

DISTINCT CACHEXIA PHENOTYPES AND THE
IMPORTANCE OF ADIPOSE TISSUE LOSS ON SURVIVAL
OF PATIENTS WITH ADVANCED PANCREATIC CANCER
ON FOLFIRINOX CHEMOTHERAPY

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Distinct Cachexia Phenotypes and the Importance of Adipose Tissue Loss on Survival of Patients with Advanced Pancreatic Cancer on FOLFIRINOX Chemotherapy

By the traditional definition of unintended weight loss, cachexia develops in ~80% of patients with pancreatic ductal adenocarcinoma (PDAC). Here we measure the longitudinal body composition changes in patients with advanced PDAC undergoing FOLFIRINOX therapy. We performed a retrospective review of 53 patients with advanced PDAC on FOLFIRINOX as first line therapy at Indiana University Hospital from July 2010 to August 2015. Demographic, clinical, and survival data were collected. Body composition measurement, trend, univariate and multivariate analysis were performed. Three cachexia phenotypes were identified. The majority of patients, 64%, had Muscle-and-Fat Wasting (MFW), while 17% had Fat-Only Wasting (FW) and 19% had No Wasting (NW). NW had significantly improved overall median survival (OMS) of 22.6 months vs. 13.0 months for FW and 12.2 months for MFW ($p=0.02$). FW (HR=5.2; 95%CI=1.5-17.3) and MFW (HR=1.8; 95%CI=1.1-2.9) were associated with an increased risk of mortality compared to NW. OMS and risk of mortality did not differ between FW and MFW. Progression of disease, sarcopenic obesity at diagnosis, and primary tail tumors were also associated with decreased OMS. On multivariate analysis cachexia phenotype and chemotherapy response were independently associated with survival. Three phenotypes of cachexia were observed. Moreover, three phenotypes suggests molecular or genetic heterogeneity of host or tumor. Identifying these differences will be vital to defining optimal treatment for cachexia. Survival among FW was as poor as MFW suggesting adipose tissue plays a crucial role in cachexia. Blunting

or possibly preventing cachexia may confer a significant survival advantage in patients with advanced PDAC.

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LIST OF ABBREVIATIONS

ANOVA – Analysis of Variance	NW – No Wasting
BMI – Body Mass Index	OMS – Overall Median Survival
CI – Confidence Interval	PDAC – Pancreatic Ductal Adenocarcinoma
CT – Computed Tomography	RECIST – Response Evaluation Criteria in Solid Tumors
ECOG – Eastern Cooperative Oncology Group	SCAT – Subcutaneous Adipose Tissue Mass
FOLFIRINOX – 5 Fluorouracil, Leucovorin, Irinotecan, Oxaliplatin	SCATI – Subcutaneous Adipose Tissue Index
FW – Fat Wasting	SD – Standard Deviation
HR – Hazard Ratio	SKM – Skeletal Muscle Mass
HU – Hounsfield Unit	SKMI – Skeletal Muscle Index
IL-6 – Interleukin 6	SKMHU – Skeletal Muscle Hounsfield Unit
IMAT – Intramuscular Adipose Tissue Mass	TAI – Total Adipose Index
IMATI – Intramuscular Adipose Tissue Index	TNF- α - Tumor Necrosis Factor-Alpha
IU – Indiana University	VAT – Visceral Adipose Tissue Mass
L3 – third lumbar vertebrae	VATI – Visceral Adipose Tissue Index
MFW – Muscle and Fat Wasting	WHO – World Health Organization

Chapter One

Introduction

Pancreatic cancer is the fourth leading cause of cancer deaths in the United States and is on the rise (1). The ductal adenocarcinoma (PDAC) subtype accounts for the majority of cases of pancreatic cancer (1). Patients with PDAC typically present late with advanced, unresectable disease and have a dismal prognosis (2,3). Gemcitabine-based chemotherapy regimens have traditionally been the standard treatment protocol for unresectable PDAC. Unfortunately, most patients will not respond and overall median survival (OMS) is only 6-9 months (2,3).

The chemotherapy combination of 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin (FOLFIRIOX) has improved survival but the overall prognosis remains exceedingly grim. Recent studies have shown FOLFIRINOX to be superior to gemcitabine, however, OMS still only approaches 15 months (4-8). Furthermore FOLFIRINOX is often poorly tolerated and dosing may need to be reduced or completely discontinued due to severe side effects (5-9).

Cachexia therapy represents a major unmet need in patients with unresectable PDAC. Cachexia is a complex metabolic derangement characterized by loss of muscle with or without the loss of fat that cannot be reversed by nutritional support (10). It has been associated with decreased tolerance of and response to treatment, decreased quality of life, decreased survival, and generally worse overall outcomes in patients with malignancies, including PDAC (10-13). The vast majority of patients with PDAC will develop cachexia during the course of their illness, even as early as at time of diagnosis, and cachexia has been implicated as a significant cause of death (14).

Body composition measurements obtained from computed tomography (CT) scans have emerged as a novel prognostic factor in many cancers, including PDAC (15-18). These measurements can be used to detect low muscle mass, known as sarcopenia, and fatty muscle infiltration, known as myosteatorsis. Sarcopenia, sarcopenic obesity, and myosteatorsis have all been associated with decreased survival in patients with malignancy (19-22). CT scans are routinely obtained in patients with unresectable PDAC for staging and to monitor disease response to treatments. These CT scans are readily available and thus body composition measurements and changes represent a potentially crucial component in the prognostic and treatment equations for patients and provide an invaluable tool for researchers.

Although the role of cachexia in PDAC is clear, to date there has been little work done to define the longitudinal development of cachexia in these patients and effort to understand the impact of FOLFIRINOX therapy on cachexia. Herein a longitudinal study of 53 patients with locally advanced and metastatic PDAC on FOLFIRINOX therapy was performed and changes in body composition and the effects on survival were examined.

