The Role of Inflammation in Patients with Intraductal Mucinous Neoplasm of the Pancreas and in those with Pancreatic Adenocarcinoma

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Abstract. *Background: There are very few data regarding inflammation in patients with intraductal papillary mucinous neoplasms (IPMNs) of the pancreas. Aim: To evaluate the circulating concentrations of placental growth factor (PlGF), transforming growth factor-alpha (TGF-α), transforming growth factor-beta1 (TGF-β1), tumour necrosis factor receptor 1 (TNF-R1) and matrix metalloproteinase-2 (MMP-2) in patients with IPMNs and in those with pancreatic adenocarcinomas. Patients and Methods: Sixty-nine patients were enrolled: 23 (33.3%) had IPMNs and 46 (66.7%) had histologically confirmed pancreatic adenocarcinomas. Thirteen healthy subjects were also studied. PlGF, TGF-α, TGF-β1, TNF-R1 and MMP-2 were determined using commercially available kits. Results: TNF-R1 (p=0.003) was the only protein significantly different among the three groups. Conclusion: Serum TNF-R1 was elevated in patients with IPMNs and in those with pancreatic adenocarcinomas, suggesting a high apoptotic activity in both groups of patients studied.*

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas have received much clinical attention in the last decade because they are slow-growing tumours which may be cured surgically in most patients (1). However, there are only few data regarding inflammation processes associated with this disease. In contrast, there is notably more information available about ductal adenocarcinomas of the exocrine pancreas. Among the causes of the aggressive behaviour of IPMNs, several passive and active strategies appear to be adopted by tumour cells to circumvent antitumour immune defenses. They include altered expression of major histocompatibility complex (MHC) class I and II antigens (2), which may impair interaction between malignant cells and potential tumour cytotoxic T lymphocytes (CTLs), and resistance to apoptosis through the Fas receptor pathway coupled with aberrant expression of the ligand, which may be considered part of the 'counterattack' of the tumour cells against the immune effector cells (3-5). Pancreatic carcinoma cells have also been shown to spontaneously secrete immunosuppressive cytokines, such as interleukin-10 and transforming growth factor-beta (TGF-β), which downregulate the host immune system and contribute to a systemic T helper 2 immune phenotype *in vivo* (3, 6); thus, cytokines play a pivotal role in the induction of cell mediated and humoral immunity (7).

Of several circulating substances which can be evaluated in patients with cancer, this study aimed to evaluate the following: placental growth factor (PlGF) (an angiogenesis-related factor (8)), transforming growth factor-alpha (TGF-α), transforming growth factor-beta1 (TGF-β1) (since TGF-α acts synergistically with TGF-β in inducing phenotypic tumoural transformation (9)), tumour necrosis factor receptor 1 (TNF-R1) (member of the TNFR superfamily, which is able to regulate a broad array of developmental processes and plays a pivotal role in numerous biological events in mammals, including induction of apoptosis, survival, differentiation and proliferation of cells (10)) and matrix metalloproteinase-2 (MMP-2) (which is a part of a family of proteolytic enzymes capable of degrading several substrates within the extracellular matrix, which is an essential step in the processes of invasion and metastasis (11)). All these

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Key Words: Placental growth factor, transforming growth factor-alpha, transforming growth factor-beta 1, tumour necrosis factor receptor 1, matrix metalloproteinase-2, pancreatic neoplasms.

substances have been studied previously in pancreatic ductal adenocarcinomas, mainly in pathological specimens, but not in patients with pancreatic IPMNs. Thus, the aims of the present study were to evaluate the circulating concentrations of PlGF, TGF-α, TGF-β1, TNF-R1 and MMP-2 in malignant diseases of the pancreas, such as IPMNs and pancreatic adenocarcinomas, and also to evaluate the concentrations of these molecules in comparison with an established serum marker of pancreatic cancer such as the carbohydrate antigen 19-9 (CA 19-9).

