Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2017, Vol. 45(1) 147–158

DOI: 10.1177/0300060516677929
journals.sagepub.com/home/imr
SAGE

Antithrombin III and D-dimer levels as indicators of disease severity in patients with hyperlipidaemic or biliary acute pancreatitis

Ning Yang[1], Jianyu Hao[2] and Donglei Zhang[2]

Abstract

Objective: To assess changes in anticoagulation and fibrinolytic systems between biliary and hyperlipidaemic acute pancreatitis (AP).

Methods: Patients with biliary or hyperlipidaemic AP were enrolled. Demographic and clinical data were collected, and antithrombin III (ATIII), protein C, protein S, and D-dimer levels were investigated.

Results: A total of 45 patients with biliary AP and 50 patients with hyperlipidaemic AP were included (68 with mild AP and 27 with moderately-severe AP). ATIII and protein C levels in the mild AP group were significantly higher, but prothrombin time and D-dimer were significantly lower, versus the moderately-severe AP group. ATIII and D-dimer were found to be risk factors for moderately-severe AP. ATIII could predict AP severity, particularly in patients with biliary AP. D-dimer was a sensitive and specific predictor for disease severity in patients with AP, particularly in patients with hyperlipidaemic AP.

Conclusion: ATIII and protein C levels decreased as severity of AP increased, particularly in cases of biliary AP. D-dimer levels increased with severity of AP, particularly in hyperlipidaemic AP. ATIII and D-dimer may be useful biomarkers for assessing AP severity in patients with biliary and hyperlipidaemic AP, respectively.

Keywords

Biliary acute pancreatitis, hyperlipidaemic acute pancreatitis, antithrombin III, protein C, D-dimer

Date received: 21 July 2016; accepted: 15 October 2016

Introduction

The prevalence of acute pancreatitis (AP) continues to increase in the Chinese population.[1] The pathogenesis of AP is founded on autodigestion of the pancreas and characterized by ischaemia/reperfusion injury

[1]Department of Endocrinology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China
[2]Department of Gastroenterology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China

Corresponding author:
Donglei Zhang, Department of Gastroenterology, Beijing Chaoyang Hospital, Capital Medical University, 8 Gongtinanlu, Chaoyang District, Beijing 100020, China.
Email: zdlzdl52973@sina.com

and infection. The common causes include alcohol consumption, overeating, cholelithiasis, and hypertriglyceridaemia.[1]

In 1952, the first case of hyperlipidaemic AP caused by primary hyperlipidaemia (also known as hyperlipoproteinemia) was reported.[2] Since then, the prevalence of hyperlipidaemic AP has greatly increased and it is presently the third leading cause of AP in China; biliary AP remains the first.[3] The prevalence of AP caused by hyperlipidaemia varies between studies, and ranges from approximately 1–12%, with 12–50% of these patients also having lipid disorders.[4,5]

The pathogenesis of biliary AP differs from hyperlipidaemic AP, in that biliary AP is caused by biliary obstruction (bile duct stone, or dysfunction of the sphincter of Oddi) and bile reflux into the pancreas. Pathogenesis of hyperlipidaemic AP is associated with obstruction of the pancreatic microcirculation by blood lipids, and injury of pancreatic acinar cells by free fatty acids and abnormally activated trypsinogen.[6–10]

Most patients with AP recover well, but 20–25% may progress onto haemorrhagic necrotizing pancreatitis or severe AP, which can lead to systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome, and even multiple organ dysfunction syndrome, with 30–50% mortality rates.[11,12] Early diagnosis and timely treatment could significantly decrease the morbidity and mortality of severe AP.[13] Thus, early and accurate evaluation of AP severity is important to prevent progression.

Hypercoagulability is observed in patients with AP, caused by activation of cytokines like interleukin (IL)-6, IL-18, and tumour necrosis factor-α during the inflammatory process.[14,15] This cascade response can lead to SIRS and multiple organ dysfunction syndrome, as well as intrinsic and extrinsic coagulation. Systemic inflammation may further impair the balance between anticoagulation and fibrinolysis. Abnormal activation of vascular endothelial cells can cause the formation of a thrombus or microthrombus in small vessels.[16,17] Thus, patients with AP may develop systemic inflammation and coagulation,[18] and dysfunction of the coagulation system in AP is associated with poor prognosis.[19]

The anticoagulation system, composed mainly of antithrombin and protein C systems, changes during SIRS. Antithrombin III (ATIII), a glycoprotein secreted by hepatocytes, has a major role in anticoagulation.[20] ATIII can significantly inhibit thrombin and clotting factor X, as well as activated clotting factor IX, XI, and XII, and plasmin, and also inhibits platelet accumulation and release.[20,21] ATIII functions by binding to heparin, and *in vivo* experiments have shown that ATIII could prevent acute pancreatitis by inhibiting cytokines, nitric oxide, and trypsin.[22] ATIII and platelet levels have been suggested as predictors of AP severity.[23]

