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Original Research

Serum JAK/STAT profile is related to the IL expression but not with the outcome in pancreatic adenocarcinoma patients

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Abstract: Current genetic characterization of pancreatic ductal adenocarcinoma (PDAC) does not integrate the host reaction to cancer cells and cannot predict the response to chemo- or immunotherapy. The JAK/STAT pathway is an important factor of cytokine-mediated cancer inflammation, but its relationship with pancreatic carcinogenesis and the role of potential biomarkers is not established yet. Our study aimed to assess the significance of serum levels of JAK/STAT3 expression and inflammatory cytokines in PDAC in relation to the clinicopathological features and prognosis. This prospective cohort study included patients with proven adenocarcinoma and a matched group of controls without any malignancies. There were evaluated the serum expression of IL2, 6, 8, 17, JAK2, and STAT3 by ELISA assays in these two groups. The PDAC patients were followed up for 24 months. A Cox regression multivariate analysis model was used to determine factors influencing survival. The study comprised 56 patients with PDAC and 56 controls. The upregulated serum JAK2/STAT3 or cytokines were present in about half of the patients with PDAC, similar to controls. The expression of JAK2 in serum of PDAC patients was significantly associated with the expression of IL2 (p=0.03) and IL6 (p=0.02) but not with survival or metastasis development. Only age and the presence of lymph node metastases were associated with reduced survival in multivariate analyses. The STAT 3/JAK2 expression, although correlated with inflammatory status (IL2, IL6) was not overexpressed in PDAC compared to controls and proved no prognostic value.

Key words: Pancreatic cancer, Inflammation; IL6; JAK/STAT; Survival; Metastasis; Prognostic.

Introduction

Pancreatic cancer is the fourth most common cause of cancer death. More than half (53%) of patients are diagnosed at an advanced tumor stage, with a 5-year survival rate of less than 6% (1).

Chronic pancreatic inflammation, especially in chronic pancreatitis, represents a risk factor for the development of pancreatic cancer, with a relative risk varying from 2 to 100 in the case of hereditary pancreatitis (2). Also, a degree of systemic inflammation is present in smoking and obese patients, factors that contribute to an increased risk of pancreatic cancer (3).

Taking into consideration the possible involvement of the inflammation pathway in pancreatic carcinogenesis, the IL-6 pathway was found in murine Kras models as important for the progression of intraepithelial pancreatic neoplasia (4). Another cytokine-mediated cancer inflammation pathway, the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway was found in many malignancies (5) and promotes cell proliferation (10) and PanIN progression to PDAC (6,7). STAT3 signaling has a role in angiogenesis, metastasis, resistance to apoptosis, and cell proliferation in multiple types of tumors, including PDAC (8). Also, inhibiting STAT3 activation can block PanIN progression and reduce the development of PDAC in mouse models (9).

Moreover, PDAC presents a paucity of neoplastic cells embedded within a dense extracellular fibrotic matrix. The cancer-associated fibroblasts have a myofibroblast or inflammatory phenotype, and the pro-inflammatory cytokine Il1, through JAK/STAT activation, plays a role in the differentiation of inflammatory fibroblasts (10). Also, TGF beta is involved in myofibroblastic differentiation (10). However, the expression of the JAK/STAT3 pathway in clinical studies on PDAC was less studied.

Our study aimed to assess the significance of JAK/STAT3 biomarkers expression in PDAC related to the clinicopathological features, including survival, and to identify if this pathway is associated with inflammatory status.

Materials and Methods

Study design and setting

Data from patients diagnosed with pancreatic cancer were collected prospectively (between January 2016 and June 2017) from a tertiary academic medical center (the Regional Institute of Gastroenterology and Hepatology in Cluj-Napoca, Romania)

Participants

Subjects of the study group were at least 18 years old, with no previous history of any other cancer in the last five years.

The diagnosis of all pancreatic cancers was based on the fine-needle aspiration endoscopic Ultrasonography (EUS) results or surgical specimens. All subjects gave informed consent before being included in the study. Patients with an unclear pathological diagnosis for pancreatic adenocarcinoma were excluded.

The subjects of the control groups were healthy people who were at least 18 years old, with no previous history of any cancer or other chronic diseases. The controls were matched to cases for sex and age (plus/minus five years).

The PDAC patients were followed up for 24 months. The date for death was noted during this interval and the survival was calculated.

The study was approved by the Ethics Committee of the Regional Institute of Gastroenterology and Hepatology in Cluj-Napoca, Romania (No. 11387) and the reporting followed the STROBE criteria.

