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RESEARCH ARTICLE

Relationships of hepatic and pancreatic biomarkers with the cholestatic syndrome and tumor stage in pancreatic cancer

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Abstract

We analyzed relationships of hepatic and pancreatic biomarkers with the cholestatic syndrome and tumor stage in exocrine pancreatic cancer ($N = 183$). Information on laboratory tests and on signs and symptoms was obtained from medical records and patient interviews. Bilirubin, aspartate aminotransferase (AST), γ-glutamyltransferase (GGT) and alkaline phosphatase were lower in tumor stage IV. The association was due to the relationship between cholestatic syndrome and earlier presentation of patients. There was no association between hepatic biomarkers and stage when adjusting by cholestatic syndrome. Relationships of hepatic and pancreatic biomarkers with pancreatic symptoms and tumor stage must be controlled in "-omics" and other studies using biomarkers.

Keywords: [MeSH terms]: pancreatic neoplasms, cholestatic syndrome, weight loss, total bilirubin, γ-glutamyltransferase, diabetes mellitus, epidemiology. [Non-MeSH terms]: semiology, symptoms, regression coefficient, glycemia, AST, blood draw, jaundice, hypocholia, choluria

Introduction

The value of signs and symptoms of disease is often forgotten in basic, clinical and epidemiologic research. This oversight may have severe consequences when a variety of biomarkers are used. Signs and symptoms play an essential role when individuals recognize health disorders and seek medical care. Relationships between signs and symptoms of disease and biochemical parameters have long been important in health research and in the practice of clinical medicine; notably, for diagnostic and prognostic purposes (Porta et al. 2003). In certain areas of biomedical research it is important to keep in mind how much symptoms and biochemical parameters are interrelated. Because they reflect pathophysiological processes and disease progression, symptoms and biochemical parameters are relevant for studies on the causes and mechanisms of diseases (Porta 2001; Porta et al. 2008, 2009; Lee & Jacobs 2009); this includes a substantial part of research on the clinical applications of proteomic, metabolomic and other "-omics" technologies (Porta et al. 2003, 2005, 2007b; Lumbreras et al. 2008, 2009; Parker et al. 2010; Gallo et al. 2011). Unfortunately, such studies rarely assess whether "-omics" results are influenced by disease signs or vary by disease stage (e.g. by tumor stage at diagnosis or at the time the study blood sample was drawn) (Lumbreras et al. 2008, 2009; Parker et al. 2010; Gallo et al. 2011).

Cholestasis (failure of normal amounts of bile to reach the intestine, resulting in obstructive jaundice)

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Abbreviations

AST (GOT), aspartate aminotransferase;
β, regression coefficient;
CI, confidence interval;
CP, chronic pancreatitis;
DM, diabetes mellitus;
EPC, exocrine pancreatic cancer;
GGT, γ-glutamyltransferase;
GLM, General linear models;
LFT, liver function test;
OCs, organochlorine compounds;
ρ, Spearman's rank correlation coefficient;
TSL, total serum lipids

and the cholestatic syndrome are caused by total or partial impairment of the bile reaching the duodenum, by inability to form bile or because the bile cannot flow. This impediment is caused by a variety of diseases (Löhr et al. 2005; Hung et al. 2007; Björnsson et al. 2008). An important cause of obstructive extrahepatic cholestasis is an adenocarcinoma in the pancreatic head (Porta et al. 1996, 2005). Jaundice occurs when excess amounts of bilirubin circulating in the blood stream dissolve in the subcutaneous fat, causing a yellowish appearance of the skin and the whites of the eyes. Jaundice is part of the cholestatic syndrome along with hypocholia (clay-colored or pale stools) and choluria (dark brown urine). Jaundice is one of the most common signs at presentation in individuals with exocrine pancreatic cancer (EPC); newly appearing painless jaundice is highly suggestive of a tumor of the pancreas head that is occluding the common bile duct (Li & Jiao 2005; Löhr et al. 2005; Porta et al. 2005, 2007b). Since jaundice is more noticeable and unusual than fatigue, anorexia and weight loss (the components of the constitutional syndrome), jaundice more commonly prompts patients to seek medical attention and is more strongly associated with an earlier diagnosis of EPC than the constitutional syndrome (Porta et al. 2005). Thus, classic cancer biomarkers are seldom independent of disease signs and symptoms and of the stage at which a disease is diagnosed (Porta et al. 2003). It would seem that these fundamental relationships should also be integrated in the new generations of diagnostic, etiologic and prognostic studies that use biomarkers (Sala et al. 2001; Li & Jiao 2005; Lee & Jacobs 2006; Crous-Bou 2009). Different studies have assessed the relationships between EPC risk and glycemia, γ-glutamyltransferase (GGT) or alkaline phosphatase (Kahan et al. 1981; Gama et al. 2003; Johansen et al. 2009). However, the methodological implications of the relationships of these factors with EPC symptoms and tumor stage have received little attention.

