Prognostic Value of Combined Detection of Serum IL6, YKL-40, and C-reactive Protein in Patients with Unresectable Pancreatic Cancer

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ABSTRACT

Background: IL6 and YKL-40 (also known as chitinase 3-like 1 protein, CHI3L1) are produced by pancreatic cancer cells and macrophages and activate inflammation. C-reactive protein (CRP) is synthesized mainly in hepatic cells and primarily stimulated by IL6. The aim of this study was to determine the prognostic value of combined detection of serum IL6, YKL-40, and CRP in patients with pancreatic cancer receiving palliative chemotherapy.

Methods: In all, 592 patients with unresectable pancreatic cancer from five hospitals in Denmark were included in the BIOPAC biomarker study between 2008 and 2017. Pretreatment and longitudinal serum values of IL6 and YKL-40 were determined. Baseline CRP and CA19-9 values were available for the whole cohort. Patients were dichotomized as low versus high using cutoffs for IL6 of >4.92 pg/mL, YKL-40 of >95% age-corrected percentile, and CRP of >10 mg/L. The main outcome was overall survival.

Results: Combined elevations of serum IL6, YKL-40, and CRP were associated with worse survival in contrast to an isolated high concentration of a single marker. Serum IL6, YKL-40, and CRP were higher in patients with advanced stage disease and in patients with poor performance status. Higher IL6 and YKL-40 levels at the time of tumor progression and serum IL6 measured over time were associated with shorter overall survival.

Conclusions: Combined high baseline serum levels of IL6, YKL-40, and CRP are associated with poor survival.

Impact: Assessment of systemic inflammation via measurements of IL6, YKL-40, and CRP may be important for pancreatic cancer prognostication.

Introduction

Pancreatic cancer is characterized by lack of early symptoms, advanced stage at the time of diagnosis, resistance to chemotherapy, and a disadvantageous tumor microenvironment due to an excess of stroma, poor vascularity, and a high grade of inflammation and immunosuppression. Pancreatic cancer's aggressive biology, genetic complexity, rapid clinical deterioration, and absence of reliable robust biomarkers yield poor survival (1). Serum cancer antigen 19-9 (CA 19-9) is the only recommended biomarker in the routine management of pancreatic cancer; however, it is limited in use due to low sensitivity and specificity. No prognostic biomarkers have yet been definitively validated in clinical practice in pancreatic cancer.

Tumor-promoting inflammation, which was first hypothesized by Rudolf Virchow in 1863 (2) and later framed into one of the classical hallmarks of the cancer concept (3), is a critical component of pancreatic cancer development and progression and markedly contributes to tumor aggressiveness and chemotherapy resistance. Activation of the systemic inflammatory response and elevated inflammatory markers, for example, C-reactive protein (CRP) and albumin (Glasgow Prognostic Score), white blood cell counts (WBC) especially absolute neutrophil count (ANC), platelet counts, and neutrophil lymphocyte and platelet lymphocyte ratios correlate with poor survival (4–6).

Risk of pancreatic cancer is associated with a variety of inflammatory conditions including smoking, high body mass index (BMI), chronic pancreatitis, heavy alcohol use, inflammatory bowel disease, and long-standing diabetes mellitus (7). Activation of inflammation is largely dependent on IL6 (8, 9), which is one of the proinflammatory cytokines secreted by activated macrophages, lymphocytes, and endothelial and tumor cells (10, 11). IL6 cytokine is a multifunctional signaling protein that facilitates the inflammation cascade and key pathways and processes within the respective tumor microenvironment, among others such as regulation of stromal desmoplasia, promotion of tumor-induced immunosuppression, and facilitation of metastasis (11, 12). Debilitating features (e.g., lack of appetite, weight loss, fatigue, anemia, fever, pain, inactivity, negative mood, cachexia), which characterize behavior in many patients with pancreatic cancer, are also partly associated with IL6 cytokine activation (13, 14). IL6 levels are elevated in the majority of patients with

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Cancer Epidemiol Biomarkers Prev 2020;29:176–84

doi: 10.1158/1055-9965.EPI-19-0672

metastatic cancer (15). However, prospectively tested IL6 and CRP were not associated with pancreatic cancer risk (16, 17).

Another marker with a role in inflammation is YKL-40 (also known as chitinase 3-like 1 protein, CHI3L1), a member of the mammalian chitinase-like proteins, and is a highly conserved glycoprotein. YKL-40 is mainly produced by cancer cells, macrophages, and neutrophils (18–21). YKL-40 elevation is exponentially age dependent (22), possibly due to an increase in age-related inflammation. YKL-40 is elevated in patients with inflammatory disorders (22) and also in subgroups of patients with many cancer types, including up to 80% in patients with pancreatic cancer (19). Furthermore, YKL-40 regulates VEGF and angiogenesis (23), cell proliferation, and differentiation (24); re-modulates extracellular matrix (21); activates Akt signaling (25); protects against apoptosis (26); and promotes metastases (27) and tumor progression (18, 20, 21, 28, 29).

Because of unfavorable prognosis of pancreatic cancer, the lack of effective treatment, and biomarkers to predict disease outcome along with association with inflammation, we explored the prognostic value of the combined detection of serum IL6, YKL-40, and CRP as potential prognostic marker in patients with pancreatic cancer receiving palliative chemotherapy.