Protein C may be protective against severe AP.[24] Protein C is a vitamin K-dependent serine protease zymogen that is mainly produced in the liver. Thrombin can activate protein C, which can not only degrade clotting factor V and VIII, but also promote fibrinolysis and anticoagulation by the endothelial cell protein C receptor through the thrombin-thrombomodulin complex. Activated protein C has been reported to suppress the inflammatory reaction and cell apoptosis by inhibiting the release of immunomediators and the adhesion of inflammatory cells and endothelial cells, preventing cell injury.[25–28] In addition, protein C has been introduced as a therapeutic target for treating SIRS. Whether activated protein C can be used to treat patients with AP remains uncertain,[29–32] as does the value of ATIII or protein C in predicting AP severity.

The association between D-dimer levels and AP severity also requires investigation. D-dimer is a specific degraded product of fibrin after the interlinking of fibrin

monomer and clotting factor XIII. Increased D-dimer levels reflect activation of coagulation and the fibrinolytic system and can be used for early and rapid diagnosis of thrombotic diseases. D-dimer is used to diagnose disseminated intravascular coagulation and to exclude the presence of venous thrombotic diseases, including deep venous thrombosis and pulmonary embolism.[33] Elevated D-dimer levels have also been reported in AP.[19,34]

The roles of coagulation and the fibrinolytic system in biliary and hyperlipidaemic AP remain uninvestigated. To clarify the clinical significance of coagulation and the fibrinolytic system in AP, the present study examined relevant changes and their association with AP severity in patients diagnosed with biliary or hyperlipidaemic AP.

Patients and methods

Study population

In this single-centre, observational cohort study, patients who were diagnosed with biliary AP or hyperlipidaemic AP between January 2014 and December 2015, at the Department of Gastroenterology, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China were sequentially enrolled. AP was diagnosed based on $\geq$2 of the following factors (determined $\geq$3 times): abdominal pain; increased serum amylase and/or lipase; and evidence of AP on contrast-enhanced computed tomography/magnetic resonance imaging or ultrasound.

Biliary AP was diagnosed in patients with AP who demonstrated the following: increased transaminase and/or bilirubin levels; bile duct stone or dilation shown by radiographic imaging; and suspected biliary obstruction in patients who required endoscopic retrograde cholangiopancreatography (ERCP). Hyperlipidaemic AP was diagnosed in patients with AP who demonstrated the following: triglycerides $\geq$11.30 mmol/l or 5.65–11.30 mmol/l with chylous serum.

All patients underwent contrast-enhanced computed tomography within 24 h of admission, and patients' demographic and clinical data were retrieved. Disease severity was graded as mild or moderately severe, based on the 2012 Atlanta classification.[35]

Patients with alcoholic AP, post-ERCP pancreatitis, chronic pancreatitis, and chronic renal dysfunction were excluded. The Ethics Committee of Beijing Chaoyang Hospital, Capital Medical University, Beijing, China approved the study protocol (No. 2012-ke-9), which was conducted in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent.

Measurements

Blood samples (15 ml) were collected from the cubital vein into VACUETTE® Z Serum Clot Activator tubes (Greiner Bio-One, Kremsmunster, Austria) within 48 h of hospital admission, and immediately sent to the hospital laboratory for testing. White blood cell counts, haematocrit, and platelet levels were determined using a Sysmex XE-2100™ automated haematology system (Sysmex, Kobe, Japan) according to the manufacturer's instructions. Liver and kidney function, blood lipids and electrolytes were determined using a Dimension® RxL Max® integrated chemistry system (Dade Behring Diagnostics, Marburg, Germany) according to the manufacturer's instructions. Laboratory parameters included the following: serum albumin, prealbumin, alanine aminotransferase (ALT), aspartate transaminase (AST), γ-glutamyltransferase (γ-GT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, total bile acids, creatinine, calcium, glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride. Prothrombin time, activated partial

thromboplastin time (APTT), fibrinogen, and thrombin time were determined by routine coagulation methods with a coagulation detector, using a Sysmex CA6000 automated analyser (Sysmex, Milton Keynes, UK) according to the manufacturer's instructions. Serum ATIII, protein C, protein S, and D-dimer were also detected using the Sysmex CA6000 automated analyser, according to the manufacturer's instructions.

