Can the matrix metalloproteinases 2 and 9 predict the course of acute pancreatitis in previously healthy patients?

Ł. Nawacki, U. Grabowska, S. Głuszek

The Faculty of Medicine and Health Sciences, Jan Kochanowski University, Kielce, Poland.

Abstract

Background : **It was confirmed that during the inflammatory process development, granulocytes are among the main groups of cells responsible for the course of acute pancreatitis. One of these substances produced by them are extracellular matrix enzymes-metalloproteinases. In the presented study, we undertook an attempt to investigate whether they may be used as an instrument to predict the course of acute pancreatitis in previously healthy patients.**

Methods : **The study included 72 patients with the first-time episode of acute pancreatitis. The 2012 Atlanta classification was used in order to divide them into 3 groups. The patients were assessed according to the most popular multifactor scoring systems and single laboratory markers. The levels of metalloproteinases 2 and 9 were determined by the ELISA method. The diagnostic value of the commonly applied scoring systems and single diagnostic markers was compared with the value of matrix metalloproteinases levels.**

Results : **A mild form of AP developed in 42 patients, a moderate form in 16, and severe in 14. All multifactor prognostic systems have high specificity and rather low sensitivity. Single laboratory markers have higher sensitivity but lower specificity than multi-factor tools. The determination of the level of MMP-2 shows specificity of 98.3%, while MMP-9-100%.**

Conclusion : **The determination of a single laboratory marker, which is the level of metalloproteinase-2 or metalloproteinase-9, is characterized by sensitivity and specificity comparable to that of multifactor prognostic scoring systems.** (Acta gastroenterol. belg., **2019**, 82, **501-505**).

Introduction

Acute pancreatitis (AP) is a disease with unpredictable course. The diagnosis of this disease is based on the guidelines in the Classification of acute pancreatitis – 2012 : revision of the Atlanta classification and definitions by international consensus (1). Classification of the severity was developed based on the two phases of the natural course of the disease-early, covering the first two weeks, and late covering the period of the subsequent several weeks or months. On this account, the disease was divided into three forms : mild (MAP), moderately severe (MSAP) and severe (SAP). The severe acute pancreatitis, which develops in 20-30% of patients, has a high mortality rate that reaches even 36-50% (2,3). Many prognostic scales have been developed and even more single laboratory markers were taken into account to predict the severity of acute pancreatitis (4-6).

Death in the first phase of acute pancreatitis is most frequently the result of acute lung injury (ALI) or acute kidney injury (AKI). ALI is caused by the impact of immune system cells, especially granulocytes, that damage the lung alveoli (7). AKI is strongly connected with damage of renal capillaries which is a result of the complement system proteins effect and direct influence of proinflammatory cytokines (8). In both above-mentioned cases the factor which intensifies the injury are extracellular matrix enzymes - matrix metalloproteinases (MMP). Among them, gelatinases : A (MMP-2) and B (MMP-9) have the greatest role in the pathomechanism of ALI and AKI (9). The second of these enzymes plays a special role in the development of acute renal failure (ARF) because its active form destroys the endothelial basal lamina, intensifying the injury and permeability of vessels (10,11).

Therefore, in our studies we decided to investigate whether determination of the level of gelatinases in blood serum of patients with acute pancreatitis may be the prognostic factor in the course of the disease.

Objective

The objective of this prospective study was to evaluate the clinical usefulness of matrix metalloproteinases (MMP-2 and -9) assays as predictive factors of the severity of acute pancreatitis, compared to currently applied markers and prognostic scoring systems.

Material and Methods

Ethical approval

This study was approved by The Ethics Committee of The Jan Kochanowski University in Kielce, Poland (26/2015 ;19.06.2015) with written informed consent obtained from each participant and/or their legal representative, as appropriate.

The study included 72 patients with the first-time episode of acute pancreatitis. The criteria for the diagnosis of acute pancreatitis were based on the 2012 revision of the Atlanta classification (1). The diagnosis of acute pancreatitis required fulfilling of at least two of the following three criteria : 1) typical physical symptoms-

Correspondence to :Łukasz Nawacki, MD, PhD, The Faculty of Medicine and Health Sciences, Jan Kochanowski University, Aleja IX Wieków Kielc 19, 25-001, Kielce, Poland. Tel. : +48-41-349 69 73;
E-mail : lukasznawacki@gmail.com

Submission date : 28/04/2019
Acceptance date : 11/06/2019

abdominal pain, often of a girdling character, radiating to the back ; 2) serum amylase or lipase activity at least three times greater than the upper limit of normal laboratory values ; 3) characteristic imaging findings of acute pancreatitis.

