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**Decreased serum platelet derived growth factor BB levels in acute and increased in chronic pancreatitis**

Stojek M *et al.* Growth factors in pancreatic diseases

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## **Abstract**

### **BACKGROUND**

#### **AIM**

To examine circulating growth factor concentrations in patients with acute pancreatitis (AP) and chronic pancreatitis (CP), and walled-off pancreatic necrosis (WOPN).

#### **METHODS**

Forty patients with mild AP, 40 patients with alcoholic CP, 33 patients with WOPN and 40 healthy subjects were examined. Serum concentrations of platelet derived growth factor BB (PDGF-BB), transforming growth factor  $\beta$ -1 (TGF $\beta$ -1), chemerin and high-mobility group box chromosomal protein 1 (HMBG1) were assayed by enzyme linked immunosorbent assay (ELISA).

#### **RESULTS**

Patients with mild AP and those with WOPN had significantly lower serum levels of PDGF-BB compared to healthy subjects ( $4.0 \pm 0.61$  ng/ mL *vs*  $6.2 \pm 0.76$  ng/ mL,  $P = 0.027$ , and  $1.60 \pm 0.31$  ng/ mL *vs*  $6.2 \pm 0.76$  ng/ mL,  $P < 0.001$ , respectively), while CP was associated with higher serum levels of PDGF-BB ( $12 \pm 1.3$  ng/ mL *vs*  $6.2 \pm 0.76$  ng/mL,  $P < 0.001$ ). Circulating TGF $\beta$ -1 and chemerin levels were elevated in CP patients ( $57 \pm 3.6$  ng/ mL *vs*  $39 \pm 3.6$  ng/mL,  $P < 0.001$  and  $73 \pm 7.2$  ng/ mL *vs*  $48 \pm 2.3$  ng/mL,  $P < 0.001$ , respectively), but not in patients with AP and WOPN. No significant changes in serum HMBG1 levels were found either in patients with AP, WOPN or CP.

**CONCLUSION:** The serum levels of some growth factors and cytokines-differ significantly in AP, WOPN and CP. These data suggest that selected growth factors and cytokines may be considered as potential diagnostic biomarkers in patients with pancreatic diseases.

**Key words:** Acute pancreatitis; Chronic pancreatitis; Walled-off pancreatic necrosis; Growth factors; Platelet derived growth factor BB; Transforming growth factor  $\beta$ -1; High-mobility group box chromosomal protein 1; Chemerin

**Core tip:** Patients with mild acute pancreatitis (AP) and patients with walled-off pancreatic necrosis (WOPN) had significantly lower serum levels of platelet derived growth factor BB (PDGF-BB) compared to healthy subjects. In contrast, alcoholic chronic pancreatitis (CP) was associated with higher serum levels of PDGF-BB. Circulating transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) and chemerin levels were elevated in CP patients, but not in patients with AP and WOPN. No significant changes in serum high-mobility group box chromosomal protein 1 (HMGB1) levels were found either in patients with AP, WOPN or CP. These data suggest that selected growth factors and cytokines may be considered as potential diagnostic biomarkers in patients with pancreatic diseases.

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## INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease triggered by intra-acinar activation of proteolytic pancreatic enzymes, leading to digestive injury of the pancreas. Inflammatory mediators—play an important role in AP, especially in the resultant multiple organ dysfunction syndrome, the primary cause of death in AP<sup>[1]</sup>. It has been shown that patients with severe AP displayed elevated levels of serum platelet derived growth factor BB (PDGF-BB)<sup>[2]</sup>. In contrast, serum transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) levels were significantly lower in patients with severe AP than in healthy subjects<sup>[3]</sup>.

Approximately 15% of patients with AP develop local complications, including pancreatic and peripancreatic collections. The most recent Atlanta classification distinguishes between acute peripancreatic fluid collections, pancreatic pseudocysts, acute necrotic collections and walled-off pancreatic necrosis (WOPN). WOPN is defined as a mature, encapsulated collection of pancreatic and/or peripancreatic necrosis with a well-defined inflammatory wall occurring more than 4 wk after onset of necrotizing pancreatitis<sup>[4]</sup>.