Conversation

This study examined the longitudinal changes in body composition and the associations of these changes with survival in patients with advanced PDAC undergoing FOLFIRINOX chemotherapy as first-line treatment. Using the traditional definition of >5% body weight loss to define cachexia, only 56.6% of patients in this cohort developed cachexia over the course of their disease. Using our definition of $\geq 5\%$ loss of muscle and/or fat mass, the prevalence of cachexia was 81%. This is consistent with previously published data and provides further evidence that cachexia treatment is a major unmet need of patients with advanced PDAC (14). The study also identified three different cachexia phenotypes, No Wasting (NW), Fat-Only Wasting (FW), and Muscle and Fat Wasting (MFW). The study identifies a subset of patients, NW, who appear resistant to cachexia and as a result have significantly improved survival. In addition, the study showed fat loss to be an equally important factor on survival as muscle loss.

To the best of the authors' knowledge this is the first paper to demonstrate different wasting patterns in patients with any cancer or chronic disease. The identification of these phenotypes is a critical detail as it suggests molecular and/or genetic heterogeneity among the hosts or the tumors as the driving force for each wasting pattern. Several cytokines have been proposed to be vital to the development of cachexia, including tumor necrosis factor-alpha, interleukin-1, interleukin-6 (IL-6), interferon-gamma, ciliary neurotrophic factor, and myostatin (23-29). It has been shown that nucleotide polymorphisms in genes that are linked to production rates are associated with the development of cachexia (30), specifically in regards to IL-6 in the setting of pancreatic cancer (31). While this study does not demonstrate molecular or genetic

differences, the distinct phenotypes are highly suggestive. Additional studies focusing on molecular and genetic difference of PDAC tumors and the patients are indicated, as therapies targeting the precise cause of cachexia are essential to achieve optimal outcomes.

The second major finding of this study is the lack of difference in survival between patients who lost muscle and fat and patients who lost only fat. The prominent theory in cachexia is that muscle loss is the major complication in cancer cachexia and to date cachexia research has mainly focused on muscle. The data presented here suggests that fat has an equivalent role, as developing the FW phenotype conferred a decreased survival that was not significantly different than the MFW phenotype.

Adipose tissue has long been seen as an energy regulating tissue with additional responsibilities of mechanical protection and temperature regulation. This changed with the discovery of leptin production by adipose tissue and it was recognized that fat had important endocrine functions as well (32). Since this discovery, adipose tissue has been shown to express and secrete a number of different signaling molecules including the cachexia-associated cytokines TNF- α and IL-6 (33). The loss of adipose tissue in cancer cachexia has also been shown to be accompanied by changes in gene expression pathways regulating energy turnover (34). Although it has been shown that fat loss is associated with decreased survival in patients with PDAC (35), the data here shows that fat loss appears to be equivalent to muscle loss.

Previous studies have shown sarcopenia, sarcopenic obesity, and myosteatorsis to be associated with decreased survival in pancreatic cancer (20-22). Of these variables, only sarcopenic obesity was shown to be associated with survival and it did not hold up

in multivariate analysis (Table 3). While not statistically significant, all of these variables did trend in the direction of having an impact on survival. In a large patient cohort these variables would likely show statistical significance and be in line with previously published data.

This study also gives insight into how we should be defining cachexia. The prevalence of cachexia defined by the traditional definition of >5% body weight loss was only 56.6%, while the prevalence using the study's definition of $\geq 5\%$ loss of muscle or fat mass was 81%. The study's definition is more in line with previously published data (14). No patient in the NW phenotype had >5% body weight loss. However, applying that definition to the FW and MFW phenotypes would have caused 5/9 and 25/34 patients to be classified as developing cachexia. This would have missed 13/43, or 30%, of patients with significant muscle or fat wasting. One explanation would be that patients with advanced PDAC are prone to developing ascites, and the ascites is contributing to maintenance of body weight despite the fact that significant muscle and/or fat wasting is occurring. Using CT body morphometric measurements is a more accurate way of determining a patient's cachexia status.

The current study is not without limitations. It is a retrospective study and therefore is subject to all limitations associated with retrospective studies. The Eastern Cooperative Oncology Group (ECOG) score was not available. Patients who are eligible for FOLFIRINOX therapy are high functioning patients with ECOG scores of 0 or 1 and therefore it can be safely assumed that there was no difference in underlying functional status between any groups analyzed. This analysis was also performed on an intent to treat basis. Data on changes to the chemotherapy regimens such as dose reductions or

changes in medications that could possibly have altered the patients' course were not available. Additionally, co-morbidities were not available and the authors recognize that some patients may have had significant co-morbidities that contributed to the outcome.

The study evaluated only 53 patients and this may have limited the study's power to detect the effects of some variables. While this is enough patients to safely say that the differences found are truly present, the authors recognize that it is not enough power to adequately eliminate type II error and that differences may be present that were not detected.

All patients in the current study presented with advanced PDAC. Given the high rate of development of cachexia in these patients it can be safely assumed that the initial CT scan was obtained after some degree of cachexia developed. The authors do not believe this would have an effect on the overall results. Closer examination of the NW phenotype shows all the patients had significant increase in muscle, fat, or both tissues during the course of treatment thus establishing a cachexia-resistant phenotype. The FW phenotype presented with lower SKMI and this is possibly due to muscle wasting occurring before the initial scan. This group, however, did not lose any additional muscle throughout the course of the disease, while the MFW phenotype lost muscle and fat congruently. Therefore, we can safely conclude that these are also two distinct phenotypes of cachexia.

This study is susceptible to length time bias. This is an unavoidable bias as there is no accurate screening test for PDAC and there is no way to know exactly when the

disease process began. Analysis of days to diagnosis of cachexia showed no difference between FW and MFW ($p=0.67$).

