auditing, quality assurance and accreditation of a center that is emerging for colorectal cancer in some European countries, should be introduced for pancreatic cancer.[76] Furthermore, these centers must have clinical research programs, with multidisciplinary teams assessing all newly diagnosis patients for eligibility to take part in clinical trials at the center or in research networks.[76]

CONCLUSIONS

Despite recent ground-breaking advances in the field of oncology as a whole, considerable gains are needed for patients diagnoses with pancreatic cancer. There is a pressing need for prognostic biomarkers to guide are selecting of surgical candidates and provide early systemic treatment to patients at high risk of micro-metastatic disease. In addition, there is mounting evidence supporting neoadjuvant therapy for resectable, borderline resectable, and locally advanced pancreatic cancer patients. Retrospective studies have shown that neoadjuvant therapy has potential to significantly increase survival, especially considering the increasing use of combined chemotherapeutic regimen and SBRT. However, the increased using of neoadjuvant therapy also introducing unique challenges and opportunities into clinical practice, including accurate re-staging, intraoperative assessment of the tumor extent, appropriate selection of systemic and radiation regimen, as well as the chance for prehabilitation. Although neoadjuvant therapy is gaining acceptance as standard of care for borderline resectable and locally advanced pancreatic cancer patients, with few exceptions, surgery first is still widely endorsed in the oncology community for patients with upfront resectable disease. Much of the considerable challenges clinicians face when deciding on the appropriate timing of treatment stems from inexact data. Therefore, well designed, properly powered, international multi-center phase III trials are pivotal to move the field forward.

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Chapter 15

Nederlandse Samenvatting

List of Publications

Curriculum Vitae

Dankwoord

INTRODUCTIE

In de laatste decennia is er veel vooruitgang geboekt in de behandeling van kanker. Kankerchirurgie is veiliger geworden, er is effectievere chemo-, immuno- en hormoontherapie beschikbaar gekomen, en screeningsprogramma's voor veel voorkomende tumoren zijn succesvol geïmplementeerd. Als gevolg van deze ontwikkelingen is de kankersterfte voor de meeste soorten kanker sterk afgenomen. Alvleesklierkanker is de enige kankersoort waarbij de overleving de afgelopen decennia niet of nauwelijks is verbeterd, met een 5-jaarsoverleving van <9%. De verwachting is dat binnen tien jaar de alvleesklierkankersterfte het aantal patiënten dat overlijdt aan darmkanker of borstkanker ruimschoots zal overtreffen.

Het compleet chirurgisch verwijderen van de alvleeskliertumor biedt de beste kans voor genezing. Helaas komt meer dan de helft van alle patiënten niet voor een operatie in aanmerking, omdat ten tijde van de diagnose de tumor al is uitgezaaid (gemetastaseerd). In patiënten waarbij een chirurg de gehele tumor uit de alvleesklier heeft kunnen verwijderen blijft de kans tevens groot dat de tumor terugkomt (recidiveert) in de alvleesklier, of op een andere plaats in het lichaam. Dit wijst erop dat in veel patiënten ten tijde van de operatie al niet-detecteerbare microscopische uitzaaiingen (metastasen) aanwezig zijn. Deze patiënten ondervinden wellicht weinig voordeel van chirurgie en hebben meer baat bij chemotherapie of chemoradiatie (een combinatie van chemotherapie en radiotherapie) voorafgaande aan de operatie, ook wel preoperatieve of neoadjuvante therapie genoemd, zodat de microscopische uitzaaiingen vroegtijdig kunnen worden behandeld.

Moleculaire markers kunnen een belangrijke rol spelen bij het identificeren van patiënten met een agressievere vorm van alvleesklierkanker die een hoog risico lopen dat de tumor terugkomt na een operatie. Moleculaire markers zijn meetbare indicatoren in tumorweefsel, bloed of urine die aan kunnen geven dat iemand ziek is, die kunnen voorspellen hoe ernstig een ziektebeloop zal zijn, of die laten zien of een behandeling werkt of niet. Bekende voorbeelden zijn het gebruik van het specifieke prostaatantigen (PSA) voor prostaatkanker en het bloedsuikergehalte om diabetes in het oog te houden. In patiënten met alvleesklierkanker wordt vaak de hoeveelheid cancer antigen 19-9 (CA 19-9) in het bloed gemeten. Helaas is deze moleculaire marker niet erg gevoelig en er is dus behoefte aan betere moleculaire markers voor alvleesklierkanker.