Chronic pancreatitis (CP) is characterized by irreversible damage to the pancreas, associated with chronic inflammation, fibrosis, and progressive destruction of both exocrine and endocrine parts of pancreas. Pancreatic fibrosis in patients with CP is associated with pancreatic parenchymal cell death followed by fibrosis. Pancreatic stellate cells (PSCs) (resident or derived from bone marrow) are activated by cytokines and growth factors (including PDGF-BB and TGF- $\beta$ ), which lead to extracellular matrix production and consequently fibrosis<sup>[5]</sup>. Recently we have shown that serum PDGF-BB and TGF- $\beta$ 1 are coordinately elevated in patients with CP<sup>[6]</sup>. Moreover, we have shown that chemerin, a pro-inflammatory chemokine, highly expressed in liver and adipose tissue is also elevated in serum of patients with CP<sup>[7]</sup>.

High-mobility group box chromosomal protein 1 (HMGB1) is a nuclear binding protein, identified as a pro-inflammatory factor and late mediator of endotoxin lethality in animal models<sup>[8]</sup>. HMGB1 is secreted by monocytes/macrophages and released from damaged and necrotic cells<sup>[9,10]</sup>. Serum HMGB1 was elevated in patients with some diseases<sup>[11-16]</sup> including advanced pancreatic cancer undergoing chemotherapy<sup>[17]</sup> and severe AP<sup>[18]</sup>. Increased HMGB1 level in serum and diseased organs (pancreas, liver kidney,

lung and ileum) was confirmed in an animal experimental model of severe AP<sup>[19,20]</sup>. No information is available about serum HMGB1 levels and its role in either WOPN or CP.

The aim of the present study was to compare serum levels of high-sensitivity C-reactive protein (hsCRP), PDGF-BB, TGF- $\beta$ 1, HMGB1 and chemerin in patients with mild AP, WOPN and CP to get more information about the possible role of these growth factors and cytokines in pathology of pancreatitis.

## **MATERIALS AND METHODS**

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Medical University of Gdansk Ethics Committee. All patients signed an informed consent form for this investigation.

### ***Patients with chronic pancreatitis***

Out of patients treated for CP in the Department of Gastroenterology and Hepatology, Medical University of Gdansk we selected 40 non-diabetic male patients, aged 32-55 yr (mean age  $45 \pm 1.2$  yr) with a history of alcoholic CP. Patients were moderate or heavy drinkers (at least 70 g of ethanol per day for more than 5 yr). Assessment of patients' alcohol intake was based solely on self-reports. The duration of disease in patients included in the study ranged from 1 to 12 yr (mean duration, 5 yr). All patients reported abdominal pain. All patients with advanced pancreatic changes (grade 5 according to the Cambridge classification) underwent endoscopic treatment (endoscopic sphincterotomy, pancreatic duct stone removal, treatment of pancreatic duct strictures and/or pancreatic pseudocysts). All patients included in the study met diagnostic criteria for CP<sup>[21]</sup>. The diagnosis was based on clinical symptoms and typical results of imaging studies. As determined by the results of endoscopic retrograde pancreatography (ERP), 34 patients displayed marked (grade 5 according to Cambridge classification), 4 moderate (grade 4) and 2 mild (grade 3) stage of disease. Most patients with CP had an exacerbation of the disease on admission. All patients included in the study were cigarette smokers.

### ***Patients with mild acute pancreatitis***

According to the most recent Atlanta classification, mild AP is defined as AP without organ failure and without either local or systemic complication<sup>[4]</sup>. 40 patients with mild AP admitted to the Department of Gastroenterology and Hepatology participated in the study. The diagnosis of AP was established in all patients based on the presence of epigastric pain and elevated amylase/lipase. Transabdominal ultrasound was performed in all cases to confirm or exclude biliary disease. Computed tomography was performed in selected cases where there was any diagnostic uncertainty. Patients received supportive treatment including intravenous fluids and pain medications. Elective ERCP was performed in selected cases. Serum samples were obtained between the 1<sup>st</sup> and 3<sup>rd</sup> day of hospitalization.