The aim of the present study was to analyze the relationships of hepatic and pancreatic biomarkers with the cholestatic syndrome and tumor stage in patients with EPC.

Subjects and methods

Methods of the PANKRAS II study have been described in detail (Porta et al. 1999, 2000, 2005, 2007a, 2008, 2009; Crous-Bou 2009; Crous-Bou et al. 2009; Lumbreras et al. 2009; Parker et al. 2011). Briefly, subject recruitment took place at five general university hospitals in eastern Spain, where 185 incident cases of EPC were prospectively identified. The present report is based on 183 EPC patients with blood measures of pancreatic and hepatic enzymes, and information on EPC signs and symptoms. Of these 183 patients, 181 had their tumor clinical stage determined, and of them 109 had results on tumor markers (see below). The ethics committees of participating hospitals approved the study protocol, and patients gave informed consent to participate.

Personal interviews and information on laboratory tests and symptoms

Patients were interviewed face-to-face by trained monitors during hospital stay (Crous-Bou 2009; Lumbreras et al. 2009). Interviews included questions about past medical conditions, symptoms, and coffee, tobacco and alcohol consumption (Porta et al. 1999, 2005; Crous-Bou 2009). A structured form was used to collect clinicopathological information from medical records, including results of laboratory procedures and semiology (Porta et al. 2000; Crous-Bou 2009). All items concerning medical conditions were further reviewed by study physicians and checked for consistency (Porta et al. 2000; Crous-Bou et al. 2009; Parker et al. 2011). Of the 183 patients, 31 had a history of diabetes mellitus and 7 of pancreatitis (chronic pancreatitis, 3 patients; acute pancreatitis, 5 patients) (Crous-Bou et al. 2009). Hospital discharge diagnoses and tumor's clinical stage were also recorded in the form (Porta et al. 1999, 2000; Crous-Bou 2009). The tumor's clinical stage at diagnosis was classified according to the tumor-node-metastasis (TNM) system. All cases were independently reviewed by the study reference pathologists, unaware of the original diagnosis. The intrapancreatic tumor site was classified for 143 patients (78%): 113 tumors were in the pancreas head, 19 in the body, and 11 in the tail (Porta et al. 2005).

Results from the following blood tests were analyzed in the present report: (i) pancreatic enzymes lipase and amylase; (ii) hepatic markers bilirubin (total and direct), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT) and alkaline phosphatase; and (iii) glycemia, total cholesterol and triglycerides. Information on laboratory tests was selected from the test performed in each hospital the date closest to hospital admission (median time from hospital admission to performance of test, 1 day). Given that the study was multicentric, laboratory procedures were those performed in daily clinical care by the participating hospitals. There were no statistically significant differences among hospitals in any of the pancreatic and liver parameters analyzed. Total cholesterol and triglycerides were analyzed at IMIM (the study coordinating center)

in a specific blood sample (Porta et al. 1999, 2007a, 2008; Lumbreras et al. 2009). The median time from hospital laboratory tests to drawing of blood to determine lipids was 12 days. The number of patients with information on total serum lipids (TSL) and glycemia was 143; 117 for direct bilirubin; 137 for total bilirubin; 143 for AST; 132 for GGT; 142 for alkaline phosphatase; 37 for serum lipase; and for amylase, 108 in serum and 18 in urine.

Based on previous research (Porta et al. 1996, 2003, 2005), detailed information on signs and symptoms was obtained from two sources: medical records (where they were registered by the attending physician at hospital admission, and abstracted by a physician into the clinicopathological form), and patient interviews. All items concerning the first sign or symptom of the disease (and its date), and presence of other signs and symptoms were further reviewed by two oncologists and checked for consistency (Porta et al. 2005). The cholestatic syndrome involved jaundice, hypocholia and choluria. The constitutional syndrome comprised asthenia, anorexia and weight loss (Porta et al. 2005, 2008). Patients with all three signs or symptoms of the same syndrome were deemed to have the complete syndrome, and patients with one or two signs or symptoms of the same syndrome to have partial syndrome.