The following enrolment criteria were adopted in the study :

1. diagnosis of first-time episode of acute pancreatitis,
2. onset of symptoms within less than 24 hours prior to hospital admission.

The following exclusion criteria were adopted in the study :

1. recurrent acute pancreatitis,
2. chronic concomitant diseases,
3. medical history of cancer,
4. surgical procedure performed within the last half year,
5. incomplete medical records, lack of determination of indispensable laboratory markers,
6 occurrence of symptoms more than 24 hours before hospital admission.

Venous blood samples were collected directly at admission to the hospital in a sitting position prior to the performance of other diagnostic (except physical examination) and therapeutic procedures. The subsequent samples were collected 24 and 48 hours after admission. Serum samples for determination of metalloproteinases level were collected once at admission to the hospital, directly after the diagnosis of acute pancreatitis. The level of metalloproteinases was determined by using the immunoenzymatic test (ELISA - enzyme-linked immunosorbent assay). The Wuhan Fine Biotech Co. Ltd. Fine Test kits were used. Metalloproteinase-2 was determined using Human MMP-2 (Matrix Metalloproteinase 2) ELISA Kit (code EH0017). This method allows specific and precise measurement with sensitivity < 0.191 ng/ml. Metalloproteinase-9 was determined using Human MMP-9 (Matrix Metalloproteinase 9) ELISA Kit (code EH0238). The method allows specific and precise measurement with sensitivity < 0.188 ng/ml. Determinations are based on the sandwich ELISA method.

The material for testing metalloproteinases was serum obtained after the centrifugation of blood collected into the Sarstedt 5.5 ml tube with a clotting activator. Before determinations, the serum was diluted in buffer provided together with the test. Due to this, a series of standards was prepared. Into each well of the microplate pre-coated with anti-MMP antibody, 0.1 millilitre of buffer solution was pipetted (zero standard), of each of the standard solutions and the examined samples. Subsequently, the plate was closed and incubated for 90 minutes at the temperature of 37 degrees Celsius. After this period, biotin-labelled anti-MMP antibody was added to each well, and the plate was incubated again at the temperature of 37 degrees Celsius for 60 minutes. The plate thus prepared was washed three times with the buffer supplied with the kit. After washing, 0.1 millilitre of horseradish peroxidase (HRP) conjugated to the streptavidin (SABC) well was added into each, and subsequently incubated for 30 minutes at the temperature of 37 degrees Celsius. Then, the plates were washed five times with the buffer supplied with the test. Subsequently, 90 µl tetramethylbenzidine (TMB) was added into each well, and incubated for 30 minutes in a dark room, at the temperature of 37 degrees Celsius. The final stage was the addition of a solution suppressing the reaction, and reading the absorbance value at the wave length of 450 nm. Based on the values obtained for standards, a calibration curve was drawn which allowed the reading of the total concentration of metalloproteinases (ng/ml) in the examined samples.

The results of MMP-2 and MMP-9 serum levels were compared with the results of the most popular prognostic scoring systems, and single laboratory markers at the usual time of their assessment :

1. APACHE II scoring system (10) : assessment on admission and 48 hours after hospital admission.
2. Ranson's score (11) : assessment on admission and 48 hours after hospital admission,
3. Glasgow scale (12) : assessment 24 hours and 48 hours after hospital admission,
4. BISAP scale (13) : assessment on admission and 48 hours after hospital admission,
5. C- reactive protein (14) : assessment on admission and after 48 hours after admission,
6. haematocrit (15), urea presented as urea nitrogen (BUN) (13), creatinine (16) : assessment on admission.

The aim of our research was to look for the earliest marker of SAP and not another one being assessed not earlier than 24 or 48 hours after onset of symptoms.