Materials and Methods

Study population and blood samples

We analyzed 1,899 serum samples from 592 consecutive patients with unresectable pancreatic cancer undergoing palliative chemotherapy who were included in the BIOPAC study (NCT03311776) from five hospitals in Denmark in the period from July 1, 2008 until December 31, 2017. Blood samples and clinical data at the time of inclusion were collected prospectively. Blood samples were collected at the initiation of palliative chemotherapy, before the second cycle, and at each timepoint of CT evaluation during treatment until cancer progression. Samples were processed according to the nationally approved standard operating procedures for blood samples (www.herlevhospital.dk/BIOPAC; ref. 15s). IL6 and YKL-40 analyses were performed prospectively and blinded to clinical data. WBC, ANC, and platelet counts as well as the majority of CRP determinations were obtained as part of routine care. CA 19-9 as the most frequently used biomarker for pancreatic cancer was measured (Siemens) in all included patients.

Serum IL6 analysis

Pretreatment and longitudinal (before the second cycle and at timepoint of first CT evaluation) serum concentrations of IL6 were determined by a commercially available human IL6 high-sensitive ELISA (Quantikine HS600B, R&D Systems) in accordance with the manufacturer's instructions. The minimal detectable limit for IL6 was 0.01 pg/mL. The intra-assay coefficient of variation (CV) was $\leq$8% and the interassay CV was $\leq$11% (30). Patients were grouped as having low or high values dichotomized using a cutoff for IL6 of >4.92 pg/mL, the 95th percentile in healthy blood donors (30).

Serum YKL-40 analysis

Pretreatment and longitudinal (before the second cycle and at timepoint of first CT evaluation) serum concentrations of YKL-40 were determined by a commercial ELISA (Quidel). The minimal detectable limit for YKL-40 was 10 μg/L. The intra-assay CV was 5% and the interassay CV was <6% (19). Elevated serum YKL-40 was defined as higher than the age-corrected 95th percentile (22).

CRP analysis

In the majority of patients, pretreatment serum CRP concentration was determined as a part of routine blood tests according to the manufacturer's instructions, using a previously validated Sentinel CRP Ultra ready-to-use, liquid assay reagent by an immunoturbidimetric method on a fully automated chemistry analyzer [Kit-test SENTINEL CRP Ultra (UD), 11508 UD-2.0/02 2015/09/23]. The measurement range for CRP is 0.3–640 mg/L, with an intra-assay CV of 3% and an interassay CV of <15%. Elevated serum CRP was conventionally defined as higher than 10 mg/L (4–6).

Statistical analysis

Results are reported in accordance with the REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) guidelines (31). Descriptive statistics were performed to describe patient demographics and baseline clinical characteristics. Spearman's correlation coefficient rank test was used to examine the inter-relationships between the median serum levels of IL6, YKL-40, and CRP and the relation of these levels to CA19-9, WBC, ANC, platelet counts, bilirubin, alkaline phosphatase (ALP), and alanine transaminase (ALT).

The outcome was 3-year overall survival (OS); thus, the patients were followed up from date of inclusion to the BIOPAC study until death from any cause, or the cohort censor date (August 3, 2018), or 3 years after date of inclusion, whichever came first. The levels of baseline serum IL6, YKL-40, and CRP were modeled by the dichotomized value using the actual cut-off point of the biomarker as described under "Materials and Methods" section. Graphical presentation using Kaplan–Meier estimates of OS was shown by grouping patients according to low versus high serum IL6, YKL-40, and CRP levels and with inclusion of the combined values of these markers. HRs and 95% confidence intervals (CI) were estimated with Cox proportional hazards regression. Variables with statistically significant association on univariate analysis were included in multivariable models.

In addition, longitudinally measured biomarkers (IL6 and YKL-40) before the second cycle and at timepoint of first CT evaluation were analyzed as time-dependent variables in a Cox model adjusted for the significant terms from the baseline analysis. Each biomarker was analyzed by including the $\log_2$-transformed baseline value as well as the $\log_2$-transformed ratio between latest value and the baseline value corresponding to HRs representing the effect of doubling the baseline value.

Finally, to evaluate the biomarkers ability to predict early death (<90 days), analysis by the ROC curve by the area under the curve (AUC) was performed. In addition, the associations between biomarkers and early death were analyzed using logistic regression.

Statistical analyses were conducted using the latest version of *R* (version 3.2.4). In all analyses, the significance level was set to 5%.

Results

Characteristics of study population

Between July 2008 and December 2017, a total of 592 consecutive patients with unresectable pancreatic cancer undergoing palliative chemotherapy were registered in the BIOPAC database. The 592 patients represent 20% of all patients with unresectable pancreatic cancer treated with chemotherapy during this period in Denmark. The median follow-up was 6.7 months, with no loss to follow-up.

Patient and disease characteristics, treatment modalities, and baseline blood test results are given in **Table 1**. The median age at inclusion was 68 years (interquartile range, 62–73 years). The cohort consisted of

Table 1. Clinical characteristics and baseline blood test results in 592 patients with unresectable pancreatic cancer.