### *Patients with walled-off pancreatic necrosis*

The third group consisted of patients with severe necrotizing pancreatitis between the 6<sup>th</sup> and 10<sup>th</sup> weeks since the onset of disease, hospitalized in the Department of Gastroenterology and Hepatology, Medical University of Gdansk. Mean age in this group was  $48 \pm 2.5$  yr. In 15 out of 33 patients there was documented alcohol abuse. 20 patients were former or current smokers. The patients presented with a variety of symptoms, such as abdominal pain, post-prandial fullness and early satiety, nausea or vomiting that were attributable to the presence of WOPN. All patients in this group underwent contrast-enhanced computed tomography both at the time of initial diagnosis and as a follow-up study leading to the diagnosis of WOPN. Endoscopic ultrasound was performed in selected cases to confirm the nature of the collection. Serum samples were obtained on the morning before the scheduled endoscopic drainage.

### *Healthy control subjects*

Thirty-five healthy male volunteers from the same demographic group as the patients with CP, AP and WOPN who received annual health examinations and reported only occasional alcohol consumption, aged 24-64 yr (mean age  $42 \pm 2.2$  yr) formed the control group.

### *Statistical analysis*

At 8 a.m. fasting blood samples were collected from patients and healthy controls. Serum PDGF-BB concentrations were determined by enzyme linked immunosorbent assay (ELISA)

assay produced by Ray Biotech, Inc., GA, USA. TGF- $\beta$ 1 concentrations were determined by ELISA assay produced by R&D System, UK. The serum chemerin concentration was determined by ELISA assay produced by Milipore Corporation, MA, USA. Serum HMGB1 concentration was measured with the ELISA assay produced by IBL International, Germany. Concentrations of CRP in serum and other biochemical parameters were measured at the Central Clinical Laboratory of Medical University of Gdansk. Statistical analysis was performed using Microsoft Excel and Statistica. The statistical significance of differences observed between patients and controls was assessed using the two-tailed *t*-test.

## RESULTS

The most important laboratory and anthropometric values in patients with mild AP, WOPN and CP and in age-matched healthy control subjects are presented in Table 1. Body weight of patients with CP and WOPN was significantly lower than in control subjects, whereas it was similar in AP patients and control subjects. BMI was significantly lower only in CP patients. Serum amylase and lipase were significantly increased in patients with AP, WOPN and CP, however the highest -levels were found in AP patients. The mean serum triacylglycerol concentration was approximately 2-fold higher in patients with AP than in healthy subjects or patients with CP. Higher serum triacylglycerol concentration was also observed in patients with WOPN compared to healthy subjects. The mean serum cholesterol concentrations were lower in WOPN and CP patients than in control subjects and AP patients.

The mean serum hsCRP concentrations were elevated in all patients with AP, WOPN and CP, with the highest values found in patients with AP (Table 2). In patients with AP the mean serum hs-CRP was about 50 time higher than in control subjects, whereas in patients with WOPN and CP approx. 20-fold higher. The most unexpected finding in this study is that serum PDGF-BB concentration was significantly lower in patients with both AP and WOPN (Table 2). As expected, based on our previously published results<sup>[6]</sup>, serum PDGF-BB concentrations in patients with advanced CP were approximately 2-fold higher than in control subjects (Table 2). The mean serum PDGF-BB concentration in patients with AP and WOPN was approximately 3 and 6-fold lower than in CP patients respectively (Table 2). Conversely, the mean serum TGF- $\beta$ 1 concentrations in patients with AP and



WOPN were essentially similar as in control subjects (Table 2). Significantly higher mean serum TGF- $\beta$ 1 concentrations were found in patients with advanced CP (Table 2). The pattern of changes in serum chemerin concentrations in patients with AP (no change), WOPN (no change) and CP (significant increase) resembled the changes in serum TGF- $\beta$ 1 concentrations (Table 2). Surprisingly, no change was found in serum concentrations of HMGB1 in patients with AP, WOPN and CP as compared to control subjects (Table 2).