In the description of quantitative data, arithmetic means were used, as well as standard deviation, median, quartiles, and ranges of values (minimum and maximum). Qualitative data were described by means of frequency and percentage. Frequencies were compared using chi-square test or Fisher's exact test. Normality of distributions was analyzed using Shapiro-Wilk test. Due to the violation of the assumption concerning normality, the distributions of continuous variables were compared by means of Mann-Whitney U test and Kruskall-Wallis test. The relationship between quantitative variables was investigated using Spearman's rank correlation coefficient. In the case of evaluation of the relationship between metalloproteinases activity and the severity of pancreatitis, the analysis was performed using receiver operating curves (ROC) for which the AUC was determined together with 95% confidence interval. All the statistical tests performed were two-tailed, and null hypothesis was rejected when p-value was less than 0.05. Calculations were performed using the software R version 3.1.2 (R Core Team (2014). R : A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http :// www.R-project.org/.), and the software STATISTICA (version 12).

Results

There were 72 patients enrolled into the study with the first-time episode of acute pancreatitis. The study group was divided according to the degree of severity and etiologic factor (Table 1).

The observation was discontinued at patient's death or discharge from hospital. There were five deaths in the SAP group : one case with alcohol and four with cholelithiasis aetiology. Thus, mortality in this group was 35.7% and 6.94% in general.

The range of normal values for MMP-2 and MMP-9 is unknown ; therefore, in the Tables below (Tabs. 2 and 3) the levels of enzymes were assessed with respect to the values observed in data, i.e. with split points determined by median (division into two groups), terciles (division into three groups), and quartiles (division into four groups).

The level of MMP-2 over 50 ng/ml did not occur in the mild form of pancreatitis. Concentration of MMP-2 above 50 ng/ml is significantly related with the advancement of the disease (p=0,0003).

The level of MMP-9 over 25 ng/ml did not occur in the mild and moderate form of pancreatitis. Concentration of MMP-9 above 25 ng/ml is significantly related with the advancement of the disease (p=0,001).

What's more four out of five patients who died had the concentration of MMP-2 above 50 ng/ml and MMP-9 above 25 ng/ml. One patient who died due to infected pancreatic necrosis after 47 days of hospitalisation had an MMP-2 level at 9,99 ng/ml and MMP-9 level at 4,99 ng/ml. Two patients

Table 1. — **The division of study group**

Degree of severity	Sex		Etiologic factor		
	Females (age, median)	Males (age, median)	Alcohol	Cholelithiasis	Other
Mild	27 (24-89 ; 63)	15 (20 – 88 ; 47)	8 (19.0%)	30 (71.4%)	4 (9.5%)
Moderate	3 (68-96 ; 68)	13 (22 – 86 ; 53)	7 (43.8%)	8 (50.0%)	1 (6.2%)
Severe	4 (47-87 ; 74.5)	10 (30 – 88 ; 55.5)	8 (57.1%)	6 (42.9%)	0 (0.0%)

Table 2. — **Level of MMP-2 according to the form of AP**

Concentration of MMP – 2 according to split point (ng/ml)	Form of AP			P-value
	Mild (n= 42)	Moderate (n=16)	Severe (n=14)	
Level of MMP-2 (split point: median)				**0.28**[1]
≤ 21.73	19 (45.2%)	11 (68.8%)	6 (42.9%)	
> 21.73	23 (54.8%)	5 (31.2%)	8 (57.1%)	
Level of MMP-2 (split points: terciles)				**0.017**[2]
gr1 : ≤ 18.80	12 (28.6%)	6 (37.5%)	6 (42.9%)	
gr2 : (18.80-30.35]	17 (40.5%)	7 (43.8%)	0 (0.0%)	
gr3 : > 30.35	13 (31.0%)	3 (18.8%)	8 (57.1%)	
Level of MMP-2 (split points: quadriles)				**0.045**[2]
gr1 : ≤ 18	10 (23.8%)	4 (25.0%)	5 (35.7%)	
gr2 : (18-21.73]	9 (21.4%)	7 (43.8%)	1 (7.1%)	
gr3 : (21.73-33.71]	13 (31.0%)	4 (25.0%)	1 (7.1%)	
gr4 : > 33.71	10 (23.8%)	1 (6.2%)	7 (50.0%)	

1. The percentage of patients with MMP-2 activity over 21.73 (median point) does not significantly differ between the groups 'mild', 'moderate', 'severe'. 2. Statistically significant differences are observed in the distributions of MMP-2 values between the three analysed groups.