Patients and Methods

Patients. Consecutive patients eighteen years of age or older who were admitted to the Unit for the Study of Pancreatic Diseases of Sant'Orsola-Malpighi Hospital of Bologna (Italy) for exocrine pancreatic neoplasms between October 2007 and March 2009 were eligible for the study.

A total of 69 patients (39 males, 30 females, mean age: 69.8±10.4 years) were enrolled: 23 (33.3%) had IPMNs and 46 (66.7%) had histologically confirmed pancreatic adenocarcinomas; the demographic and clinical characteristics of the patients studied are reported in Table I. The body mass index (BMI) was stratified according to the WHO classification (12) and pancreatic insufficiency was defined as faecal elastase-1 concentrations less than 200 μg/g (13). At the time of the study, none of the patients had had any treatment for their disease. Of the 23 patients with IPMNs, 10 (43.5%) had branch type IPMNs and the remaining 13 (56.5%) had main duct type IPMNs.

Finally, 13 blood donors were also studied as healthy controls (7 males, 6 females, p=1.000 *vs*. all patients; mean age: 57.0±14.6 years, p=0.003 *vs*. all patients).

Methods. Blood specimens were obtained in the morning in a fasting state from each subject. Laboratory personnel were unaware of the clinical diagnoses or the details of the patient clinical history. The serum specimens were stored at –20˚C until analysis.

PlGF, TGF-α, TGF-β1, TNF-R1 and MMP-2 were determined using commercially available kits (R&D Systems, Minneapolis, MN, USA). The intra-assay CVs were <3.9% and the inter-assay CVs were <7.9%. The reference limits used were: 0.2-26 pg/ml for PlGF, 0.5-32 pg/ml for TGF-α, 18,289-63,416 pg/ml for TGF-β1, 749-1,966 pg/ml for TNF-R1 and 117-410 ng/ml for MMP-2. Furthermore, CA 19-9 was also assayed using an electrochemical luminescence immunoassay (reference limits: 0-37 U/ml).

Ethics. The study was approved by the Clinical Committee of the Department of Internal Medicine of Sant'Orsola Hospital of Bologna (Italy) and was carried out in accordance with the Helsinki Declaration of the World Medical Association. All subjects gave written informed consent to participate in the study.

Statistical analysis. Means, standard deviations and frequencies were used as descriptive statistics. Data were analysed by means of non-parametric tests: Kruskal-Wallis test, Spearman rank correlation, Fisher's exact test, Pearson chi-square, and linear-by-linear association chi-square. SPSS v. 13.0 (SPSS Inc., Chicago, IL, USA) was used to analyse the data. Two-tailed p-values of less than 0.05 were considered statistically significant.

Table I. *Demographic and clinical characteristics of the patients with malignant pancreatic diseases according to the final diagnosis. Data are reported as mean±SD or frequencies.*

	Pancreatic intraductal papillary mucinous neoplasm (N=23)	Pancreatic adeno-carcinoma (N=46)	p-Value
Gender			0.797[a]
Male	14 (60.9%)	25 (54.3%)	
Female	9 (39.1%)	21 (45.7%)	
Age (years)	72.1±7.6	68.6±11.5	0.165[b]
Body mass index (kg/m^2)	22.4±1.9	20.8±3.0	0.010[b]
BMI classes			
Underweight (<18.5 kg/m^2)	-	12 (26.1%)	0.062[c]
Normal (18.5-24.9 kg/m^2)	22 (95.7%)	32 (69.6%)	
Pre-obese (25-29.9 kg/m^2)	1 (4.3%)	1 (2.2%)	
Obese (30 kg/m^2 or more)	-	1 (2.2%)	
Localisation			
Head	15 (65.2%)	36 (78.3%)	0.174[d]
Body	1 (4.3%)	4 (8.7%)	
Body/tail	0	2 (4.3%)	
Tail	3 (13.0%)	2 (4.3%)	
Diffuse	4 (17.4%)	2 (4.3%)	
Metastasis	2 (8.7%)	31 (67.4%)	<0.001[a]
Pain	4 (17.4%)	44 (95.7%)	<0.001[a]
Jaundice	3 (13.0%)	26 (56.5%)	0.001[a]
Diabetes	8 (34.8%)	40 (87.0%)	<0.001[a]
Pancreatic insufficiency	7 (30.4%)	14 (30.4%)	1.000[a]
Pancreatic surgery	9 (39.1%)	21 (45.7%)	0.797[a]

[a]Fisher's exact test; [b]Kruskal-Wallis test; [c]linear-by-linear association chi-square; [d]Pearson chi-square.

