Thrombopoietin as early biomarker of disease severity in patients with acute pancreatitis

Pancreas

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ABSTRACT

Objectives: To study the concentrations of thrombopoietin (TPO), a growth factor recently involved in the pathogenesis of experimental acute pancreatitis (AP), and its potential role as early diagnostic and prognostic biomarker in patients with AP.

Methods: TPO was measured in 44 AP patients, 18 patients with non-pancreatic acute abdominal pain, and 18 healthy volunteers.

AP severity was classified on the basis of the 2012 International Atlanta Symposium on Acute Pancreatitis criteria.

Results: TPO levels did not differ between AP patients and controls, whereas were higher in patients with moderately severe or severe AP compared to mild AP. ROC analysis of TPO for severe AP diagnosis showed an area under the curve (AUC) of 0.80. A cut-off value of 31.48 pg/ml showed the highest sensitivity, allowing to rule-out severe AP when TPO was lower, whereas TPO higher than 98.23 pg/mL was associated with severe AP with high specificity (93.5%).

Furthermore, TPO levels were greater in AP patients developing organ dysfunction or sepsis, and in non-survivors compared to survivors.

Conclusions: Our data provide the first evidence for TPO as potential early prognostic biomarker in AP patients. High TPO levels at hospital admission may predict organ dysfunction, sepsis, and fatal outcome in AP patients.

Key words: acute pancreatitis, thrombopoietin, biomarker, disease severity.

Introduction

Acute pancreatitis (AP) is a relatively common critical disease, whose incidence, in developed countries, may reach 10-44 cases per 100,000 [1].

The original Atlanta Classification distinguished two forms of the disease, interstitial edematous or mild AP, and necrotising or severe AP [2]. Most patients experience only mild, self-limited AP; however, up to 20% have the more severe form of illness, characterized by the development of local complications and/or organ dysfunction, and 10 to 30% of these patients experience a fatal outcome [3, 4]. Recently, an international web-based consensus revised the Atlanta criteria in order to allow a more consistent classification worldwide, and to improve clinical assessment of severity, distinguishing between mild, moderately severe or severe AP [5].

Early identification of patients with severe AP remains a common challenge in daily clinical practice, especially at the time of diagnosis and first evaluation, and has important consequences on patient prognosis, since appropriate early treatment profoundly affects morbidity and mortality[3, 4, 6, 7].

Following the publication of Ranson's score [8], many clinical score systems, aimed to promptly identify patients with severe AP, have been proposed [9-12]. Among these, the "Acute Physiology And Chronic Health Evaluation II" (APACHE II) score [9] has been the most widely adopted in several guidelines, although its application may be somewhat limited by its complexity, and by the poor sensitivity at the time of initial presentation.

Moreover, many candidate biomarkers of disease severity have been studied, including cytokines and inflammatory mediators, adhesion molecules, hormones, and proteins produced and released by inflammatory cells [6, 7, 13-19]. Of these, C-reactive protein (CRP) showed the most promising results, although it significantly raised only after 48 hours from the onset of symptoms [19].

Thrombopoietin (TPO) is a humoral growth factor mainly involved in the proliferation and differentiation of megakariocytes [20, 21]. TPO is also able to enhance platelet aggregation in response to several agonists [22], and it boosts platelet-leukocyte adhesion via P-selectin expression [23].

Elevated TPO levels have been demonstrated in patients with critical diseases [24], such as unstable angina [25], severe trauma [26], ischemic stroke [27], disseminated intravascular coagulation [28], and burn injury, in particular after development of sepsis [29]. In addition, it has been shown that sepsis severity is the major determinant of elevated TPO levels in septic patients, suggesting that TPO represents a potential marker of disease severity [30].

A recent study reported that TPO levels are increased in a murine model of AP induced by L-arginine, and that TPO enhances pancreatic damage and AP-associated lung injury in this model [31].

Aim of this study was to investigate TPO levels in patients with AP at the time of diagnosis and first evaluation, and to evaluate TPO as an early biomarker for the diagnosis and for the assessment of disease severity in AP patients.