Dit proefschrift is verdeeld in twee delen. Het eerste deel focust op het identificeren van moleculaire markers voor alvleesklierkanker die gebruikt kunnen worden voor het herkennen van patiënten die een hoog risico lopen op het terugkeren van de tumor na een operatie. Het tweede deel onderzoekt de waarde van het geven van chemotherapie of chemoradiatie voorafgaande aan de operatie in patiënten met alvleesklierkanker.

MOLECULAIRE MARKERS

In **Hoofdstuk 2** wordt de voorspellende waarde van Human Leukocyte Antigen (HLA) expressie voor de overleving van patiënten met alvleesklierkanker onderzocht doormiddel van immuunhistochemie. HLA expressie is betrokken bij immunologische herkenning van lichaamsvreemde componenten, waaronder kankercellen, door het lichaam. Classical HLA klasse I, HLA-G, en HLA-E expressie werd gevonden in respectievelijk 78%, 21%, en 96% van de patiënten met alvleesklierkanker. HLA-G expressie was geassocieerd met een verminderde overleving (mediane overleving, 11 vs. 18 maanden; HR, 1.86; 95% CI, 1.12 – 3.09; p=0.016). Classical HLA klasse I en HLA-E expressie waren niet geassocieerd met overleving. Deze bevindingen laten zien dat het immuunsysteem een belangrijke rol speelt bij de progressie van alvleesklierkanker.

Naast het immuunsysteem speelt ook angiogenesis, het vormen van nieuwe bloedvaten door de tumor, een belangrijke rol bij de groei en uitzaaiing van tumoren. Integrin $\alpha_v\beta_6$, vascular endothelial growth factor receptor 2 (VEGFR2), epithelial growth factor receptor (EGFR), en c-MET zijn essentieel voor tumor angiogenesis. **Hoofdstuk 3** onderzoekt de klinische significantie van integrin $\alpha_v\beta_6$, VEGFR2, EGFR en c-MET expressie in patiënten met alvleesklierkanker door middel van immuunhistochemie. Integrin $\alpha_v\beta_6$, VEGFR2, EGFR, en cMET expressie werd geobserveerd in respectievelijk 89%, 73%, 69%, en 87% van de alvleesklierkankerpatiënten. Patiënten met integrin $\alpha_v\beta_6$ (mediane overleving, 15 vs. 35 maanden; log-rank p=0.012), en cMET (mediane overleving, 15 vs. 41 maanden; log-rank p=0.003) expressie hadden een significante kortere overleving. EGFR en VEGFR2 expressie bleek niet geassocieerd met overleving.
Deze resultaten suggereren dat integrin $\alpha_v\beta_6$ en cMET expressie mogelijk gebruikt kunnen worden voor het identificeren van patiënten met een hoog risico op het terugkeren van de tumor na een alvleesklierkankeroperatie.

Hoofdstuk 4 onderzoekt de prognostische waarde van urokinase receptor (uPAR) in patiënten met alvleesklierkanker doormiddel van immuunhistochemie. uPAR is een onderdeel van het plasminogeenactiveringssysteem dat betrokken is bij weefselorganisatie. In 66% van de alvleesklierkankerpatiënten werd uPAR expressie gedetecteerd. uPAR expressie werd geassocieerd met een kortere overleving (mediane overleving, 14 vs. 23 maanden; HR, 1.72; p=0.009). Deze resultaten bevestigen de belangrijke rol van uPAR in de metastasering van alvleesklierkanker en de waarde van uPAR bij het identificeren van een subgroep patiënten met een hogere risico op tumor recidief.