Results

The two groups of patients were not statistically different regarding the gender (p=0.797), age (p=0.165) and localisation of the tumour (p=0.174), whereas the BMI of patients with pancreatic ductal adenocarcinomas was significantly lower than that of patients having IPMNs (p=0.010; Table I). In addition, the frequencies of metastases (p<0.001), pain (p<0.001), jaundice (p=0.001) and diabetes (p<0.001) were significantly higher in patients with pancreatic ductal adenocarcinomas than in those having IPMNs. The frequency of pancreatic exocrine insufficiency at the time of the study and the frequency of patients who underwent pancreatic surgery after the diagnosis were not different between the groups of patients studied (p=1.000 and p=0.797, respectively).

The mean and standard deviation values of serum concentrations of PlGF, TGF-α, TGF-β1, TNF-R1, MMP-2 and CA 19-9 in the two patient groups and the healthy subjects studied are reported in Table II.

Table II. *Circulating levels of the various moleades studied in the three groups of subjects. Data are reported as mean±SD and the Kruskal-Wallis test was applied.*

	Pancreatic intraductal papillary mucinous neoplasm (N=23)	Pancreatic adeno-carcinoma (N=46)	Healthy subjects (N=13)	*p*-Value§
PlGF (pg/ml)	21.6±19.7	18.9±16.3	30.1±25.6	0.499
TGF-α (pg/ml)	19.3±25.8	21.0±38.3	1.8±1.2	0.153
TGF-β1 (pg/ml)	14,132±4,074	14,298±4,452	15,732±3,089	0.408
TNF-R1 (ng/ml)	1,987±940[b]	1,872±722[a]	1,170±505	0.003
MMP-2 (ng/ml)	280±78	267±68	233±73	0.237
CA 19-9 (U/ml)	1,237±4,905*	1,268±2,828[$c]	21.4±8.8	0.007

§Comparisons among the three groups of patients studied; [a]p=0.001; [b]p=0.004; [c]p=0.003: *vs*. healthy subjects; [$]$p$=0.044 *vs*. intraductal papillary mucinous neoplasms; *One patient had serum CA 19-9 concentrations of 23,688 U/ml. In the other 22 patients, CA 19-9 serum concentrations ranged from 0.53 to 1,151 U/ml, with a mean value±SD of 172±269 U/ml.

Table III. *The significance of the relationships between the clinical parameters and the serum concentrations of TNF-R1 and CA 19-9 in 23 patients with pancreatic intraductal papillary mucinous neoplasms (IPMNs) and in 46 patients with pancreatic adenocarcinomas (ADACs). The Kruskal-Wallis test was applied (with the exception of BMI) and the significant p-values are reported in bold.*

	TNF-R1		CA 19-9	
	IPMN	*ADAC*	*IPMN*	*ADAC*
Gender (males *vs*. females)	0.166	0.100	0.257	0.749
Class of BMI[a]	0.098	0.209	0.559	0.489
Localisation of the tumour	0.669	0.553	0.553	0.484
Metastases (yes *vs*. no)	0.585	**0.034**	0.081	0.648
Pain (yes *vs*. no)	0.417	0.332	1.000	0.085
Jaundice (present *vs*. absent)	0.201	0.063	0.235	0.947
Diabetes (yes *vs*. no)	0.220	0.514	0.156	0.744
Pancreatic exocrine insufficiency (yes *vs*. no)	0.204	0.053	0.204	0.305
Pancreatic surgery (yes *vs*. no)	0.801	0.051	0.801	0.087
Branch type *vs*. main duct type	0.495	-	0.620	-

[a]Spearman rank correlation.

