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Circulating cytokines in predicting development of severe acute pancreatitis

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Abstract

Introduction: Severe acute pancreatitis (AP) is associated with high morbidity and mortality. Early prediction of severe AP is needed to improve patient outcomes. The aim of the present study was to find novel cytokines or combinations of cytokines that can be used for the early identification of patients with AP at risk for severe disease.

Methods: We performed a prospective study of 163 nonconsecutive patients with AP, of whom 25 had severe AP according to the revised Atlanta criteria. Admission serum levels of 48 cytokines and growth factors were determined using Bio-Plex Pro Human Cytokine Assay 21-plex and 27-plex magnetic bead suspension panels. Admission plasma levels of C-reactive protein (CRP), creatinine and calcium were measured for comparison. In subgroup analyses, we assessed the cytokine profiles of patients with severe AP ($n = 14$) who did not have organ dysfunction (OD) upon admission (modified Marshall score <2).

Results: Of 14 cytokines elevated in the severe AP group, interleukin 6 (IL-6) and hepatocyte growth factor (HGF) levels were independent prognostic markers of severe AP. IL-6, HGF and a combination of them predicted severe AP with sensitivities of 56.0%, 60.0% and 72.0%, respectively, and specificities of 90.6%, 92.8% and 89.9%, respectively. The corresponding positive likelihood ratio (LR+) values were 5.9, 8.3 and 7.1, respectively. The predictive values of CRP, creatinine and calcium were comparable to those of the cytokines. In subgroup analyses of patients with severe AP and without OD upon admission, we found that IL-8, HGF and granulocyte colony-stimulating factor (G-CSF) levels predicted the development of severe AP, with G-CSF being the most accurate cytokine at a sensitivity of 35.7%, a specificity of 96.1% and a LR+ of 9.1.

Conclusions: IL-6 and HGF levels upon admission have prognostic value for severe AP which is similar to levels of CRP, creatinine and calcium. Although IL-6 and HGF, as either single or combined markers, were not perfect in identifying patients at risk for severe AP, the possibility that combining them with novel prognostic markers other than cytokines might improve prognostic accuracy needs to be studied. The accuracy of IL-8, HGF and G-CSF levels in predicting severe AP in patients without clinical signs of OD upon admission warrants larger studies.

Introduction

Acute pancreatitis (AP) is usually a mild disease, resolving within days. Severe AP, as defined according to the revised Atlanta criteria [1], accounts for about 20% of cases. The mortality rate in severe AP ranges from 9% to 24% [2,3] and can be as high as 47% to 69% among patients who develop multiple organ dysfunction syndrome [4,5].

In AP, premature activation of trypsin within pancreatic acinar cells causes pancreatic autodigestion, leading to a

local inflammatory process. This process is characterized by the release of pro- and anti-inflammatory cytokines and other inflammatory mediators, which recruit neutrophils, monocytes and lymphocytes into the pancreas. In severe AP, the local inflammatory process is amplified and spreads through the circulation throughout the body, resulting in a systemic inflammatory response [6,7]. Systemic inflammation is thought to contribute to the development of organ dysfunction (OD), which may be transient (<48 hours) or persistent (>48 hours). The latter is associated with a 35% to 50% mortality rate [8,9]. Accurate prediction of persistent OD is needed

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because these patients will benefit from general supportive care, including early fluid resuscitation, in the ICU [10,11].

Although levels of a variety of cytokines, determined upon admission to the hospital, may predict the course of AP [12-15], no cytokine has proved to be useful enough to be incorporated into routine clinical use. Furthermore, to the best of our knowledge, no attempt has been made to discriminate the predictive power of cytokines in patients with OD upon admission from those who are bound to develop OD during hospitalization.

In this study, we analyzed 48 different cytokines in a total of 25 patients with severe AP to determine, upon admission to hospital, novel markers specific for such patients. We used plasma levels of C-reactive protein (CRP), creatinine and calcium as conventional biomarkers. In further analyses of the data, we excluded patients with modified Marshall scores ≥ 2 , whom, we reasoned, already had OD upon admission, compared with the respective patients with modified Marshall scores < 2 , who developed OD later during hospitalization.

Methods

Patients and definitions

In this prospective study conducted at the Helsinki University Central Hospital between June 2003 and January 2008, we assessed 163 nonconsecutive patients with AP admitted within 72 hours of the onset of symptoms. Patients with the signs of chronic pancreatitis were excluded.

AP was diagnosed if two of the following three features were observed: typical abdominal upper epigastric pain, serum or plasma amylase level at least three times greater than the upper limit of normal and characteristic findings of AP on transabdominal ultrasonography, computed tomography or magnetic resonance imaging. The severity of AP was categorized retrospectively according to the revised Atlanta classification system as mild, moderately severe (local complication or transient OD) or severe (persistent OD) [1]. Two patients with severe AP were referred to Helsinki University Central Hospital because they needed to be admitted to the ICU.

The Marshall score [16] was calculated according to the method described by Banks *et al.* [1] to assess the presence of OD upon admission. Thus, OD was deemed to be present if the modified Marshall score was ≥ 2 for one of the three organ systems (respiratory, renal or cardiovascular). A flowchart of the patient distribution is presented in Figure 1.

All patients, or their next of kin, gave their informed consent to participate. The Ethics Committee of the Helsinki University Central Hospital Department of Surgery approved the study.

