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Circulating interleukin-6 is associated with disease progression, but not cachexia in pancreatic cancer

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ABSTRACT

Background: Cachexia is a wasting syndrome characterized by involuntary loss of >5% body weight due to depletion of adipose and skeletal muscle mass. In cancer, the pro-inflammatory cytokine interleukin-6 (IL-6) is considered a mediator of cachexia and a potential biomarker, but the relationship between IL-6, weight loss, and cancer stage is unknown. In this study we sought to evaluate IL-6 as a biomarker of cancer cachexia while accounting for disease progression.

Methods: We retrospectively studied 136 subjects with biopsy-proven pancreatic ductal adenocarcinoma (PDAC), considering the high prevalence of cachexia is this population. Clinical data were abstracted from subjects in all cancer stages, and plasma IL-6 levels were measured using a multiplex array and a more sensitive ELISA. Data were evaluated with univariate comparisons, including Kaplan-Meier survival curves, and multivariate Cox survival models.

Results: On multiplex, a total of 43 (31.4%) subjects had detectable levels of plasma IL-6, while by ELISA all subjects had detectable IL-6 levels. We found that increased plasma IL-6 levels, defined as detectable for multiplex and greater than median for ELISA, were not associated with weight loss at diagnosis, but rather with the presence of metastasis ($p < 0.001$ for multiplex and $p = 0.007$ for ELISA). Further, while >5% weight loss was not associated with worse survival, increased plasma IL-6 by either methodology was.

Conclusion: Circulating IL-6 levels do not correlate with cachexia (when defined by weight loss), but rather with advanced cancer stage. This suggests that IL-6 may mediate wasting, but should not be considered a diagnostic biomarker for PDAC-induced cachexia.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the second leading cause of cancer-related deaths with a poor 5-yr survival of <10% [1]. While these symptoms are due to local tumor effects, patients also

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Abbreviations:

BMI	body mass index
IL-6	Interleukin-6
PDAC	pancreatic ductal adenocarcinoma

frequently present with systemic such as weight loss. Tumor-induced inflammation leads to anorexia and has been suggested as the cause of the hypermetabolic state observed in many cancer patients [2,3]. Additionally, anatomic compression from the mass can cause gastric outlet obstruction and/or exocrine pancreatic insufficiency leading to impaired oral intake and malabsorption, respectively. This combination of factors can lead to cachexia, which is characterized by weight loss due to the loss of adipose and skeletal muscle tissues. Although objective data are available for body weight in electronic medical records for patients, pre-morbid body weights are often unavailable, and self-reported weights are at risk for recall bias [4–6]. This is a key limitation in the field of cancer cachexia research and illustrates the need for a more objective disease biomarker.

Among pro-inflammatory cytokines, there has traditionally been specific interest in interleukin-6 (IL-6) as a biomarker for PDAC-induced cachexia. Early studies in a murine model of cachexia demonstrated a positive correlation with weight loss and increased serum IL-6 levels [7]. The abrogation of cachexia in mice with an IL-6 antibody has suggested that IL-6 may be a primary mediator of cachexia. Similarly, IL-6 levels have been reported to be elevated in the sera of subjects with PDAC-induced cachexia compared to patients without cachexia [8,9]. However, other studies have failed to find an association between circulating IL-6 and cachexia, and importantly, many studies demonstrating an association between IL-6 and cachexia have not considered differences in tumor and cancer stage as potential confounding variables [10]. We and others have reported that increased serum levels of IL-6 in treatment-naïve subjects with PDAC are associated with disease progression and decreased survival [11–13], but whether cachexia is associated with increased circulating IL-6 independent of these factors has not been assessed. Therefore, in the current study we sought to determine if elevated IL-6 levels in subjects with PDAC-induced cachexia is related to weight loss or simply reflective of an advanced cancer stage.

