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Mateusz Sporek[1], Witold Kolber[2], Beata Kuśnierz-Cabala[3], Paulina Dumnicka[4], Anna Gurda-Duda[5], Marek Kuźniewski[6], Bogdan Solnica[3], Jan Kulig[5]

DETERMINATION OF HEPATOCYTE GROWTH FACTOR AT EARLY PHASE OF ACUTE PANCREATITIS

Abstract: Aim: The aim of this study was to assess the diagnostic value of hepatocyte growth factor (HGF) as a new predictor of severity in patients with acute pancreatitis (AP) at early phase of disease. Materials and Method. The studied group involved 40 patients (16 women and 24 men) with AP admitted to I[st] Dept. of Surgery Jagiellonian University Medical College in Krakow. Twenty-four patients had mild and twelve severe form of AP. Glasgow and Imrie scores were calculated to evaluate severity of AP. HGF concentrations were measured by ELISA (R&D Systems) on days 1, 3 and 5 after admission within 48 hours after onset of symptoms.
Results: Serum median concentrations of HGF was significantly higher in patients with severe versus mild clinical course of AP on each of the study days (7.61 vs 3.30 ng/mL, p = 0.05 on day 1; 7.19 vs 3.43, p = 0.04 on day 3 and 5.76 vs 2.42, p = 0.02 on day 5). HGF positively correlated with Glasgow and Imrie scores (R = 0.57 and R = 0.51). HGF negatively correlated with fetuin A, a negative acute phase protein (R = –0.60 on day 3 and R = –0.45 on day 5) and positively with CRP (R = 0.93; R = 0.80), SAA (R = 0.78; R = 0.82), IL-6 (R = 0.61; R = 0.77; R = 0.85 on day 1, 3 and 5, respectively) and PMN-elastase (R = 0.58; R = 0.64; R = 0.77). On day 1 of the study, HGF reached the diagnostic sensitivity of 100% and specificity of 50% for the detection of severe and moderate AP.
Conclusions: Serum HGF correlates with several inflammatory markers and clinical scores (Glasgow, Imrie) in patients with AP and may be considered a new promising tool in assessing the severity of acute pancreatitis.

Key words: acute pancreatitis, cytokines, hepatocyte growth factor.

INTRODUCTION

Despite recent advances in medical sciences, acute pancreatitis (AP) is still associated with severe complications resulting in high mortality (20–30%) [1–2]. It is necessary to evaluate the severity of AP and the risk of life-threatening complications at early phase [1, 3–4]. In 1996, Ueda et al. [5] have shown high hepatocyte growth factor (HGF) concentrations in patients with organ (liver, kidney, or lung) dysfunction.

HGF, also known as hepatotrophin, is a multifunctional cytokine involved in cell proliferation, differentiation and migration as well as angiogenesis. HGF is produced and delivered to injured tissues via systemic (endocrine) and local

(paracrine) mechanism and acts on various types of cells through its receptor c-Met [3, 6]. HGF serum concentration significantly increases in the course of acute inflammation, such as sepsis or viral infection (e.g. viral hepatitis). As other growth factors, HGF secretion is regulated by pro-inflammatory cytokines, including interleukin 6 (IL-6) that also stimulates acute phase proteins synthesis, regeneration of hepatocytes, as well as proliferation and differentiation of megakaryocytic hematopoietic cells and T and B lymphocytes [5–7].

HGF is a potent anti-inflammatory agent suppressing NF-κB activation in numerous types of cells, including macrophages (i.e. induces macrophage differentiation with an anti-inflammatory phenotype — type M2) and lymphocytes and inhibiting LPS-mediated production of pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), IL-1β, IL-6 and IL-18 [6–8]. Sanada et al. [9] showed that HGF exert an anti-oxidant effect on vascular smooth muscle cells (VSMCs). HGF might represent a novel integrated multiple-target approach to suppress inflammation and to lower the risk of complications from inflammatory injury [10].

The aim of this study was to assess diagnostic value of HGF as a predictor of severity in patients with AP at early phase of disease.

MATERIALS AND METHODS

STUDIED GROUP

The studied group involved 28 patients with mild (MAP) and 12 with severe and moderate form of AP (SAP) admitted to the Ist Department of General and Gastrointestinal Surgery, Jagiellonian University Medical College in Krakow. All patients in the studied group were admitted to the hospital within 48 hours following the onset of acute disease signs and symptoms. A diagnosis of AP was established based on clinical history, ultrasound scan and serum amylase/lipase activity (at least three times above the reference limit). The progression of morphological changes within the pancreas and in the surrounding tissues was evaluated using ultrasound imaging. In all patients ultrasound was performed every day during the study period. All patients with the moderate and severe form of AP underwent computed tomography (CT). In a few cases, the CT was performed more than once and showed the evolution of necrotic changes in the parenchyma of the pancreas and in the retroperitoneal and peritoneal spaces. The severity of AP was determined according to clinical and laboratory parameters; AP classification met the Atlanta criteria revised in 2012 [11]. Patients with formerly diagnosed liver or kidney failure and patients who refused to give the informed consent were excluded.