Samples and cytokine measurements

Serum samples were collected upon admission to the hospital and stored at -70°C until used for analyses. Each sample (20 μl) was studied by magnetic bead suspension array using the Bio-Plex Pro Human Cytokine 21- and 27-plex panels (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions except that the assay reagents were used at half of their recommended concentrations. The 21-plex panel contains interleukin 1 α (IL-1 α), IL-2 receptor α (IL-2R α), IL-3, IL-12p40, IL-16, IL-18, cutaneous T-cell attracting chemokine, growth-regulated oncogene α , hepatocyte growth factor (HGF), interferon $\alpha 2$ (IFN- $\alpha 2$), leukemia inhibitory factor (LIF), monocyte chemotactic protein 3 (MCP-3), macrophage colony-stimulating factor (M-CSF), macrophage migration inhibitory factor, monokine induced by IFN- γ , β -nerve growth factor, stem cell factor, stem cell growth factor β , stromal cell-derived factor 1 α , tumor necrosis factor β (TNF- β) and TNF-related apoptosis inducing ligand. The 27-plex contains IL-1 β , IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, basic fibroblast growth factor, eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN- γ , IFN- γ -induced protein 10, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1 α (MIP-1 α), MIP-1 β , platelet-derived growth factor BB, regulated on activation, normal T cell expressed and secreted (that is, RANTES), TNF- α and vascular endothelial growth factor. The samples were analyzed using the Bio-Plex 200 System, and the results were calculated using Bio-Plex Manager 6.0 software (Bio-Rad Laboratories).

No measurable values for IFN- $\alpha 2$ were obtained from any sample, and thus this cytokine was excluded from data analyses. Also, the majority of the values for LIF, eotaxin, GM-CSF, IL-1 α , IL-3, IL-12p40, IL-15 and TNF- β were below the detectable limit, and therefore these cytokines were scored on a dichotomous scale of detectable or undetectable value. The persons doing the cytokine measurements were unaware of the clinical status of the patients.

Plasma levels of CRP (normal reference range < 10 mg/L), creatinine (50 to 90 $\mu\text{mol/L}$) and calcium (2.15 to 2.51 mmol/L) were determined in accordance with our hospital's laboratory routine.

Statistical analysis

Statistical analysis was performed using IBM SPSS version 19 statistical software (SPSS, Chicago, IL, USA). The results are given as medians with ranges and interquartile ranges (IQR). Spearman's rank correlation was used to test correlations between two continuous variables. For univariate analysis, comparisons between groups were made using the Mann-Whitney *U* test. The level of significance was adjusted using the Bonferroni method by dividing the

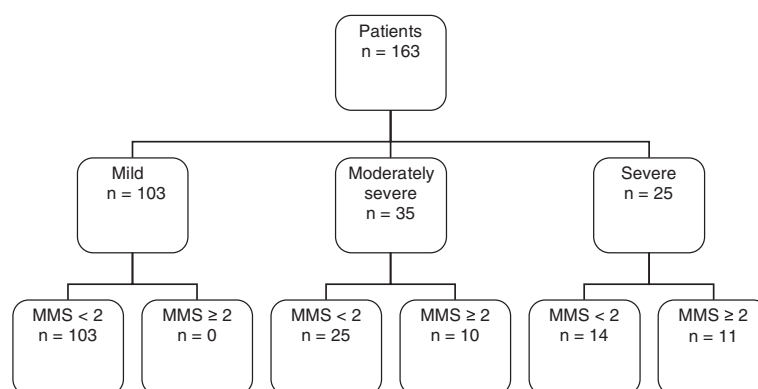


Figure 1 Patient classifications according to modified Marshall score [20] and revised Atlanta criteria [1] upon admission. MMS, Modified Marshall score.

significance level 0.05 by the number of simultaneous tests. After univariate analysis, parameters were entered into multivariate analysis, and logistic regression analysis was performed to identify independent markers predictive of persistent OD. In the *post hoc* analysis, we determined clinically optimal cutoff values for each cytokine using receiver operating characteristic (ROC) curves, with corresponding sensitivities, specificities, positive likelihood ratios (LR+), negative likelihood ratios (LR-) and diagnostic odd ratios (DORs) with 95% confidence intervals [17].

The DOR is the ratio of the odds of positive test results (OD) among patients with OD to the odds of a positive test result (OD) among the patients without OD. The higher the value, the better the discriminatory test performance is [18]. The clinically optimal cutoff value was defined as the point on the curve where the number or false positives is as low as possible (specificity $\geq 90\%$), with the maximum sensitivity, to avoid overtreatment of patients in the ICU. Areas under the ROC curve were also calculated.

Table 1 Characteristics of the patients^a

Characteristics	All patients (N = 163)	Mild or moderately severe AP (n = 138)	P-value ^b	Severe AP (n = 25)			
				All (N = 25)	MMS <2 (n = 14)	P-value	MMS ≥ 2 (n = 11)
Men	120 (73.6)	96 (69.6)	0.006	24 (96.0)	13 (92.9)	NS	11 (100.0)
Age, years	48 (18 to 87)	49 (18 to 87)	NS	43 (29 to 81)	41 (29 to 81)	NS	51 (29 to 69)
Etiology							
Alcohol	113 (69.3)	91 (65.9)	0.028	22 (88.0)	11 (78.6)	NS	11 (100)
Biliary	35 (21.5)	34 (24.6)	0.021	1 (4.0)	1 (7.1)	NS	0
Other cause	11 (6.7)	9 (6.5)	NS	2 (8.0)	2 (14.3)	NS	0
Idiopathic	4 (2.5)	4 (2.9)	NS	0	0	NS	0
Onset of symptoms, hr	24 (1 to 72)	24 (1 to 72)	NS	24 (3 to 72)	18 (3 to 48)	<0.001	48 (24 to 72)
C-reactive protein, mg/L	19 (3 to 435)	16 (3 to 426)	0.002	94 (3 to 435)	30 (3 to 229)	<0.001	294 (22 to 435)
Creatinine, $\mu\text{mol/L}$	64 (31 to 1086)	62 (31 to 313)	<0.001	92 (47 to 1086)	68 (47 to 147)	<0.001	248 (92 to 1086)
Calcium, mmol/L	2.2 (1.27 to 2.73)	2.23 (1.27 to 2.73)	<0.001	1.86 (1.39 to 2.30)	2.04 (1.49 to 2.30)	0.013	1.67 (1.39 to 2.17)
Length of hospital stay, days	6 (1 to 93)	5 (1 to 41)	<0.001	26 (1 to 93)	28 (1 to 93)	NS	17 (1 to 49)
Mechanical ventilation	25 (15.3)	2 (1.4)	<0.001	23 (92.0)	13 (92.9)	NS	10 (90.9)
Renal replacement therapy	16 (9.8)	0	<0.001	16 (64.0)	7 (50.0)	NS	9 (81.8)
OD within 24 hours	30 (18.4)	10 (7.2)	<0.001	20 (80.0)	9 (64.3)	NS	11 (100)
OD on days 1 to 7	5 (3.1)	0	<0.001	5 (20.0)	5 (37.5)	NS	0 (0)
Mortality	8 (4.9)	1 (0.7)	0.014	7 (28.0)	2 (14.3)	NS	5 (45.5)