Statistical analyses

Statistical analyses were performed using SPSS software, version 17.0 (SPSS, Chicago, IL, USA). Categorical data are presented as *n* (%) prevalence, and between-group differences were assessed using χ^2-test, Yates' continuity correction, or Fisher's exact test, as appropriate. Continuous data are presented as mean ± SD or median (interquartile range), and between-group differences were compared by independent Student's *t*-test (normally distributed variables) or Mann-Whitney *U*–test (not normally distributed variables). Backward conditional logistic regression analyses were performed to identify risk factors for moderately-severe AP. Receiver operating characteristic (ROC) curve analyses were used to determine the sensitivity, specificity, positive and negative predictor values, area under the curve (AUC) and 95% confidence intervals (CIs). A P value < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of the study population

A total of 95 patients were enrolled (65 male and 30 female; mean age of onset, 47.6 ± 15.5 years, range, 23–89 years), diagnosed with biliary AP ($n = 45$) or hyperlipidaemic AP ($n = 50$). Of these patients, 68 were classified with mild AP, and 27 patients were classified with moderately-severe AP. Age of onset in the biliary AP group was higher (56.1 ± 16.6 versus 38.7 ± 7.7 years) but the incidence of fatty liver was lower (28.9% versus 92.0%) compared with the hyperlipidaemic AP group ($P < 0.01$). Fewer male patients (27/45 versus 38/50) and patients with type II diabetes mellitus (8/45 versus 13/50), and a lower mild AP/moderately-severe AP ratio (2.21:1 versus 2.85:1) were found in the biliary AP versus hyperlipidaemic AP group, but these differences were not statistically significant ($P > 0.05$).

The following were significantly higher in the biliary versus hyperlipidaemic AP group: ALT (230.44 ± 149.19 versus 45.22 ± 37.94 U/l), AST (297.44 ± 209.73 versus 58.84 ± 49.47 U/l), γ-GT (464.82 ± 312.98 versus 86.54 ± 65.15 U/l), ALP (157.42 ± 66.39 versus 97.32 ± 27.62 U/l), total bilirubin (40.35 ± 32.38 versus 23.89 ± 15.56 μmol/l), creatinine (82.02 ± 20.58 versus 73.64 ± 26.42 μmol/l), and calcium (2.17 ± 0.19 versus 1.92 ± 0.29 mmol/l; all $P < 0.05$). The following were significantly lower in the biliary versus hyperlipidaemic AP group: prealbumin (0.243 ± 0.07 versus 0.333 ± 0.09 g/l), glucose (9.51 ± 3.37 versus 14.54 ± 5.49 mmol/l), total cholesterol (4.49 ± 1.08 versus 7.76 ± 2.16 mmol/l), LDL-C (2.35 ± 0.76 versus 2.93 ± 1.45 mmol/l), and triglyceride. (1.13 ± 0.61 versus 24.59 ± 13.15 mmol/l; all $P < 0.05$).

Coagulation, anticoagulation, and fibrolytic markers were compared between the biliary and hyperlipidaemic AP groups. Prothrombin and thrombin times were significantly higher in the biliary versus hyperlipidaemic AP group (12.20 ± 1.32 versus 11.35 ± 1.02 s and 19.38 ± 1.54 versus 17.38 ± 1.25 s, respectively; all $P < 0.05$). Fibrinogen, ATIII, protein C, and protein S levels were all lower in the biliary versus hyperlipidaemic AP group (368.59 ± 111.61 versus 498.34 ± 115.08 mg/dl; 75.60 ± 13.49

versus $80.63 \pm 9.59\%$; 79.53 ± 18.21 versus $117.44 \pm 28.54\%$; and 73.42 ± 19.40 versus $86.52 \pm 25.72\%$, respectively; all $P < 0.05$). There was no statistically significant difference in D-dimer levels between patients with biliary versus hyperlipidaemic AP.

Relative to patients with moderately-severe AP, calcium levels were significantly higher and total bilirubin and glucose were lower in patients with mild AP (2.09 ± 0.25 versus 1.92 ± 0.32 mmol/l; 21.40 ± 19.49 versus 32.41 ± 15.38 μmol/l; 11.42 ± 5.13 versus 14.01 ± 5.13 mmol/l, respectively; all $P < 0.05$). No statistically significant differences were found between patients with mild or moderately-severe AP in terms of other blood chemistry findings. In terms of coagulation, anticoagulation and fibrinolytic factors, ATIII and protein C levels were higher and prothrombin time and D-dimer were lower in patients with mild AP versus those with moderately-severe AP ($P < 0.05$; Table 1).

Subgroup analyses

Patients were categorized into the following 4 subgroups: mild biliary AP, moderately-severe biliary AP, mild hyperlipidaemic AP and moderately-severe hyperlipidaemic AP. Age of onset was higher but incidence of fatty liver disease was lower in the mild or moderately-severe biliary AP groups compared with the mild or moderately-severe hyperlipidaemic AP groups ($P < 0.01$). The moderately-severe hyperlipidaemic AP group had the highest percentage of patients with type II diabetes mellitus. Male/female ratios and cholecystectomy rates were comparable among the different groups ($P > 0.05$). White blood cell count in the moderately-severe biliary AP and mild hyperlipidaemic AP groups were higher than in the mild biliary AP group ($P < 0.05$). Prealbumin, glucose, total cholesterol, and triglyceride levels in the mild and moderately-severe biliary AP groups were lower than those the hyperlipidaemic AP subgroups, while ALT, AST, γ-GT, ALP, direct bilirubin, and calcium levels were higher ($P < 0.05$). Patients with mild biliary or mild hyperlipidaemic AP had higher calcium and protein C levels but lower glucose and D-dimer than patients with moderately-severe biliary or

Table 1. Coagulation, anticoagulation and fibrinolytic factors in patients with mild or moderately-severe acute pancreatitis (AP).