In **Hoofdstuk 5** worden geschikte moleculaire targets voor de tumor-specifieke beeldvorming van alvleesklierkanker geëvalueerd op basis van target selectiecriteria en immuunhistochemie. Integrin $\alpha_v\beta_6$, CEA, EGFR, en uPAR expressie bleek significant ($p<0.001$) hoger in alvleesklierkanker tumorweefsel vergeleken met normaal alvleesklierweefsel en hadden als gevolg de hoogste targetscore. Deze resultaten laten zien

dat integrin $\alpha_v\beta_6$, CEA, EGFR en uPAR geschikte targets zouden kunnen zijn voor de tumor-specifieke beeldvorming van alvleeskliertumoren.

PREOPERATIEVE THERAPIE

Het operatief verwijderen van de alvleeskliertumor alleen is niet genoeg. De meeste patiënten ontvangen daarom chemotherapie of chemoradiatie na de operatie, ook wel postoperatieve of adjuvante therapie genoemd, om de kans op een recidief te verkleinen. Operaties bij alvleesklierkanker zijn echter erg ingrijpend en hebben een hoge kans op ernstige complicaties, waardoor meer dan een kwart van de patiënten na de operatie niet in staat is om tijdig aanvullende therapie te ondergaan. Het geven van chemotherapie of chemoradiatie voor de operatie zorgt ervoor dat de onzichtbare micrometastasen zo vroeg mogelijk behandeld kunnen worden zonder vertraging of afstel door mogelijk chirurgische complicaties. Daarnaast kan preoperatieve therapie de tumor doen krimpen waardoor het makkelijker wordt om de tumor in zijn geheel te verwijderen. Preoperatieve therapie wordt al door de richtlijnen aangeraden voor patiënten met borderline resectable alvleeskliertumoren, waarbij de tumor reeds in de omliggende bloedvaten is gegroeid, maar de literatuur met betrekking tot patiënten met alvleeskliertumoren die nog niet in de omliggende bloedvaten zijn gegroeid, is nog onduidelijk.

In **Hoofdstuk 6** is een Markov besluitvormingsmodel gebruikt om de levensverwachting en de kwaliteit van leven gecorrigeerde levensverwachting te vergelijken voor patiënten die chirurgie met en zonder neoadjuvante therapie hebben ondergaan. De kansen gebruikt in het besluitvormingsmodel waren afkomstig van een systematische review van de literatuur gepubliceerd op PubMed tussen 2000 en 2015. De standaardanalyse van het model laat een hogere levensverwachting (mediane overleving, 32.2 vs. 26.7 maanden) en kwaliteit van leven gecorrigeerde levensverwachting (mediane overleving, 25.5 vs. 20.8 maanden) zien voor patiënten die preoperatieve therapie hebben ondergaan vergeleken met patiënten die geen preoperatieve therapie hebben ondergaan voor hun operatie. Deze bevindingen bleven robuust bij het uitvoeren van verschillende sensitiviteitsanalyses. De bevinding van deze studie laten een substantiële overlevingswinst zien voor patiënten die preoperatieve chemoradiatie hebben ondergaan.

In **Hoofdstuk 7** is een grote nationale dataset gebruikt om de overleving van alvleesklierkankerpatiënten die een operatie hebben ondergaan met en zonder neoadjuvante therapie te vergelijken. In patiënten met stadium 3 alvleesklierkanker was preoperatieve therapie geassocieerd met significante overlevingswinst (mediane overleving, 22.9 vs. 17.3 maanden; log-rank $p<0.0001$). In patiënten met stadium I (mediane overleving, 26.2 vs. 25.7 maanden; $p=0.4418$) en stadium II (mediane overleving, 23.5 vs. 23.0 maanden; $p=0.7751$) alvleesklierkanker had preoperatieve therapie geen invloed op de overleving. De resultaten van deze studie laten zien dat de impact van preoperatieve therapie op de

overleving van patiënten met alvleesklierkanker mogelijk afhankelijk is van het tumorstadium.