Results

Clinical and demographic data

In our study using potentially resectable subjects (Cohort A) and those with known metastatic PDAC (Cohort B), a total of 74 (54.4%) subjects were classified as Stage III or IV at study enrollment (Table 1). Twenty-seven (33.8%) subjects in cohort A had received neoadjuvant treatment, generally chemotherapy followed by chemoradiation, prior to surgery (and collection of plasma). The mean age at diagnosis in the overall study population was 65.5 years with an even gender distribution. Subjects in cohort B were younger at diagnosis and were more likely to be Caucasian, but otherwise had a similar demographic profile compared to cohort A. The relative weight change at diagnosis was −6.2% (interquartile range (IQR) −12.2–0.0), which was similar in the two study cohorts. Furthermore, the proportion of subjects losing >5% and >10% of their usual body weight was similar. These comparisons were essentially unchanged when only the 53 treatment-naïve subjects in Cohort A were analyzed (Supplemental Table).

Factors associated with increased circulating IL-6 levels

We initiated our analysis of IL-6 using a multiplex platform. Results showed that only 43/136 (31.4%) subjects had levels of plasma IL-6 above the detection threshold, so data were dichotomized as detectable or undetectable. Detectable plasma IL-6 levels were associated with increased primary tumor size, the presence of metastases, as well as higher serum CA-19-9, AST, and absolute neutrophil counts (Table 2). Importantly, detectable plasma IL-6 levels using multiplex were not associated with the degree of weight loss (relative or absolute) at diagnosis or usual adult BMI. Comparisons remained unchanged when performing a sensitivity analysis excluding those in the surgery cohort receiving therapy prior to surgery, with the exception that differences in serum AST and absolute neutrophil counts were no longer statistically different (*data not shown*).

To confirm these findings, we performed a similar analysis using a high-sensitivity ELISA detection method. Using this platform, all subjects had measurable IL-6 and were dichotomized as greater than or less than the median value (2.28 pg/mL). Subjects with high IL-6 were heavier overall, including higher usual adult BMI and BMI at diagnosis. Importantly, high IL-6 was associated with the presence of metastases (Table 2). Even though subjects with high IL-6 had greater absolute weight loss at the time of diagnosis, the rates of cachexia and relative weight loss were not different between the groups.

Next, a multivariable logistic regression model was fit to assess the relationship between increased plasma IL-6 and clinical factors of interest. By multiplex, the presence of detectable plasma IL-6 levels was most strongly associated with the presence of metastases (OR 8.25, 95% CI 3.09–21.95, $p < 0.001$) (Table 3, Model 1). Although detectable plasma IL-6 was also associated with higher BMI and baseline neutrophil counts, the associated effect sizes were negligible. Similarly, the presence of metastases was strongly associated with increased IL-6 values by ELISA (OR 3.44, 95% CI 1.41–8.41, $p = 0.007$). Otherwise, female sex, usual body weight, and AST levels were statistically significant, but had small effect sizes (Table 3, Model 2). Sensitivity analyses were performed for both models excluding subjects from cohort A (potentially resectable subjects) with previous chemotherapy exposure, and showed similar results, including effect sizes (*data not shown*).

Increased circulating IL-6 levels, but not weight loss, correlate with poorer survival

We evaluated the association between weight loss and IL-6 with survival. As anticipated, those subjects with an advanced cancer stage and poor performance status had decreased survival. Consistent with our previous findings and the work of others [11–13], increased plasma IL-6 levels were also associated with poorer survival, irrespective of the method used to measure IL-6 (Fig. 1). Interestingly, weight loss >5% was not associated with shorter survival, however weight loss >10% showed a trend towards significance ($p = 0.074$). These results remained largely unchanged when subjects with prior therapy were excluded (*data not shown*).

Finally, Cox survival models were fit for both IL-6 detection methods. Increased plasma IL-6 was associated with increased mortality irrespective of the detection method (Table 4). As expected, an advanced cancer stage was also associated with mortality. However, a relative weight loss >5% was again not associated with mortality.

Table 1
Clinical and demographic data for subjects with biopsy-proven pancreatic ductal adenocarcinoma (PDAC) in this study. Cohort A denotes subjects enrolled from a prospective study in those undergoing surgery, while cohort B indicates those who were selected for study from a clinical trial in metastatic PDAC. Categorical variables are shown as n (%) and continuous variables are summarized with means and standard deviations unless otherwise noted. dx, diagnosis; BMI, body mass index.