Finally, this study only applies to patients who received FOLFIRINOX as first-line treatment. It has previously been shown that certain chemotherapy regimens can induce cachexia alone (36). To the best of the authors' knowledge there is no data linking the administration of FOLFIRINOX to the development of cachexia. It has, however, been shown that cachexia develops in the vast majority of patients with PDAC (14). Thus it is unlikely that FOLFIRINOX was solely responsible for the cachexia that was observed. Additionally, the NW phenotype patients also received FOLFIRINOX. This strengthens the conclusion that the cachexia was disease driven and not treatment driven. Nevertheless, similar studies need to be performed involving patients undergoing other chemotherapy regimens and in other malignancies before these results can be generalized.

To the best of the authors' knowledge, this is the first study to show distinct cachexia phenotypes in any cancer or chronic disease. The fact that multiple phenotypes emerged raises suspicion that molecular and/or genetic heterogeneity is present in the hosts or the tumors and discovery of these differences could lead to more targeted therapies. Individualized therapy will be crucial in order to optimize care of patients with cachexia. The study also demonstrates the important role that adipose tissue plays in cachexia. Research focusing on fat's role in cachexia will be vital to understanding the entire disease process.

Chapter Two

Methods

This study was approved by the Indiana University Institutional Review Board and was carried out in compliance with the IU Standard Operating Procedures for Research Involving Human Subjects. All data collection occurred between September 1, 2015 and November 29, 2016.

All patients presenting to Indiana University Hospital between the dates of July 1, 2010 and August 31, 2015 with advanced PDAC treated with FOLFIRINOX as first-line therapy with available survival data and adequate CT images for analysis were eligible for inclusion in the study. Advanced PDAC was defined as PDAC that was not amenable to surgical resection. Sixty-six patients were identified. Upon review 13 patients were excluded: four with missing CT scans, six with only a single CT scan, two with poor quality CT scans unable to be analyzed, and one treated with an alternative chemotherapy regimen prior to presentation at Indiana University. This resulted in 53 total subjects in the study. Demographic, clinical, and survival data along with CT scans were collected on these 53 subjects.

CT images were analyzed for cross-sectional area (cm²) for skeletal muscle (SKM), intramuscular adipose tissue (IMAT), visceral adipose tissue (VAT), and subcutaneous adipose tissue (SCAT) mass at the level of the 3rd lumbar vertebrae (L3) (37) using Slice-O-Matic® software V4.3 (Tomovision, Montreal, Quebec, Canada)(Figure 1). Hounsfield unit thresholds were set at -29 to +150 HU for SKM, -30 to -190 HU for IMAT and SCAT, and -50 to -150 HU for VAT (38). Two consecutive images were analyzed on all CT scans by a single investigator (JKK). The mean of the

two images was normalized to height in meters-squared to establish tissue specific indices. Total adipose index (TAI) was calculated by adding IMAT index (IMATI), VAT index (VATI) and SCAT index (SCATI). Sarcopenia was defined as a SKMI <52.4 cm²/m² for males and <38.5 cm²/m² for females (19). Myosteatorosis was defined as a mean skeletal muscle radiodensity of <33 HU for patients with a BMI ≥25 kg/m² and <41 HU for patients with a BMI <25 kg/m² (39). Estimates of whole body stores for skeletal muscle, total adipose, and each adipose compartment were obtained by applying the following regression equations by Mourtzakakis et al (25).

$$\text{Total body muscle mass (kg)} = 0.3 \times (\text{skeletal muscle at L3 (cm}^2\text{)} + 6.06$$

$$\text{Total body adipose mass (kg)} = 0.042 \times \text{adipose tissue at L3 (cm}^2\text{)} + 11.2$$

Obesity was defined by the World Health Organization (WHO) criteria (40). Patients were classified as having sarcopenic obesity if they met the criteria for sarcopenia and obesity. Total muscle measurements included the rectus abdominus, external and internal oblique, transversus abdominus, psoas, erector spinae, and quadratus lumborum.

Disease response to chemotherapy was determined by using Response Evaluation Criteria in Solid Tumors (RECIST) criteria (41) by a single investigator (SS). Patients were classified into three groups based on best response: regression of disease, stable disease, and progression of disease.

Serial CT scans were performed every three months per institution protocol. All CT scans from the time of diagnosis until the end of the study were analyzed and included in the data analysis. A total of 298 CT scans were analyzed. Tissue mass measurements were graphed versus time to identify any trends. Trends in skeletal muscle index (SKMI) and TAI were noted and patients were divided into three categories: No

Wasting (NW), Fat-Only Wasting (FW), and Muscle and Fat Wasting (MFW). Cut offs for significant change was set at $\geq 5\%$ increase or decrease from the initial CT measurement to the final CT measurement. The subjects were then re-categorized as needed based on this cutoff threshold. Patients with a significant increase in one category but significant decrease in another category were defined as NW.