Hoofdstuk 8 onderzoekt of het nodig is om postoperatieve chemotherapie te geven in patiënten die reeds preoperatieve therapie gevolgd door een alvleesklierkankeroperatie hebben ondergaan. De bevindingen van deze studie laten zien dat het geven van postoperatieve therapie in patiënten die al preoperatieve therapie en een alvleesklierkankeroperatie hebben ondergaan, niet resulteert in een betere overleving (mediane overleving, 27.5 vs. 27.1 maanden; log-rank p=0.5409).

In **Hoofdstuk 9** wordt de impact van positieve snijvlakken geëvalueerd in patiënten met alvleesklierkanker die reeds preoperatieve chemotherapie of chemoradiatie hebben ondergaan. Positieve snijvlakken werden gevonden in 17.7% van de patiënten die neoadjuvante therapie hadden ondergaan en in 25.7% die geen neoadjuvante therapie hadden ondergaan. Na preoperatieve therapie bleven positieve snijvlakken geassocieerd met een verminderde overleving (mediane overleving, 18.5 vs. 25.9 maanden; HR, 1.58). Deze bevindingen suggereren dat preoperatieve therapie niet de noodzaak wegneemt om alvleeskliertumoren compleet te verwijderen.

Hoofdstuk 10 valideert de prognostische waarde van de nieuwe 8ste editie American Joint Commission on Cancer (AJCC) stagering voor alvleesklierkanker in patiënten die preoperatieve chemotherapie of chemoradiatie gevolgd door een operatie hebben ondergaan. De 8ste editie AJCC stagering was instaat om een significant verschil aan te tonen tussen stadium II vs. I (HR, 1.157; p=0.017) en stadium III vs. II (HR, 1.187; p=0.0142). Daarnaast verbeterde de c-statistiek van 0.54 voor de 7de editie stagering naar 0.56 voor de 8ste editie stagering. De resultaten van deze studie laten zien dat de 8ste editie AJCC stagering klinisch relevant blijft in alvleesklierkankerpatiënten die preoperatieve chemotherapie of chemoradiatie hebben ondergaan.

In **Hoofstuk 11** wordt de rol van postoperatieve chemoradiatie vergeleken met postoperatieve chemotherapie in patiënten die een operatie hebben ondergaan voor alvleesklierkanker door gebruik te maken van een internationaal cohort bestaand uit patiënten behandeld in Boston of Leiden. In totaal werden 350 patiënten geïncludeerd, waarvan 39.4% van de patiënten postoperatieve chemoradiatie hadden ondergaan. Postoperatieve chemoradiatie was niet geassocieerd met overlevingswinst vergeleken met postoperatieve chemotherapie alleen. Patiënten met positieve lymfeklieren (lymfeklieren waarin tumorcellen werden gevonden) lieten echter een significant betere overleving zien na behandeling met postoperatieve chemoradiatie vergeleken met postoperatieve chemotherapie alleen (log-rank p=0.04). De resultaten van deze studie laten zien dat het geven van postoperatieve radiatie alleen in een specifieke subset alvleesklierkankerpatiënten tot overlevingswinst leidt.

In **Hoofdstuk 12** wordt de waarde van stereotactische radiotherapie (SBRT) onderzocht in patiënten die niet in aanmerking komen voor een alvleesklierkankeroperatie. Vier verschillende behandelingsgroepen werden geïdentificeerd: chemotherapie alleen, of

chemotherapie gecombineerd met external beam radiotherapie (EBRT), intensity-modulated radiotherapie (IMRT), en SBRT. De mediane overleving was 9,9 maanden voor patiënten die alleen chemotherapie hebben ondergaan, 10.9 maanden na chemotherapie en EBRT, 12.0 maanden na chemotherapie en IMRT, en 13.9 maanden na chemotherapie en SBRT. In aparte analyses bleek de overleving voor alvleesklierkankerpatiënten die SBRT hadden ondergaan beter dan patiënten die chemotherapie alleen (log-rank $p<0.0001$) of chemotherapie en traditionele EBRT (log-rank $p=0.0180$) hadden ondergaan. De overleving was vergelijkbaar voor patiënten die IMRT en SBRT hadden ondergaan (log-rank $p=0.0492$). De resultaten van deze studie benadrukken dat SBRT een veelbelovende behandelstrategie is voor alvleesklierkanker.