Characteristic	Patients with AP		Statistical significance
	Mild ($n = 68$)	Moderately severe ($n = 27$)	
Prothrombin time, s	11.57 ± 1.13	12.26 ± 1.41	$P = 0.016$
APTT, s	28.79 ± 4.81	29.78 ± 6.64	NS
Fibrinogen, mg/dl	430.78 ± 124.70	450.45 ± 145.53	NS
Thrombin time, s	18.14 ± 1.70	18.89 ± 1.64	NS
ATIII, %	81.13 ± 11.24	70.98 ± 10.11	$P < 0.01$
Protein C, %	104.46 ± 31.53	86.96 ± 24.83	$P = 0.011$
Protein S, %	79.49 ± 25.71	82.39 ± 18.17	NS
D-dimer, mg/l	1.39 ± 0.86	3.61 ± 1.29	$P < 0.01$

Data presented as the mean ± SD.

APTT, activated partial thromboplastin time; ATIII, antithrombin III.

NS, no statistically significant between-group difference ($P > 0.05$; independent Student's *t*-test).

hyperlipidaemic AP ($P < 0.05$). ALT in the mild hyperlipidaemic AP group was significantly lower than in the moderately-severe hyperlipidaemic AP ($P < 0.05$) (Table 2). Patients with mild biliary AP had higher ATIII than patients with moderately-severe biliary AP ($P < 0.01$). Protein C was higher but thrombin time was shorter in the mild and moderately-severe hyperlipidaemic AP groups compared with both biliary AP subgroups ($P < 0.01$). The moderately-severe biliary AP group had lower ATIII, protein C, and protein S levels than did the moderately-severe hyperlipidaemic AP group ($P < 0.01$; Table 3). There were no other statistically significant differences in coagulation, anticoagulation and fibrinolytic factors.

Anticoagulation and fibrinolytic markers for predicting AP severity

A backward, conditional logistic regression model was applied to the data and risk factors for moderately-severe AP were analysed. Logistic regression analysis showed that D-dimer was an independent risk factor for AP (relative risk [RR] 4.504), moderately-severe biliary AP (RR 3.147), and moderately-severe hyperlipidaemic AP (RR 9.824). ATIII was an independent risk factor for moderately-severe AP (RR 1.071) and moderately-severe biliary AP (RR 1.104; Table 4).

Receiver operating characteristic curve analyses were performed for ATIII and D-dimer in the AP, biliary AP, and hyperlipidaemic AP groups. The accuracy of ATIII for predicting moderately-severe AP in the total AP and biliary AP groups was moderate (Table 5), particularly in the biliary AP group. D-dimer had moderate diagnostic accuracy for predicting moderately-severe biliary AP and excellent diagnostic accuracy for predicting moderately-severe AP in the total AP group and particularly in patients with hyperlipidaemic AP (Table 5).

Discussion

Acute pancreatitis is a result of inflammation caused by the activation of trypsin in pancreatic tissue, which may result in dysfunction of other organs.[3] The two main AP subtypes are biliary and hyperlipidaemic, and both types were included for analyses in the present study. Hyperlipidaemic AP often occurs in young men, and amylase is usually normal or slightly elevated.[36–39] The present results concur with similar studies, with young males predominant in hyperlipidaemic AP (although the proportions of males were not significantly different between biliary and hyperlipidaemic AP). Compared with other categories of AP, hyperlipidaemic AP is more likely to progress to moderately severe and severe, accompanied by acute respiratory distress syndrome, acute renal failure, or multiple organ failure.[12] Furthermore, the course of hyperlipidaemic AP is longer than that of other AP subtypes.[36,40] In patients with hyperlipidaemic AP, insulin and heparin administration can help lower triglyceride levels,[41] and blood purification may prevent complications when routine treatments fail.[42,43] Thus, reduction of triglyceride levels is important to prevent the recurrence of hyperlipidaemic AP.[44]

In the present study, patients with AP were first stratified into biliary or hyperlipidaemic AP subgroups, and compared for AP severity. ATIII was found to be lower in the biliary AP group than in the hyperlipidaemic AP group, and ATIII was lower in patients with mild AP versus those with moderately-severe AP. To the best of the authors' knowledge, this is the first report showing that ATIII in patients with moderately-severe biliary AP was significantly lower than in patients with moderately-severe hyperlipidaemic AP. Furthermore, logistic regression analyses showed that ATIII was inversely correlated with moderately-severe AP in all patients with AP, and particularly in those with biliary AP.

Table 2. Demographic, clinical and routine blood parameters in patients with biliary or hyperlipidaemic acute pancreatitis (AP), stratified into mild and moderately-severe groups.