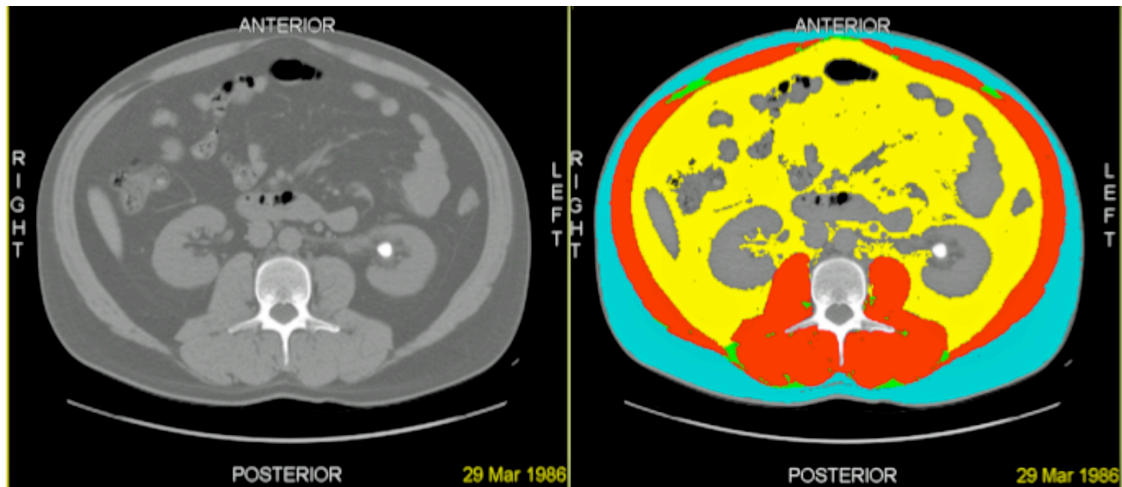


Figure 1. Representative Slice-O-Matic Example. The left image is representative of a CT image at the L3 level as obtained during routine monitoring of the disease. The right image is the same CT image at the L3 level with the Slice-O-Matic tag overlay. Red represents skeletal muscle, yellow represents visceral adipose tissue, green represents intramuscular adipose tissue and blue represents subcutaneous adipose tissue. ive Slice-O-Matic Example. The left image is representative of a CT

Statistical Analysis

The primary outcome measure was overall survival, calculated in months from the time of diagnosis to the time of death or last follow up. The main prognostic factor was cachexia phenotype, however, age, sex, disease extent, best chemotherapy response, presence of sarcopenia, obesity, sarcopenic obesity, and myosteatorsis at diagnosis, and tumor location were also examined. Age was dichotomized at the mean for Kaplan-Meier and Cox proportional analysis. Results of Kaplan-Meier survival analysis are reported as median survival with log base p values. Results of Cox proportional analysis are reported as hazard ratios with 95% confidence intervals.

Mean age, BMI, tissue indices, changes in tissue indices, Hounsfield units, total number of CT scans, and months between initial and final CT scan were compared between cachexia phenotypes using ANOVA. Tukey's method was performed when the ANOVA revealed a difference to identify where the difference occurred. Changes in BMI, tissue indices, and Hounsfield units were analyzed by one-sample T test to evaluate for difference from zero. Sex, disease extent, disease response, tumor location, obesity, sarcopenia, sarcopenic obesity and myosteatorsis were compared between cachexia phenotypes using Pearson's chi-square test. All continuous variables are reported as the mean with standard deviation, with the exception of total number of CT scans and months between initial and final CT scan, which are reported with the range. All categorical variables are reported as the true measurement with percentage.

Multivariate analysis was performed using generalized linear regression. All variables were included in the multivariate analysis except sarcopenia and obesity. Age was dichotomized at the mean for multivariate analysis. Results are reported as difference

in months of survival compared to the stated reference with 95% confidence intervals and p values.

All statistical analysis was performed using IBM SPSS Statistics for Windows version 23.0 (SPSS, Chicago, IL). Images for Kaplan-Meier curves and tissue trend graphs were created using GraphPad Prism version 7 (GraphPad Software, LaJolla, CA). Waterfall plots were created using Microsoft Excel for Windows 2016 (Microsoft Corporation, Redman, WA). Significance was set at a p value < 0.05 for all results.

Results

Demographic and disease data are summarized in Table 1. Overall mean age was 59.5 (SD=9.9) years. The majority of the patients were male, 62.3%. Patients presented equally with locally advanced and metastatic disease, 49% and 51% respectively. The majority of patients had positive response to chemotherapy with 43% having tumor regression and 40% having stable disease compared to 17% with tumor progression. The pancreatic head (51%) was the most common place for the primary tumor followed by the body (38%) then the tail (11%). Overall patients were less likely to be obese (44%) or have sarcopenic obesity (11%) and equally likely to have sarcopenia (49%) at presentation. A total of 296 CT scans were analyzed for an overall mean of 5.6 (range=2.0-18.0) scans per subject. Mean time between initial CT scan and final CT scan analyzed was 11.1 months. Overall median survival was 14.7 months for the entire cohort (Figure 2A). History of weight loss prior to presentation was unknown.

Based on SKMI and TAI trend analysis (Figure 3) three distinct wasting patterns were identified: The majority of patients developed MFW, 64%, followed by NW, 19%, and FW, 17%. There was no difference between phenotypes in age, sex, disease extent, disease response, tumor location, obesity, sarcopenia, myosteatorsis, mean number of CT scans or time between CT scan. Sarcopenic obesity was more likely to be present in the FW phenotype.

Initial body composition measurements and changes are summarized in Table 2. There was no difference in initial BMI, IMATI, VATI, SCATI, or TAI. A difference was observed in initial SKMI and SKM HU between the groups ($p=0.036$ and $p=0.045$). Post-hoc analysis revealed the FW phenotype started with lower SKMI and SKM HU

measurements than the MFW phenotype. Figure 4 illustrates the tissue changes for each patient. The NW phenotype showed no significant losses in any measured variable and actually showed significant increases in BMI, IMAT, SCAT, and TAI. The FW phenotype showed significant losses in all adipose tissue compartments and in total adipose tissue. The MFW phenotype showed significant losses in all measured variables including SKM HU.

Univariate analysis showed that cachexia phenotype, chemotherapy response, and tumor location were associated with survival. FW phenotype had an OMS of which was significantly longer than FW and MFW (Figure 2B). FW and MFW were associated with an increased of mortality when compared to NW (Table 3). Presence of sarcopenic obesity on initial CT scan was associated with a significantly decreased OMS (Figure 2C). Other variables associated with decreased OMS and increased risk of mortality were progression of disease while on FOLFIRINOX therapy and primary tumors located in the tail of the pancreas (Table 3).