Characteristic	Number (%)
Median age, (IQR) years	68.00 (62.00–73.00)
71–89 years	226 (38.2)
Gender, female	278 (47.0)
ECOG PS	
0	200 (33.8)
1	303 (51.2)
2	88 (14.9)
3	1 (0.2)
BMI	
Normal weight	341 (57.6)
Underweight	51 (8.6)
Overweight	177 (29.9)
NA	23 (3.9)
Weight loss	
<2%	116 (19.6)
≥2%	327 (55.2)
NA	149 (25.2)
Diabetes	139 (23.5)
Smoking	
No	161 (27.2)
Yes	362 (61.1)
NA	69 (11.7)
Alcohol	
No	389 (65.7)
Yes	132 (22.3)
NA	71 (12.0)
Stage	
II and III	162 (27.4)
IV	428 (72.3)
NA	2 (0.3)
Number of metastatic sites	
0	163 (27.5)
1	302 (51.0)
2	77 (13.0)
3+	12 (2.0)
NA	38 (6.4)
Metastatic sites	
Liver	488 (82.4)
Peritoneum	221 (37.3)
Chemotherapy type, first-line	
Gemcitabine	413 (69.8)
Platinum-based combination	113 (19.1)
Gemcitabine plus *nab*-paclitaxel	52 (8.8)
Capecitabine-based combination	14 (2.4)
Median CA19-9 (IQR) U/mL	1,000.00 (132.00–7,285.00)
<59 × ULN	325 (54.9)
≥59 × ULN	215 (36.3)
NA	52 (8.8)
Median WBC (IQR; × 10^9/L)	8.70 (6.80–10.80)
Median ANC (IQR; × 10^9/L)	6.20 (4.68–8.10)
Median platelets (IQR; × 10^9/L)	301 (232–392)
IL6 median (IQR) pg/mL	6.45 (3.20–14.85)
>4.92 (high)	355 (60.0)
≤4.92 (low)	237 (40.0)
CRP median (IQR) mg/L	15.65 (3.61–48.18)
>10 (high)	339 (57.3)
≤10 (low)	253 (42.7)
YKL-40 median (IQR) μg/L	138.00 (78.50–233.50)
>95th age percentile (high)	224 (37.8)
≤95th age percentile (low)	367 (62.0)
Low IL6, YKL-40, and CRP (type 0)	152 (25.7)
High IL6 only (type I)	39 (6.6)
High YKL-40 only (type I)	29 (4.9)
High CRP only (type I)	55 (9.3)
High IL6 and YKL-40, or IL6 and CRP, or YKL-40 and CRP (type II)	154 (26.0)
High IL6, YKL-40, and CRP (type III)	162 (27.4)

(Continued on the following column)

Table 1. Clinical characteristics and baseline blood test results in 592 patients with unresectable pancreatic cancer. (Cont'd)

Note: Baseline serum values of IL6, CRP, and YKL-40 were dichotomized using cutoffs for IL6 of >4.92 pg/mL, CRP mg/L of >10, and YKL-40 of >95% age-corrected percentile as reference. Patients with low values of IL6, YKL-40, and CRP (type 0); high value of one (type I) or two biomarkers (type II); or high values of all three biomarkers (type III). The baseline concentrations of IL6, CRP, and YKL-40 were available for all patients except one with a missing YKL-40 value.
Abbreviations: IQR, interquartile range; NA, not available; ULN, upper limit of normal.

more men (53%) than women (47%). The majority of patients (303/51.2%) were classified with an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) of 1, while 200 (33.8%) and 88 (14.9%) were registered with a PS 0 and 2, respectively. According to the WHO classification of BMI subgroups, 51 patients (8.6%) were underweight (BMI < 18.5), 341 patients (57.6%) were of normal weight BMI from 18.5 to 25), and 177 patients (29.9%) were overweight (BMI > 25). The majority of patients (324/54.7%) had lost more than 5% of their body weight before inclusion in the study. Most patients were diagnosed with stage IV cancer (72.3%), 82.4% of whom had liver metastases, and 37.3% had peritoneal carcinomatosis. All patients were given first-line chemotherapy as follows: gemcitabine $n = 413$ (69.8%), FOLFIRINOX $n = 113$ (19.1%), gemcitabine + nab-Paclitaxel $n = 52$ (8.8%), or capecitabine-containing regimens $n = 14$ (2.4%). One hundred forty-eight patients (25%) received second-line chemotherapy.

One hundred sixty-two (27.4%) patients had concomitant high values of serum IL6, CRP, and YKL at the time of inclusion (type III). High values of two biomarkers were observed in 154 (26.0%) patients (type II). One hundred twenty-three (20.8%) patients, including 39 (high IL6), 55 (high CRP), and 29 (high YKL-40) patients had an isolated high concentration of a single marker (type I). One hundred fifty-two (25.7%) patients had low serum values of all three biomarkers (type 0). Slightly higher prevalence of patients with stage II–III and PS 0 was observed in this group (Supplementary Table S1). More than 50% of the patients had high IL6 or CRP levels based on the described cutoffs at baseline and 38% of patients had high YKL-40.