	Patient group			
	Biliary AP		Hyperlipidaemic AP	
Parameter	Mild ($n = 31$)	Moderately severe ($n = 14$)	Mild ($n = 37$)	Moderately severe ($n = 13$)
Sex, male	20 (64.5)	7 (50.0)	26 (85)	12 (72.7)
Age of onset, years	56.1 ± 16.6	63.4 ± 10.3	38.7 ± 7.7[a]	35.5 ± 5.2[c]
Diabetes mellitus type 2	5 (16.1)	3 (21.4)	7 (18.9)	6 (46.2)[c]
Fatty liver disease	9 (29.0)	4 (28.6)	34 (91.2)[a]	12 (92.3)[c]
Cholecystectomy	2 (6.5)	1 (7.1)	0 (0.0)	0 (0.0)
White blood cell count, $\times 10^9$/l	12.26 ± 4.48	15.21 ± 3.24[b]	14.43 ± 4.45[b]	14.21 ± 3.32
Haematocrit, %	0.41 ± 0.05	0.44 ± 0.04	0.43 ± 0.04	0.45 ± 0.04
Platelets, $\times 10^9$/l	217.94 ± 67.84	202.00 ± 123.10	237.08 ± 71.85	213.62 ± 32.48
Albumin, g/l	39.09 ± 5.10	38.79 ± 4.31	38.79 ± 3.97	37.55 ± 5.79
Prealbumin, g/l	0.25 ± 0.08	0.23 ± 0.07	0.33 ± 0.09[a]	0.33 ± 0.09[d]
ALT, U/l	256.42 ± 148.82	172.93 ± 138.03	37.95 ± 32.13[a]	65.92 ± 46.39[b,e]
AST, U/l	303.84 ± 189.37	283.29 ± 256.53	55.84 ± 52.89[a]	67.38 ± 38.66[d]
γ-GT, U/l	507.94 ± 320.29	369.36 ± 283.83	85.54 ± 61.93[a]	89.38 ± 76.24[d]
ALP, U/l	163.29 ± 72.05	144.43 ± 51.69	97.89 ± 25.34[a]	95.69 ± 34.42[d]
Total bilirubin, μmol/l	30.10 (14.60, 59.70)	34.33 ± 17.89	21.67 ± 16.02[a]	30.35 ± 12.54
Direct bilirubin, μmol/l	17.60 (6.40, 3.30)	18.01 ± 11.73	2.20 (1.05, 3.59)[a]	1.79 (0.90, 6.76)[d]
Total bile acids, μmol/l	24.00 (4.30, 126.50)	27.50 (4.40, 89.35)	1.90 (1.45, 3.25)[a]	1.60 (1.10, 1.90)[d]
Creatinine, μmol/l	79.24 ± 19.49	88.19 ± 22.33	73.84 ± 27.42	73.07 ± 24.37
Calcium, mmol/l	2.21 ± 0.15	2.08 ± 0.25[b]	1.99 ± 0.26[a]	1.74 ± 0.29[c,d]
Glucose, mmol/l	8.79 ± 3.13	11.10 ± 3.44[b]	13.62 ± 5.47[a]	17.14 ± 4.87[b]
Total cholesterol, mmol/l	4.40 ± 1.13	4.72 ± 0.96	7.69 ± 2.21[a]	7.96 ± 2.07[d]
HDL-C, mmol/l	1.42 ± 0.36	1.46 ± 0.78	1.52 ± 1.27	1.86 ± 1.83
LDL-C, mmol/l	2.30 ± 0.76	2.46 ± 0.78	2.83 ± 1.31	3.26 ± 1.81
Triglyceride, mmol/l	1.05 ± 0.52	1.31 ± 0.75	22.82 ± 12.44[a]	29.62 ± 14.31[d]

Data presented as *n* (%) prevalence, mean ± SD or median (quartile 1, quartile 3).

ALT, alanine aminotransferase; AST, aspartate transaminase; γ-GT, g-glutamyltransferase; ALP, alkaline phosphatase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

[a]$P < 0.01$ and [b]$P < 0.05$, compared with mild biliary AP; [c]$P < 0.01$, compared with mild hyperlipidaemic AP; [d]$P < 0.01$, compared with moderately-severe biliary AP; [e]$P < 0.05$, compared with mild hyperlipidaemic AP (χ^2-test, Yates' continuity correction, Fisher's exact test, independent Student's *t*-test, or Mann–Whitney *U*-test, as appropriate).

Lower ATIII may be related to abnormal liver function caused by inflammation and increased consumption during AP, which is consistent with published findings.[23,45]

To the best of the authors' knowledge, the present study is also the first to investigate differences in protein C levels between biliary and hyperlipidaemic AP. Protein C

Table 3. Coagulation, anticoagulation, and fibrinolytic factors in patients with biliary or hyperlipidaemic acute pancreatitis (AP), stratified into mild and moderately-severe groups.