Table 3. — **Level of MMP-9 according to the form of AP**

Concentration of MMP – 9 according to split point (ng/ml)	Form of AP			P-value
	Mild (n= 42)	Moderate (n=16)	Severe (n=14)	
Level of MMP-9 (split point: median)				**0.45**[1]
≤ 12.80	23 (54.8%)	8 (50.0%)	5 (35.7%)	
> 12.80	19 (45.2%)	8 (50.0%)	9 (64.3%)	
Level of MMP-9 (split points: terciles)				**0.91**[1]
gr1 : ≤ 10.76	15 (35.7%)	5 (31.2%)	4 (28.6%)	
gr2 : (10.76-14.97]	15 (35.7%)	5 (31.2%)	4 (28.6%)	
gr3 : > 14.97	12 (28.6%)	6 (37.5%)	6 (42.9%)	
Level of MMP-9 (split points: quadriles)				**0.90**[1]
gr1 : ≤ 5.97	12 (28.6%)	3 (18.8%)	3 (21.4%)	
gr2 : (5.97-12.90]	11 (26.2%)	5 (31.2%)	2 (14.3%)	
gr3 : (12.90-16.10]	10 (23.8%)	4 (25.0%)	4 (28.6%)	
gr4 : > 16.10	9 (21.4%)	4 (25.0%)	5 (35.7%)	

1. No statistically significant differences are observed in the distributions of MMP-9 values between the three analysed groups.

Table 4. — **Comparison of clinical usefulness of metalloproteinases and single laboratory parameters**

Laboratory marker	MMP-2 > 50 ng/ml	MMP-9 > 25 ng/ml	CRP			HCT >44%	BUN >20 mg/dl	Creatinine >1,4 mg/dl
			> 47 mg/l	> 120 mg/l	> 150 mg/l			
Od admission								
Sensitivity	35.7%	28.6%	78.6%	50%	42.9%	64.3%	71.4%	64.3%
Specificity	98.3%	100%	63.8%	74.1%	82.8%	69%	74.1%	86.2%
AUC	0.592	0.60	0.58	0.74	0.71	0.54	0.71	0.65

Table 5. — **Comparison of clinical usefulness of metalloproteinases and prognostic scoring systems**

Prognostic scoring system	MMP – 2 > 50 ng/ml	MMP – 9 > 25 ng/ml	APACHE II	Ranson
Od admission				
Sensitivity	35.7%	28.6%	42.9%	35.7%
Specificity	98.3%	100%	91.4%	84.5%
AUC	0.592	0.60	0.75	0.73
24 hours after admission				
Prognostic scoring system	**Glasgow**		**BISAP**	
Sensitivity	50%		14.3%	
Specificity	91.4%		100%	
AUC	0.80		0.70	
48 hours after admission				
Prognostic scoring system	**Glasgow**	**APACHE II**	**BISAP**	**Ranson**
Sensitivity	35.7%	57.1%	35.7%	14.3%
Specificity	100%	93.1%	100%	100%
AUC	0.84	0.83	0.81	0.89

who died due to acute renal failure in the second day of hospitalisation had the level of MMP-2 130,21 ng/ml and 144,4 ng/ml. Their MMP-9 serum concentration was 58,11 ng/ml and 78,78 ng/ml, respectively. One patient died in the sixth day of hospitalisation because of acute lung injury. His MMP-2 and MMP-9 levels were 87,5 ng/ml and 39,44 ng/ml, respectively. The cause of death of last patient (after seven days of hospitalisation) was also acute lung injury. In this case the levels of MMP-2 and MMP-9 were 68,14 ng/ml and 27,9 ng/ml, respectively. In conclusion, the concentration level of MMP-2 and MMP-9 is strictly correlated with acute lung injury, acute kidney injury and time of death.

Table 4 presents a comparison of clinical usefulness of determination of the level of 2 and 9 metalloproteinases, and the most common single laboratory markers. The concentration of these markers were determined according to previous researches (13-15).

The CRP level over 270 mg/l measured 48 hours after admission is characterized by the highest diagnostic strength (AUC 0.77, sensitivity 21,4%, specificity 94,8%). Nevertheless, its sensitivity and specificity is lower than that of matrix metalloproteinases. Laboratory markers assessed on admission are characterized by higher sensitivity but lower specificity than matrix metalloproteinases. What's more we need to realize that patients enrolled to the study were previously healthy. The clinical usefulness of CRP, haematocrit, creatinine and BUN will be lower if the patient will suffer from any chronic diseases.