The study protocol has been approved by Jagiellonian University Medical College Bioethics Committee (KBET/86/B/2012). The study was conducted in accordance with the Declaration of Helsinki.

Demographic characteristics were presented in Table 1.

Table 1

Characteristics of studied groups in terms of sex and age.

Group	n	Sex, n (%)		Age (years) mean ± SD
		Women	Men	
MAP	28	14 (50%)	14 (50%)	51,8 ± 15,3*
SAP	12	2 (16,6%)	10 (83,3%)	42,1 ± 11,5*
Total	40	16 (40%)	24 (60%)	46,9 ± 13,4

MAP — mild acute pancreatitis; SAP — severe and moderate acute pancreatitis; n — number of patients; *p <0.05

LABORATORY METHODS

Median and range values of HGF in patients with MAP and SAP were determined in samples collected on day 1, day 3 and day 5 of hospitalization (Table 2). The samples were taken together with the blood used for routine biochemical tests in order to avoid additional burden for the patients. Twenty minutes after drawing, the blood was centrifuged (4000 rotations/minute; 10 minutes), the serum was frozen and kept in temperature –70°C until measured. The measurements were carried out when all the samples have been collected. The following methods were used:

— Interleukin 6, soluble TNFa receptor II (sTNFRII) and HGF: ELISA with the use of R&D Systems kits (Minneapolis, USA). The reference intervals established for these methods (manufacturer's information) were: 0.70–12.5 pg/mL for IL-6; 1.0–3.17 ng/mL for sTNFRII and 0.67–1.99 ng/mL for HGF, respectively.
— Fetuin A and PMN-elastase: ELISA with the use of BioVendor kits (Brno, Czech Republic). The reference range established for fetuin A was 0.260–0.348 g/L, according to author's study. The reference interval for PMN-elastase was 25.8–48.6 ng/mL for PMN-elastase, according to the manufacturer.

Table 2

HGF serum concentration in patients with MAP and SAP during 5 days of observation.

Day of study	MAP	SAP	p
day 1	3.30 (2.28–5.20)	7.61 (4.49–8.17)	0.05
day 3	3.44 (2.29–6.26)	7.20 (4.92–10.22)	0.04
day 5	2.42 (2.00–4.93)	5.76 (3.37–8.58)	0.02

HGF — hepatocyte growth factor; MAP — mild acute pancreatitis; SAP — severe and moderate acute pancreatitis

Interleukin 18 (IL-18): ELISA with use of MBL kits (Nagoya, Japan). The reference range established for this method was 36.1–257.8 pg/mL (manufacturer's information).

The measurements of all ELISA micro-plates were performed using Automatic Reader BIO-TEK® Instruments, Inc., USA.

Serum amylase, cholinesterase (CHE), aspartate aminotransferase (AST) activities as well as albumin concentrations were measured with Modular P Clinical Analyzer (Roche Diagnostica, Mannheim, Germany). Reference range values for serum amylase ranged from 62 to 220 U/L, for CHE 5320–12920 U/L, for AST from 10–37 U/L and for albumin 35–50 g/L. CRP and serum amyloid A (SAA) concentrations were measured using immunonephlometric method on Nephelometer BN II (Siemens Healthcare Diagnostics, Germany); the reference range for SAA was <6.40 mg/L (the lower detection limit was 0.8 mg/L) and for CRP 0.16–5.0 mg/L. Hematology parameters were measured using the Sysmex XE 2100 Hematological Analyzer (Sysmex Corp., Japan).

STATISTICAL ANALYSIS

Data are presented as mean ± SD for normally distributed variables and median (lower-upper quartile) for non-normal distributed variables ($p < 0.05$ in Kolmogorov–Smirnov test for normality). Mann–Whitney U test was used to assess differences between MAP and SAP groups. Correlations between variables were assessed with Spearman rank coefficients. The cut-off value together with diagnostic sensitivity and specificity for the prediction of SAP were assessed using the receiver operating characteristic (ROC) analysis. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed using statistical package Statistica 9.0 (Statsoft Inc. Tulsa, USA).