Comparison of median serum IL6, CRP, and YKL-40 by stage, PS, and presence of liver metastases and peritoneal carcinomatosis

IL6, CRP, and YKL-40 levels increased in accordance with increasing stage and worsening PS (**Table 2**). In contrast to CRP [8.86 mg/L (3.00–21.50) and 17.00 mg/L (4.00–47.94), $P = 0.01$ for patients without versus with metastases, respectively], no difference was found in IL6 and YKL-40 values stratified by presence of liver metastases. Baseline levels of IL6, CRP, and YKL-40 for patients with peritoneal carcinomatosis were lower compared with those diagnosed with liver metastasis.

Relationships between serum IL6, CRP, and YKL-40 and CA19-9, liver serum parameters, and inflammatory biomarkers

IL6, CRP, and YKL-40 were all correlated with each other and with the other examined inflammatory biomarkers (**Table 3**). IL6 and CRP but not YKL-40 were positively correlated with CA19-9. Serum levels

Table 2. Baseline median serum levels of IL6, CRP, and YKL-40 stratified by stage, performance status, and presence of liver metastases.

Baseline serum IL6, CRP, and YKL-40 stratified by stage					
Stage	**II and III**		**IV**		***P***
Number of patients	162		428		
IL6 median (IQR) pg/mL	4.90 (2.60–9.30)		7.45 (3.40–17.92)		<0.001
CRP median (IQR) mg/L	7.50 (3.00–30.00)		19.00 (5.00–59.50)		<0.001
YKL-40 median (IQR) μg/L	107.00 (67.25–173.50)		151.00 (85.00–255.00)		<0.001
Baseline serum IL6, CRP, and YKL-40 stratified by PS					
PS	**0**	**1**	**2**	**3**	***P***
Number of patients	200	303	88	1	
IL6 median (IQR) pg/mL	5.60 (2.40–11.72)	6.90 (3.45–14.00)	10.50 (4.20–21.27)	44.00 (44.00–44.00)	<0.001
CRP median (IQR) mg/L	11.00 (3.00–38.25)	18.00 (4.00–49.00)	27.89 (7.90–76.27)	177.00 (177.00–177.00)	0.001
YKL-40 median (IQR) μg/L	109.00 (67.75–202.75)	148.00 (85.25–232.25)	167.00 (100.00–299.25)	1,474.00 (1,474.00–1,474.00)	<0.001
Baseline serum IL6, CRP, and YKL-40 stratified by presence of liver metastases					
Liver metastases	**No**		**Yes**		***P***
Number of patients	66		488		
IL6 median (IQR) pg/mL	6.25 (2.62–12.70)		6.40 (3.20–14.33)		0.339
CRP median (IQR) mg/L	8.86 (3.00–21.50)		17.00 (4.00–47.94)		0.011
YKL-40 median (IQR) μg/L	144.00 (82.25–225.00)		137.00 (79.00–228.00)		0.870
Baseline serum IL6, CRP, and YKL-40 stratified by presence of peritoneal carcinomatosis					
Peritoneal carcinomatosis	**No**		**Yes**		***P***
Number of patients	333		221		
IL6 median (IQR) pg/mL	7.40 (3.50–18.00)		5.00 (2.60–10.30)		<0.001
CRP median (IQR) mg/L	21.00 (5.00–63.37)		10.00 (3.00–32.00)		<0.001
YKL-40 median (IQR) μg/L	148.50 (84.00–255.25)		125.00 (72.00–186.00)		0.003
Baseline serum IL6, CRP, and YKL-40 stratified by presence of metastasis in: liver versus peritoneum versus both versus other					
PS	**Liver**	**Peritoneum**	**Both**	**Other**	***P***
Number of patients	304	37	184	29	
IL6 median (IQR) pg/mL	7.50 (3.50–18.00)	6.40 (2.70–13.00)	5.00 (2.60–9.93)	6.10 (2.40–11.00)	<0.001
CRP median (IQR) mg/L	23.00 (6.00–65.24)	9.00 (3.00–24.00)	10.00 (3.00–32.55)	8.71 (3.00–18.36)	<0.001
YKL-40 median (IQR) μg/L	146.00 (84.50–262.50)	132.00 (83.00–201.00)	119.00 (70.50–185.25)	206.00 (74.00–227.00)	0.025

Abbreviation: IQR, interquartile range.

of liver parameters were correlated with all three biomarkers, except for IL6 and YKL-40 in relation to ALT.

Univariate and multivariate analyses

In survival analyses, patients with combined high serum values of two or three biomarkers including IL6, YKL-40, and CRP had worse OS compared with patients with low values or with an isolated high concentration of one biomarker (**Table 4**). When IL6, YKL-40, or CRP were analyzed separately, patients with serum levels above cutoff had increased overall mortality in a univariate Cox model and reduced survival in Kaplan–Meier plots (**Fig. 1**).

Mortality was increased in the patients with higher PS; substantial weight loss; higher stage; number of metastatic sites; increased baseline CA 19.9, WBC, ANC, platelet counts, and capecitabine-containing regimens. Combination chemotherapy with either FOLFIRINOX or gemcitabine + nab-Paclitaxel was associated with better survival

Table 3. Spearman correlation coefficients between baseline IL6, CRP, and YKL-40 and CA19-9, WBC, ANC, platelets, and liver serum parameters.