Table 5 compares the diagnostic value of matrix metalloproteinases in comparison with the most common multifactor prognostic scoring systems.

As we can see, Ranson's score 48 hours after admission shows the highest diagnostic strength (AUC 0.89, specificity 100%). Its specificity is comparable with that of MMP-2 and MMP-9. However, sensitivity is lower. Furthermore, the predictive ability is determined as late as after 48 hours, while at that time, it might be necessary to treat patient in an intensive care unit. The multifactor prognostic scales assessed at the time of admission are characterised by lower specificity and comparable sensitivity in comparison to matrix metalloproteinases.

Discussion

Autoactivation of pancreatic enzymes leads to damage of the organ itself, as well as the peripancreatic tissue. This leads to infiltration of immune cells and exposure of collagen, which results in an increased production of matrix metalloproteinases (16). Therefore, we undertook an attempt to check whether the determination of the levels of MMP-2 and MMP-9 may be used as a marker of SAP (17).

The majority of currently available scoring systems require assay of many parameters. In the study by Wu et al. (13), who analysed medical histories of 18,256 patients with acute pancreatitis, all parameters in the APACHE II scoring system were assessed only in 2.2% of cases. What's more, the Ranson and Glasgow scales require 48 hours for full assessment. Therefore, the best diagnostic instrument would be a scoring system with a small number of parameters, or a single prognostic marker. Many studies focus on the latter.

One of the factors most frequently taken into account as a tool prognosticating the severe form of pancreatitis

is the level of C- reactive protein (CRP), with various cut-off points. However, the level of CRP increases as late as within 24-48 hours after onset of symptoms so it cannot be an early predictive factor.

Valverde-Lopez et al. (14) analysed also the usefulness of creatinine concentration and BUN (18) level as prognostic factors of SAP. In our study we received comparable sensitivity and specificity in both parameters.

Pallisera et al. (15) analyzed the value of haematocrit exceeding 44% as a prognostic factor of severe form of acute pancreatitis, obtaining sensitivity and specificity on the levels 45% and 63%. These values were 64.3% and 69% in our own study.

While comparing all the above-mentioned laboratory markers with the level of metalloproteinases it may be noted that all of them are characterized by a considerably lower specificity, but show a higher sensitivity. In the case of multifactor prognostic scoring systems (Tab. 5) it is possible to obtain specificity similar to that of metalloproteinases. However, it is necessary to consider the fact that each of them requires the determination of several parameters, some of which (Glasgow, Ranson) require the determination twice, and a similar specificity is obtained 48 hours after hospitalization.

The limitation of the presented study is primarily a small number of patients, especially those with the severe form of pancreatitis. Nevertheless, it should be taken into account that the study was conducted in one ward with a unified system of classification of patients, diagnostic methods, and treatment. In addition, the enrolled study group included patients without medical history of neither pancreatic nor chronic diseases. This was also related with the limitation of the number of patients who fulfilled the enrolment criteria.

Conclusion

Multifactor prognostic scoring systems for prognosticating the severe form of pancreatitis are characterized by high specificity, but low diagnostic sensitivity. In addition, full analysis is obtained as late as after 48 hours. The use of a single laboratory marker as a predictive factor of SAP is characterized by higher sensitivity, but lower specificity, compared to multifactor prognostic scoring systems. The results obtained and the data from literature indicate that it is necessary to conduct further studies concerning the role of metalloproteinases in the pathology of acute pancreatitis, and their importance as prognostic factors of the severe form of this disease.

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article. All authors declare no conflict of interest.