Methods

Study design

This was a prospective observational study. All patients signed informed consent form for the participation in the study. The study was conducted according to the Helsinki Declaration, and approved by our Institutional Ethical Committee.

Patients

The study group comprised 44 patients with AP, 18 patients with non-pancreatic acute abdominal pain and normal values of serum amylase, and 18 healthy volunteers, used as controls.

Patients with AP and with non-pancreatic acute abdominal pain were prospectively enrolled at the time of hospital admission in the Emergency Department (ED) of the "Città della Salute e della Scienza di Torino" Hospital (Turin, Italy).

The diagnosis of AP in the ED was based on suggestive symptoms and a minimum three-fold increase of pancreatic amylase serum levels above the upper normal values [4]. AP diagnosis was

then confirmed by measuring lipase serum levels, not available in the ED, upon patient admission.
Other possible causes of elevated pancreatic amylase serum levels were excluded.
Another control group consisted of healthy volunteers, receiving no medications. None of them had shown any evidence of febrile illness during the previous two weeks. Their haematological indices, and liver and kidney function tests were within normal ranges.
Exclusion criteria were: age<18 years; known haematological diseases with consequences on the coagulation, the platelet count or the TPO production [32], and known malignancies in chemioterapic treatment.

Data collection

Age and sex were noted for all patients and control subjects. For all AP patients we recorded also the etiology and the occurrence of sepsis. For the purpose of this study, the diagnosis of sepsis was defined on the basis of positive bacterial blood culture during the clinical course. Total hospital lenght of stay and in-hospital mortality rate were used as outcome parameters.
Disease severity for AP patients was evaluated on the basis of the criteria proposed by the 2012 revision of the International Atlanta Symposium on Acute Pancreatitis [5]. The APACHE II score [9], calculated both at the time of admission and after 24h, the Ranson's score [8] at 48h, the BISAP score[33], and the CRP concentrations [34] at 48h were also collected for evaluation of the severity of the disease.
Imaging methods, treatment, and ICU admission were decided at the discretion of the attending physicians, independently from participation to this study.

Biochemical analyses

In patients with AP and with non-pancreatic abdominal pain, TPO levels were determined on blood samples drawn at the admission in the emergency room, before any therapeutic intervention was started.

To obtain plasma samples, EDTA-anticoagulated tubes were centrifuged at 1,600 g for 10 minutes at 4°C within 2 hours after blood sampling. All plasma samples were stored at −20°C until analysis.
TPO concentrations were measured in duplicate using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota), following the manufacturer's instructions.
CRP was assayed by high-sensitivity nephelometry (Dade Behring, Marburg, Germany).

Analysis of data

Data are presented as median and 25%-75% Percentile, as the distribution of all biochemical variables in the study population was not normal. Comparisons between groups were carried-out by nonparametric Mann-Whitney test or nonparametric Kruskal-Wallis test followed by Dunn's test for multiple comparisons, as appropriate.
We used receiver-operating-characteristic (ROC) and area under the curve (AUC) statistics to test TPO accuracy and, for comparison, the accuracy of APACHE II (at ED admission and after 24 hours) and BISAP scores [35, 36]. We used a generalized U-statistics to compare the AUCs [36].
A *P* value of < 0.05 was considered significant.
All statistics have been performed using GraphPad Prism 4.00 for Windows (GraphPad Software, La Jolla, CA, USA), and Stata Statistical Software: Release 11 (StataCorp LP , College Station, TX).

Results

Patient Characteristics

The prospective study population consisted of 44 non-consecutive patients with AP (18 men, 26 women; median age 70.0 years; 54.5 to 77.0 years), 18 patients with non-pancreatic acute abdominal pain and normal serum amylase levels (8 men, 10 women; median age 58.5 years; 41.0

to 76.5 years), and 18 healthy volunteers (7 men, 11 women; median age 61.0 years; 51.5 to 80.5 years). No significant differences for age and gender were found between the three study groups.

In the control group of the patients with non-pancreatic acute abdominal pain and normal serum amylase levels, 6 had renal colic pain, 3 biliary colic pain, 2 inflammatory bowel disease, whereas 3 and 4 patients were diagnosed with gastritis and irritable bowel syndrome, respectively.