^aAP, Acute pancreatitis; MMS, Modified Marshall score; NS, Not significant; OD, organ dysfunction. ^bSignificance of the difference between mild or moderately severe AP (n = 138) and severe AP (n = 25). The severe AP group is categorized into two subgroups: admission modified Marshall Score <2 or ≥ 2 . Data are median (range) or n (%).

Results

Patients

The characteristics of the patients are shown in Table 1. The severity of AP was classified as mild in 103 patients (63%), moderately severe in 35 patients (17%) and severe in 25 patients (15%). All of the patients with severe AP developed persistent OD and required invasive mechanical ventilation and/or renal replacement therapy.

OD was present upon admission in 21 patients (13%). Ten of these cases were transient OD, which responded rapidly to treatment and resolved within 48 hours. In these cases, the severity of AP was classified as moderately severe. Eleven patients with severe AP had OD upon admission, and another nine patients developed OD within 24 hours after admission. Five patients developed OD within 1 to 7 days after admission. There were no cases of new-onset OD after the first week of treatment.

The overall mortality rate was 4.9%. Seven (28%) of the twenty-five patients with severe AP died. Two of them were not admitted to the ICU because of their old age and comorbidities, and they died within 48 hours. One patient with moderately severe AP died as a result of carbon dioxide retention.

Cytokine profiles of patients with severe acute pancreatitis

The levels of 14 of 47 cytokines measured were significantly higher in the severe AP group than in the mild and moderately severe AP groups ($P < 0.001$ after Bonferroni

adjustment) (Table 2). The corresponding differences in the levels of the other 33 cytokines were not significant (Table 3).

By stepwise forward logistic regression analysis of the 14 cytokines, we identified IL-6 ($P = 0.006$) and HGF ($P < 0.001$) as independent prognostic markers of severe AP. Many of the other 14 cytokines (M-CSF, HGF, IL-8, MCP-1 and G-CSF) had a strong positive Spearman's correlation with IL-6 ($r > 0.6$) (Table 4). M-CSF, IL-8, MCP-1 and G-CSF did not improve the explanatory power of the regression model and thus were excluded from the stepwise analysis.

IL-6 and HGF levels predicted severe AP with sensitivities of 48.0% and 60.0%, respectively, and specificities of 93.5% and 92.8%, respectively (Table 5). In the combined regression model of IL-6 and HGF, sensitivity was improved to 72.0% and specificity remained high at 89.9%. The LR+ values for IL-6, HGF and the combined model were 7.4, 8.3 and 7.1, respectively, and the DOR values were 13.2, 19.8 and 22.8, respectively. None of the differences between IL-6, HGF and the combined model were statistically significant (Table 5). Additional file 1 shows the diagnostic performance of all of the 14 cytokines that could differentiate mild or moderately severe AP from severe AP.

The discriminatory power of plasma calcium <1.91 mmol/L, creatinine >141 μ mol/L and CRP >227 mg/L was comparable to that of IL-6 and HGF (Table 5).

Table 2 The 14 cytokines that differed between mild or moderately severe acute pancreatitis patients and severe acute pancreatitis patients^a

Cytokines	All patients (N = 163)			Admission MMS <2 (n = 142)		
	Mild or moderately severe AP (n = 138)	P-value ^b	Severe (n = 25)	Mild or moderately severe AP (n = 128)	P-value ^b	Severe AP (n = 14)
G-CSF	119.7 (66.6-198.2)	<0.001	260.2 (132.5 to 1011.8)	118.41 (66.6 to 174.3)	0.0007	234.0 (151.2 to 531.1)
GRO- α	58.8 (35.7-92.3)	<0.001	128.2 (98.4 to 169.1)	54.5 (33.2 to 82.5)	0.002 (NS)	118.5 (62.4 to 158.4)
HGF	1055.5 (764.7-1730.6)	<0.001	3613.0 (2055.3 to 6348.8)	989.6 (753.6 to 1501.6)	<0.001	2202.6 (1554.3 to 3305.5)
IL-2R α	214.2 (150.6-316.7)	<0.001	483.3 (385.6 to 673.3)	209.5 (145.5 to 290.4)	0.0015 (NS)	449.7 (228.1 to 504.3)
IL-6	59.7 (15.3-202.1)	<0.001	428.9 (138.5 to 1796.3)	49.9 (14.7 to 185.6)	0.0013 (NS)	234.5 (66.2 to 1931.3)
IL-8	26.6 (19.3-41.9)	<0.001	82.4 (46.4 to 115.6)	25.41 (18.0 to 37.6)	<0.001	59.7 (32.0 to 102.8)
IL-18	139.6 (91.6-187.1)	<0.001	202.8 (151.8 to 305.4)	131.5 (90.7 to 181.5)	0.062 (NS)	162.4 (135.3 to 201.6)
LIF ^c	0 (0)	<0.001	1 (0 to 1)	0 (0)	0.002 (NS)	1 (0 to 1)
M-CSF	14.5 (8.1-28.1)	<0.001	40.2 (18.6 to 68.6)	12.4 (5.5 to 21.5)	0.007 (NS)	28.7 (12.2 to 52.0)
MCP-1	49.0 (21.0-111.2)	<0.001	125.0 (64.6 to 199.6)	47.6 (30.0 to 100.6)	0.032 (NS)	92.1 (36.3 to 185.2)
MCP-3	17.4 (0.57-53.6)	<0.001	54.58 (24.3 to 98.7)	16.3 (0.2 to 47.9)	0.006 (NS)	52.0 (16.5 to 102.1)
β -NGF	5.79 (3.65-9.1)	<0.001	11.3 (7.4 to 15.9)	5.5 (3.4 to 8.5)	0.007 (NS)	10.4 (4.5 to 15.5)
SCF	114.1 (85.2-147.0)	0.0008	155.8 (109.2 to 256.0)	111.6 (82.0 to 143.3)	0.012 (NS)	148.5 (107.3 to 218.9)
SDF-1 α	87.6 (53.5-151.5)	<0.001	163.6 (104.4 to 226.3)	86.8 (52.8 to 148.5)	0.010 (NS)	171.3 (85.1 to 234.8)