Table 1. Results of laboratory tests by tumor stage.

	Tumor stage					
	Total	I	II	III	IV	
Laboratory tests	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)	*p* value
Total	183	45 (24.6)	23 (12.6)	23 (12.6)	92 (50.3)	
Direct bilirubin (mg/dL)	147 (80.3)	41 (91.1)	18 (78.3)	19 (82.6)	67 (72.8)	0.084[a]
Mean (SD)	6.6 (6.4)	8.5 (5.9)	7.1 (7.4)	7.9 (5.8)	4.8 (6.2)	
Median	5.7	8.0	6.0	9.1	1.2	0.009[b]
Minimum	0.0	0.0	0.1	0.1	0.0	
Maximum	26.3	19.1	22.2	17.8	26.3	
Total bilirubin (mg/dL)	176 (96.2)	45 (100)	21 (91.3)	22 (95.7)	86 (93.5)	0.219[a]
Mean (SD)	8.4 (9.3)	12.0 (9.0)	8.6 (11.2)	9.9 (7.5)	5.8 (8.7)	
Median	5.5	10.3	1.4	11.3	1.1	0.004[b]
Minimum	0.1	0.1	0.3	0.3	0.2	
Maximum	47.7	32.0	38.7	22.6	47.7	
AST (U/L)	182 (99.5)	45 (100)	22 (95.7)	23 (100)	90 (97.8)	0.688[a]
Mean (SD)	122.1 (147.3)	178.5 (209.1)	121.5 (167.5)	132.0 (141.6)	90.9 (90.3)	
Median	81.0	102.0	79.5	87.0	54.5	0.019[b]
Minimum	9.0	10.0	10.0	9.0	10.0	
Maximum	1012	1012	784.0	561.0	391.0	
GGT (U/L)	166 (90.7)	41 (91.1)	19 (82.6)	19 (82.6)	85 (92.4)	0.310[a]
Mean (SD)	498.8 (614.1)	607.7 (630.6)	395.4 (374.5)	454.4 (376.4)	479.0 (692.1)	
Median	297.5	382.0	370.0	403.0	206.0	0.237[b]
Minimum	8.0	12.0	15.0	14.0	8.0	
Maximum	4282	2869	1258	1553	4282	
Alkaline phosphatase (U/L)	181 (98.9)	45 (100)	22 (95.7)	22 (95.7)	90 (97.8)	0.402[a]
Mean (SD)	874.0 (872.0)	1076 (916.3)	930.4 (1338)	882.5 (707.9)	758.2 (738.4)	
Median	638.0	985.0	563.0	845.0	419.5	0.065[b]
Minimum	48.0	61.0	158.0	114.0	48.0	
Maximum	5980	5360	5980	3133	3285	
Glycemia (mg/dL)	182 (99.5)	45 (100)	22 (95.7)	23 (100)	90 (97.8)	0.688[a]
Mean (SD)	136.3 (61.4)	152.7 (78.4)	135.6 (69.4)	125.9 (50.6)	131.8 (51.4)	
Median	114.0	122.0	109.5	109.0	112.5	0.417[b]
Minimum	58.0	58.0	78.0	72.0	67.0	
Maximum	447.0	447.0	386.0	262.0	331.0	
Serum amylase (U/L)	138 (75.4)	35 (77.8)	18 (78.3)	19 (82.6)	65 (70.7)	0.639[a]
Mean (SD)	199.1 (578.5)	204.3 (228.1)	124.2 (113.8)	151.2 (187.8)	231.6 (819.8)	
Median	72.0	104.0	83.5	56.0	70.0	0.468[b]
Minimum	8.0	20.0	12.0	13.0	8.0	
Maximum	6574	966.0	380.0	706.0	6574	

[a]Fisher's exact test (two-tail).
[b]Kruskal–Wallis test.

Table 2. Correlations between laboratory tests, symptoms and lipids.