The clinical characteristics of the patients with AP are summarized in Table 1.

Age was lower in the mild AP group compared to the severe AP group, whereas the ratio male/female was higher in the severe AP patients than in mild AP patients (Table 1).

Gallstones were the most common cause of AP in all patient groups.

Thirty-one patients (70.45%) were diagnosed as having mild AP, 5 (11.36%) moderately severe AP, and 8 (18.18%) severe AP (Table 1).

The overall in-hospital mortality rate in patients with AP was 9.1% (4/44). All of the deceased patients belonged to the group of patients with severe AP. Three patients died within 10 days due to multi-organ failure (MOF), and 1 patient died due to late complications (sepsis).

Patients with severe AP had longer hospitalization than those with less severe forms of the disease (Table 1).

Seven patients (16%) developed sepsis during the course of the disease, two in the moderately severe and five in the severe AP groups, respectively (Table 1).

All the examined traditional predictors of disease severity and mortality, *i.e.* the APACHE II score at the admission and after 24 hours, the Ranson's score, the BISAP score, and the CRP values measured at 48 hours from the admission, were significantly higher in the severe AP patients (Table 1).

TPO levels

TPO levels were not significantly different in patients with AP compared to the two control groups, although a progressive increase in median TPO concentrations was observed from normal subjects

to patients with non-pancreatic abdominal pain, and from patients with non-pancreatic abdominal pain to patients with AP (Figure 1A).

No differences in TPO levels were observed in patients with AP because of alcohol, gallstone, and other causes (not shown).

Median TPO concentrations were 105.5 pg/ml (78.23-109.27) in patients with severe AP, 68.29 pg/ml (57.59 to 257.90) in patients with moderately severe AP, and 45.25 pg/ml (28.11 to 58.67) in patients with mild AP ($P < 0.01$ for moderately severe AP *versus* mild AP; $P < 0.05$ for severe AP *versus* mild AP) (Figure 1B). TPO levels did not statistically differ between patients with moderately severe AP and those with severe AP (Figure 1B).

Prediction of severity and relationship with prognosis

The receiver-operating characteristics (ROC) curves of TPO for the diagnosis of severe AP are presented in Figure 2. The area under the curve (AUC) on ROC analysis for patients with severe AP was 0.80 (95% CI, 0.65 to 0.94) versus patients classified as having moderately severe or severe AP. For the purpose of this analysis, we considered together the patients having moderately severe AP or severe AP according to the 2012 revision of Atlanta's classification, since for both groups the at least transient development of organ dysfunction would require the admission to intensive care unit.

A cut-off value of 51.55 pg/ml for TPO concentrations showed the best relationship between sensitivity and specificity, although they resulted sensibly lower than those of the severity scores currently most employed (Table 2). However, setting the cut-off value to 31.48 pg/ml reached 100% sensitivity and allowed to definitely rule-out severe AP when TPO levels resulted lower than this threshold (Table 2). On the contrary, TPO concentrations higher than 98.23 pg/mL were associated with the presence of severe AP with high specificity (Table 2).

When we compared the AUCs for the detection of severe AP of the different diagnostic tests, sensitivity and specificity were significantly higher, compared to TPO, only for the APACHE II score at ED admission ($P < .01$).

In patients who developed organ dysfunction (one or more) during the course of their disease, TPO levels were greater than in those without organ dysfunction (Figure 3A). In particular, TPO was significantly increased in AP patients who developed hepatic or renal dysfunction during the course of the disease, but not in those who developed pulmonary dysfunction (Figure 3B). In addition, we detected significantly more elevated TPO levels in AP patients who developed sepsis compared to those without infection (Figure 3C), and in non-survivors compared to survivors (Figure 3D).

Discussion

Our study is the first to investigate TPO levels, and to evaluate TPO as an early biomarker of diagnosis and disease severity in patients with AP. The results obtained show that TPO, whereas it is not a useful marker to distinguish patients with AP from controls with acute abdominal pain, is significantly more elevated, at the time of the first clinical evaluation, in patients with severe or moderately severe AP than in those with mild AP. Furthermore, higher TPO levels in AP patients are associated with the development of organ dysfunction and sepsis, and with fatal outcome.