## DISCUSSION

In this paper, we show that serum concentrations of PDGF-BB decreased significantly in patients with mild AP. This is in contrast to the results reported recently, which show that serum PDGF-BB is elevated in AP, especially in patient with unfavorable clinical evolution of the disease<sup>[2]</sup>. There are several possible explanations for this discrepancy. The first and possibly most important is the degree of severity of AP. Our patients with AP were classified as having mild disease according to the current Atlanta classification. The patients included in the study reported by Espinosa *et al*<sup>[2]</sup> had predicted: (1) mild AP (approximately 75% of study participants) and (2) severe AP (25% of participants) as determined by the Ranson score. Pooling data from patients with mild and severe AP was probably contributing to huge dispersion of the results ( $12 \pm 11$  ng/L serum PDGF-BB concentration). The dispersion of the results in our study was much lower. Secondly, it is not clear why in the study by Espinosa *et al*<sup>[2]</sup>, the reported concentration of serum PDGF-BB in control subjects was relatively low ( $0.99 \pm 0.95$  ng/L). Several authors using the same system for serum PDGF-BB concentration determination (ELISA, R&D System, Minneapolis MN, USA), reported the median serum concentrations of PDGF-BB in healthy subjects to be approximately 4 ng/mL<sup>[22-24]</sup>, which is close to the values found in our control group. However, a much higher median value for serum PDGF-BB in healthy subjects (approx. 13 ng/mL; range 1.08 to 76.9 ng/mL) has also been reported<sup>[25]</sup>. Together, these studies point to a huge variability in serum PDGF-BB in healthy humans, which may lead to misleading interpretation of the data. Considering: (1) that our assays were performed by one person; (2) that serum samples from each patient (or healthy subject) were tested several times using the same determination system; and (3) that more homogenous groups of patients with AP and WOPN were included in the study, we

believe that serum PDGF-BB levels in mild AP patients are indeed slightly but significantly lower compared to healthy subjects and patients with CP. Moreover, the results presented here suggest that serum concentrations of PDGF-BB in patients with WOPN are significantly lower than in healthy subjects. To our best knowledge, this is the first report of a decreased serum level of PDGF-BB in WOPN. Thus, the question arises about the particular molecular mechanism which causes the decrease in serum PDGF-BB concentrations in patients with AP and WOPN and the possible consequences of the decrease in serum PDGF-BB. Based on the data presented here we can only speculate that decreased synthesis or increased degradation of PDGF-BB (or both) may occur during the course of AP. PDGF signaling modulates both fibrosis and angiogenesis<sup>[26]</sup>. It has been proposed that PDGF is involved in regulating maturation of the infarct vasculature<sup>[26]</sup>. By analogy, one may suppose that decreased levels of PDGF in AP patients may lead to impairment in vascular maturation during resolution of the inflammatory process which is an important step in pancreatic healing. Therefore the decrease in PDGF-BB could be one possible factor leading to pancreatic necrosis in WOPN patients.

In this paper, we also show that serum TGF- $\beta$ 1 and chemerin concentrations did not change significantly in patients with mild AP. This is in contrast to the results reported recently, which show that serum TGF- $\beta$ 1 is significantly lower in patients with severe AP<sup>[3]</sup>. A possible explanation for these discrepancies is the degree of severity of AP. As far as serum chemerin is concerned, to our best knowledge this is the first report about serum concentrations of this adipokine in mild AP.

The results for serum PDGF-BB, TGF- $\beta$ 1 and chemerin concentrations in patients with CP are similar to those reported previously<sup>[6,7]</sup>.