		Hypocholia	Choluria	Jaundice	Pruritus	Cholestatic syndrome	Steatorrhea	Total cholesterol	Triglycerides	Total lipids
Direct bilirubin (mg/dL)	ρ	0.605	0.676	0.765	0.478	0.758	0.161	0.373	0.512	0.496
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	0.051	<0.001	<0.001	<0.001
	N	147	147	147	147	147	147	117	117	117
Total bilirubin (mg/dL)	ρ	0.618	0.712	0.786	0.567	0.790	0.219	0.404	0.538	0.522
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001
	N	175	175	175	175	176	175	137	137	137
AST (U/L)	ρ	0.447	0.574	0.630	0.390	0.613	0.149	0.393	0.338	0.428
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	0.045	<0.001	<0.001	<0.001
	N	181	181	181	181	182	181	143	143	143
GGT (U/L)	ρ	0.463	0.588	0.600	0.312	0.590	0.172	0.417	0.432	0.481
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	0.027	<0.001	<0.001	<0.001
	N	166	166	166	166	166	166	132	132	132
Alkaline phosphatase (U/L)	ρ	0.401	0.585	0.577	0.319	0.581	0.167	0.395	0.410	0.454
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	0.025	<0.001	<0.001	<0.001
	N	180	180	180	180	181	180	142	142	142
Glycemia (mg/dL)	ρ	0.074	0.068	0.032	0.012	0.062	−0.100	0.008	<0.001	0.001
	p value	0.320	0.361	0.667	0.877	0.409	0.182	0.923	1.000	0.991
	N	181	181	181	181	182	181	143	143	143
Serum amylase (U/L)	ρ	0.012	0.015	0.091	0.012	0.026	0.081	−0.047	0.116	−0.009
	p value	0.885	0.864	0.292	0.886	0.763	0.348	0.631	0.231	0.925
	N	137	137	137	137	138	137	108	108	108

		Cachexia	Asthenia	Anorexia	Weight loss	Constitutional syndrome	Diabetes mellitus	Coffee consumption	Alcohol consumption	Tobacco smoking
Direct bilirubin (mg/dL)	ρ	−0.033	0.080	0.134	0.013	0.086	0.004	−0.038	−0.068	0.099
	p value	0.696	0.333	0.106	0.880	0.299	0.961	0.670	0.449	0.262
	N	146	147	147	147	147	144	130	128	131
Total bilirubin (mg/dL)	ρ	0.028	0.035	0.116	−0.061	0.046	0.103	−0.040	−0.065	0.045
	p value	0.710	0.644	0.126	0.423	0.543	0.181	0.619	0.426	0.578
	N	175	175	175	175	176	172	156	152	157
AST (U/L)	ρ	0.009	0.144	0.157	−0.053	0.074	0.089	0.004	−0.152	−0.068
	p value	0.902	0.053	0.035	0.478	0.319	0.239	0.964	0.056	0.390
	N	181	181	181	181	182	177	162	158	163
GGT (U/L)	ρ	−0.031	0.084	0.073	−0.098	0.004	0.127	−0.066	−0.054	0.079
	p value	0.694	0.284	0.350	0.208	0.962	0.108	0.428	0.516	0.337
	N	165	166	166	166	166	162	147	145	148
Alkaline phosphatase (U/L)	ρ	−0.038	0.120	0.099	−0.030	0.084	0.177	−0.035	−0.113	−0.025
	p value	0.616	0.108	0.184	0.690	0.262	0.018	0.659	0.158	0.755
	N	180	180	180	180	181	176	161	157	162
Glycemia (mg/dL)	ρ	0.069	0.049	0.026	−0.054	0.070	0.415	0.127	−0.058	−0.142
	p value	0.357	0.509	0.731	0.467	0.345	<0.001	0.108	0.472	0.070
	N	181	181	181	181	182	177	162	158	163
Serum amylase (U/L)	ρ	0.066	0.091	0.051	−0.076	−0.033	−0.002	0.011	0.101	0.056
	p value	0.441	0.288	0.552	0.376	0.703	0.986	0.903	0.274	0.539
	N	138	137	137	137	138	134	121	118	122

ρ, Spearman's rank correlation coefficient;
N, Number of patients.
Total cholesterol, triglycerides, and total lipids in mg/dL. Diabetes mellitus and coffee consumption in two categories, tobacco smoking in three categories and alcohol consumption in grams.

Analyses of serum concentrations of lipids

Blood concentrations of total cholesterol and triglycerides were determined enzymatically (CHOD-PAP and GPO-PAP methods, respectively) (Porta et al. 1999, 2008; Lumbreras et al. 2009). TSL were calculated from total cholesterol and triglycerides using the standard short-formula: TSL = 2.27 × Total Cholesterol + Triglycerides + 62.3 (Porta et al. 1999, 2009; Lumbreras et al. 2009).

Table 3. Relationship between laboratory tests, tumor stage and other factors.