^a β -NGF, β -nerve growth factor; G-CSF, granulocyte colony-stimulating factor; GRO- α , Growth-related oncogene α ; HGF, Hepatocyte growth factor; IL, Interleukin; IL-1R α , Interleukin 1 receptor antagonist; IL-2R α , Interleukin 2 receptor α ; SDF, stromal derived factor; LIF, Leukemia inhibitory factor; MCP, Monocyte chemotactic protein; M-CSF, Macrophage colony-stimulating factor; SDF, Stromal derived factor; SCF, Stem cell factor; SDF, Stromal cell-derived factor IQR, interquartile range; NS, not significant. ^bLevel of significance is defined as $P < 0.001$ (Bonferroni-adjusted). ^c0 = Undetectable value, 1 = Detectable value. Cytokine levels (pg/ml) are expressed as median (IQR).

Table 3 Levels of 33 cytokines showing no significant difference between mild or moderately severe and severe acute pancreatitis^a

Cytokines	Mild or moderately severe AP (n = 138)	P-value ^b	Severe AP (n = 25)
CTACK	993.1 (746.4 to 1,556.1)	0.033 (NS)	693.3 (652.5 to 1,150.6)
Eotaxin ^c	1 (0 to 1)	0.133 (NS)	1 (0 to 1)
FGF	25.9 (8.6 to 44.6)	0.123 (NS)	19.4 (0.4 to 35.2)
GM-CSF ^c	0 (0)	0.663 (NS)	0 (0 to 1)
IFN-γ	99.5 (50.7 to 151.0)	0.772 (NS)	82.4 (50.9 to 149.5)
IP-10	379.3 (244.5 to 594.0)	0.475 (NS)	446.2 (222.6 to 825.3)
IL-1α ^c	0 (0 to 1)	0.473 (NS)	1 (0 to 1)
IL-1β	1.9 (0.8 to 3.0)	0.961 (NS)	1.9 (0.5 to 3.4)
IL-1Ra	121.1 (64.8 to 260.0)	0.131 (NS)	202.6 (70.9 to 669.8)
IL-2	7.6 (2.2 to 13.4)	0.796 (NS)	6 to 7 (1.7 to 12.9)
IL-3 ^c	0 (0)	0.008 (NS)	0 (0 to 1)
IL-4	5.1 (2.6 to 7.5)	0.261 (NS)	3.8 (2.3 to 6.4)
IL-5	2.7 (1.6 to 4.1)	0.091 (NS)	1.8 (1.0 to 3.4)
IL-7	5.9 (3.9 to 10.5)	0.482 (NS)	5.2 (3.9 to 9.4)
IL-9	19.5 (7.3 to 37.8)	0.410 (NS)	16.1 (1.7 to 38.7)
IL-10	8.2 (3.9 to 17.7)	0.012 (NS)	15.8 (6.2 to 42.3)
IL-12p70	18.7 (11.2 to 40.4)	0.442 (NS)	15.5 (9.0 to 36.5)
IL-12p40 ^c	0 (0)	0.691 (NS)	0 (0)
IL-13	7.2 (3.9 to 13.5)	0.249 (NS)	6.5 (2.6 to 12.1)
IL-15 ^c	0 (0)	0.018 (NS)	0 (0 to 1)
IL-16	220.2 (99.9 to 786.8)	0.120 (NS)	341.3 (157.2 to 980.1)
IL-17A	79.8 (33.2 to 155.9)	0.084 (NS)	45.8 (9.2 to 98.4)
MIP-1α	6.2 (3.7 to 8.0)	0.215 (NS)	4.6 (2.4 to 7.1)
MIP-1β	67.1 (42.6 to 90.2)	0.830 (NS)	63.6 (40.1 to 95.8)
MIF	695.8 (348.5 to 1,553.9)	0.004 (NS)	1461.2 (713.4 to 4,529.4)
MIG	1,415.5 (821.0 to 2,348.2)	0.021 (NS)	2,147.5 (1,257.4 to 4,268.7)
PDGF-BB	1,183.6 (360.4 to 2,475.2)	0.063 (NS)	583.3 (207.8 to 1,427.3)
RANTES	2,905.6 (1,110.3 to 4,511.1)	0.093 (NS)	1,877.2 (787.5 to 3,090.9)
SCGF-β	31452.4 (21610.3 to 46978.0)	0.004 (NS)	47129.9 (30743.1 to 56343.2)
TRAIL	57.4 (33.3 to 92.0)	0.042 (NS)	84.8 (51.8 to 122.7)
TNF-α	20.8 (10.3 to 31.9)	0.730 (NS)	18.2 (9.7 to 31.5)
TNF-β ^c	0 (0 to 1)	0.187 (NS)	0 (0)
VEGF	29.9 (17.7 to 71.1)	0.436 (NS)	23.6 (17.4 to 64.2)