The only two parameters showing significant differences among the three groups were TNF-R1 (p=0.003) and CA 19-9 (p=0.007). In particular, TNF-R1 concentrations were significantly higher in both patient groups than in healthy subjects (IPMN, p=0.004; pancreatic adenocarcinoma, p=0.001). In contrast, serum CA 19-9 concentrations were significantly higher in patients with pancreatic adenocarcinomas than in those with IPMNs (p=0.044) and healthy subjects (p=0.003). It should be noted that a serum CA 19-9 concentration of 23,688 U/ml was found in one patient with a main duct IPMN and distant metastases; in the other 22 patients, CA 19-9 serum concentrations were 172±269 U/ml (mean±SD) and ranged from 0.53 to 1,151 U/ml.

The significance of the relationships between the clinical parameters and the serum concentrations of TNF-R1 and CA 19-9 are reported in Table III. Within the group of patients with pancreatic adenocarcinomas, patients with metastases had serum concentrations of TNF-R1 significantly higher than those without (p=0.034) (Figure 1).

Discussion

It is well known that patients with pancreatic adenocarcinomas have a poor outcome; on the other hand, IPMNs of the pancreas are slow-growing neoplasms which may be cured surgically in most patients (1). The differences between the two neoplasms are also confirmed by the clinical data of this study. In fact, patients with ductal adenocarcinomas had a frequency of pain, diabetes, jaundice and metastases that was significantly higher than those with IPMNs, as well as a significantly lower BMI. Unfortunately, in this study, only few data were available regarding inflammation in pancreatic IPMNs. Thus, the present study evaluated PlGF, TGF-α, TGF-β1, TNF-R1 and MMP-2 in patients with malignant chronic diseases of the pancreas, such as pancreatic adenocarcinomas and IPMNs, since a better understanding of the basis of molecular cross-talk between tumour cells and the immune system will be helpful in developing immunotherapeutic approaches to pancreatic neoplasms, motivated by the lack of conventional immune therapeutic options for these patients, especially for those having an IPMN.

PlGF is implicated in several pathological processes, including the growth and spread of cancer. In agreement with a previous report (14), this study found that the serum levels of PlGF in both patient groups (those with pancreatic adenocarcinomas and those with IPMNs) were not significantly different from those in control subjects. These results are not surprising given that PlGF has been associated with arteriogenesis (15) and it is up-regulated after anti-vascular endothelial factor therapy as this substance is involved in the rapid restoration of the tumour blood supply after treatment (16). Thus, in this study, the patients were not medically treated at the time of the study and this explains, at least in part, the results obtained.

A behaviour similar to that of PlGF was found for TGF-α, TGF-β1 and MMP-2. In particular, TGF-α is involved in cancer progression. Gastrointestinal cancer cells produce and secrete TGF-α *in vitro* (17), and immunohistochemical

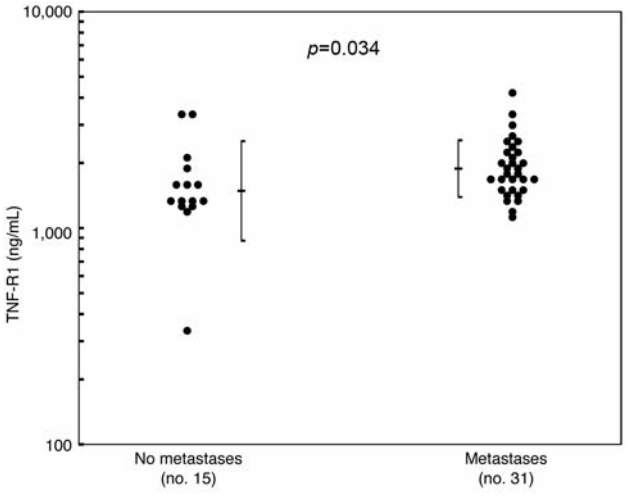

Figure 1. *Distribution of TNF-R1 in patients with pancreatic adenocarcinomas with and without metastases. The Kruskal-Wallis test was applied. Mean and SD values are also shown.*

staining of sections of human tumours has shown that these tumours express TGF-α (18). Thus, the tumours themselves may contribute to the increase in TGF-α. This study confirmed the previously reported results showing that TGF-α is a normal constituent of human serum (19), lending support to the hypothesis that TGF-α plays a role as a growth factor in normal cellular physiology. TGF-α is also thought to play a role in inflammatory response (20) and wound healing (21). Thus, physiological response to the presence of tumours, to treatment or to both may also contribute to increased serum TGF-α levels in these patients.