	Patient group			
	Biliary AP		Hyperlipidaemic AP	
Characteristic	Mild ($n = 31$)	Moderately severe ($n = 14$)	Mild ($n = 37$)	Moderately severe ($n = 13$)
Prothrombin time, s	11.96 ± 1.36	12.75 ± 1.11	11.24 ± 0.77^{a}	11.68 ± 1.55
APTT, s	28.20 ± 4.88	30.39 ± 7.05	29.31 ± 4.75	29.07 ± 6.37
Fibrinogen, mg/dl	360.20 ± 99.91	387.16 ± 136.32	488.92 ± 112.87^{b}	524.29 ± 122.98^{a}
Thrombin time, s	19.17 ± 1.61	19.81 ± 1.30	17.24 ± 1.22^{b}	17.80 ± 1.32^{d}
ATIII, %	79.90 ± 12.98	66.07 ± 9.24^{b}	82.16 ± 9.62	76.27 ± 8.40^{d}
Protein C, %	82.73 ± 19.84	72.44 ± 11.68^{a}	122.66 ± 27.84^{b}	$102.59 \pm 26.03^{c,d}$
Protein S, %	73.25 ± 20.52	73.81 ± 17.38	84.73 ± 28.59	91.63 ± 14.47^{d}
D-dimer, mg/l	1.47 ± 0.90	3.13 ± 11.28^{b}	1.32 ± 0.82	$4.12 \pm 1.14^{a,c}$

Data are presented as mean ± SD.

APTT, activated partial thromboplastin time; ATIII, antithrombin III.

$^{a}P < 0.05$ and $^{b}P < 0.01$, compared with mild biliary AP; $^{c}P < 0.05$, compared with mild hyperlipidaemic AP; $^{d}P < 0.01$, compared with moderately-severe biliary AP (independent Student's *t*-test).

Table 4. Logistic regression analyses to assess whether ATIII and D-dimer were risk factors for moderately-severe acute pancreatitis (AP) in all patients with AP, biliary AP, or hyperlipidaemic AP.

Patient group	Parameter	β	SE	Statistical significance	OR	95% CI
AP ($n = 95$)	ATIII	−0.068	0.033	$P = 0.036$	0.934	0.876, 0.996
	D-dimer	1.505	0.327	$P < 0.001$	4.504	2.372, 8.553
Biliary AP ($n = 45$)	ATIII	−0.099	0.045	$P = 0.028$	0.906	0.829, 0.990
	D-dimer	1.146	0.407	$P = 0.005$	3.147	1.416, 6.992
Hyperlipidaemic AP ($n = 50$)	ATIII	—	—	—	—	—
	D-dimer	2.285	0.692	$P = 0.001$	9.824	2.530, 38.146

ATIII, antithrombin III; SE, sensitivity; OR, odds ratio; CI, confidence interval.

levels were found to be lower in patients with biliary AP versus those with hyperlipidaemic AP, and protein C levels were lower in patients with moderately-severe AP versus patients with mild AP. Protein C levels also correlated with AP severity in the biliary and hyperlipidaemic AP groups. Protein C levels in the mild or moderately-severe biliary AP groups were lower than in the mild or moderately-severe hyperlipidaemic AP groups. The reason for lower protein C may be lower production by the liver and increased consumption in patients with AP.[46] There was no statistically significant difference in prothrombin time or APTT between moderately-severe and mild AP in biliary AP and hyperlipidaemic AP groups, however, fibrinogen in the mild and moderately-severe hyperlipidaemic AP groups was significantly higher than in the mild and moderately-severe biliary AP groups. Although protein C in hyperlipidaemic AP

Table 5. Receiver operating characteristic (ROC) curve analyses of plasma ATIII and D-dimer concentrations for the diagnosis of moderately-severe acute pancreatitis (AP) in all patients with AP, biliary AP, or hyperlipidaemic AP.

		Patient group		
Characteristic	Parameter	AP ($n = 95$)	Biliary AP ($n = 45$)	Hyperlipidaemic AP ($n = 50$)
ATIII	Cut-off value, %	<73.75	<71.45	<73.95
	Sensitivity, %	66.7	71.4	53.8
	Specificity, %	75.0	80.6	83.8
	Positive predictor value, %	51.4	62.5	53.8
	Negative predictor value, %	85.1	86.2	83.8
	AUC	0.744	0.803	0.686
	95% CI	0.640, 0.849	0.674, 0.932	0.526, 0.846
D-dimer	Cut-off value, mg/l	>1.87	>2.85	>1.995
	Sensitivity, %	92.6	81.8	100
	Specificity, %	77.69	95.8	83.8
	Positive predictor value, %	62.5	90.0	68.4
	Negative predictor value, %	96.4	92.0	100
	AUC	0.922	0.865	0.973
	95% CI	0.865, 0.979	0.752, 0.978	0.000, 1.000

ATIII, antithrombin III; AUC, area under the curve; CI, confidence interval.