	Overall (n = 136)		Cohort A (n = 80)		Cohort B (n = 56)		p-value
Age at dx	65.5	10.3	67.5	10.1	62.6	10.0	0.007
Male sex	74	54.4	39	48.8	35	62.5	0.113
Caucasian Race[a]	124	91.2	71	88.8	53	94.6	0.034
Usual BMI (kg/m^2)	30.6	6.5	30.7	6.9	30.4	6.0	0.817
BMI at dx (kg/m^2)	27.7	5.4	27.7	6.1	27.7	4.4	0.974
Weight Change at dx (%)[b]	−6.2	−12.2–0	−7.9	−12.0–0	−5.6	−12.4–0	0.688
Weight Loss >5%	70	56.9	43	61.4	27	50.9	0.245
Weight Loss >10%	38	30.9	23	32.9	15	28.3	0.588
ECOG at dx[a]							<0.001
0	51	38.9	15	20.0	36	64.3	
1	70	53.4	51	68.0	19	33.9	
2	8	6.1	7	9.3	1	1.8	
3	1	0.8	1	1.3	0	0.0	
4	1	0.8	1	1.3	0	0.0	
Tumor Size (cm)	3.8	1.8	3.5	1.7	4.4	1.8	0.003
Tumor Location[a]							0.001
Head/Uncinate	75	55.6	53	67.1	22	39.3	
Body/Tail	60	44.4	26	32.9	34	60.7	
AJCC Cancer Stage[a]							<0.001
IA	6	4.4	6	7.5	0	0.0	
IIA	7	5.2	7	8.8	0	0.0	
IIB	49	36.0	48	60.0	0	0.0	
III	10	7.4	10	12.5	0	0.0	
IV	64	47.1	9	11.3	56	100	
Surgery Type							<0.001
None	38	27.9	0	0.0	38	67.9	
Whipple	45	33.1	43	53.8	2	3.6	
Distal Pancreatectomy	13	9.6	13	16.3	0	0.0	
Total Pancreatectomy	8	5.9	8	10.0	0	0.0	
Other (includes palliative bypasses)	32	23.5	16	20.0	16	28.6	

[a] Analyzed with Fisher's exact test.
[b] Summarized with median and interquartile range (IQR) and analyzed with Wilcoxon's rank sum test.

Discussion

In subjects with PDAC from all cancer stages increased IL-6 levels are not associated with weight loss >5%, suggesting that IL-6 should not be broadly considered as a biomarker for PDAC-induced cachexia. Relative weight loss was not associated with increased IL-6 levels even when a higher threshold (>10%) was considered. Rather, increased IL-6 levels are strongly associated with an advanced cancer stage and independently associated with mortality. In this study, there was a low proportion of subjects with detectable IL-6 levels measured by multiplex, which may be a consequence of the diverse stage distribution of subjects; this challenge was overcome by using ELISA as a more sensitive detection method. These data suggest that when considering all cancer stages, increased plasma IL-6 is not a biomarker of PDAC-induced cachexia.

Multiple groups have evaluated the use of increased IL-6 levels as a diagnostic biomarker for PDAC compared to a variety of controls, including benign biliary obstruction, chronic pancreatitis, and healthy controls [14–18]. Not all studies have demonstrated accurate diagnostic classification, which may reflect differences in study design. For example, the selection of hospitalized control subjects, who may have other diseases leading to systemic inflammation and elevated IL-6 levels, would decrease the likelihood of observing a difference in IL-6 levels between PDAC and controls [18].

Among patients with PDAC, IL-6 levels have also been evaluated as a prognostic marker. Importantly, elevated IL-6 has been associated with tumor burden, the presence of liver metastases, and shorter overall survival [11,12,15]. Circulating IL-6 as a prognostic biomarker of PDAC is consistent with preclinical data showing reduced tumor progression with mono- or combination IL-6 blockade, demonstrating a role for IL-6 in disease progression [19,20]. Our data agrees with previous reports that across all stages of PDAC, elevated circulating levels of IL-6 are associated with advanced disease and decreased overall survival.

The increased circulating IL-6 levels observed in PDAC, particularly in patients with worse clinical outcomes, is assumed to promote a pro-inflammatory state leading to the development of PDAC-induced cachexia. This hypothesis was fueled by preclinical data including those from the murine colon-26 model of colorectal cancer, which demonstrated that IL-6 was associated with weight loss, and this effect was abrogated when the mice were treated with an IL-6 monoclonal antibody [7].