Dependent variable	Factor	Model 1			Model 2		
		β	CI 95%	*p* value	β	CI 95%	*p* value
Direct bilirubin	Tumor stage						
	Stage II vs. stage I	−0.71	−1.77, 0.36	0.192	−0.88	−1.98, 0.23	0.118
	Stage III vs. stage I	−0.26	−1.29, 0.78	0.624	−0.63	−1.69, 0.42	0.238
	Stage IV vs. stage I	−1.33	−2.07, −0.59	0.001	−1.33	−2.12, −0.54	0.001
Total bilirubin	Tumor stage						
	Stage II vs. stage I	−0.76	−1.57, 0.06	0.069	−0.85	−1.64, −0.06	0.035
	Stage III vs. stage I	−0.16	−0.95, 0.64	0.697	−0.39	−1.17, 0.39	0.323
	Stage IV vs. stage I	−1.09	−1.65, −0.52	<0.001	−1.09	−1.66, −0.52	<0.001
AST	Tumor stage						
	Stage II vs. stage I	−0.47	−1.03, 0.10	0.103	−0.41	−0.99, 0.17	0.166
	Stage III vs. stage I	−0.25	−0.80, 0.29	0.359	−0.36	−0.93, 0.22	0.220
	Stage IV vs. stage I	−0.60	−0.99, −0.20	0.003	−0.69	−1.12, −0.27	0.001
GGT	Tumor stage						
	Stage II vs. stage I	−0.49	−1.34, 0.35	0.253	−0.39	−1.25, 0.47	0.377
	Stage III vs. stage I	−0.13	−0.96, 0.70	0.762	−0.41	−1.27, 0.44	0.337
	Stage IV vs. stage I	−0.64	−1.22, −0.07	0.029	−0.75	−1.35, −0.15	0.015
Alkaline phosphatase	Tumor stage						
	Stage II vs. stage I	−0.30	−0.81, 0.21	0.253	−0.12	−0.65, 0.41	0.656
	Stage III vs. stage I	−0.16	−0.67, 0.34	0.527	−0.22	−0.75, 0.31	0.408
	Stage IV vs. stage I	−0.42	−0.77, −0.06	0.023	−0.43	−0.82, −0.05	0.028

β, multiple linear regression coefficient. TSL, total serum lipids.
Serum concentrations of laboratory tests were log-transformed. Models 1 are adjusted by age and sex; Models 2 are further adjusted by TSL and time from blood draw for laboratory analyses to blood draw for analyses of lipids.

Statistics

Univariate statistics were computed as customary (Armitage et al. 2002). For comparisons between continuous variables Kruskal–Wallis was used. When a tendency was observed the Jonckheere–Terpstra test for linear trend was used. Fisher's exact test for homogeneity was applied to assess the relationship between two categorical variables. To analyze the relationships of serum concentrations of biomarkers and glycemia with tumor stage and syndromes, general linear models (GLM) were applied (Armitage et al. 2002). The main effects of all predictors were independently explored in base models, which included age and sex; the results when the variable of interest tumor is stage are shown in Table 3, while the results for cholestatic syndrome are shown in Table 4. Serum concentrations of pancreatic enzymes, hepatic markers and glycemia were log-transformed when dependent variables were in multivariate models. Other variables – e.g. symptoms, TSL, time intervals – were retained in the models when they materially altered the estimates (Porta 2001; Porta et al. 2008, 2009). We used heuristic diagrams to summarize the main results; such diagrams represent different relationships, not necessarily causal. The level of statistical significance was set at 0.05, and all tests are two-tailed. Analyses were performed with SPSS, version 12.0 (SPSS Inc., Chicago, IL, 2003) and the R Project for Statistical Computing, version 2.7.2 (2008).

Results

Concentrations of direct bilirubin, total bilirubin, AST and alkaline phosphatase were lower in patients with tumor stage IV than in patients with tumors in stages I, II and III (all medians $p < 0.02$ except for the alkaline phosphatase) (Table 1). Concentrations of GGT, glycemia and amylase did not significantly differ by tumor stage, although median concentrations were higher in stage I than in other tumor stages. No statistically significant differences were observed in concentrations of direct bilirubin, AST, GGT, lipase and amylase between patients with and without diabetes mellitus (DM) (data not shown). The median glycemia in patients with DM was 175 mg/dL and in patients without DM, 108 mg/d L ($p < 0.001$); the median value of alkaline phosphatase in patients with DM was 1057 U/L, whereas in patients without DM it was 565 U/L ($p = 0.019$).