It has been shown previously that serum HMGB1 levels were elevated in patients with severe AP<sup>[18]</sup>. Moreover, increased HMGB1 levels in the serum and diseased organs (pancreas, liver kidney, lung and ileum) have been demonstrated in animal experimental models of severe AP<sup>[19,20]</sup>. This is in contrast to the results reported in this paper, where serum HMGB1 levels did not change significantly compared to healthy subjects. One possible explanation for these discrepancies is the degree of severity of AP. We performed our assessment in patients with mild AP. The elevated level of serum HMGB1 was observed in patients with severe AP<sup>[18]</sup>. Moreover, in contrast to the previously reported

results<sup>[18]</sup> we could not find any association between serum HMGB1 levels and serum hs-CRP concentrations. We could not find the significant changes in serum HMGB1 levels in patients with either WOPN or CP. This is in agreement with the observation that serum HMGB1 in patients with severe AP declined to normal levels on day 3 after admission and did not increase during the following two weeks<sup>[18]</sup>. To our best knowledge this is the first report about serum HMGB1 levels in these pathologies. Overall, the results presented here suggest that serum HMGB1 does not change significantly in patients with mild AP, WOPN and CP.

In conclusion, the results presented here indicate that serum PDGF-BB concentrations are lower in patients with mild AP and WOPN compared to healthy subjects, and significantly elevated in patients with CP. In contrast, serum TGF $\beta$ -1 and chemerin concentrations did not change significantly in patients with AP and WOPN and were significantly elevated in patients with CP. We did not find significant changes in serum HMGB1 levels in patients with AP, WOPN and CP. The present study indicates that the serum PDGF-BB level is significantly different in AP, WOPN and CP, whereas serum TGF $\beta$ -1 and chemerin levels are elevated only in patients with CP.

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**Table 1 Selected laboratory values in controls, patients with acute pancreatitis, walled-off pancreatic necrosis and chronic pancreatitis (mean  $\pm$  SEM)**

	<b>Control</b>	<b>Acute pancreatitis</b>	<b>Walled-off necrosis</b>	<b>Chronic pancreatitis</b>
Number of control subjects/patients	35	40	33	40
Age (yr)	42 $\pm$ 2.2	52 $\pm$ 2.6 <sup>a</sup>	48 $\pm$ 2.5 <sup>a</sup>	45 $\pm$ 1.2
Body weight (kg)	79 $\pm$ 2.6	78 $\pm$ 2.7	72 $\pm$ 3.0 <sup>a</sup>	68 $\pm$ 1.5 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	25 $\pm$ 0.46	25 $\pm$ 0.62	24 $\pm$ 0.78	22 $\pm$ 0.49 <sup>a</sup>
Serum amylase (U/L)	49 $\pm$ 6.1	991 $\pm$ 554 <sup>a</sup>	124 $\pm$ 26 <sup>a</sup>	107 $\pm$ 13 <sup>a</sup>
Serum lipase (U/L)	26 $\pm$ 3.0	519 $\pm$ 105 <sup>a</sup>	87 $\pm$ 26 <sup>a</sup>	157 $\pm$ 45 <sup>a</sup>
ALT (U/L)	22 $\pm$ 1.5	120 $\pm$ 27 <sup>a</sup>	30 $\pm$ 7.5	31 $\pm$ 32 <sup>a</sup>
AST (U/L)	22 $\pm$ 6.5	96 $\pm$ 22 <sup>a</sup>	29 $\pm$ 6.5	32 $\pm$ 1.1 <sup>a</sup>
Total protein (g/L)	76 $\pm$ 0.84	63 $\pm$ 1.3 <sup>a</sup>	70 $\pm$ 1.2 <sup>a</sup>	72 $\pm$ 1.1 <sup>a</sup>
Albumin (g/L)	45 $\pm$ 0.75	31 $\pm$ 1.3 <sup>a</sup>	34 $\pm$ 1.4 <sup>a</sup>	44 $\pm$ 1.1
Hemoglobin, (g/dL)	15 $\pm$ 0.44	13 $\pm$ 0.32 <sup>a</sup>	13 $\pm$ 0.30 <sup>a</sup>	14 $\pm$ 0.24 <sup>a</sup>
BUN (mg/dL)	12 $\pm$ 0.45	13 $\pm$ 1.2	13 $\pm$ 0.72	13 $\pm$ 1.1
Bilirubin (mg/dL)	0.87 $\pm$ 0.07	2.0 $\pm$ 0.39 <sup>a</sup>	2.0 $\pm$ 0.81 <sup>a</sup>	0.79 $\pm$ 0.11
Serum triacylglycerol (mg/dL)	103 $\pm$ 5.9	307 $\pm$ 80 <sup>a</sup>	131 $\pm$ 6.5 <sup>a</sup>	115 $\pm$ 8.4
Serum cholesterol (mg/dL)	189 $\pm$ 3.9	220 $\pm$ 24	172 $\pm$ 9.7 <sup>a</sup>	179 $\pm$ 7.0 <sup>a</sup>