Although IL-6 has been studied as a potential biomarker of PDAC-induced cachexia, results from such studies have been mixed. There is substantial variability in the inclusion criteria used in these studies according to PDAC cancer stage, as well as heterogeneity in study definitions for “weight loss”, use of different detection methods of IL-6, and previous exposure to chemotherapy with or without radiation, which make interpretation of these findings challenging. An early study compared IL-6 levels in PDAC to disease (chronic pancreatitis) and healthy controls, and observed higher levels in those with PDAC and weight loss [9]. In another study of treatment-naïve subjects with advanced PDAC, IL-6 was also associated with weight loss following diagnosis [21]. Yet another study examining subjects from all cancer stages showed a trend toward increased weight loss in those with high IL-6, but this did not reach statistical significance [22]. However, none of these studies have attempted to determine if circulating IL-6 was more strongly associated with tumor progression or cachexia. Our data strongly suggest that instead of serving as a biomarker of PDAC-induced cachexia, IL-6 is instead closely associated with disease

Table 2
Weight patterns and PDAC-related characteristics associated with increased IL-6 measured by Multiplex and ELISA. Continuous variables are summarized with means and standard deviations unless otherwise noted. dx, diagnosis; BMI, body mass index; AST, aspartate transaminase.

	IL-6 measured by multiplex					IL-6 measured by ELISA				
	Undetectable (n = 93)		Detectable (n = 43)		p-value	IL-6 < median (n = 65)		IL-6 >median (n = 66)		p-value
Age at dx	65.6	10.8	65.1	9.3	0.787	64.9	11.0	65.6	9.8	0.718
Male Sex	49	52.7	25	58.1	0.553	34	52.3	37	56.1	0.666
Usual BMI (kg/m^2)	30.1	6.8	31.5	5.8	0.249	28.1	5.3	32.6	6.8	<0.001
BMI at dx (kg/m^2)	27.3	5.6	28.7	4.8	0.165	25.9	4.6	28.9	5.4	0.002
Weight Loss at dx (kg)[b]	4.6	0–11.0	4.5	0–11.9	0.954	4.5	0–9.0	8.0	2.0–15.0	0.031
Weight Loss at dx (%)[b]	6.5	0–12.0	5.3	0–12.4	0.805	6.0	0–10.3	8.7	1.9–15.4	0.082
Weight Loss >5%	48	59.3	22	52.4	0.465	32	55.2	38	63.3	0.367
Weight Loss >10%	25	30.9	13	31.0	0.992	14	24.1	24	40.0	0.065
ECOG at dx[a]					0.031					0.448
0	29	32.2	22	53.7		21	33.3	27	42.9	
1	55	61.1	15	36.6		38	60.3	31	49.2	
2	4	4.4	4	9.8		3	4.8	4	6.4	
3	1	1.1	0	0.0		0	0.0	1	1.6	
4	1	1.1	0	0.0		1	1.6	0	0.0	
Tumor Size (cm)	3.6	1.7	4.3	1.9	0.040	3.7	1.5	4.1	2.1	0.208
T Stage[a]					0.172					0.845
1	3	4.2	2	20.0		3	7.1	1	2.9	
2	4	5.6	0	0.0		2	4.8	2	5.9	
3	53	74.7	8	80.0		30	71.4	27	79.4	
4	11	15.5	0	0.0		7	16.7	4	11.8	
Tumor Location[a]					0.741					0.583
Head/Uncinate	52	56.5	23	53.5		37	57.8	35	53.0	
Body/Tail	40	43.5	20	46.5		27	42.2	31	47.0	
M Stage					<0.001					0.044
No	62	66.7	10	23.3		39	60.0	28	42.4	
Yes	31	33.3	33	76.7		26	40.0	38	57.6	
Stage Detailed[a]					<0.001					0.266
IA	4	4.3	2	4.7		4	6.2	1	1.5	
IIA	7	7.5	0	0.0		3	4.6	3	4.6	
IIB	41	44.1	8	18.6		26	40.0	20	30.3	
III	10	10.8	0	0.0		6	9.2	4	6.1	
IV	31	33.3	33	76.7		26	40.0	38	57.6	
Neutrophil count (K/uL)	5.0	1.7	6.0	2.7	0.033	5.2	2.1	5.4	2.2	0.602
AST (U/L) [b]	25.5	17.0–87.5	35.0	23.0–75.0	0.058	23.0	16.0–40.0	46.5	23.0–126	<0.001
Albumin (g/dL)	4.0	0.5	3.8	0.4	0.052	4.0	0.5	3.8	0.5	0.052
CA19-9 (U/mL) [b]	214	50.0–970	2156	193–21550	<0.001	224	32.3–1685	605	111–4741	0.091
Enrollment in Cohort A	69	74.2	11	25.6	<0.001	41	63.1	34	51.5	0.181