Direct bilirubin, total bilirubin, AST, GGT and alkaline phosphatase were highly correlated with jaundice, choluria and hypocholia (ρ between 0.40 and 0.79) and, therefore, with the cholestatic syndrome (ρ between 0.58 and 0.79); to a lesser extent (but statistically significant), bilirubin and hepatic enzymes were also associated with the three measures of lipids (all ρ > 0.33) (Table 2). Direct bilirubin, total bilirubin, AST, GGT, alkaline phosphatase and serum amylase were not significantly correlated with pancreatitis, nor with coffee, alcohol and tobacco consumption.

Multivariate models show an inverse relationship between tumor stage and direct bilirubin, total bilirubin, AST, GGT and alkaline phosphatase (Table 3). As compared to patients with stage I tumors, patients with tumors in stage IV had significantly lower concentrations of direct and total bilirubin, AST, GGT and alkaline

Table 4. Relationship between laboratory tests and cholestatic syndrome.

Dependent variable	Model	β	95% CI	*p* value	R^2	*p* value model
Direct bilirubin (mg/dL)					0.701	<0.001
	Cholestatic syndrome					
	Partial vs. absent	2.61	2.05, 3.17	<0.001		
	Complete vs. absent	3.93	3.49, 4.41	<0.001		
	Age	0.01	−0.01, 0.03	0.128		
	Coffee intake	−0.48	−1.02, 0.07	0.084		
Total bilirubin (mg/dL)					0.730	<0.001
	Cholestatic syndrome					
	Partial vs. absent	1.73	1.35, 2.11	<0.001		
	Complete vs. absent	3.13	2.82, 3.45	<0.001		
	Age	0.02	0.01, 0.03	0.006		
	Coffee intake	−0.33	−0.72, 0.06	0.097		
AST (U/L)					0.463	<0.001
	Cholestatic syndrome					
	Partial vs. absent	1.12	0.78, 1.47	<0.001		
	Complete vs. absent	1.60	1.31, 1.89	<0.001		
	Age	0.01	0.00, 0.02	0.030		
	Coffee intake	−0.08	−0.43, 0.28	0.662		
GGT (U/L)					0.492	<0.001
	Cholestatic syndrome					
	Partial vs. absent	2.04	1.51, 2.57	<0.001		
	Complete vs. absent	2.32	1.91, 2.73	<0.001		
	Age	0.01	−0.01, 0.02	0.544		
	Coffee intake	−0.67	−1.21, −0.13	0.016		
Alkaline phosphatase (U/L)					0.399	<0.001
	Cholestatic syndrome					
	Partial vs. absent	0.98	0.65, 1.32	<0.001		
	Complete vs. absent	1.35	1.07, 1.63	<0.001		
	Age	0.01	0.00, 0.02	0.034		
	Coffee intake	−0.20	−0.55, 0.14	0.247		
Serum amylase (U/L)					0.021	0.776
	Cholestatic syndrome					
	Partial vs. absent	0.02	−0.49, 0.53	0.933		
	Complete vs. absent	0.09	−0.33, 0.52	0.661		
	Age	−0.01	−0.02, 0.01	0.267		
	Coffee intake	−0.03	−0.58, 0.52	0.913		

β, multiple linear regression coefficient. R^2, coefficient of determination.
Serum concentrations of laboratory tests were log-transformed. All models are further adjusted by sex.

phosphatase. This was so even when including TSL in the model (Table 3, models 2). In models 2 of Table 3 (which included tumor stage adjusted by age, sex, TSL, and the interval from blood draw for laboratory analyses to blood draw for lipid determination), TSL and the interval were always statistically significant. Notably, in all models for the biomarkers adjusted for age and the cholestatic syndrome, tumor stage was not statistically significant. When adjusting for age, sex and coffee consumption, the linear coefficients for stage were even lower (e.g. for GGT, $\beta_{\text{stage IV vs. stage I}} = -0.58$, $p = 0.077$).

Total bilirubin was significantly higher in cases with complete cholestatic syndrome than in patients with partial or no cholestatic syndrome ($p < 0.001$) regardless of tumor stage (Figure 1). Equally, total bilirubin was similar in stages I, II, III and IV in patients with the complete cholestatic syndrome ($p = 0.956$, Kruskal–Wallis test). There was no association between direct bilirubin and stage upon stratification by cholestatic syndrome ($p = 0.386$ without syndrome, $p = 0.472$ in partial syndrome and $p = 0.650$ in complete syndrome, Kruskal–Wallis test).