TPO is a cytokine/growth factor mainly characterized for its ability to stimulate the proliferation and differentiation of platelet precursors in the bone marrow [20, 21]. TPO also exerts several other biological activities that may be implicated in sustaining and amplifying inflammatory reactions [24]. For instance, TPO primes platelet aggregation and adhesion to circulating monocytes [22], enhances IL-8 and reactive oxygen species production from polymorphonuclear leukocytes [38], stimulates the angiogenic processes [39], and modulates apoptosis [40, 41]. Moreover, an increase of TPO levels has been described in patients affected by several critical diseases [24-29], and, in some instance, a correlation between clinical severity and TPO concentrations has also been proven, for instance in patients with sepsis [30]. No data is currently available on the levels of TPO in patients with AP.

However, a recent experimental study has demonstrated that TPO levels are increased in a model of necrotizing pancreatitis induced by L-arginine in mice, and that blocking TPO with a neutralizing antibody reduces the severity of pancreatic damage and of pancreatitis-associated lung injury in this model [31]. In our study, TPO levels in patients with AP did neither differ from those measured in patients admitted to the ED with non-pancreatic abdominal pain, nor in normal healthy subjects. Therefore, TPO levels are not helpful as a marker for the diagnosis of AP. However, whereas AP diagnosis is usually straightforward, early risk stratification of patients with AP remains a clinical need and an open research challenge [6]. The severe form of AP account, indeed, for the morbidity and mortality caused by AP, which is divided in two phases, the early phase, related to the development of multi-organ failure (MOF), within the first week, and the late phase, usually related to infectious complications, appearing after the first two weeks [1, 3, 4]. In recent years, a great research effort has been made in the attempt to identify objective markers that can predict the severity of AP at the time of hospital admission [6, 13, 19]. Because there is no specific pharmacologic treatment for AP, an ideal prognostic marker would guide physicians in the appropriate management of AP patients, for instance leading to vigorous fluid resuscitation or to triage patients for admission to the intensive care unit. The proposed approaches have included clinical scoring systems, such as the Ranson's criteria [8], APACHE II score [9], and others [10-12, 33], as well as the use of inflammatory markers, such as C-reactive protein or other inflammatory cytokines [6, 7, 13, 19], although none of them has gained widespread acceptance in clinical practice [6, 7]. In this study, we evaluated TPO levels in patients with AP at the time of first clinical evaluation and diagnosis in the ED, and found that TPO was significantly more elevated in patients with severe or moderately severe AP than in patients with mild AP. For the purpose of this study, we classified patients with AP on the basis of 2012 revised version of Atlanta classification [5]. The characteristics of our patients were similar to those of analogous studies for etiology, development of organ dysfunction, and mortality, making our results easily generalizable. Therefore, the present results indicate that TPO may be proposed as a marker of disease severity in patients with AP, at times as early as that

of first clinical evaluation and diagnosis in the ED. The performance of TPO levels as prognostic marker in AP patients is slightly inferior or similar to those of other clinical scoring systems or circulating biomarkers, but the advantage of obtaining such prognostic information earlier could heavily influence the management and the outcome of patients with AP, especially those with the most severe forms of the disease. In particular, the identification of two cut-off points, one with high sensitivity and the other with high specificity, could help the clinicians to easily rule-out severe AP in the presence of very low TPO levels, on one hand, and to early identify the most severe AP patients when TPO levels result very high, on the other hand. In addition, our results confirm and extend to human disease the evidence coming from experimental data suggesting that TPO may be implicated in the pathogenesis of acute pancreatic damage [31].

We cannot precisely define the origin of the rise in TPO level observed in AP patients. It is known that the liver is the main site of TPO production [20, 21]; therefore, TPO levels could increase as a consequence of the liver acute-phase response accompanying acute inflammatory diseases, since IL-6, the main acute-phase reactant produced in the liver, enhances TPO synthesis from hepatocytes [42]. However, activated platelets themselves could also be a major source for TPO release, since they are known to release full-length biological active TPO upon stimulation [43]. A recent study has shown that the blockade of platelets with platelet glycoprotein (GP)-1bα antibody attenuated AP in two animal models of AP induced by caerulein and L-arginine [44]. Therefore, it can be hypothesized that TPO may intervene in the pathogenesis of organ damage in AP by increasing platelet activation and platelet-monocyte adhesion, thus facilitating leukocyte recruitment in the microvasculature [45], and that its blockade may prevent the development of microvascular thrombosis and subsequent organ failure. This hypothesis is in agreement with previous studies demonstrating that leukocyte-dependent tissue injury is reduced by platelet depletion in lung and liver [46, 47].