[a] Analyzed with Fisher's exact test.
[b] Summarized with median and interquartile range (IQR) and analyzed with Wilcoxon's rank sum test.

Table 3
Multivariable logistic regression models demonstrating the odds ratio for having increased plasma IL-6 levels in 136 subjects with pancreatic ductal adenocarcinoma. Model 1 represents detectable IL-6 (measured by multiplex) and model 2 represents IL-6 greater than the median value (measured by ELISA). dx, diagnosis; BMI, body mass index; AST, aspartate transaminase.

	Model 1			Model 2		
	Odds Ratio	95% CI	p-value	Odds Ratio	95% CI	p-value
Age at dx	1.02	(0.97, 1.06)	0.470	1.02	(0.98, 1.07)	0.274
Sex			0.673			0.039
Male	Reference			Reference		
Female	1.21	(0.50, 2.89)		2.67	(1.05, 6.76)	
M Stage			<0.001			0.007
No	Reference			Reference		
Yes	8.23	(3.09, 21.95)		3.44	(1.41, 8.41)	
BMI at dx (kg/m^2)	1.09	(1.00, 1.18)	0.045	–		
Neutrophil (K/uL)	1.23	(1.01, 1.50)	0.044	–		
Usual Weight (kg)	–			1.05	(1.02, 1.07)	0.001
AST (U/L)	–			1.01	(1.00, 1.01)	0.007

progression.

In this study, there was a low proportion of subjects with detectable IL-6 levels measured by multiplex; this challenge was overcome by using ELISA as a more sensitive detection method. This illustrates how measurement techniques for circulating IL-6 may influence the study results, particularly when the analyte of interest has a relatively low abundance. Multiplex assays have become an increasingly utilized tool in translational research due to the ability to more rapidly and economically perform unbiased explorations on a panel of cytokine and chemokine expression levels. However, it is important to emphasize that these results should be considered 'hypothesis-generating' and require