Direct bilirubin, total bilirubin, GGT, AST and alkaline phosphatase were the biochemical parameters most strongly associated with the cholestatic syndrome (Table 4). The respective age and sex-adjusted regression coefficients for patients with complete cholestatic syndrome were 3.77, 3.01, 2.15, 1.52 and 1.29 (all $p < 0.001$). As also shown in the table by the coefficient of determination (R^2), age, sex, coffee intake and the cholestatic syndrome explained statistically about 70% of the variability in the concentrations of direct and total bilirubin ($p < 0.001$). When included in the models shown in Table 4, stage was not associated with any of the biochemical parameters. When hospital of admission was included in

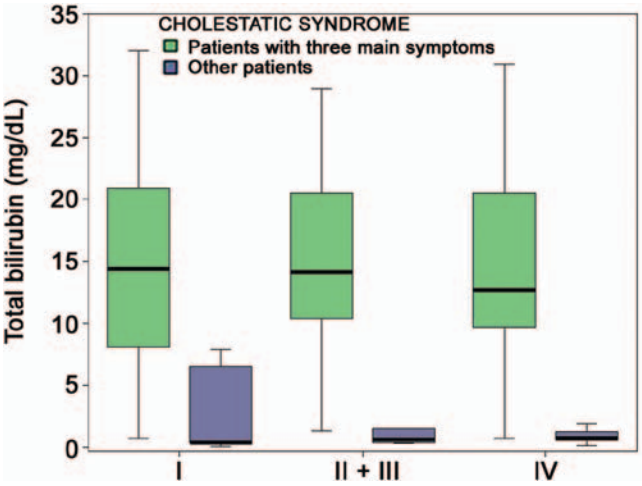

Figure 1. Concentration of total bilirubin (mg/dL) by tumor stage and presence or absence of full cholestatic syndrome. The box itself contains the middle 50% of the data (the inter-quartile range). The upper edge of the box indicates the 75th percentile of the data set, and the lower edge indicates the 25th percentile. The thick line inside the box indicates the median value of the data. The ends of the vertical lines or "whiskers" represent the minimum and maximum data values, unless outliers are present, in which case the whiskers extend to a maximum of 1.5 times the inter-quartile range.

the multivariate models, estimates for tumor stage or for cholestatic syndrome were not altered (data not shown).

Discussion

As compared to patients with tumors in stage I, patients with tumors in stage IV had statistically significantly lower concentrations of direct and total bilirubin; this was so both in simple and multivariate models, which adjusted by potential confounders. However, the association between bilirubin and stage was entirely due to the relationships among bilirubin, cholestatic syndrome and earlier stage at presentation. Specifically,

(i) as previously reported (Porta et al. 2005), in our patients all cholestatic signs and symptoms were individually associated with more localized tumors ($p < 0.001$), and there was a clear trend towards more localized tumors with increasing number of cholestatic signs ($p < 0.001$);
(ii) as expected, direct bilirubin and total bilirubin were highly correlated with jaundice, choluria and hypocholia, and, hence, with the cholestatic syndrome, regardless of tumor stage; and
(iii) consequently, there was no association between bilirubin and stage when we stratified by cholestatic syndrome (Figure 1).

These relationships are graphically summarized in Figure 2:

- The location of the tumor within the pancreas causally influences the occurrence of signs as well as the concentrations of liver and pancreatic enzymes (top 2 single-headed arrows of Figure 2). For instance, a tumor in the pancreatic head may occlude the common bile duct, hence causing cholestatic syndrome and increasing bilirubin and other liver enzymes (top 2 single-headed arrows of Figure 2, Diagram A). By contrast, patients with a tumor in the pancreatic body or tail are more likely to present with the constitutional syndrome (upper right single-headed arrow of Figure 2, Diagram B).
- Through its association or not with the cholestatic syndrome, pancreatic tumor site influences stage (Porta et al. 2005); i.e. stage is influenced by the presence or absence of symptoms (point 1 in the previous paragraph above and single-headed arrow in the lower right side of Figure 2).
- Therefore, the association between bilirubin (and other hepatic and pancreatic biomarkers) and stage is non-causal (double-headed arrow in the lower left side of Figure 2, Diagram A). The double-headed arrow in the middle of Figure 2 is based on point 2 in the previous paragraph.