A clear limitation of our study is represented by the small number of patients enrolled. Whereas we believe it provides convincing preliminary evidence on the potential utility of TPO as early

biomarker for severe AP, its results need to be confirmed in larger multi-institutional randomized trials.

In conclusion, our results show that TPO, although not an useful marker for the diagnosis of AP, is significantly more elevated, at the time of first clinical evaluation, in patients with severe or moderately severe AP than in those with mild AP. These data provide the first evidence for the potential of circulating TPO as an early biomarker useful to timely identify patients affected by the most severe forms of AP. Further, high TPO levels at the time of hospital admission may help to predict organ dysfunction, sepsis, and fatal outcome in patients with AP.

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FIGURE LEGENDS

Figure 1. TPO concentrations in patients with acute pancreatitis (AP), patients with non-pancreatic acute abdominal pain, and healthy subjects. Plasma TPO levels were determined by ELISA.
Panel A. Comparison of TPO levels, determined at the time of hospital admission, in patients with AP, patients with non-pancreatic acute abdominal pain, and healthy subjects.
Panel B. TPO levels in patients with AP according to disease severity evaluated on the basis of 2012 revision of the 1992 Atlanta classification [5].
NS = not significant.

Figure 2. Receiver operating characteristic (ROC) curves of TPO, measured at time of hospital admission in patients with acute pancreatitis (AP) according to disease severity evaluated on the basis of the 2012 revision of Atlanta's classification. For the purpose of this analysis, we considered together the patients having moderately severe AP or severe AP, since for both groups the at least transient development of organ dysfunction would require the admission to intensive care units.

Figure 3. Plasma TPO levels at time of hospital admission in patients with acute pancreatitis (AP) in relationship with overall organ dysfunction (**Panel A**), hepatic, renal, and pulmonary dysfunction (**Panel B**), sepsis development (**Panel C**), and mortality (**Panel D**).
NS = not significant.

Table 1

Table 1. Clinical characteristics of patients with acute pancreatitis (AP).

		2012 Revised Atlanta Criteria		
		Mild	***Moderately severe***	***Severe***
Age *– year*		63.0 (50.0-74.0)	72.0 (61.5-85.5)	77.0 (74.5-85.0)*
Sex *- M/F*		11/20	2/3	5/3
Etiology *– no. (%)*	*Gallstones*	15/31 (48.38%)	1/5 (20.00%)	7/8 (87.50%)
	Alcohol	6/31 (19.35%)	0/5 (0%)	0/8 (0%)
	Others	10/31 (32.25%)	4/5 (80.00%)	1/8 (12.5%)
In-hospital mortality *– no.*		0/31	0/5	4/8
Survivors LOS *– Days*		12.0 (10.0-15.0)	16.0 (12.0-21.0)	42.0 (24.5-60.5) [#]
Organ dysfuction *– no. (%)*	*Hepatic*	0/31 (0%)	0/5 (0%)	6/8 (75.00%)
	Renal	0/31 (0%)	2/5 (40.00%)	6/8 (75.00%)
	Pulmonary	0/31 (0%)	3/5 (60.00%)	5/8 (62.50%)
Sepsis development *– no. (%)*		0/31 (0%)	2/5 (40.00%)	5/8 (62.50%)
APACHE II Score *(at ED admission)*		5.0 (3.0-7.0)	9.0 (9.0-12.5)**	11.5 (10.5-15.0) [#]
APACHE II Score *(at 24 h)*		5.0 (3.0-7.0)	11.0 (8.0-13.5)**	14.5 (11.0-21.0) [#]
Ranson's Score *(at 48 h)*		2.0 (1.0-2.0)	4.0 (4.0-4.5)*	5.0 (4.0-6.5) [#]
BISAP Score		1.0 (0.0-1.0)	1.0 (1.0-2.0)	3.0 (2.5-3.0)[#]
C-reactive protein *(at 48 h) – mg/L*		69.4 (17.8-118.1)	198.0 (183.5-244.8)**	294.8 (241.8-361.3) [#]
TPO *– pg/mL*		45.25 (27.36-55.23)	105.5 (77.13-110.3)*	68.29 (63.39-305.5)**