^aAP, Acute pancreatitis; CTACK, Cutaneous T-cell attracting chemokine; FGF, Basic fibroblast growth factor; GM-CSF, Granulocyte-macrophage colony-stimulating factor; IFN, Interferon; IL, Interleukin; IL-1Ra, Interleukin 1 receptor antagonist; IP-10, Interferon-γ-induced protein 10; VEGF, vascular endothelial growth factor; MIF, Macrophage migration inhibitory factor; MIG, Monokine induced by interferon γ; MIP, Macrophage inflammatory protein; VEGF, vascular endothelial growth factor; PDGF-BB, Platelet-derived growth factor, two BB chains; RANTES, Regulated on activation, normal T cell expressed and secreted; SCGF-β, Stem cell growth factor β; TNF, Tumor necrosis factor; TRAIL, Tumor necrosis factor-related apoptosis-inducing ligand; VEGF, Vascular endothelial growth factor IQR, interquartile range; NS, Not significant. ^bLevel of significance is defined as $P < 0.001$ (Bonferroni-adjusted). ^c0 = Undetectable value, 1 = Detectable value. Data are median (IQR).

When calcium, creatinine and CRP were added into the logistic regression analysis with IL-6 and HGF, calcium was also able to predict severe AP independently ($P = 0.023$), as were IL-6 ($P = 0.025$) and HGF ($P = 0.027$), but creatinine ($P = 0.86$) and CRP ($P = 0.45$) were not. The ROC curves for the logistic regression model of IL-6 and HGF and that for CRP are presented in

Figure 2, and their clinically optimal cutoff points are listed in Table 5.

Predicting severe acute pancreatitis in patients with modified Marshall score <2 upon admission

In the patients who developed persistent OD after admission to the hospital ($n = 14$), compared with patients who

Table 4 Cytokines showing strong correlation with interleukin 6^a

Cytokines	Spearman's r	P-value
M-CSF	0.62	<0.001
HGF	0.67	<0.001
IL-8	0.67	<0.001
MCP-1	0.71	<0.001
G-CSF	0.72	<0.001

^aG-CSF, Granulocyte colony-stimulating factor; HGF, Hepatocyte growth factor; IL, Interleukin; MCP, Monocyte chemotactic protein.

did not ($n = 128$), the levels of IL-8, HGF and G-CSF were significantly higher ($P < 0.001$, Bonferroni-adjusted) (Table 2). We did not perform multivariate analysis in this subgroup, owing to the low number of patients. We therefore determined how many of these 14 OD patients could be identified using the same clinically optimal cutoff points that we used earlier for the whole patient cohort (Table 5). G-CSF correctly predicted severe AP in 5 of 14 patients (Table 6). IL-8 detected one patient, and HGF identified two additional patients. Thus, the combination of these three cytokines detected eight of fourteen OD patients. The diagnostic performance of each cytokine (IL-8, HGF and G-CSF) and that of the combination are presented in Table 7.

In the subgroup of patients who developed persistent OD after admission, calcium had a sensitivity of 36.4%, a specificity of 94.3%, a LR+ value of 6.4 and a DOR value of 9.5. These values were similar to the respective values for G-CSF (Table 6). The sensitivity of CRP and creatinine each was 7.1%, indicating poor performance in predicting OD. The ROC curves of G-CSF and CRP are presented in Figure 3.

Predicting severe acute pancreatitis in patients with admission modified Marshall scores ≥ 2

When comparing the levels of 14 cytokines in the patients with transient OD ($n = 10$) or persistent OD ($n = 11$), we found that the levels of HGF were higher

in the patients with persistent OD ($P = 0.007$); however, the difference was not significant after we applied the Bonferroni correction. IL-6 and IL-8 had P -values of 0.067 and 0.057, respectively, and the rest of the cytokines had P -values >0.1 .