Hoofdstuk 13 beschrijft de waarde van gerandomiseerd dubbelblind klinisch onderzoek en van observationele studies die gebruik maken van grote landelijke en internationale registerdatabases bij het beantwoorden van onderzoeksvragen binnen het chirurgisch vakgebied. Gerandomiseerd dubbelblind klinisch onderzoek wordt beschouwd als de ‘Gouden Standaard” waar het gaat om bewijsvoering binnen de geneeskunde, maar is tevens duur en tijdrovend. Grote database studies geven de mogelijkheid om een meer diverse patiëntenpopulatie te bestuderen en onderzoeksvragen te beantwoorden die niet of moeilijk door gerandomiseerd dubbelblind onderzoek kunnen worden beantwoord, inclusief vragen met betrekking tot het navolgen van behandelrichtlijnen en zeldzame uitkomsten. Daarnaast maken prospectieve dataregisters kwaliteitsbenchmarking en audits mogelijk.

CONCLUSIES

De conclusie af te leiden uit de resultaten van de studies beschreven in deze thesis is tweeledig. In de eerste plaats laten deze studies de potentie van moleculaire markers, zoals HLA-G, integrin $\alpha v\beta 6$, cMET, en uPAR, zien om patiënten met alvleesklierkanker te identificeren met een hoog risico op recidief na een alvleesklierkankeroperatie, die wellicht meer baat zouden kunnen hebben bij het vroegtijdig behandelen met chemotherapie of chemoradiatie. Daarnaast laten de studies beschreven in deze thesis zien dat preoperatieve chemoradiatie overlevingswinst en een betere kwaliteit van leven kan opleveren in het merendeel van de patiënten met alvleesklierkanker die voor een operatie in aanmerking komen. Internationale gerandomiseerde dubbelblinde klinisch studies zijn nodig om deze bevindingen te valideren.

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CURRICULUM VITAE

Susanna Willemina Leuntje de Geus werd in 2009 ingeloot voor haar studie Geneeskunde aan de Universiteit Leiden. In 2011 is zij in het kader van het Honours College traject begonnen met haar onderzoek naar biomarker expressie in alvleesklierkanker op de afdeling Heelkunde van het LUMC onder leiding van Dr. A.L. Vahrmeijer en Dr. P.J.K. Kuppen. In 2014-2016 heeft zij onderzoek gedaan op de afdeling Heelkunde van het Beth Israel Deaconess Medical Center, een Harvard Medical School geaffilieerd academisch centrum, in Boston onder leiding van Prof. J.F. Tseng naar de invloed van preoperatieve chemoradiatie op de overleving van resectabele alvleesklier patiënten. Haar coschappen deed zij onder meer op de afdeling kinderchirurgie en hepato-pancreato-biliare chirurgie van het Massachusetts General Hospital, en de afdeling oncologische chirurgie van Boston Medical Center, beide in Boston. Gedurende haar studie Geneeskunde heeft Susanna tevens een studie Bestuurskunde afgerond aan de Universiteit Leiden met de thesis getiteld, 'The international diffusion of organ donation policy reform: a cross-country comparison'. Sinds haar afstuderen is Susanna werkzaam als postdoctoraal onderzoeker op de afdeling Heelkunde van Boston Medical Center onder leiding van Prof. J.F. Tseng, In 2020 ontving zij de American College of Surgeons (ACS) Commission on Cancer Paper Competition award voor haar manuscript getiteld, 'Volume of Pancreas-Adjacent Operation Favorably Influence Pancreaticoduodenectomy Outcomes at Lower Volume Pancreas Center'. In hetzelfde jaar heeft zij mee gesolliciteerd voor de Heelkunde opleiding in de Verenigde Staten, waarvan ten tijde van publicatie van deze thesis het resultaat nog niet bekend is.

DANKWOORD

Dit proefschrift is mede tot stand gekomen dankzij het werk en de steun van velen. Alle mensen die -elk op hun eigen manier- deze periode voor mij zo onvergetelijk hebben gemaakt, wil ik hierbij dan ook graag bedanken.