Disease severity was evaluated on the basis of the 2012 revision of Atlanta classification.

Data are presented as median and 25%-75% Percentile.

[*] $P < 0.01$ *versus* patients with mild AP.

[#] $P < 0.001$ *versus* patients with mild AP.

[**] $P < 0.05$ *versus* patients with mild AP.

Table 2

Table 2. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-), and accuracy of plasma Thrombopoietin concentrations, and of APACHE II (at ED admission and after 24 hours) and BISAP scores, as prognostic markers of disease severity in patients with acute pancreatitis classified on the basis of the 2012 Revision of Atlanta classification.

	Cut-off	***Sensitivity***	***Specificity***	***PPV***	***NPV***	***LR+***	***LR-***	***Accuracy***
Thrombopoietin *(pg/mL)*	≤ 31.48	100%	35.5%	39.4%	100%	1.55	0	54.5%
Thrombopoietin *(pg/mL)*	≥ 51.55	76.9%	64.5%	47.6%	87%	2.168	0.358	68.2%
Thrombopoietin *(pg/mL)*	≥ *98.23*	46.2%	93.5%	75%	80.6%	7.15	0.58	79.5%
APACHE II Score *(at ED admission)*	≥ *8*	100%	83.9%	72.2%	100%	6.2	0	88.6%
APACHE II Score *(at 24 h)*	≥ *8*	92.3%	83.9%	70.6%	96.3%	5.72	0.09	86.4%
BISAP Score	≥ *2*	69.2%	93.5%	81.8%	87.9%	10.73	0.33	86.4%

Figure 1

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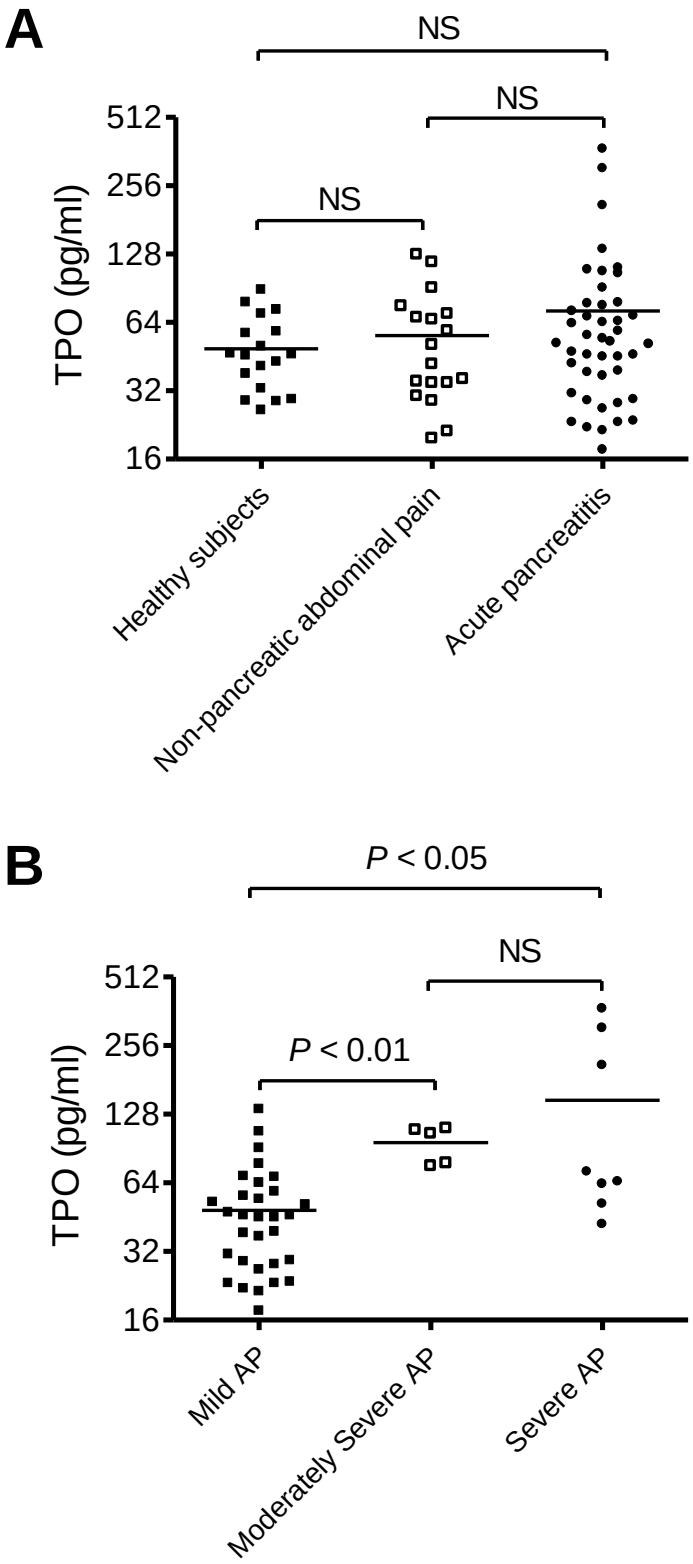