Data collection

We prospectively collected information regarding demographic data, diagnosis, staging, therapy, and survival. Demographic data included the age and gender of patients.

Cancer-related data included the date of diagnosis, the extension of the disease, location of the primary tumor, histological type.

Diagnosis and staging of pancreatic cancer were based on imaging tests, including Computer Tomography (CT) and EUS. A primary resectable tumor was distinguished between locally advanced and metastatic disease.

Survival was defined as the number of months between the date of diagnosis and the date of death. The date of diagnosis was defined as the time from the first imaging modality (CT, MRI, or EUS) giving the diagnosis of pancreatic cancer.

The Nutritional and functional assessment

Current body weight and height were measured at the time of inclusion. Diabetes was diagnosed if fasting glucose values met the ADA criteria (11) and the duration since diabetes onset was recorded.

Blood sampling

Blood samples were collected at the time of diagnosis. Peripheral venous blood was collected into a tube containing Ethylenediaminetetraacetic Acid (EDTA) and was prepared by centrifugation at 5000 × g for 5 min. The serum samples were stored at - 80°C until use. The selected protein was quantified from serum using Elisa analyses.

ELISA methods

JAK2, STAT3, IL2, 6, 8 and 17 serum levels were quantitatively determined by sandwich enzyme-linked immunosorbent assays (ELISA). Samples were individually measured in duplicates following kits' instructions (JAK2: MyBioSource catalog number MBS2515858, sensitivity 75.00 pg/mL, intra-assay precision CV = 4.24-6.27 % and inter-assay precision CV =3.15-6.92 %; STAT3: Fine Test catalog number EH0602, sensitivity <0.1800 ng/mL, intra-assay precision CV <8 % and inter-assay precision CV <10 %; IL-2: BioVendor catalog number RGP011R, sensitivity 0.97 pg/mL, intra-assay precision CV = 4.2 % and inter-assay precision CV =9.0%; IL-6: BioVendor catalog number RGP013R, sensitivity 0.81 pg/mL, intra-assay precision CV = 4.4 % and inter-assay precision CV =9.1%; IL-8: BioVendor catalog number RD194558200R, sensitivity 0.5 pg/mL, intra-assay precision CV = 3.7-5.2 % and inter-assay precision CV =6.1-8.2%; IL-17: R&D Systems catalog number D1700, sensitivity 15 pg/mL, intra-assay precision CV = 4.1-4.7 % and inter-assay precision CV =7.0-8.4%). A calibration curve was generated for each parameter using the protein standard provided by the kit. Absorbance was measured with a microplate reader (ClarioStar, BMGLabtech), data acquisition and processing were done by using the integrated Mars software. A 4-parameter fit calibration curve was used for the quantification and the final concentration was calculated as the mean of the two measurements.

Statistical analyses

The Chi-square test or Fisher exact test were used for categorical data. Comparisons between two groups of continuous data were performed with a t-test for independent samples for data with a normal distribution or a Wilcoxon rank-sum test otherwise. Univariate and multivariate Cox proportional hazard models with each protein expression variable adjusted for age, stage (III, IV vs. I, II), metastasis, tumor size >=3 cm, and diabetes were built. The Cox proportional hazard assumption and multicollinearity assumptions were checked. Similarly, univariate and multivariate logistic regression models (adjusted for age and N1) were built to predict metastasis. We checked the models for multicollinearity, misspecification and the goodness of fit. Associations between quantitative variables were assessed with the Spearman correlation coefficient.

For all statistical tests, a two-tailed p-value was used, along with a 0.05 significance level. All analyses were performed in the R environment for statistical computing and graphics, version 4.0.2.

Results

Patients' characteristics

We included 56 patients with PDAC (28 patients with PDAC and diabetes, 28 patients with PDAC without diabetes), and 56 controls. The patients and controls were matched for age (62.57±9.99 years old vs. 62.39±10 years old, p=0.9) and sex (male/female ratio 33/ 23 vs. 29/27, p=0.4). When comparing the group with diabetes and without diabetes to controls, no differ-

Table 1. Demographic and clinic characteristics of the patients in the adenocarcinoma and the control group, n (%).