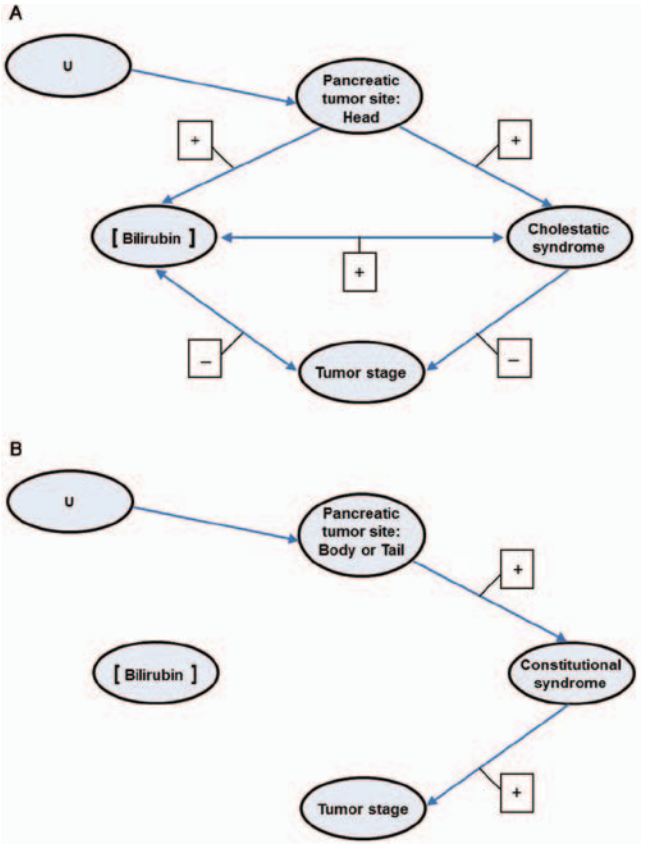

Figure 2. Relationships between pancreatic tumor site, bilirubin concentrations, syndromes (diagram A: cholestatic, diagram B: constitutional), and tumor stage in patients with exocrine pancreatic cancer. Heuristic diagrams representing associative and causal relationships between the syndromes, bilirubin blood concentration and tumor stage. The double-headed arrows indicate association (not causal) between the factors included. U, Unmeasured factors. [Bilirubin], Blood concentration of bilirubin.

There were no significant differences across hospitals in the concentrations of the biomarkers. Laboratory procedures were those performed in daily clinical care

by the participating hospitals. The objectives of the PANKRAS II study did not include to evaluate the analytical methodology of each hospital; thus, this methodology was not registered. When multivariate models (Tables 3 and 4) included the hospital of admission as a potential confounder, the associations of tumor stage and of cholestatic syndrome with the levels of the biomarkers analyzed did not change.

Results on signs and symptoms must always be interpreted with caution. First, extensive clinical evidence shows that some symptoms (e.g. jaundice) are less insidious and, hence, more easily perceived and reported by patients than others (e.g. anorexia); thus, some amount of symptom misclassification is inevitable. Second, the precision of estimates for very frequent or infrequent symptoms may be low. Third, syndromes may better reflect the relevant dimensions of the underlying pathophysiological processes. And fourth, pathophysiologic processes underlying syndromes are considerably different.

The study size was small and some of our estimates were statistically imprecise. Size also limited the number of variables that models could account for. However, data had enough power to detect associations of relevant magnitude and scientific relevance. Furthermore, this is the largest study of its type in pancreatic cancer, and findings deserve to encourage further studies along the same lines; i.e. integrating knowledge from internal medicine, clinical epidemiology and molecular epidemiology (Porta & Alvarez-Dardet 1998).

Cholestatic and constitutional symptoms reflect pathophysiological processes and disease progression; they are associated with pancreatic and liver parameters, as well as with tumor stage; they may also influence biomarkers of exposure (Porta et al. 2009). For instance, in diseases with high lipid mobilization, as EPC, liver and pancreatic tests can be helpful to assess changes in lipid concentrations and in concentrations of lipophilic biomarkers of environmental factors, such as certain vitamins and organochlorine compounds (Porta 2001; Porta et al. 2003, 2008, 2009, 2012). These relationships need to be considered by a number of studies using biomarkers, including reports on the etiologic, diagnostic and prognostic applications of "-omics" technologies (Gallo et al. 2011).

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Declaration of interest

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