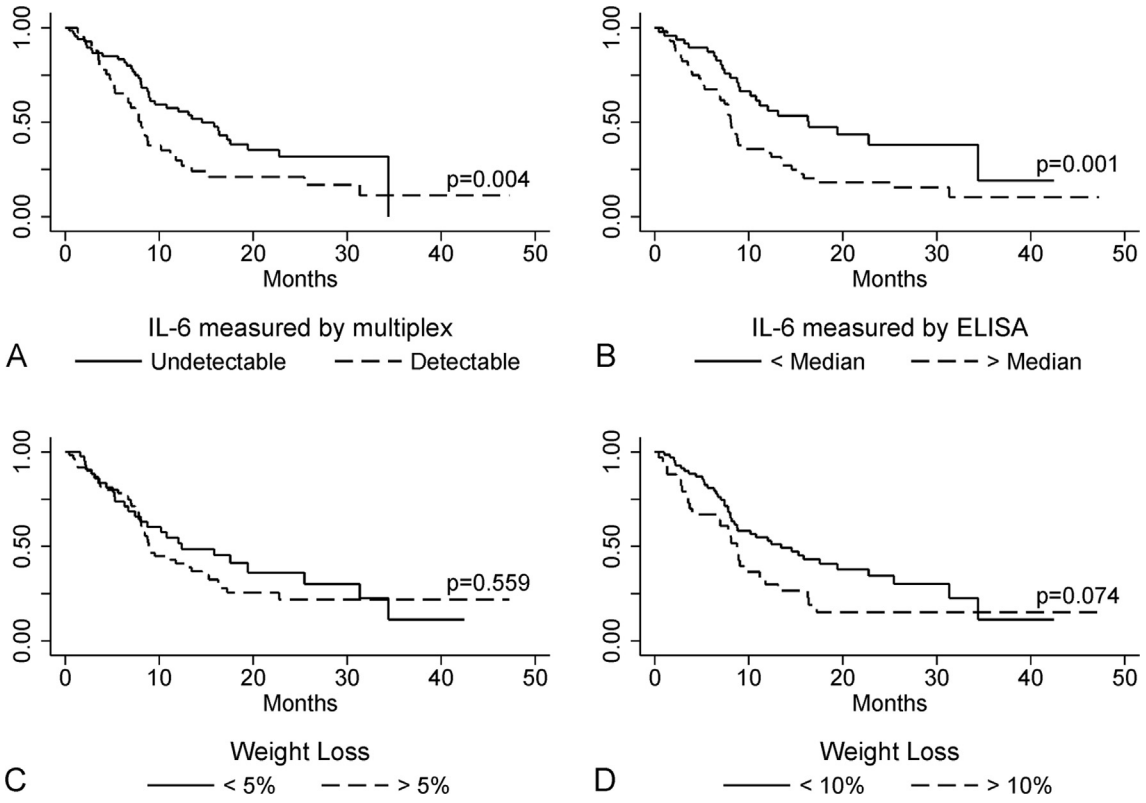

Fig. 1. Kaplan-Meier plots depicting survival comparisons for subjects with pancreatic ductal adenocarcinoma and increased plasma IL-6 levels detected using Multiplex (panel A) and ELISA (panel B) and relative weight loss at the time of diagnosis >5% (panel C) and >10% (panel D). The median survival times were compared using log-rank tests.

Table 4
Multivariate Cox models for survival in pancreatic ductal adenocarcinoma using multiplex (Model 1) and ELISA (Model 2) for detecting increased plasma IL-6 levels. dx, diagnosis.

	Model 1			Model 2		
	HR	95% CI	p-value	HR	95% CI	p-value
Age at dx			0.474			0.606
≤Median	Reference			Reference		
>Median	1.19	(0.74, 1.89)		1.13	(0.71, 1.80)	
Sex			0.576			0.426
Female	Reference			Reference		
Male	1.14	(0.72, 1.81)		1.21	(0.76, 1.94)	
Weight Loss			0.556			0.691
≤5%	Reference			Reference		
>5%	1.16	(0.71, 1.88)		1.11	(0.66, 1.86)	
ECOG at dx			0.368			0.344
0	Reference			Reference		
≥1	1.26	(0.76, 2.10)		1.29	(0.76, 2.18)	
Cancer Stage			0.042			0.027
Early (I or II)	Reference			Reference		
Advanced (III or IV)	1.71	(1.02, 2.85)		1.82	(1.07, 3.10)	
Plasma IL-6 (Multiplex)			0.030	–	–	
Undetectable	Reference			–	–	
Detectable	1.69	(1.05, 2.71)		–	–	
Plasma IL-6	–	–				0.005
≤ Median	–	–		Reference		
>Median	–	–		1.97	(1.23, 3.14)	

validation with a second more sensitive method. In the current study, plasma IL-6 was only detectable in 32% of PDAC subjects when analysis was performed with a multiplex platform, but detectable in 100% of the subjects using an ELISA platform. The use of a more sensitive detection method also permitted greater discrimination between subjects with low values.

Another important aspect that is considered in our study is the potential confounding effect of prior systemic chemotherapy with or without radiation. However, due to the cross-sectional design of this study, serial IL-6 levels were not available for intra-subject comparisons, so sensitivity analyses were performed excluding subjects from cohort A who had received therapy prior to surgery (and blood collection) for key statistical comparisons.

While in the current study increased circulating IL-6 was not

associated with PDAC-induced cachexia when defined according to weight loss, it remains possible that circulating IL-6 levels may be associated with skeletal muscle and adipose loss, as we only assessed change in body weight without consideration of body composition. Future studies comparing circulating IL-6 levels with changes in individual body compartments (i.e., muscle mass, subcutaneous fat, and visceral fat) are needed to further understand this relationship. Furthermore, it is also important to note that we did not assess the expression levels of IL-6 in the tumor or muscle/adipose microenvironments, so it remains possible that local expression of IL-6 may have effects on weight loss or skeletal muscle and adipose loss that cannot be captured by measurement of circulating IL-6 levels. Lastly, there is possibility of confounding from an unmeasured variable, such as diabetes mellitus, that may be independently associated with differences in IL-6 levels. Unfortunately, the retrospective nature of the study design and lack of access to historical records for all study subjects did not allow us to evaluate the effects of diabetes; this challenge should be considered in future study designs.