	PDAC with diabetes (n=28)	PDAC without diabetes (n=28)	Controls (n=56)	P-value
Age (years), mean (SD)	63.29 (9.77)	61.86 (10.33)	62.39 (10)	0.9
Age >=50 years, n (%)	25 (89.3)	23 (82.1)	48 (85.7)	1
Sex (female), n (%)	9 (32.14)	14 (50)	27(48.2)	0.4
BMI (kg/m^2), median (IQR)	26.18±4.19(17.3-33)	24.72±5.75 (16.5-49.1)	-	0.07
Weight				
Underweight	3(10.71)	9(32.14)		0.05
Normal	6(21.43)	7(25)	--	0.75
Overweight	19(67.9)	12(48.9)		0.06
Obesity	7 (25)	4 (14.29)		0.31
Location				
Head+ uncinated, n (%)	20 (71.4)	16 (57.1)		0.26
Body+tail, n (%)	8 (28.6)	12 (42.9)		0.40
Smoking	15 (53.6)	13 (46.4)	-	0.59
T stage				
T3, n (%)	16 (57.1)	13 (46.4)	-	0.59
T4, n (%)	13 (46.4)	14 (50)		
N1 stage, n (%)	26 (92.9)	24 (85.7)	-	0.66
Metastasis, n (%)	6 (21.4)	9 (32.1)	-	0.36

PDAC, pancreatic cancer; SD, standard deviation; IQR, interquartile range; BMI, body mass index, T, tumor; N, adenopathy.

Table 2. Biomarkers expression in PDAC patients compared to controls**.**

Characteristic	PDAC with diabetes (n=28)	PDAC without diabetes (n=28)	P	PDAC (n=56)	Control (n=56)	P
JAK2 (pg/mL), median (IQR)	66014.36 (43812)	64435.14 (54144)	0.91	65189.21 (46983)	66346.69 (70792)	0.46
STAT3 (ng/mL), median (IQR)	50.75 (45.51 - 55.04)	55.74 (45.67 - 60.14)	0.52	51.5 (45.67 - 58.43)	48.92 (45.16 - 55.62)	0.57
IL-2 (pg/mL), median (IQR)	5836.08 (8468)	5950.1 (8805)	0.89	5950.1 (8620)	6728.97 (8824)	0.94
IL-6 (pg/mL), median (IQR)	274.42 (85.4)	264.65 (47.05)	0.19	268.6 (63.9)	259.42 (40.7)	0.19
IL-8 (pg/mL), median (IQR)	698.65 (352.9)	660.61 (285.5)	0.35	689.9 (307.6)	614.1 (302.2)	0.65
IL-17 (pg/mL), median (IQR)	3598.81 (1073.05)	3771.62 (1487.4)	0.9	3598.81 (1297.2)	3761.2 (1085.5)	0.7

IQR, interquartile range; PDAC, pancreatic ductal adenocarcinoma; JAK2, Janus kinase 2; STAT3, Signal Transducer and Activator of Transcription 3; IL, interleukin.

ence was seen for BMI patients, smoking status, location of the tumors, or TNM staging (Table 1).

Biomarkers expression in PDAC with or without diabetes compared to controls

The biomarkers values in the PDAC with or without diabetes compared to controls were higher than the median value in 27/56 patients vs. 30/56 patients for JAK2, 24/56 patients vs. 16/56 patients for STAT3, 47/56 patients vs. 52/56 patients for IL2 and 27/56 patients vs. 26/56 patients for IL6, 35/56 patients vs. 36/56 patients for IL8, 28/56 patients vs. 28/56 patients for IL17. None of the four markers were differentially expressed compared to controls (Table 2).

Also, no difference of expression of the four markers was proved for PDAC patients with or without diabetes (Table 2).

The risk of metastasis and mortality associated with protein expression

At 24 months of follow-up, almost 43% of patients with PDAC had died. The median overall survival (OS) was 18 months for the 56 patients with PDAC. Survival was significantly different in relation to the lymph node metastases (p=0.02) and age (p=0.04) in a Kaplan-Meier analysis, but with none from the biomarkers studied (Table 3).

No associations were found between biomarkers and metastases in the univariate, nor in the multivariate logistic regression analyses (Table 3).

Association of STAT3/JAK2 biomarkers with the inflammatory status

The expression of JAK2 in serum of patients with PDAC was significantly associated with the expression of IL2 and IL6 (Table 4).

Discussion

This prospective study shows that the JAK/STAT3 pathway has no serum overexpression in PDAC patients compared to controls and no association with the survival or metastasis during 24 months of follow-up.