Discussion

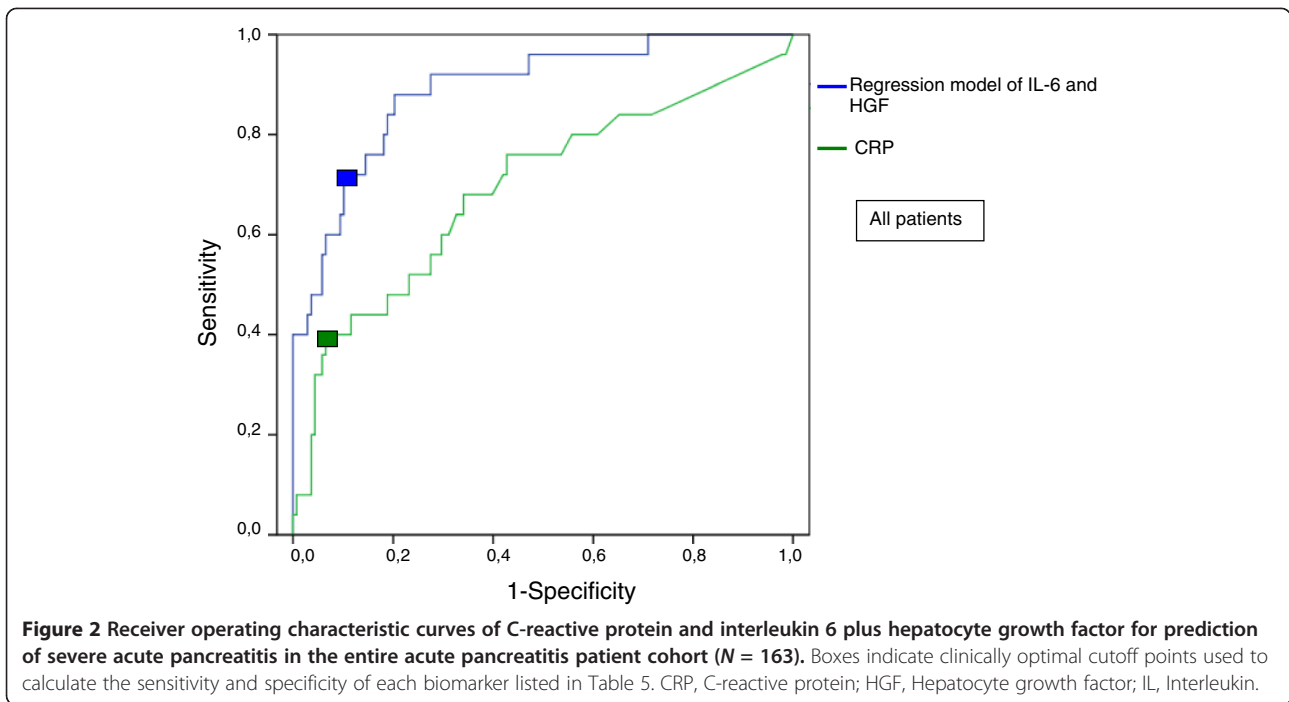
The results of our present study show that the levels of 14 of 47 cytokines were significantly higher in the severe AP group than in the patients with mild or moderately severe AP. Regression analysis, however, revealed that only IL-6 and HGF were independent predictive markers of severe AP. Both IL-6 and HGF are multifunctional cytokines that play many roles in inflammation, and the serum levels of these cytokines reflect the magnitude of the inflammatory response.

IL-6 is a proinflammatory cytokine released by macrophages in response to tissue injury, and it stimulates the synthesis of acute phase proteins (for example, CRP) in the liver. HGF stimulates mitogenesis, cell motility and matrix invasion, and thus it has a central role in angiogenesis, tumorigenesis and tissue regeneration [19]. Both IL-6 [12,20] and HGF [21,22] have been demonstrated to have prognostic value for AP upon admission. These studies, together with the results of the present study, indicating LR+ values 7.4 and 8.3 for IL-6 and HGF, respectively, for predicting severe AP, thus give credence to the view etc., thus giving credence to the view that single cytokines such as IL-6 and HGF may not be perfect predictors of severe AP. Our results also show that the LR+ value for the combination of IL-6 and HGF was 7.1, indicating that combining the cytokines did not improve their predictive accuracy for severe AP. One reason for this may be the strong Spearman's correlation between IL-6 and HGF, and, indeed, with many other cytokines (Table 4). Moreover, the sensitivities, specificities and LR+ were determined using *post hoc* analysis in which the cutoff points were set in the same population. If the analyses had been done prospectively with preset cutoff values, the predictive power of biomarkers would most

Table 5 Diagnostic performances of interleukin 6 and hepatocyte growth factor and conventional markers in predicting severe acute pancreatitis in all patients ($N = 163$)^a

Predictive marker with optimal cutoff point	AUC	Sensitivity (%)	Specificity (%)	LR+	LR-	DOR
IL-6 (>501.6 pg/ml)	0.81 (0.72 to 0.90)	48.0 (30.0 to 66.5)	93.5 (88.1 to 96.5)	7.4 (3.5 to 15.6)	0.56 (0.38 to 0.81)	13.2 (4.7 to 37.3)
HGF ($>3,020.1$ pg/ml)	0.87 (0.81 to 0.94)	60.0 (40.7 to 0.77)	92.8 (87.1 to 96.0)	8.3 (4.2 to 16.3)	0.43 (0.27 to 0.70)	19.2 (6.9 to 53.6)
IL-6 + HGF	0.89 (0.82 to 0.96)	72.0 (52.4 to 85.7)	89.9 (83.7 to 93.9)	7.1 (4.1 to 12.3)	0.31 (0.17 to 0.59)	22.8 (8.1 to 64.0)
CRP (>227 mg/L)	0.692 (0.57 to 0.82)	40.0 (23.4 to 59.3)	93.5 (88.0 to 96.5)	6.1 (2.8 to 13.6)	0.64 (0.47 to 0.89)	9.6 (3.4 to 27.2)
Creatinine (>141 μ mol/L)	0.76 (0.65 to 0.88)	40.0 (23.4 to 59.3)	96.3 (91.7 to 98.4)	10.9 (4.1 to 29.1)	0.62 (0.45 to 0.86)	17.5 (5.3 to 57.9)
Calcium (<1.91 mmol/L)	0.81 (0.70 to 0.91)	57.9 (36.3 to 76.9)	93.2 (87.6 to 96.4)	8.5 (4.1 to 17.8)	0.45 (0.27 to 0.77)	18.8 (6.0 to 58.4)

^aIL, interleukin; CRP, C-reactive protein; IL, interleukin; HGF, Hepatocyte growth factor; IL, Interleukin; AUC, Area under curve; DOR, Diagnostic odds ratio; LR-, Negative likelihood ratio; LR+, Positive likelihood ratio; 95% confidence intervals are given in parentheses.



probably have been even lower. Taken together, the results suggest that none of the cytokines alone or combinations of them would be useful in clinical practice, which would require LR+ values >10.