References

1. BANKS P.A., BOLLEN T.L., DERVENIS C., GOOSZEN H.G., JOHNSON C.D., SARR M.G. *et al.* Classification of acute pancreatitis-2012 : revision of the Atlanta classification and definitions by international consensus. *Gut.,* 2013, **62** : 102-111.
2. GŁUSZEK S., KOZIEŁ D. Prevalence and progression of acute pancreatitis in the Świętokrzyskie Voivodeship population. *Pol Przegl Chir.*, 2012, **84** : 618-625.
3. HE W-H., ZHU Y., ZHU Y., JIN Q., XU H-R., XION Z-J. *et al.* Comparison of multifactor scoring systems and single serum markers for the early prediction of the severity of acute pancreatitis. *J Gastroenterol Hepatol*., 2017, **32** : 1895-1901.
4. MUNIGALA S., YADAV D. Case-fatality from acute pancreatitis is decreasing but its population mortality shows little change. *Pancreatology*., 2016, **16** : 542–550.
5. LEE K.J., KIM H.M., CHOI J.S., KIM Y.J., KIM Y.S., CHO J.H. Comparison of Predictive Systems in Severe Acute Pancreatitis According to the Revised Atlanta Classification. *Pancreas*., 2016, **45** : 46-50.
6. ANDERSSON B, ANSARI D, ANDERSSON E, PERSSON S, ANDERSSON R. Fatal acute pancreatitis occurring outside of the hospital : clinical and social characteristics. *World J Surg*., 2010, **34** : 2286-2291.
7. ZHANG X.P., WANG L., ZHOU Y.F. The pathogenic mechanism of severe acute pancreatitis complicated with renal injury : a review of current knowledge. *Dig Dis Sci*., 2008, **53** : 297-306.
8. NUKARINEN E., LINDSTRÖM O., KUULIALA K., KYLÄNPÄÄ L., PETTILÄ V., PUOLAKKAINEN P. *et al.* Association of Matrix Metalloproteinases -7, -8 and -9 and TIMP -1 with Disease Severity in Acute Pancreatitis. A Cohort Study. *PLoS ONE*., 2016, **11** : 1-11.
9. LI H., QIAN Z., LIU Z., LIU X., HAN X., KANG H. Risk factors and outcome of acute renal failure in patients with severe acute pancreatitis. *J Crit Care*., 2010, **25** : 225-229.
10. KNAUS W.A., DRAPER E.A., WAGNER D.P., ZIMMERMAN J.E. APACHE II : a severity of disease classification system. *Crit Care Med*., 1985, **13** : 818-829.
11. RANSON J.H., RIFKIND K.M., ROSES D.F., FINK S.D., ENG K., SPENCER F.C. Prognostic signs and the role of operative management in acute pancreatitis. *Surg Gynecol Obstet*., 1974, **139** : 69-81.
12. BLAMEY S.L., IMRIE C.W., O'NEILL J., GILMOUR W.H., CARTER D.C. Prognostic factors in acute pancreatitis. *Gut*., 1984, **25** : 1340-1346.
13. WU B.U., JOHANNES R.S., SUN X., TABAK Y., CONWELL D.L., BANKS P.A. The early prediction of mortality in acute pancreatitis : a large population-based study. *Gut*., 2008, **57** : 1698-1703.
14. VALVERDE-LÓPEZ F., MATAS-COBOS A.M., ALEGRÍA-MOTTE C., JIMÉNEZ-ROSALES R., ÚBEDA-MUÑOZ M., REDONDO-CEREZO E. BISAP, RANSON, lactate and others biomarkers in prediction of severe acute pancreatitis in a European cohort. *J Gastroenterol Hepatol*., 2017, **32** : 1649-1656.
15. PALLISERA A., JORBA R., RAMIA J., RODRIGUEZ J., SUBIRANA H., ZÁRATE L. *ET AL*. Biological markers of severity in acute pancreatitis. *Cent. Eur. J. Med*., 2014, **9** : 550-555.
16. VAN DOREN S.R. Matrix metalloproteinase interactions with collagen and elastin. *Matrix Biol*., 2015, **44-46** : 224-231.
17. WERESZCZYNSKA-SIEMIATKOWSKA U., SIEMIATKOWSKI A., SWIDNICKA-SIERGIEJKO A., MROCZKO B., DABROWSKI A. The imbalance between matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in acute pancreatitis. *Z Gastroenterol*., 2015, **53** : 199-204.
18. WU B.U., BAKKER O.J., PAPACHRISTOU G.I., BESSELINK M.G., REPAS K., VAN SANTVOORT H.C. *et al.* Blood urea nitrogen in the early assessment of acute pancreatitis : an international validation study. *Arch Intern Med*., 2011, **171** : 669-676.