	IL6	**CRP**	**YKL-40**
IL6	—	$R_s = 0.69$; $P < 0.01$	$R_s = 0.42$; $P < 0.01$
CRP	$R_s = 0.69$; $P < 0.01$	—	$R_s = 0.41$; $P < 0.01$
YKL-40	$R_s = 0.42$; $P < 0.01$	$R_s = 0.41$; $P < 0.01$	—
CA19-9	$R_s = 0.25$; $P < 0.01$	$R_s = 0.23$; $P < 0.01$	$R_s = 0.07$; $P = 0.12$
WBC	$R_s = 0.50$; $P < 0.01$	$R_s = 0.46$; $P < 0.01$	$R_s = 0.29$; $P < 0.01$
ANC	$R_s = 0.50$; $P < 0.01$	$R_s = 0.47$; $P < 0.01$	$R_s = 0.29$; $P < 0.01$
Platelets	$R_s = 0.20$; $P < 0.01$	$R_s = 0.23$; $P < 0.01$	$R_s = 0.14$; $P < 0.01$
Alkaline phosphatase	$R_s = 0.41$; $P < 0.01$	$R_s = 0.48$; $P < 0.01$	$R_s = 0.26$; $P < 0.01$
Bilirubin	$R_s = 0.18$; $P < 0.01$	$R_s = 0.20$; $P < 0.01$	$R_s = 0.10$; $P = 0.02$
Alanine transaminase	$R_s = 0.06$; $P = 0.16$	$R_s = 0.16$; $P < 0.01$	$R_s = 0.04$; $P = 0.32$

Abbreviation: R_s, correlation coefficient from Spearman rank test.

Table 4. Univariate and multivariate HRs for OS according to clinical characteristics and baseline serum values of IL6, CRP, and YKL-40, dichotomized using cutoffs for IL6 of >4.92 pg/mL, CRP mg/L of >10, and YKL-40 of >95% age-corrected percentile as reference.

	Univariate analysis		Multivariate analysis	
Variables	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age >70 years	1.14 (0.96–1.35)	0.13	–	–
Sex (female)	0.95 (0.80–1.12)	0.52	–	–
PS 1	1.59 (1.32–1.92)	<0.01	1.20 (0.92–1.55)	0.18
PS 2	2.67 (2.06–3.46)	<0.01	3.11 (2.12–4.56)	<0.01
BMI (underweight)	1.30 (0.96–1.75)	0.09	–	–
BMI (overweight)	1.12 (0.93–1.35)	0.22	–	–
Weight loss ≥2%	1.28 (1.03–1.60)	0.03	1.17 (0.89–1.53)	0.25
Diabetes	0.89 (0.73–1.08)	0.25	–	–
Smoking	1.02 (0.85–1.24)	0.82	–	–
Alcohol	0.89 (0.73–1.09)	0.26	–	–
Stage II + III	0.58 (0.48–0.70)	<0.01	[a]	[a]
Number of metastatic sites (1)	1.60 (1.31–1.95)	<0.01	1.21 (0.77–1.89)	0.41
Number of metastatic sites (2)	1.78 (1.34–2.35)	<0.01	1.40 (0.83–2.37)	0.20
Number of metastatic sites (3+)	3.93 (2.17–7.12)	<0.01	2.31 (1.03–5.22)	0.04
Liver metastasis	1.09 (0.83–1.41)	0.54	–	–
Peritoneum metastasis	0.60 (0.51–0.72)	<0.01	0.73 (0.49–1.07)	0.10
Platinum-based combination	0.53 (0.43–0.66)	<0.01	0.55 (0.42–0.74)	<0.01
Gemcitabine plus *nab*-paclitaxel	0.55 (0.39–0.76)	<0.01	0.44 (0.29–0.67)	<0.01
Capecitabine-based combination	0.66 (0.39–1.13)	0.13	–	–
CA19-9 U/mL ≥59 x ULN	1.54 (1.29–1.84)	<0.01	1.27 (0.99–1.63)	0.06
WBC × 10^9/L (per unit increase)	1.11 (1.09–1.14)	<0.01	0.94 (0.84–1.05)	0.29
ANC × 109/L (per unit increase)	1.14 (1.11–1.17)	<0.01	1.13 (0.99–1.29)	0.08
Platelets × 10^9/L (per unit increase)	1.00 (1.00–1.00)	0.04	1.00 (1.00–1.00)	0.29
Low IL6, YKL-40, and CRP (Type 0)	1.00 (Ref.)	Ref.	Ref.	Ref.
High IL6 only (Type I)	1.08 (0.75–1.55)	0.69	–	–
High CRP only (Type I)	1.41 (1.03–1.94)	0.03	1.17 (0.77–1.78)	0.46
High YKL-40 only (Type I)	1.33 (0.89–1.99)	0.16	–	–
High IL6 and YKL-40 or IL6 and CRP or YKL-40 and CRP (Type II)	1.94 (1.54–2.45)	<0.01	1.41 (1.02–1.93)	0.04
High IL6, YKL-40, and CRP (Type III)	2.93 (2.32–3.69)	<0.01	1.87 (1.34–2.62)	<0.01

Note: Patients with low values of IL6, YKL-40, and CRP (type 0); high value of one (type I) or two biomarkers (type II); or high values of all three biomarkers (type III). The significant terms are included in the multivariate analyses.