Multivariate analysis showed cachexia phenotype, chemotherapy response, and tumor location to be independently associated with overall survival. Development of FW is associated with a mean decrease in survival of 9.9 months while development of MFW is associated with a mean decrease in survival of 8.8 months when compared to NW. Progression of disease while on FOLFIRINOX was associated with a mean decrease in survival of 7.0 months when compared with regression of disease (Table 3).

Median time to onset of cachexia was 71.5 days. Comparing survival of patients who had early onset cachexia, before the median, and those that had late onset cachexia,

after the median, showed no difference, 16.1 vs. 12.2 months (p=0.88), or risk of mortality (HR=1.06; 95%CI=0.50-2.24).

Table 1. Patient Characteristics (N=53)

Variable	Overall	NW (19%)	FW (17%)	MFW (64%)	P Value
Age, years (SD)	59.5 (9.9)	58.8 (7.7)	61.0 (12.4)	59.4 (10.0)	0.88
Sex					
Male	33	7	5	13	0.81
Female	20	3	4	21	
Disease Extent					
Locally Advanced	26	3	6	17	0.28
Metastatic	27	7	3	17	
Disease Response					
Regression	23	6	5	12	0.52
Stable	21	2	3	16	
Progression	9	2	1	6	
Tumor Location					
Head	27	2	6	19	0.22
Body	20	6	3	11	
Tail	6	2	0	4	
Obesity at Dx					
Yes	18	2	4	12	0.51
No	35	8	5	22	
Sarcopenia at Dx					
Yes	26	5	7	14	0.15
No	27	5	2	20	
Sarcopenic Obesity					
Yes	6	0	4	2	0.002
No	47	10	5	32	
Myosteotosis					
Yes	28	6	7	15	0.18
No	25	4	2	19	
Mean # CT scans (range)	5.6 (2-18)	6.9 (2- 18)	4.2 (2-7)	5.6 (2-13)	0.21
Mean Months between initial and final CT scan (range)	11.1 (0.7 - 45-6)	13.1 (6.7- 24.7)	11.2 (0.9- 45.6)	10.5 (0.7- 28.2)	0.71

Table 2. Baseline Body Composition and Changes

Variable	Overall (SD)	NW	P Value	FW	P Value	MFW	P Value	P Value among cachexia phenotypes
Initial BMI (kg/m ²)	28.8 (7.3)	26.3 (4.8)		28.7 (12.4)		29.5 (6.1)		0.48
BMI change (kg/m²)	-2.9 (5.9)	2.2 (2.7)	0.028	-5.5 (11.2)	0.129	-3.7 (3.5)	<0.001	0.005
BMI % change	-8.3 (15.0)	8.3 (9.4)	0.021	-12.5 (19.5)	0.058	-12.1 (11.7)	<0.001	<0.001
Initial SKMI (cm²/m²)	46.9 (9.5)	44.4 (8.3)		40.8 (7.1)		49.2 (9.6)		0.036
SKM change (kg)	-3.5 (5.9)	3.7 (5.4)	0.056	0.7 (1.4)	0.144	-6.8 (3.9)	<0.001	<0.001
SKMI % change	-7.2 (13.3)	10.1 (14.6)	0.056	2.2 (3.5)	0.911	-14.8 (6.5)	<0.001	<0.001
Initial IMATI (cm ² /m ²)	4.1 (2.6)	4.5 (2.9)		4.1 (2.9)		3.9 (2.6)		0.85
IMAT change (kg)	-0.09 (0.3)	0.1 (0.1)	0.003	-0.2 (0.2)	0.034	-0.1 (0.3)	0.006	0.005
IMATI % change	-13.0 (78.2)	44.6 (52.6)	0.025	-40.0 (25.5)	0.001	-22.7 (86.1)	0.133	0.027
Initial VATI (cm ² /m ²)	49.6 (34.6)	48.5 (41.8)		31.9 (18.0)		54.6 (34.9)		0.22
VAT change (kg)	-2.1 (2.8)	0.8 (2.0)	0.240	-2.0 (1.9)	0.016	-2.9 (2.7)	<0.001	<0.001
VATI % change	-28.5 (64.9)	50.3 (103.0)	0.157	-49.6 (33.9)	0.001	-46.1 (33.8)	<0.001	<0.001
Initial SCATI (cm ² /m ²)	71.3 (46.2)	57.5 (28.4)		53.0 (30.4)		80.2 (51.8)		0.169
SCAT change (kg)	-2.8 (3.9)	1.3 (1.0)	0.003	-2.0 (2.2)	0.026	-4.3 (4.0)	<0.001	<0.001
SCATI % change	-27.8 (47.9)	36.0 (62.8)	0.103	-34.3 (27.4)	0.002	-44.8 (28.6)	<0.001	<0.001
Initial TAI (cm ² /m ²)	120.9 (61.7)	106.0 (59.1)		84.9 (41.3)		134.8 (63.4)		0.066
TA change (kg)	-5.0 (5.9)	2.2 (2.6)	0.028	-4.1 (4.0)	0.015	-7.3 (5.2)	<0.001	<0.001
TAI % change	-29.5 (49.8)	37.8 (69.1)	0.118	-46.2 (27.1)	<0.001	-46.2 (27.1)	<0.001	<0.001
Initial SKM HU	35.0 (7.1)	34.3 (6.5)		30.0 (7.3)		36.5 (6.8)		0.045
SKM HU change	-0.7 (8.3)	-1.6 (5.0)	0.34	9.6 (12.5)	0.089	-3.2 (5.3)	0.001	<0.001
SKM HU % change	-0.04	-6.3 (16.2)	0.247	38.1 (48.7)	0.078	-8.3 (15.1)	0.003	<0.001

Table 3. Survival Analysis (Overall Median Survival = 14.7 months)