RESULTS

Mean hospital stay of patients with diagnosis of moderate and severe acute pancreatitis was 20 (12–47) days and was statistically significantly longer compared to patients with mild form of disease (6 (5–8) days; $p = 0.01$).

DIAGNOSTIC UTILITY OF HGF IN AP

Serum HGF concentrations were significantly higher in severe and moderate than in mild AP on each of the study days (Table 2). Based on ROC analysis, HGF cut-off value for detecting SAP was 3.28 ng/mL on day 1 of the study. Diagnostic sensitivity and specificity equaled 100% and 50%, respectively and area under the ROC curve was 0.75 (Figure 1). Additionally, HGF concentrations in patients with

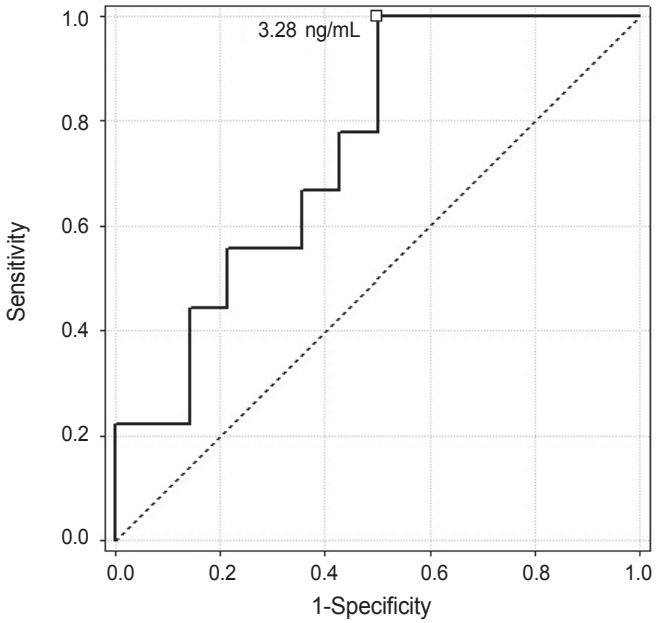

Fig. 1. ROC curve showing the diagnostic performance of HGF measured on the day 1 of the study for the detection of SAP. HGF concentration at the chosen cut-off point is shown on the graph. HGF — hepatocyte growth factor; SAP — severe and moderate acute pancreatitis

SAP were more than 3-fold higher on the days 1 and 3, and 2-fold higher on the day 5 of the study when compared to the values obtained in healthy population.

RELATIONSHIP BETWEEN HGF AND CLINICAL SEVERITY SCORES

During first 48 hours, HGF concentration significantly correlated with both Glasgow (R = 0.57; p = 0.007) and Imrie (R = 0.51; p = 0.02) scores routinely used for assessing prognosis in AP. Average values for each prognostic scale in patients with SAP and MAP are show in Table 3. Score greater than 3 in the Glasgow and Imrie scale were found in 3 patients (25%) of the SAP group. Both scales had

Table 3

Glasgow and Imrie scores in severe and mild AP patients.

Scoring scale	mean ± SD		p
Glasgow	MAP	1 (0–1)	0.02
	SAP	2 (1–3)	
Imrie	MAP	1 (0–2)	0.04
	SAP	2 (1–3)	

MAP — mild acute pancreatitis; SAP — severe and moderate acute pancreatitis

a low diagnostic sensitivity rate of 25% and maximal 100% specificity at cut-off value of 3 points.

RELATIONSHIP BETWEEN HGF AND INFLAMMATORY MARKERS

Positive correlation between HGF and interleukin 6 concentration was significant already on the first day of the study (i.e. at the time of admission to the ward) and it was even stronger on days 3 and 5. Also, positive correlations were noted between HGF concentration and direct neutrophil count as well as PMN-elastase concentration (Table 4). On day 3 after admission to hospital, HGF concentrations correlated positively with most of the inflammatory markers studied: IL-6, soluble TNFRII, SAA, CRP and direct monocyte count. Correlation between HGF concentration and total white blood cells count was observed on the day 5 of the study. Statistically significant negative correlation was noted between HGF levels and negative acute phase proteins (fetuin A and albumin) as well as cholinesterase activity (Table 4).

Table 4

The most important statistically significant correlations between HGF and selected hematological and biochemical markers in patients with AP during consecutive 5 days of observation.