Abbreviation: ULN, upper limit of normal.

[a]Because both number of metastasis and stage are significant, only number of metastasis is included.

compared with gemcitabine alone. The following exposures were not associated with mortality: age, sex, BMI, history of diabetes, smoking, alcohol consumption, and presence of liver or peritoneum metastases.

Significant variables in univariate analysis were subsequently analyzed in a multivariate model. Combined high values of two or three of the biomarkers IL6, YKL-40, and CRP remained independently associated with a poorer OS (**Table 4**).

In addition, we performed ROC curve analysis to describe the ability of the three biomarkers compared with CA19-9 to predict early death, defined as survival duration <90 days from the time of diagnosis (**Fig. 2**). For baseline serum IL6, CRP, YKL-40, and CA19-9, the area under the ROC curve was 0.74, 0.72, 0.69, and 0.68, respectively (Supplementary Table S2). The AUC for combined high values of IL6, CRP, YKL-40 was higher (AUC = 0.86; 95% CI, 81.4–89.7) compared with the high value of each marker separately. Notably, combining the values of these three markers with CA19-9 did not increase the AUC further.

In contrast to an isolated high value of one biomarker, having more than one biomarker was associated with early death in the univariate analyses along with PS 1–2, stage IV cancer, higher baseline CA 19.9, WBC, and ANC (Supplementary Table S3). Combined high baseline values of IL6, YKL-40, and CRP remained independently associated with early death after further adjustment for significant covariates in multivariate analysis. Patients with high levels of two or three biomarkers were at 6.3-fold increased risk of early death compared with patients without increased levels of IL6, CRP, and YKL-40.

Changes in serum IL6 and YKL-40 during first-line chemotherapy

Longitudinal serum levels of IL6 and YKL-40 were available in 398 patients before the second cycle of chemotherapy and in 260 patients at the time of first CT evaluation. This analysis showed that IL6 values, both the baseline $\log_2$-transformed value and the $\log_2$-transformed ratio between current value and the baseline value, were associated with OS (HR: 1.34; 95% CI, 1.21–1.47 and HR: 1.40; 95% CI, 1.29–1.52, respectively; Supplementary Table S4). YKL-40 levels in a time-varying model were not prognostic for survival.

Finally, the biomarkers were dichotomized based on the ≥25% versus <25% increase of IL6 and YKL-40 levels measured before the second cycle of chemotherapy and prior to first CT evaluation (Supplementary Fig. S1). For IL6, a significantly shorter OS was observed among patients with an increase of at least 25% from baseline level ($P = 0.012$). In addition, we analyzed IL6 and YKL-40 concentrations 281 patients with available samples collected at the time of cancer progression as determined by CT. We found statistically significant associations between survival and serum IL6 and YKL-40 levels, also after adjustment (Supplementary Table S5).