Serum glucose (mmol/L)	5.1 ± 0.10	6.8 ± 0.6 <sup>a</sup>	6.1 ± 0.37 <sup>a</sup>	6.2 ± 0.25 <sup>a</sup>
White blood cell count (× 10 <sup>9</sup> /L)	6.7 ± 0.25	11 ± 0.73 <sup>a</sup>	8.2 ± 0.57	8.6 ± 0.49 <sup>a</sup>
Systolic BP (mmHg)	123 ± 1.0	139 ± 3.6 <sup>a</sup>	128 ± 22	121 ± 2.4
Diastolic BP (mmHg)	80 ± 1.2	85 ± 2.2 <sup>a</sup>	78 ± 14	79 ± 1.1
Platelets (× 10 <sup>9</sup> /L)	250 ± 8.4	224 ± 18	289 ± 27 <sup>a</sup>	249 ± 24

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<sup>a</sup>*P* < 0.05 *vs* controls. BMI: Body mass index; ALT: Alanine transaminase; AST: Aspartate transaminase; BUN: Blood urea nitrogen; BP: Blood pressure.



**Table 2 Serum concentrations of high-sensitivity C-reactive protein, platelet derived growth factor BB, transforming growth factor  $\beta$ , chemerin and high-mobility group box chromosomal protein 1 in controls, patients with acute pancreatitis, walled-off pancreatic necrosis and chronic pancreatitis (mean  $\pm$  SEM)**

	Control	Acute pancreatitis	Walled-off necrosis	Chronic pancreatitis
hsCRP (mg/dL)	1.7 $\pm$ 0.19	141 $\pm$ 16 <sup>a</sup>	40 $\pm$ 13 <sup>a</sup>	23 $\pm$ 6.2 <sup>a</sup>
PDGF-BB (ng/mL)	6.2 $\pm$ 0.76	4.0 $\pm$ 0.61 <sup>a</sup>	1.6 $\pm$ 0.31 <sup>a</sup>	12 $\pm$ 1.3 <sup>a</sup>
TGF- $\beta$ (ng/mL)	39 $\pm$ 3.6	39 $\pm$ 3.1	34 $\pm$ 4.1	57 $\pm$ 3.6 <sup>a</sup>
Chemerin (ng/mL)	48 $\pm$ 2.3	47 $\pm$ 2.9	54 $\pm$ 3.3	73 $\pm$ 7.2 <sup>a</sup>
HMGB1 (ng/mL)	3.4 $\pm$ 0.61	3.4 $\pm$ 0.64	3.5 $\pm$ 0.58	3.0 $\pm$ 0.55

<sup>a</sup> $P < 0.05$  vs controls. hsCRP: High-sensitivity C-reactive protein; PDGF-BB: Platelet derived growth factor BB; TGF- $\beta$ : Transforming growth factor  $\beta$ ; HMGB1: High-mobility group box chromosomal protein 1.