In agreement with previous studies [23-25], our results show that many severe AP patients already have OD upon presentation. Consequently, to find true prognostic markers, such patients should be excluded from further

analysis. We did so in the subgroup analysis with a limited number of severe AP patients ($n = 14$). When we compared the severe AP groups with admission modified Marshall scores <2 or ≥ 2 , we found that the latter group had had symptoms significantly longer and had significantly higher CRP and creatinine levels upon admission, but otherwise no other clinical marker or symptom differed between the groups. In the group of severe AP patients

Table 6 Performance of biomarkers to predict severe acute pancreatitis in patients with admission modified Marshall Score <2 ($N = 14$)^a

Severe AP patients	IL-8 >88.1 pg/ml	HGF >3,020.1 pg/ml	G-CSF >477.7 pg/ml	CRP >227 mg/L	Creatinine >141 μ mol/L	Calcium <1.91 mmol/L
1	-	-	-	-	-	-
2	-	+	+	-	-	+
3	-	-	-	-	-	-
4	+	-	-	-	-	b
5	-	+	+	-	-	b
6	-	+	-	+	-	b
7	-	-	-	-	+	b
8	-	-	-	-	-	+
9	-	-	-	-	-	-
10	+	-	+	-	-	+
11	-	-	-	-	-	-
12	+	-	+	-	-	-
13	-	+	-	-	-	-
14	+	-	+	-	-	+

^aAP, Acute pancreatitis; CRP, C-reactive protein; G-CSF, Granulocyte colong-stimulating factor; HGF, Hepatocyte growth factor; IL, Interleukin. +, Value above cutoff point (except for calcium below cutoff point). -, Value below cutoff point (except for calcium above cutoff point). ^bMissing data.

Table 7 Diagnostic performances of biomarkers markers in predicting severe acute pancreatitis in patients with admission modified Marshall score <2 (N = 142)^a

Predictive marker and optimal cutoff point	AUC	Sensitivity (%)	Specificity (%)	LR+	LR-	DOR
IL-8 (>88.1 pg/ml)	0.79 (0.68 to 0.90)	25.6 (11.7 to 54.7)	95.3 (90.2 to 97.8)	6.1 (2.0 to 19.0)	0.75 (0.54 to 1.05)	8.1 (2.0 to 33.6)
HGF (>3,020.1 pg/ml)	0.83 (0.73 to 0.92)	28.6 (11.7 to 54.7)	95.3 (90.2 to 97.8)	6.1 (2.0 to 19.0)	0.75 (0.54 to 1.05)	8.1 (2.0 to 33.6)
G-CSF (>477.7 pg/ml)	0.78 (0.65 to 0.90)	35.6 (16.3 to 61.2)	96.1 (91.2 to 98.3)	9.1 (3.0 to 27.7)	0.67 (0.45 to 0.99)	13.7 (3.3 to 56.1)
CRP (>227 mg/L)	0.54 (0.37 to 0.70)	7.1 (1.3 to 31.5)	95.3 (90.2 to 97.8)	1.5 (0.2 to 11.8)	0.97 (0.2 to 11.8)	1.6 (0.2 to 14.0)
Creatinine (>141 μmol/L)	0.62 (0.45 to 0.79)	7.1 (1.3 to 31.5)	99.2 (95.7 to 99.9)	9.1 (0.6 to 37.2)	0.94 (0.91 to 1.08)	9.7 (0.57 to 164.2)
Calcium (<1.91 mmol/L)	0.74 (0.60 to 0.88)	36.4 (15.2 to 64.6)	94.3 (88.7 to 97.2)	6.4 (2.2 to 18.5)	0.68 (0.43 to 1.06)	9.5 (2.2 to 40.2)
G-CSF + IL-8 + HGF		57.1 (32.6 to 78.6)	90.6 (84.3 to 94.6)	6.1 (3.0 to 12.3)	0.47 (0.26 to 0.87)	12.9 (3.8 to 43.4)

^aIL, Interleukin; CRP, C-reactive protein; IL, interleukin; G-CSF, Granulocyte colony-stimulating factor; HGF, Hepatocyte growth factor; IL, Interleukin; AUC, Area under the curve; DOR, Diagnostic odds ratio; LR-, Negative likelihood ratio; LR+, Positive likelihood ratio. 95% confidence intervals are given in parentheses.

who presented with modified Marshall scores <2, HGF, as well as also IL-8 and G-CSF, measured upon admission predicted later development of OD. The combination of HGF, G-CSF and IL-8 identified 8 of 14 of these severe AP patients. Thus, in 6 of 14 patients, none of these cytokines were raised above the cutoff level, although there was no difference in the clinical course of the disease. The reason for this may derive from variations in immune responses between individuals, the kinetics of the cytokines and/or the phase of AP. This raises the question whether combining cytokines with other, non-cytokine-related prognostic markers might improve the predictive power for severe

AP. Several markers, other than cytokines, have been found to be of prognostic value in OD associated with systemic inflammation [26-30]. Furthermore, novel biomarkers will most probably be discovered by means of systems biology, as reviewed by Skibsted *et al.* [31].

The crucial question in clinical practice is how to distinguish, upon admission to the hospital, those patients with transient OD from those with persistent OD. At present, no means of identifying such patients exist. In our present study, the limited number of persistent OD patients had higher levels of HGF, IL-6 and IL-8, but the differences were not statistically significant. Whether