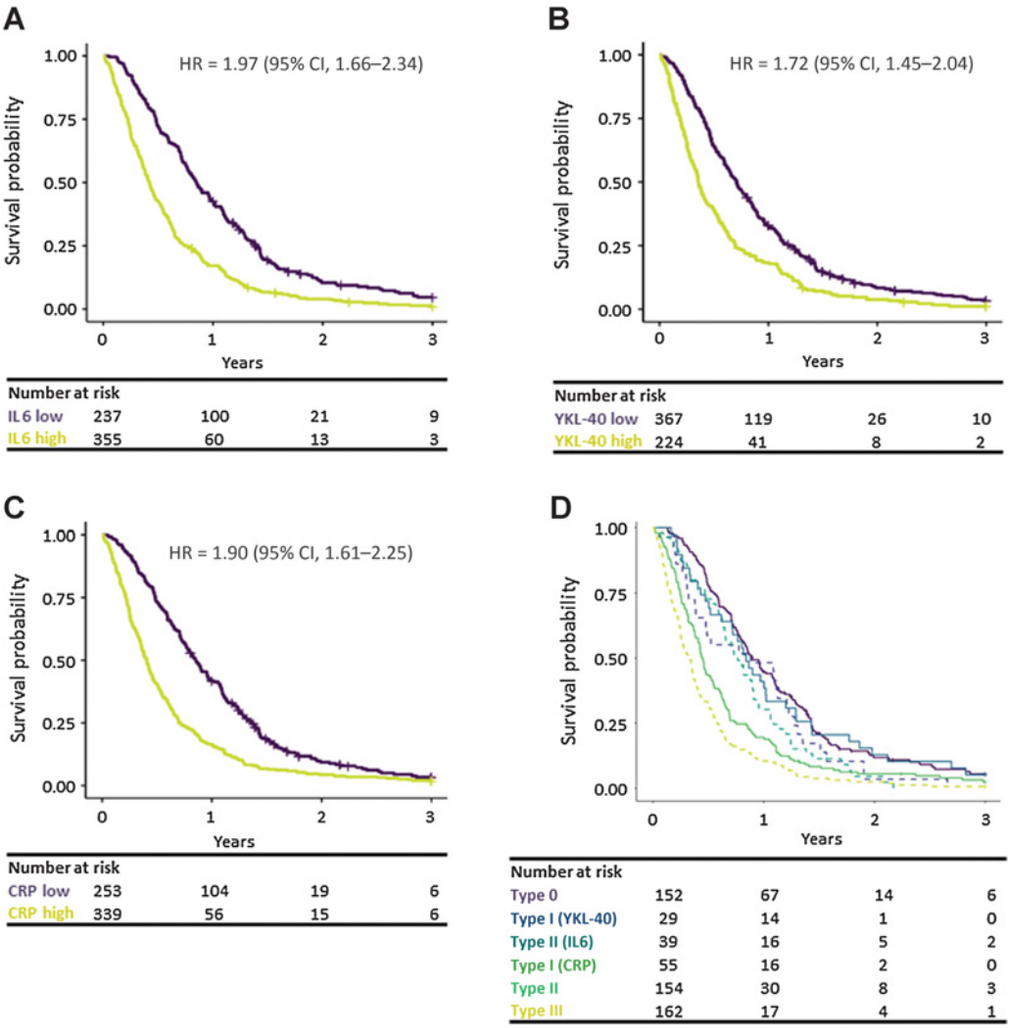

Figure 1.

Kaplan–Meier survival curves showing baseline serum IL6 (**A**), YKL-40 (**B**), and CRP (**C**) and combined IL6, YKL-40, and CRP (**D**), dichotomized as high and low using cutoffs of >4.92 pg/mL, >95th age percentile, and >10 mg/L, respectively. In the combined model, Kaplan–Meier curves show patients with low values of IL6, YKL-40, and CRP (type 0), high value of one (type I) or two biomarkers (type II), or high values of all three biomarkers (type III).

Discussion

In this large cohort of patients with pancreatic cancer undergoing palliative chemotherapy, we found that combined high serum levels of IL6, YKL-40, and CRP were associated with a worse OS, independently of other prognostic covariates. The majority of patients had concomitant high serum values of all or at least two of the biomarkers IL6, YKL-40, and CRP and a minority was registered with either low serum values or an isolated high value of a single marker. Higher values of IL6, YKL-40, or CRP were seen in patients with worse PS and higher stage. High levels of at least two biomarkers were associated with increased 90-day mortality. Accordingly, high serum IL6 and YKL-40 values at the time of cancer progression were negatively correlated with survival. Moreover, patients with an increase in IL6 greater than 25% 2–3 months after start of palliative chemotherapy had worse outcomes. These findings support the notion that the inflammatory response is a complex systemic reaction and is associated with poor outcome in patients with advanced pancreatic cancer.

Pancreatic cancer is one of the most aggressive of all malignant tumors (7), and only minor improvements in treatment have taken place over the past 20 years (32). Almost one-third of the BIOPAC cohort patients die within 3 months after start of palliative chemotherapy (Supplementary Fig. S2). Although pancreatic cancer implicates a rich pool of genetic alterations, no biomarker has yet been definitively validated for use in the clinical setting for prognostic, predictive, or diagnostic applications. In fact, the complex heterogeneity of the cancer at the molecular level may complicate identification of a single reliable biomarker that can be readily measured or utilized. Application of CA19-9, a serum-based protein biomarker, which is the most extensively validated and commonly used in daily clinical practice, is restricted because of false-positive elevations in other benign conditions, as well as not being detectable in individuals with a Lewis antigen–negative phenotype. CA19-9 above the median of 59× upper limit of normal (ULN) U/mL was reported to be associated with the poorest prognosis among a large group of patients with advanced pancreatic cancer (33, 34). However, the impact of high CA19-9 in our study was dependent on

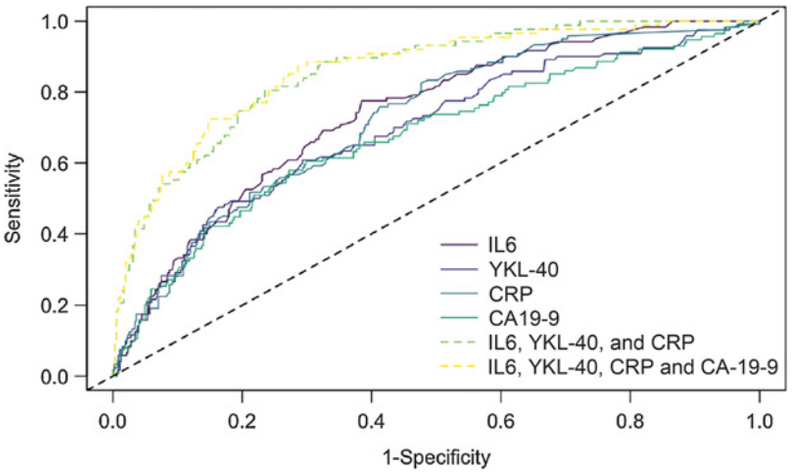

Figure 2.
ROC curve of single biomarker, IL6, CRP, and YKL-40, and combined marker detection in predicting early death, defined as survival duration <90 days from the time of diagnosis.

other covariates. Indeed, a PS of 2 was confirmed to be the strongest negative factor in all analyses, as expected (34).