was normal, levels of protein C were lower in the mild versus moderately-severe groups (i.e., decreased as AP progressed). The present findings suggest that patients with hyperlipidaemic AP may develop hypercoagulation and require anticoagulation therapy, and are consistent with published studies.[19,24] Unlike the present study, however, one study was conducted in a population of patients diagnosed with severe necrotizing pancreatitis,[19] thus, differed from the present group which had milder forms of AP, and the other study was conducted using an animal model,[24] with the associated limitations of animal studies. Activated protein C has been introduced as treatment for severe sepsis with promising results and relatively low mortality rates,[47,48] however, some studies have shown that activated protein C may increase the risk of bleeding or ineffective therapy.[49,50] The present study suggests that activated protein C may enhance the therapeutic efficacy for treating patients with biliary AP and moderately-severe hyperlipidaemic AP, since protein C was significantly decreased, but activated protein C may not be appropriate for treating mild hyperlipidaemic AP.

In the present study, and in accordance with other published studies,[51–54] patients with moderately-severe biliary or hyperlipidaemic AP had higher D-dimer levels than those with mild AP, and those with moderately-severe hyperlipidaemic AP had higher D-dimer levels versus moderately-severe biliary AP. Logistic regression analyses indicated that elevated D-dimer levels were a risk factor for development of moderately-severe biliary or hyperlipidaemic AP. D-dimer had moderate diagnostic accuracy in predicting moderately-severe biliary AP, and exhibited excellent diagnostic accuracy in predicting moderately-severe AP in the whole cohort and in hyperlipidaemic AP. D-dimer levels were comparable

between the mild biliary and hyperlipidaemic AP groups, but higher in the moderately-severe hyperlipidaemic versus moderately-severe biliary AP group, suggesting that patients with hyperlipidaemic AP have more serious coagulation and fibrinolysis disorders than those with biliary AP. Elevated D-dimer in AP may be caused by inflammation and LDL-C,[34,45] however, it should be noted that D-dimer is an acute phase reactant that is typically elevated in any systemic inflammation, including AP.[34]

The present results may be limited by the fact that this was a single centre study with a relatively small patient cohort. A larger-scale clinical trial is required to verify the therapeutic potential of activated protein C in treating AP. In addition, further studies with larger sample sizes are needed to validate the usefulness of D-dimer as a predictor of moderately-severe AP. Patients with severe AP, who may develop severe complications and who have high mortality rates, were not investigated in the present study due to insufficient patients, and should be evaluated in future studies if possible. Any conclusions drawn from the present study require further validation in a larger-scale, multicentre study. In addition, the potential molecular mechanisms involved in coagulation and anticoagulation induced by immunomediators should be investigated.

In summary, the present study showed that coagulation and fibrinolysis disorders were associated with AP severity and aetiology. Biliary AP was associated with lower ATIII and protein C levels versus hyperlipidaemic AP, and patients with hyperlipidaemic AP had higher D-dimer levels. For patients with AP, detection of ATIII and D-dimer levels may help estimate AP severity and serve as a marker for monitoring AP progression. During early AP, patients with greatly decreased ATIII and increased D-dimer levels may be more likely to develop moderately-severe AP, and assessment of these factors would enable administration of appropriate treatments to enhance the efficacy of therapy and improve patient outcomes.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for- profit sectors.

References

1. Qian JM. Reviewing the etiology, diagnosis and treatment of acute pancreatitis in China. *Zhonghua Nei Ke Za Zhi* 2007; 46: 979–980. [in Chinese, English abstract].
2. Klastkin G and Gordon M. Relationship between relapsing pancreatitis and essential hyperlipemia. *Am J Med* 1952; 12: 3–23.
3. Bai Y, Liu Y, Jia L, et al. Severe acute pancreatitis in China: etiology and mortality in 1976 patients. *Pancreas* 2007; 35: 232–237.
4. Chang MC, Su CH, Sun MS, et al. Etiology of acute pancreatitis–a multi-center study in Taiwan. *Hepatogastroenterology* 2003; 50: 1655–1657.
5. Athyros VG, Giouleme OI, Nikolaidis NL, et al. Long-term follow-up of patients with acute hypertriglyceridemia-induced pancreatitis. *J Clin Gastroenterol* 2002; 34: 472–475.
6. van Geenen EJ, van der Peet DL, Bhagirath P, et al. Etiology and diagnosis of acute biliary pancreatitis. *Nat Rev Gastroenterol Hepatol* 2010; 7: 495–502.
7. Weiner S, Gramatica L, Voegle LD, et al. Role of the lymphatic system in the pathogenesis of inflammatory disease in the biliary tract and pancreas. *Am J Surg* 1970; 119: 55–61.
8. Chen EZ, Huang J, Xu ZW, et al. Clinical features and outcomes of patients with severe acute pancreatitis complicated with gangrenous cholecystitis. *Hepatobiliary Pancreat Dis Int* 2013; 12: 317–323.