JAK genes code tyrosine kinases, which are required for the signaling of a host of immune modulators in tumor, stromal and immune cells. They are activated by cytokines such as Il6 through phosphorylation of STAT3 (Signal Transducer and Activator of Transcription 3 alterations in this family have been associated with an immune evasion by tumor cells (5,12). Data from the literature have shown that STAT 3 contributes to the initiation and progression of PDAC (13–15). STAT 3

Table 3. Metastasis at the time of diagnosis and patients' survival in association with clinical, demographic and serologic parameters in PDAC patients.

	Metastasis risk at the diagnosis			***Patients' Survival***		
Univariate analysis	**OR unadjusted**	**95%CI**	***P* value**	**HR unadjusted**	**95%CI**	***P* value**
Age	1.07	(1 - 1.15)	0.08	1.05	(1 - 1.1)	0.047
Age > 50 yr	2.88	(0.45 - 56.49)	0.342	250449495.77	(0 - Inf)	0.998
Sex (male *vs* female)	0.73	(0.22 - 2.46)	0.607	1.12	(0.49 - 2.57)	0.784
Weight status						
Obese vs normal	0.33	(0.04 - 1.65)	0.213	0.7	(0.23 - 2.18)	0.543
Overweight vs normal	0.26	(0.05 - 1.05)	0.075	0.84	(0.34 - 2.06)	0.708
Smoking	0.35	(0.1 - 1.18)	0.101	0.83	(0.37 - 1.84)	0.638
Diabetes	0.58	(0.17 - 1.89)	0.368	1.32	(0.59 - 2.95)	0.497
Tumor size ≥ 3 cm	2.5	(0.37 - 49.77)	0.418	1.37	(0.4 - 4.68)	0.613
T4	3.13	(0.93 - 11.65)	0.072	1.06	(0.48 - 2.37)	0.881
N1	0.32	(0.05 - 1.9)	0.191	0.32	(0.12 - 0.87)	0.025
M1	-	-	-	0.79	(0.32 - 2)	0.627
Stages III, IV vs. I, II	-	-	-	1.52	(0.64 - 3.61)	0.348
JAK2 (pg/mL)	1	(1 - 1)	0.595	1	(1 - 1)	0.841
STAT3 (ng/mL)	0.9724	(0.9139 - 1.0231)	0.332	1.0126	(0.98 - 1.0463)	0.453
IL-2 (pg/mL)	1	(0.9999 - 1.0001)	0.872	1	(0.9999 - 1)	0.836
IL-6 (pg/mL)	1.0092	(1.001 - 1.0207)	0.066	0.9993	(0.9971 - 1.0016)	0.562
IL-8 (pg/mL)	1.0001	(0.9982 - 1.0016)	0.882	0.9994	(0.9981 - 1.0008)	0.429
IL-17 (pg/mL)	1.0002	(0.9996 - 1.0007)	0.496	1	(0.9996 - 1.0003)	0.953
Multivariate analysis	**OR adjusted**			**HR adjusted**		
JAK2 (pg/mL)	1	(1 - 1)	0.712	1	(1 - 1)	0.781
STAT3 (ng/mL)	0.9759	(0.9141 - 1.029)	0.419	1.0183	(0.9851 - 1.0526)	0.284
IL-2 (pg/mL)	1	(0.9999 - 1.0001)	1	1	(0.9999 - 1)	0.811
IL-6 (pg/mL)	1.0072	(1.0002 - 1.0195)	1.0072	0.9984	(0.9927 - 1.004)	0.568
IL-8 (pg/mL)	0.9999	(0.9981 - 1.0015)	0.9999	0.9993	(0.9981 - 1.0006)	0.281
IL-17 (pg/mL)	1.0002	(0.9997 - 1.0008)	1.0002	1.0001	(0.9997 - 1.0004)	0.751

OR, odds ratio; HR, hazard ratio; CI, confidence interval; T, tumor; N, adenopathy; M, metastasis; JAK2, Janus kinase 2; STAT3, Signal Transducer and Activator of Transcription 3; IL, interleukin; The multivariate logistic regression models were adjusted for age and N1; The multivariate Cox models were adjusted for age and stage III, IV vs. I, II.

Table 4. The association of STAT3/JAK2 biomarkers with inflammatory status.

	IL2	**IL6**	**IL8**	**IL17**
STAT3, rho (p value)	0.03 (0.81)	0.07 (0.60)	0.12 (0.39)	0.07 (0.59)
JAK2, rho (p value)	0.29 (0.03)	-0.31 (0.02)	0.11 (0.42)	-0.01 (0.97)

Rho, Spearman correlation coefficient; JAK2, Janus kinase 2; STAT3, Signal Transducer and Activator of Transcription 3; IL, interleukin.

mediates a series of biological responses involved in proliferation, apoptosis, and inflammation (5). The involvement of STAT 3 in tumor-genesis-associated inflammation has been demonstrated in the lung (16) and colon cancer (17).