Our findings should be interpreted in the context of the increased understanding of the interaction between cancer and activation of systemic inflammation (35), which can be measured by CRP, a nonspecific acute-phase protein synthesized in the liver in response to stimulation mainly from IL6 (35). The discovery of IL6-mediated protumor inflammation (7, 8), which is one of the players in the immune cell traffic (36, 37) and activation, provides further evidence of communication between pancreatic cancer–induced immunosuppression and inflammation (38). Although our findings can be placed in the context of immune system communication, the specific details of this process and applicable therapeutic targeting, as they relate to IL6-induced signaling, are yet to be determined. Our results indicating that high IL6, CRP, and YKL-40 levels are associated with increased mortality in the extended cohort of patients with pancreatic cancer undergoing palliative chemotherapy are consistent with previous studies (15) and in line with the known association between pancreatic cancer and IL6-induced inflammation (39). Individual and combined higher levels of CRP, IL6, and macrophage-inhibitory cytokine-1 were associated with significant reductions in overall mortality in a large prospective study of 446 patients with pancreatic cancer (40). Inflammation plays an important role in cancer progression (3, 37, 41). IL6 is an immunomodulating cytokine with pleiotropic properties and is produced by many cell types (10, 11). Several reports have highlighted the integral role of IL6 in facilitating tumor-promoting processes including inflammation (8, 9, 42). IL6-mediated inflammation is thought to be a component of tumor-induced immunosuppression (11, 12) and resistance to immunotherapy (38, 43). Greater activation of the systemic inflammatory response and high plasma IL6 levels have been reported in the majority of patients with advanced pancreatic cancer (5, 6, 13, 15). Preclinical pancreatic cancer models have clearly shown that the anti-IL6 receptor antibody, tocilizumab, in combination with chemotherapy reduces tumor growth, number of distant metastases, and local recurrence rate (44). Furthermore, blockade of IL6-regulated signaling pathways has been shown to inhibit liver metastasis (45), immunomodulate suppressive microenvironment, and enhance the efficacy of anti-programmed death-1 ligand 1 checkpoint inhibitor therapy (38, 43). Targeting of IL6 in combination with chemotherapy is actively under investigation in patients with advanced pancreatic cancer (NCT02767557).

In addition, our findings that YKL-40 had a negative prognostic value and that it was correlated with other inflammation markers are consistent with previous studies (15, 46). YKL-40 regulates key pathways and processes within the respective tumor microenvironment, including inflammation, angiogenesis, cell proliferation, differentiation, and remodeling of the extracellular matrix and thus promotes tumor progression (29, 47). Ablation of YKL-40 expression promotes CTL activation and, thus, antitumor immunity (18). YKL-40 may be a new therapeutic target for patients with cancer, because antibodies against YKL-40 are able to inhibit tumor angiogenesis and cancer progression (18, 23, 28). Tumor-associated macrophage-secreted YKL-40 could promote metastasis via activation of MAPK signaling (27). Previous studies characterize the majority of patients with pancreatic cancer as having high plasma YKL-40 (19). Further studies in different populations are needed to confirm our results.

The primary limitation of this study is that the patients with pancreatic cancer in the BIOPAC study represent only 20% of all newly diagnosed cases of pancreatic cancer in Denmark according to Danish Cancer Registry. The biomarkers of interests were prespecified in the BIOPAC project from the beginning and analyzed prospectively. However, due to the way clinical data were obtained from the patient files, recall bias was an inevitable potential source of bias due to the differences in the accuracy or completeness of the recollections retrieved from the patient files, although our records were thoroughly quality-checked by two separate investigators. The analyzed cohort in this study was restricted to patients treated in a palliative setting, thus survival prediction is limited by generally short survival in this population and cannot be directly applied to patients with resectable pancreatic cancer, or patients with much longer survival. Further longitudinal prospective studies (5) are required before inflammatory markers might be used routinely in the management of pancreatic cancer across all stages (5).

In conclusion, our findings suggest that combined high baseline serum levels of IL6, YKL-40, and CRP are associated with poor prognosis in patients with unresectable pancreatic cancer. A high concentration of IL6 measured before chemotherapy and over time is a strong independent prognostic biomarker in patients with unresectable pancreatic cancer. Detection of inflammation markers including IL6, YKL-40, and CRP is likely to help clinicians prognosticate outcomes in patients with pancreatic cancer. Further investigation and development of anti-inflammation therapeutic strategies in patients with pancreatic cancer are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acknowledgments

The authors thank the biomedical laboratory scientists Charlotte Falk, Vibeke H. Holm, Dorte B. Nielsen, Helle B. Sørensen, Ulla Kjærulff-Hansen, Teresa Rozenfeld, and Mie B. Krüger for their assistance. The authors acknowledge the nurses Hanne M. Michaelsen, Lars M. Larsen, Lene T. Voxen, Finn Hejlesen, Signe Gamborg Nielsen, and Jette L. Hansen; the physician Dan Calatayud; and the medical students Camilla Palmquist and Karin Bagni for their contribution to inclusions of patients, data registration, or sample collection.

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Received June 10, 2019; revised August 29, 2019; accepted October 29, 2019; published first November 4, 2019.

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