Regarding TGF-β1, it was found that serum concentrations of this substance in the patients with pancreatic adenocarcinomas were not significantly different from those found in patients with IPMNs and in healthy subjects; the mechanisms linking levels of serum TGF-β1 and pancreatic cancer are unclear because of the complex, dual role of TGF-β in carcinogenesis (22). Furthermore, TGF-β is produced by and can act on nearly every cell type and on normal cells; thus, it is possible that, in humans, elevated concentrations of this substance can be found in pancreatic tissue (23) but not in the circulation. Another possible explanation is that TGF-β is involved in the process of carcinogenesis (24) and, when the cancer becomes evident, its serum levels tend to normalise.

The overexpression of MMP-2 has largely been described in human tumours and the presence of its active form at the invasion front correlates with its invasive potential (25). Immunohistochemical studies showed MMP-2 in tumoural as well as in stromal cells, whereas *in situ* hybridisation demonstrated *MMP-2* mRNA in stromal cells (26). It has been suggested that peritumoural fibroblasts deliver MMP-2 to cancer cells which could bind (27) and activate gelatinase A. To date, there have been no studies on the circulating levels of this molecule in pancreatic cancer patients to the authors knowledge. This study found its circulating levels to be similar in pancreatic cancer patients and in healthy subjects. Furthermore, they were also similar in patients with IPMNs and in those with pancreatic adenocarcinomas.

The biological activities of TNF are mediated through two TNF receptors (TNF-Rs), namely TNF-R1 and TNF-R2. TNFR trimerisation is necessary for the functional activities mediated by the receptors, and this self-assembly occurs in the absence of the ligand (28). Engagement of TNF-R1 by TNF activates several transcription factors which include NF-κB and c-Jun/activator protein 1, leading to upregulation of a large number of genes involved in inflammatory and immune responses (29, 30). To date, TNF-R1 signaling studies have been difficult to perform due to the proapoptotic consequences of overexpression, and some researchers have resorted to the use of cytoplasmic truncations of TNF-R1 which do not reflect the physiological events or the natural structure of these molecules (31). Furthermore, the identification of the proteins involved in the control of apoptosis may provide leads for novel therapeutic approaches; finally, there are no studies exploring the behaviour of TNF-R1 in patients with pancreatic neoplasms. Thus, the circulating levels of this substance were evaluated in adenocarcinoma patients and IPMN patients and it was found that serum concentrations of TNF-R1 were significantly higher in both patients with pancreatic adenocarcinomas and those with IPMNs as compared to the control subjects, suggesting a high apoptotic activity both in patients with adenocarcinomas and those with IPMNs. Most importantly, the serum levels of TNF-R1 in patients with pancreatic adenocarcinomas were higher in patients with metastases than in those without, suggesting that this protein may be utilised in clinical practice for selecting those patients who can be surgically resected.

The relatively small sample size in each group may be considered as a weakness of this study; however, it should be noted that exocrine pancreatic neoplasms have not a high incidence and that the patients were consecutively enrolled.

In conclusion, serum TNF-R1 was elevated in patients with IPMNs and in those with pancreatic adenocarcinomas suggesting a high apoptotic activity in both groups of patients studied; further studies should be performed in order to confirm the findings that serum TNF-R1 is also a potentially good marker for selecting patients with pancreatic ductal adenocarcinomas having distant metastases.

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Received May 12, 2010
Revised June 1, 2010
Accepted June 8, 2010