In vivo studies showed that this pathway might activate the dendritic cells, followed by T cell activation (20). In mice, the loss of P53 function activates JAK2-STAT3 signaling to promote pancreatic tumor growth and stroma modification facilitating the immune evasion of PDAC cells, but the influence of STAT3 activation would be only transient (18).

Only a few studies revealed the biomarker potential of the JAK/STAT 3 pathway in the pancreas. Denley et al. demonstrated that a high expression of the JAK/STAT3 pathway was noted from 86 tissues of the resected PDAC and correlated with inflammatory markers such as PCR(19). The comparison of patients with high JAK/STAT3 expression with those with moderate or low expression proved an association with a reduced overall survival (hazard ratio=1.68) and with a reduction in the density of the local tumoral immune response (19), However, the serum profile of JAK/STAT3 expression was not assessed. In the current work we proved that the serum level is similar in PDAC patients to controls, it correlates with the inflammatory status represented by Il2 and Il8, but we found no association with survival or metastases development. Also, JAK2 was found in 62

patients with resectable PDAC analyzed by immunohistochemistry and a prognostic role was shown (20), but the therapeutic test with the JAK1/JAK2 inhibitor ruxolitinib has not proved an improvement of survival in patients with metastatic PDAC unless a high CRP was present (21). Another study has shown that the presence of JAK3 was seen in "immune exhausted "PDAC patients with microsatellite instability (22), without influence on prognosis (23), while a large work on 3594 PDACs targeting genomic alterations, did not report JAK/STAT modifications (24). These conflicting data and the present study on serum JAK2/STAT3 expression in PDAC patients raise the question about its involvement in the carcinogenetic process or only in the inflammatory status of the patient.

More facts about the gain-of-function mutations in JAKs which activate the JAK2/STAT3 pathway are known in hematologic malignancies (25). Also, JAK1 and JAK2 deficiency can be fatal due to neurological defects and erythropoiesis deficiencies (26).

Concerning the JAK3 mutations, this was associated with immunodeficiency syndromes (26) and the IL6 / JAK3 / STAT3 signaling pathway is also involved in tumor growth and progression and prevents antitumor immunity (27). IL-6 promotes the development of many solid tumors (breast, cervical, colorectal, esophageal, head-and-neck, ovarian, pancreatic, prostate, renal, and non-small cell lung cancers) (25).

Elevated levels of STAT3 have been observed in both solid and hematologic tumors (28), with a role in tumor growth and proliferation (29), antiapoptosis (30), angiogenesis (31), and metastasis (32). In preclinical studies, STAT3 inhibition results in increased apoptosis and decreased proliferation. STAT 3 is involved in resistance to chemotherapy. There is a close link between STAT3 activation and chemoresistance, and STAT3 inhibition has been shown to restore the sensitivity of tumors to chemotherapy (33–36), but we failed to find any clinical influence, too.

PDAC is associated with the presence of peritumoral inflammation, with inflammatory pathways being involved in tumorigenesis, with an unfavorable evolution (37–40). Increased systemic inflammation was independently associated with reduced survival after pancreaticoduodenectomy in patients with pancreatic cancer (24). Our findings proved that the JAK2 serum profile was correlated with Il2 and Il6 status, but no association with prognosis was found (Table 4). These negative results raised the question of the utility in clinical practice to use this biomarker in the case of PDAC, although initial studies considered it a promising target for cancer treatment (41-45).

There are limitations to our study. First of all, there was a limited number of patients included. Second, discouraged by the negative results in the serum sampling for JAK2/ STAT3, we did not perform the immunohistochemistry from the EUS fine-needle aspiration samples.

In conclusion, we have provided data that JAK2 is related to the inflammatory status in PDAC patients, but their serum level is not overexpressed compared to controls. Also, we were unable to prove an influence on the prognosis of such patients. However, further studies are required in order to better understand the mechanism and consequence of JAK and STAT3 activation in PDAC.

Conflict-of-interest

The authors have no conflicts of interest to declare

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None

Author contributions

Petrusel L, Seicean A and Seicean R conceived and designed the study; Petrusel L, Rusu I, Seicean A, Ilies M and Iuga C performed the research; Leucuta DC analysed data; Petrusel L, Seicean A and Seicean R wrote the paper.

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