Figure 2

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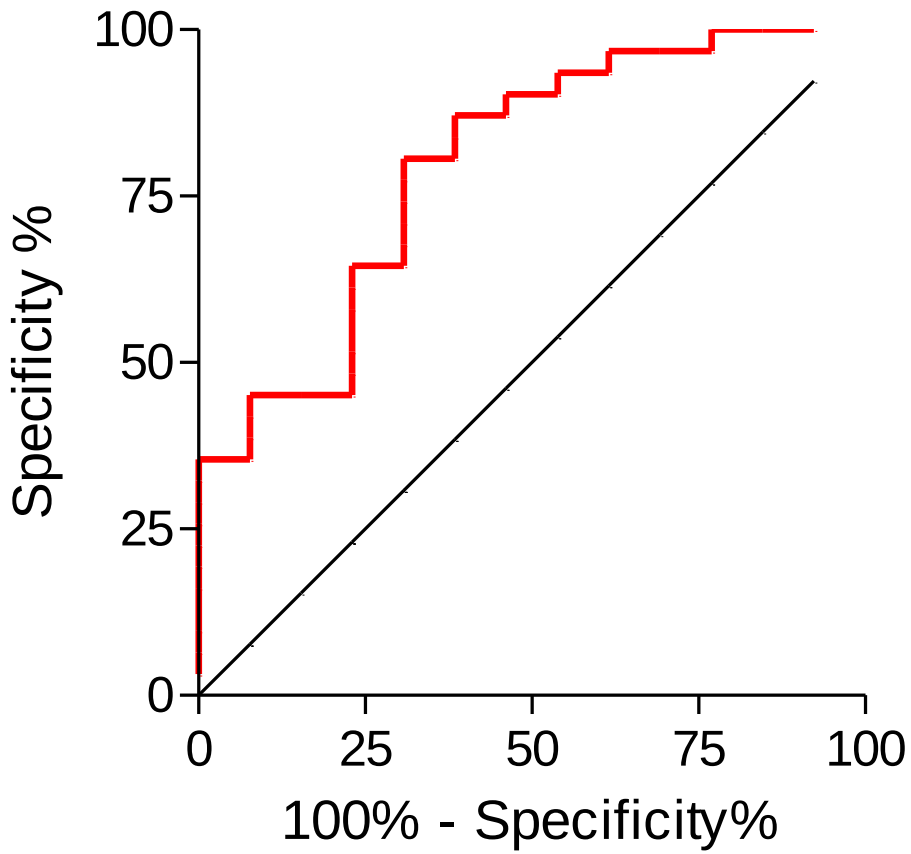

Figure 3