Variable	Survival			Univariate Analysis			Multivariate Analysis		
	Median OS (months)	P value	Hazard Ratio	95% CI	P value	Months Difference	95% CI	P value	
Age, years									
<59.5	16.1	0.066	Reference	Reference	0.07	Reference	Reference	0.50	
≥59.5	12.8		1.76	0.96 – 3.23		-1.41	-5.5 – 2.7		
Gender									
Female	18.9	0.56	Reference	Reference	0.56	Reference	Reference	0.91	
Male	13.0		1.2	0.65 – 2.21		-0.94	-5.2 – 3.4		
Disease Extent									
Locally Advanced	17.0	0.23	Reference	Reference	0.23	Reference	Reference	0.91	
Metastatic	13.0		1.43	0.8 – 2.64		0.30	-4.7 – 5.3		
Chemotherapy Response									
Regression	18.0		Reference	Reference		Reference	Reference		
Stable	16.1	0.001	1.3	0.7 – 2.5	0.46	1.56	-3.3 – 2.5	0.53	
Progression	8.3		2.1	1.4 – 3.3	0.001	-6.98	-12.8 – -1.1	0.02	
Sarcopenia									
No	17.0	0.32	Reference	Reference	0.32	N/I			
Yes	13.0		0.35	0.96					
Obesity									
No	14.2	0.90	Reference	Reference	0.90	N/I			
Yes	17.0		0.96	0.50 – 1.85					
Sarcopenic Obesity									
No	16.1	0.04	Reference	Reference	0.05	Reference	Reference	0.87	
Yes	10.4		2.47	1.0 – 6.1		-0.69	-8.7 – 7.3		
Myosteatosis									
No	17.8	0.21	Reference	Reference	0.21	Reference	Reference	0.09	
Yes	14.2		1.48	0.8 – 2.74		-3.9	-8.3 – 0.54		
Tumor Location									
Head	17.8		Reference	Reference		Reference	Reference		
Body	14.7	0.02	1.2	0.6 – 2.3	0.58	-2.7	-7.5 – 2.2	0.28	
Tail	10.4		2.1	1.2 – 3.8	0.01	-7.1	-14.5 – 0.34	0.06	
Cachexia Phenotype									
No Wasting	22.6		Reference	Reference		Reference	Reference		
Fat Only Wasting	13.0	0.02	5.2	1.5 – 17.3	0.008	-10.4	-18.5 – -2.2	0.01	
Muscle and Fat Wasting	12.2		1.8	1.1 – 2.9	0.02	-9.9	-15.6 – -4.3	0.001	

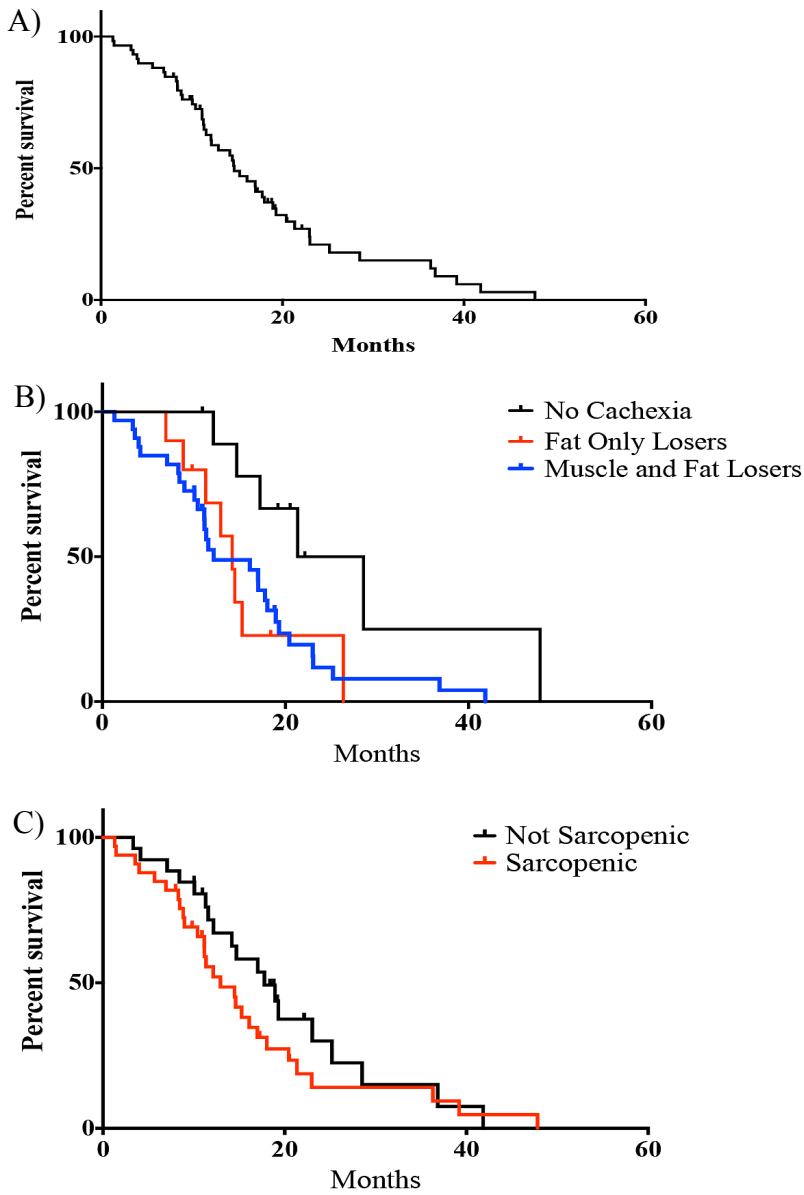


Figure 2. Kaplan-Meier Survival Curves. A) Overall median survival for the entire cohort was 14.7 months. B) Patients with NW had significantly increased median overall survival of 22.6 months compared to those with FW and MFW with median overall survivals of 13.0 and 12.2 months, respectively. C) Absence of sarcopenia on initial CT scan did not result in statistically significant increased survival compared to sarcopenia being present on initial CT scan, 17.0 and 13.0 months respectively.