In our study including subjects with PDAC from all cancer stages, increased circulating IL-6 levels are not associated with weight loss >5%, suggesting that IL-6 should not be broadly considered as a biomarker for PDAC-induced cachexia. Furthermore, relative weight loss is not associated with increased IL-6 levels, even when using a higher threshold (>10%). Rather, increased IL-6 levels are strongly associated with an advanced cancer stage and independently associated with mortality, suggesting the ongoing need to further examine the role of IL-6 in the pathogenesis of PDAC. Further studies to identify novel, objective markers of PDAC-induced cachexia are needed to permit investigation of targeted therapies to address this ongoing clinical need.

Methods

Study subject recruitment

This study was approved by our local Institutional Review Board. The diagnosis of PDAC was confirmed in all subjects by histology from either the primary pancreatic mass or a metastatic site. A total of 136 subjects with pancreatic ductal adenocarcinoma (PDAC) were identified for further study from two independent studies from January 1, 2011–July 1, 2016 at our institution (Fig. 2). The first study is an ongoing prospective registry of patients with PDAC who undergo surgical intervention [23]. Eighty-five subjects were initially identified with potentially resectable PDAC for the current study (designated as cohort A); five subjects were excluded from final analyses as depicted in Fig. 1. The second study was a multicenter clinical trial which included 76 subjects with treatment naïve metastatic PDAC [24]. Of these, 17 were treated at outside institutions, so clinical data and biosamples were unavailable. An additional 3 subjects were excluded due to insufficient quantities of remaining plasma for additional analyses, so a total of 56 subjects were selected for the current study (designated as cohort B), which involves additional analyses regarding weight loss and validation of plasma IL-6 levels with a second detection method (12). None of the subjects enrolled had a preceding diagnosis of chronic pancreatitis.

Data collection and study definitions

Serial data were obtained regarding body weight including the usual body weight and weight at cancer diagnosis, treatment start (either surgery or chemotherapy), and last available follow up. The term “usual body weight” is used to denote a subject's typical weight prior to the onset of cancer-related symptoms, and was typically more than 12 months prior to symptom onset. Usual body weight was derived from subjects' self-reported weight loss for 110/125 (88.0%) of participants. For the purposes of this study, and in line with the consensus definition, cachexia was defined as weight loss >5% of usual body weight at the time of cancer diagnosis [25]. Clinical laboratory values were abstracted within 2 weeks preceding the date of surgery (cohort A) or date of chemotherapy initiation (cohort B). The date of PDAC diagnosis was defined as the date of first pathologic diagnosis. Cancer staging for