Parameter	Spearman correlation coefficient R ($p < 0.05$)		
	Days of observation		
	Day 1	Day 3	Day 5
CRP	NS	0.93	0.80
SAA	NS	0.78	0.82
sTNFRII	NS	0.48	0.61
Fetuin A	NS	–0.60	–0.45
Albumin	NS	–0.72	NS
IL-6	0.61	0.77	0.85
IL-18	NS	NS	0.62
WBC	NS	NS	0.74
Neutrophil direct count	0.47	0.46	0.74
Monocyte direct count	NS	0.45	0.76
N/L ratio	0.54	0.45	0.70
PMN-elastase	0.58	0.64	0.77
Amylase	NS	NS	0.57
AST	NS	NS	0.75
CHE	NS	–0.81	–0.57

IL-6 — interleukin 6; CRP — C reactive protein; SAA — serum amyloid A; sTNFRII — soluble TNFa receptor; WBC — white blood cells; CHE — cholinesterase; PMN-elastase — polymorphonuclear elastase; AST — aspartate aminotransferase; IL-18 — interleukin 18; N/L ratio — neutrophils/lymphocytes ratio; NS — not statistically significant

DISCUSSION

According to the newest 2012 revision of Atlanta classification, acute pancreatitis begins at the time of the onset of pain in patient and its course may include two phases: the early phase comprises the first week of the disease (maximum 10 days) and the late phase may last for several weeks or even months [11]. During the first 7 days of AP, it is recommended to start the appropriate therapeutic interventions within the first 48 hours ("therapeutic window") to lower the risk of complications [1, 11–14]. Consequently, the diagnostic tests that help predict the severity of AP should be offered within this first 48 hours of disease. Studies in this area included early acute phase markers such as TNF-α (tumour necrosis factor-alpha), IL-1β, IL-6, IL-8, PAF (platelet activating factor) and MCP-1 (monocyte chemoattractant protein 1) as well as adhesion molecules ICAM-1 (intracellular adhesion molecule 1) and selectins, however, laboratory techniques used (based on ELISA) may significantly reduce their availability [14]. CRP is used as a gold standard marker, but its most significant increase in AP is noted after about 72 hours from the onset of the disease [3]. About 12 hours before CRP, a peak in SAA concentration is observed, however SAA testing is much less popular and offered only by selected laboratories [15]. Both acute phase proteins mentioned show significant correlations with numerous inflammatory markers, including HGF. According to Ueda et al. [16], serum HGF is as useful as C-reactive protein and more useful than IL-6 for detecting severe pancreatitis and for predicting hepatic dysfunction [16]. Also, correlation between plasma HGF level and the severity of AP has been observed in animal models [4]. Pancreatitis associated elevations in HGF suggest that adhesion molecules and growth factors could be the markers of AP. According to Warzecha et al. [4], HGF is not only a marker of acute inflammation but also a result of pancreatitis and the increase in HGF levels during inflammation plays a role in self-defense mechanism reducing tissue damage. Pezzilli et al. [17] and Barassi et al. [18] confirmed that HGF levels in AP patients are higher comparing to healthy population and that peak levels are observed after 2 days of disease. However, in these studies HGF concentration was not useful to predict the severity of AP. To the contrary, in our study HGF levels were significantly increased already on the first day of AP, and SAP patients presented higher levels than those with MAP (Table 3). Our results are consistent with the study of Pezzilli et al. [17] that showed HGF levels about 3-times higher in AP patients comparing to controls.

CONCLUSIONS

Serum HGF correlates with several inflammatory markers and clinical scores (Glasgow, Imrie) in patients with AP and can be considered as a new promising tool in assessing severity of acute pancreatitis.

CONFLICT OF INTEREST STATEMENT

None declared.

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[1] Department of Anatomy
Jagiellonian University Medical College, Kraków, Poland
Head: prof. Jerzy Walocha MD, PhD

[2] Department of Surgery
General Hospital in Wadowice, Poland
Head: Zygmunt Łabudziński, MD, PhD

[3] Department of Diagnostics,
Chair of Clinical Biochemistry
Jagiellonian University Medical College, Kraków, Poland
Head: prof. Bogdan Solnica, MD, PhD

[4] Department of Medical Diagnostics,
Faculty of Pharmacy
Jagiellonian University Medical College, Kraków, Poland
Head: Ryszard Drożdż PhD

[5] I[st] Chair of General and Gastrointestinal Surgery
Jagiellonian University Medical College, Kraków, Poland
Head: prof. Jan Kulig MD, PhD

[6] Department of Nephrology
Jagiellonian University Medical College, Kraków, Poland
Head: prof. Władysław Sułowicz MD, PhD

Corresponding author:
Beata Kuśnierz-Cabala, PhD
Department of Diagnostics,
Chair of Clinical Biochemistry
Jagiellonian University Medical College
ul. Kopernika 15A, 31-501 Krakow, Poland
Phone/Fax: +48 12 424 83 65
E-mail: mbkusnie@cyf-kr.edu.pl