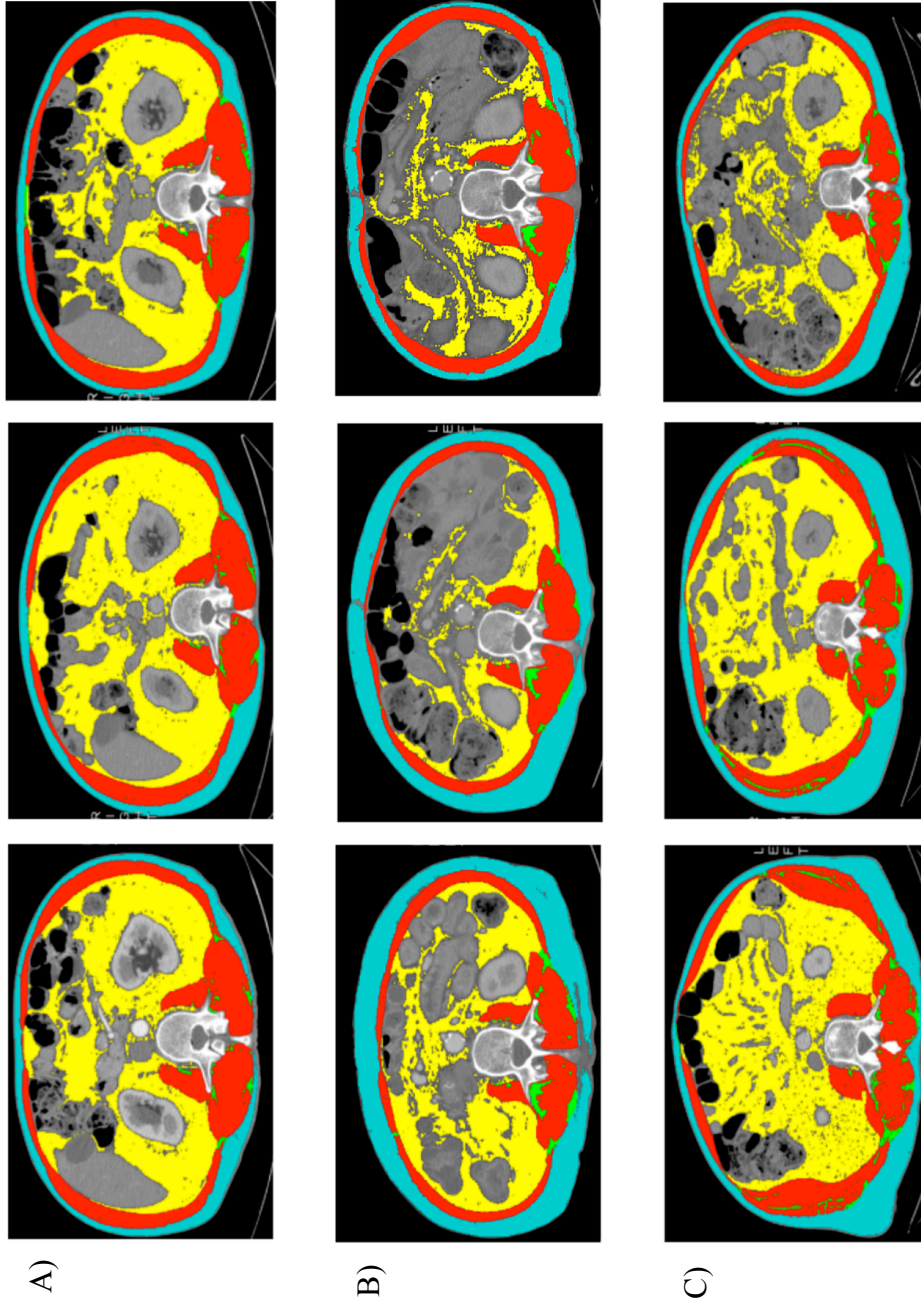


Figure 3. Single Patient Serial Slice-O-Matic Images. A) Representative images from a patient with NW phenotype demonstrates consistency in tissue masses between images B) Representative images from a patient in the FW phenotype clearly shows significant decrease in VAT and SCAT. C) Representative images from a single patient in the MFW phenotype shows marked decreases in SKM, VAT, and SCAT.