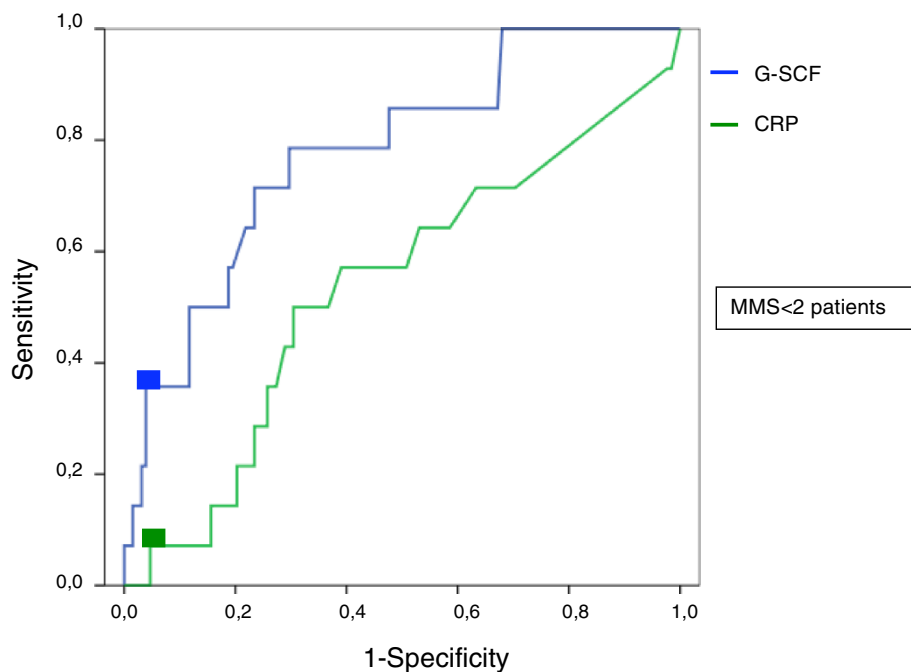


Figure 3 Receiver operating characteristic curves of granulocyte colony-stimulating factor and C-reactive protein for prediction of severe acute pancreatitis in patients with admission modified Marshall scores <2 (N = 142). Boxes indicate clinically optimal cutoff points used to calculate the sensitivity and specificity of each biomarker given in Table 7. CRP, C-reactive protein; G-CSF, Granulocyte colony-stimulating factor; MMS, Modified Marshall score.

these cytokines, as individual or combined markers, can aid in distinguishing between transient and persistent OD needs to be studied in a larger number of OD patients upon admission to the hospital.

Although no specific treatment for AP exists so far, concomitantly with better understanding of the underlying pathophysiology, immunomodulatory treatment has become a matter of interest. However, many patients already have OD upon admission [23]. In addition, AP patients may develop immune suppression rapidly [32]. In such patients, anti-inflammatory therapy may be detrimental. In some patients, immune suppression is confined mainly to the circulation and the inflammatory stage proceeds in other body compartments, such as the lungs [33]. In this complex setting, the determination of immune inflammatory status may aid in the selection of appropriate immunomodulating therapy to prevent or alleviate OD [34].

Conclusions

We analyzed the levels of 48 cytokines in 163 patients with AP upon admission to the hospital. IL-6 and HGF were independent predictive markers of severe AP; however, neither cytokine nor the combination of them was perfect in identifying AP patients at risk for severe disease. In fact, the results are similar to those for CRP, creatinine and calcium. IL-8, HGF and G-CSF levels could be used to predict severe AP in patients without clinical signs of OD upon admission. To the best of our knowledge, this study is the largest to date with regard to the number of cytokines screened in a group of AP patients. The possibility that combining cytokines with prognostic markers other than cytokines improves the prediction of severe AP warrants further studies.

Key messages

- The levels of 14/47 cytokines upon admission to the hospital were higher in patients with severe AP than in those with mild or moderately severe AP.
- IL-6 and HGF were independent predictors of severe AP.
- The predictive value of IL-6 and HGF was comparable to that of CRP, creatinine and calcium.
- Many patients who will develop severe AP, did not have signs of OD upon admission to hospital.
- IL-8, HGF and G-CSF is able to predict severe AP in patients without clinical signs of OD at the time of hospital admission.

Additional file

Additional file 1: Diagnostic performances of the 14 cytokines that could differentiate mild or moderately severe AP from severe AP.

Abbreviations

AP: Acute pancreatitis; AUC: Area under the curve; β -NGF: β nerve growth factor; CRP: C-reactive protein; CTACK: Cutaneous T-cell attracting chemokine; DOR: Diagnostic odds ratio; FGF: Basic fibroblast growth factor; G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony-stimulating factor; HGF: Hepatocyte growth factor; IFN: Interferon; IL: Interleukin; IL-1Ra: Interleukin 1 receptor antagonist; IL-2Ra: Interleukin 2 receptor α ; IP-10: Interferon- γ -induced protein 10; LIF: Leukemia inhibitory factor; LR: Likelihood ratio; MCP: Monocyte chemotactic protein; M-CSF: Macrophage colony-stimulating factor; MIF: Macrophage migration inhibitory factor; MIG: Monokine induced by interferon γ ; MIP: Macrophage inflammatory protein; OD: Organ dysfunction; PDGF-BB: Platelet-derived growth factor, two BB chains; RANTES: Regulated on activation, normal T cell expressed and secreted; ROC: Receiver operating characteristic curve; SCF: Stem cell factor; SCGF- β : Stem cell growth factor β ; SDF: Stromal cell-derived factor; TNF: Tumor necrosis factor; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; VEGF: Vascular endothelial growth factor.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AN and LKyh collected clinical data and participated in data analyses. AN and HR drafted the manuscript. MM analyzed the cytokine levels. PM was a supervisor of the data analyses and participated in designing the study. LKyl, EK and PP participated in designing and coordinating the study and provided supervision. MS and HR designed and supervised the study. All authors critically revised the manuscript and read and approved the final version.

Acknowledgements

Mrs. Teija Kanasuo is thanked for her excellent technical assistance. The work was supported by the Martti I Turunen Foundation and the Emil Aaltonen Foundation (to AN) and by the Academy of Finland (to MS).

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Received: 18 February 2014 Accepted: 1 May 2014

Published: 21 May 2014

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doi:10.1186/cc13885

Cite this article as: Nieminen *et al.*: Circulating cytokines in predicting development of severe acute pancreatitis. *Critical Care* 2014 **18**:R104.

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