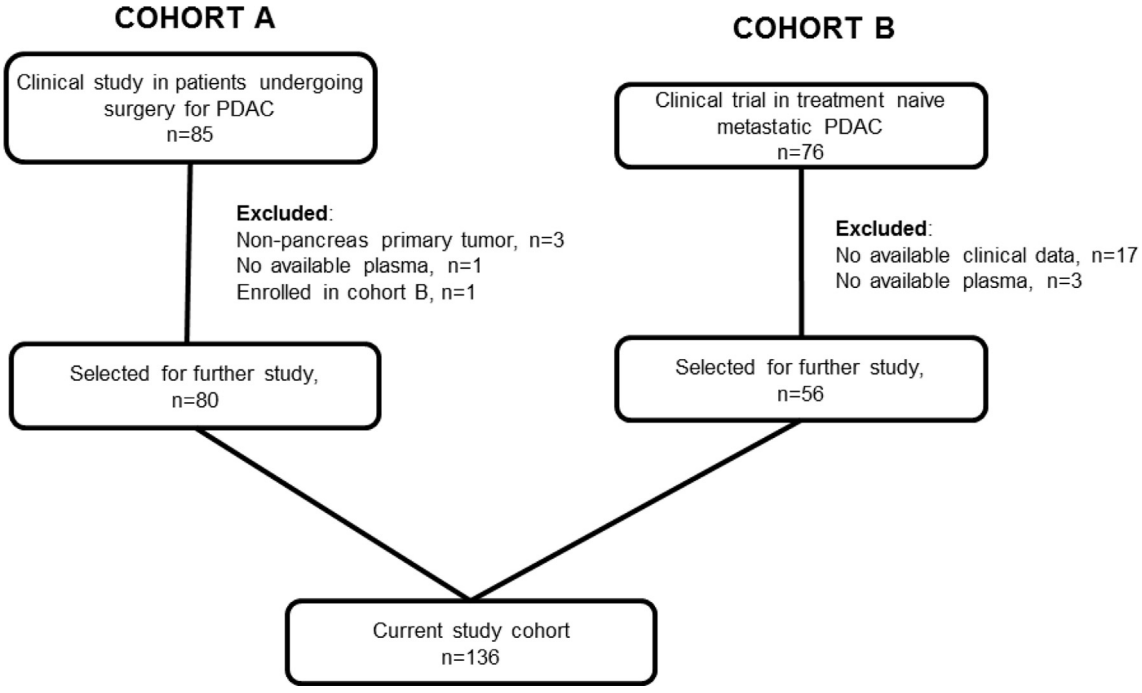

Fig. 2. Study participant flowchart.

the subjects from Cohort A was based on definitive postoperative staging. The date of censoring for survival analyses was the date of last clinical follow-up.

Analyses of plasma cytokine levels

Peripheral blood samples were obtained from all study subjects intraoperatively for those undergoing surgery or immediately prior to the first cycle of chemotherapy for those with metastatic disease. Samples were centrifuged at 1500 rpm for 10 min, and then plasma was aliquoted and stored at −80 °C. Plasma cytokine levels were initially analyzed using Luminex Multiplex Cytokine Kits (Procarta, Cytokine Assay, eBioscience) including IL-6. Samples from each of the two study cohorts were batch processed independently, and all samples were run in duplicate. Since only 32% (43/136) of subjects had a detectable IL-6 level with this method, a higher sensitivity platform was selected for additional comparisons. Thus, all available samples from both cohorts were batch analyzed using an enzyme-linked immunosorbent assay (ELISA) for IL-6 according to the manufacturer's specifications (Human IL-6 ELISA Kit, High Sensitivity, eBioscience).

Statistics

Continuous variables were analyzed using t-tests or Wilcoxon rank sum tests, and categorical variables were analyzed with chi-square or Fisher's exact tests, as appropriate. For comparisons using IL-6 levels, groups were dichotomized into either detectable or undetectable levels (when using the multiplex platform) and greater or less than the median value (when using ELISA). Multivariate logistic regression models were fit including statistically significant and clinically relevant variables (defined *a priori* as age and gender) to identify factors associated with increased plasma IL-6 with both detection methods. Terms in the model were determined through stepwise selection where race (dichotomized to white vs. other), ECOG status (dichotomized to 0 vs ≥ 1), tumor location (dichotomized to head/uncinate vs. body/tail), M stage, any surgery ever, any chemo ever, chemo type, stage (dichotomized to early (stage I or II) vs late (stage III or IV)), weight change at diagnosis, percent weight change at diagnosis, weight at diagnosis, BMI at diagnosis, usual weight, usual BMI, self-reported weight loss, weight at treatment start, weight at last follow-up, tumor size, hemoglobin, neutrophil, lymphocyte, AST, albumin, INR, and CA19-9 were eligible for inclusion in addition to age and sex. Kaplan–Meier curves were used to assess survival differences and estimate median survival among patients who did and did not have increased plasma IL-6 levels. Cox models for survival were also fit, including: age, sex, tumor size, cancer stage, weight loss at cancer diagnosis, and increased plasma IL-6. Sensitivity analyses were performed using an identical methodology, but excluding subjects from Cohort A with previous chemotherapy with or without radiation to assess for confounding from neoadjuvant treatment. All analyses were completed using SAS version 9.4 (Cary, NC). A p-value of <0.05 was considered statistically significant.

Study approval

The Ohio State University IRB reviewed and approved this study of human subjects. Written informed consent was obtained from all subjects.

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Conflicts of interest/disclosures

The authors have declared that no conflict of interest exists.

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Appendix A. Supplementary data

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