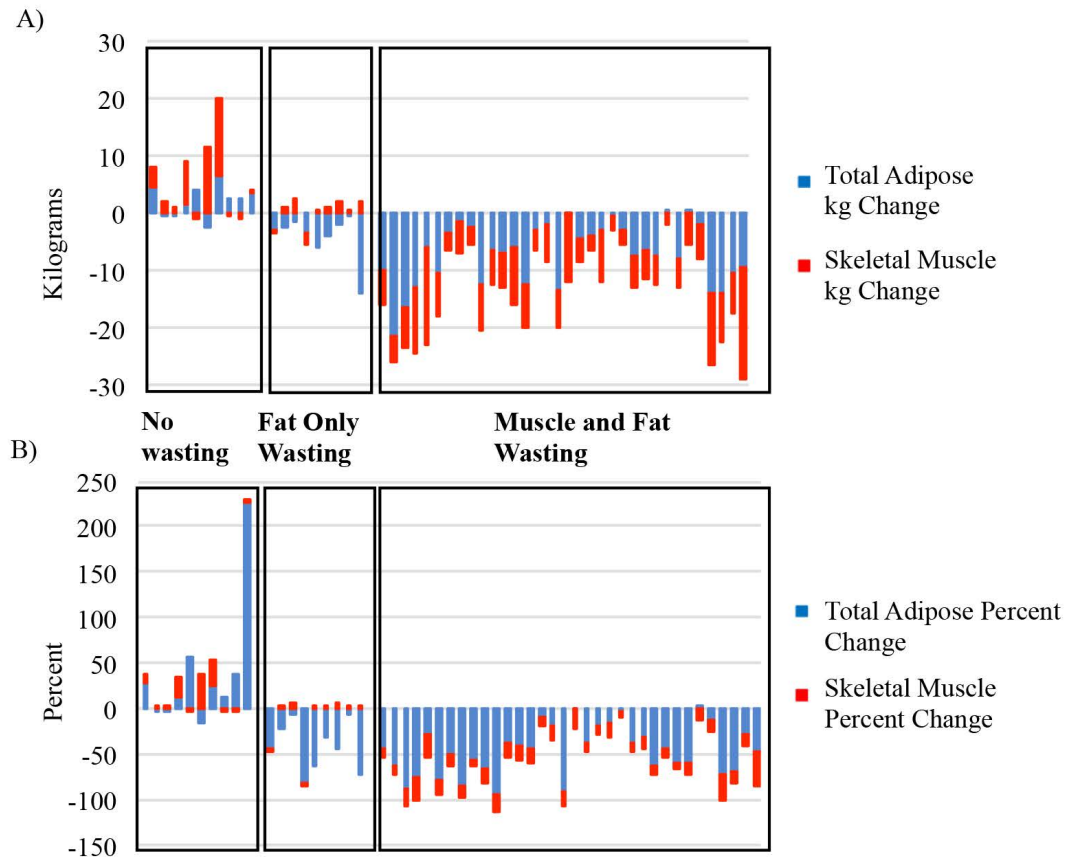


Figure 4. Muscle and Adipose Changes by Cachexia Phenotype. NW phenotype had nonsignificant loss of muscle or fat and most actually gain muscle and/or fat, while the FW group shows loss of only fat and the MFW shows major loss of muscle and fat when measured as kilograms (A) and as percent of initial measurement (B).

Chapter Three

Conclusion

Cachexia has been associated with decreased response and tolerance to therapy and decreased survival in patients with cancer. This study identifies three distinct cachexia phenotypes that are present in patients with advanced PDAC undergoing FOLFIRINOX therapy. The data demonstrates that there is a subset of patients that are resistant to cachexia, the NW group, and that this resistance confers a survival advantage. Additionally, the data shows no difference in survival between patients who lose only adipose tissue, FW, and those that lose muscle and adipose tissue, MFW, suggesting adipose tissue plays a crucial role in cachexia. Three phenotypes suggests molecular or genetic heterogeneity of the host or tumor and identifying these differences will be vital to defining optimal treatment for cachexia. Blunting or possibly preventing cachexia may confer a significant survival advantage in patients with advanced PDAC.

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Curriculum Vitae Joshua Kays

Education

University of Illinois
Doctor of Medicine

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Indiana University
Masters of Science in Translational Research

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Northern Illinois University
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Professional Training

Indiana University
General Surgery Resident

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July 2013 – current

Indiana University
Oncology Research Fellow
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July 2015 – July 2017

Research Focus: Description of the clinical course and identification of clinical markers of cancer cachexia with special focus on pancreatic ductal adenocarcinoma

Licensing

Physician License State of Indiana – Active
USMLE Step 1 – passed June 2011
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Credentials/Certifications

Basic Life Support – up to date
Advance Cardiac Life Support – up to date
Advance Trauma Life Support – up to date

Research/Publications

Kays JK, et al. Resection or transplantation for hepatocellular carcinoma: is the decision clear beyond the Milan criteria? *Hepatobiliar Surg Nutr.* August 2017; 6(4):284-286

Dolejs SC, Beane JD, **Kays JK**, et al. The model for end-stage liver disease predicts outcomes in patients undergoing cholecystectomy. *Surgical Endoscopy.* May 2017: 1-9

Bonetto A, **Kays JK**, et al. Differential bone loss in mouse models of cancer cachexia. *Frontiers of Physiology.* January 2017; 7(679):1-10

Valsangkar N, **Kays JK**, et al. The impact of the members of the Society of University Surgeons on the scholarship of American surgery. *Surgery*. July 2016;160(1):47-53

Professional Presentations

Kays JK, et al. Distinct cachexia phenotypes and the importance of adipose tissue loss on survival of patients with advanced pancreatic cancer on FOLFIRINOX. *Indiana University Department of Surgery Research Day*. Indianapolis, IN. 2017.

Kays JK, et al. Subcutaneous fat mass is independently associated with survival in women with ovarian cystadenocarcinoma. *Academic Surgical Congress*. Las Vegas, NV. 2017

Kays JK. et al. Three distinct cachexia phenotypes are associated with survival in advanced pancreatic ductal adenocarcinoma. *3rd Cancer Cachexia Conference*. Washington, DC. 2016

Kays JK, et al. Muscle wasting as a critical evolutionary conserved response to liver regeneration. *Indiana University Department of Surgery Research Day*. Indianapolis, IN. 2016

Kays JK, et al. Changes in Body Composition Correlate With Survival In Patients With Pancreatic Adenocarcinoma on FOLFIRINOX Therapy. *Indiana University Simon Cancer Center Research Day*. Indianapolis, IN. 2016. (Poster)

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Teaching Experience

Indiana University School of Medicine Clinical Educator	Indianapolis, IN July 2013 – current
Responsible for clinical education of medical students and physician assistant students rotating on General Surgery services service	

University of Illinois College of Medicine M3 Orientation Instructor	Peoria, IL July 2012
Taught incoming third year medical students basic suturing and knot tying techniques	

University of Illinois College of Medicine Peoria Manual High School Enrichment Program Instructor	Peoria, IL 2011-2013
Taught high school students interested in medical careers various healthcare topics	

Awards

Indiana University Simon Cancer Center Research Day 2016

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