9. Hackert T, Pfeil D, Hartwig W, et al. Platelet function in acute experimental pancreatitis induced by ischaemia-reperfusion. *Br J Surg* 2005; 92: 724–728.
10. Gan SI, Edwards AL, Symonds CJ, et al. Hypertriglyceridemia-induced pancreatitis: a case-based review. *World J Gastroenterol* 2006; 12: 7197–7202.
11. Al Mofleh IA. Severe acute pancreatitis: pathogenetic aspects and prognostic factors. *World J Gastroenterol* 2008; 14: 675–684.
12. Frossard JL, Steer ML and Pastor CM. Acute pancreatitis. *Lancet* 2008; 371: 143–152.
13. Milian JW, Portugal SJ, Laynez Ch R, et al. Necrotic acute pancreatitis in the intensive care unit: a comparison between conservative and surgical medical treatment. *Rev Gastroenterol Peru* 2010; 30: 195–200. [in Spanish, English abstract].
14. Park J, Chang JH, Park SH, et al. Interleukin-6 is associated with obesity, central fat distribution, and disease severity in patients with acute pancreatitis. *Pancreatology* 2015; 15: 59–63.
15. Janiak A, Leśniowski B, Jasińska A, et al. Interleukin 18 as an early marker or prognostic factor in acute pancreatitis. *Prz Gastroenterol* 2015; 10: 203–207.
16. Bhatia M. Acute pancreatitis as a model of SIRS. *Front Biosci (Landmark Ed)* 2009; 14: 2042–2050.
17. Zhou J, Ke L, Tong Z, et al. Risk factors and outcome of splanchnic venous thrombosis in patients with necrotizing acute pancreatitis. *Thromb Res* 2015; 135: 68–72.
18. Raraty MG, Connor S, Criddle DN, et al. Acute pancreatitis and organ failure: pathophysiology, natural history, and management strategies. *Curr Gastroenterol Rep* 2004; 6: 99–103.
19. Radenković D, Bajec D, Karamarkovic A, et al. Disorders of hemostasis during the surgical management of severe necrotizing pancreatitis. *Pancreas* 2004; 29: 152–156.
20. Quinsey NS, Greedy AL, Bottomley SP, et al. Antithrombin: in control of coagulation. *Int J Biochem Cell Biol* 2004; 36: 386–389.
21. Olson ST, Björk I, Sheffer R, et al. Role of the antithrombin-binding pentasaccharide in heparin acceleration of antithrombin-proteinase reactions. Resolution of the antithrombin conformational change contribution to heparin rate enhancement. *J Biol Chem* 1992; 267: 12528–12538.
22. Hagiwara S, Iwasaka H, Shingu C, et al. Antithrombin III prevents cerulein-induced acute pancreatitis in rats. *Pancreas* 2009; 38: 746–751.
23. Maeda K, Hirota M, Ichihara A, et al. Applicability of disseminated intravascular coagulation parameters in the assessment of the severity of acute pancreatitis. *Pancreas* 2006; 32: 87–92.
24. Ottesen LH, Bladbjerg EM, Osman M, et al. Protein C activation during the initial phase of experimental acute pancreatitis in the rabbit. *Dig Surg* 1999; 16: 486–495.
25. Chen P, Zhang Y, Qiao M, et al. Activated protein C, an anticoagulant polypeptide, ameliorates severe acute pancreatitis via regulation of mitogen-activated protein kinases. *J Gastroenterol* 2007; 42: 887–896.
26. Babu BI, Genovese T, Mazzon E, et al. Recombinant human activated protein C (Xigris) attenuates murine cerulein-induced acute pancreatitis via regulation of nuclear factor κB and apoptotic pathways. *Pancreas* 2012; 41: 619–628.
27. Ping C, Yongping Z, Minmin Q, et al. Activated protein C improves the severity of severe acute pancreatitis via up-regulating the expressions of endothelial cell protein C receptor and thrombomodulin. *Dig Dis Sci* 2010; 55: 1599–1609.
28. Esmon CT. Inflammation and the activated protein C anticoagulant pathway. *Semin Thromb Hemost* 2006; 32(Suppl 1): 49–60.
29. Shankar-Hari M and Wyncoll D. Activated protein C in severe acute pancreatitis without sepsis? Not just yet. *Crit Care* 2010; 14: 188.
30. Miranda CJ, Babu BI and Siriwardena AK. Recombinant human activated protein C as a disease modifier in severe acute pancreatitis: systematic review of current evidence. *Pancreatology* 2012; 12: 119–123.
31. Miranda CJ, Mason JM, Babu BI, et al. Twenty-four hour infusion of human recombinant activated protein C (Xigris) early in severe acute pancreatitis: The XIGAP 1 trial. *Pancreatology* 2015; 15: 635–641.

32. Kyhälä L, Lindström O, Kylänpää L, et al. Activated protein C retards recovery from coagulopathy in severe acute pancreatitis. *Scand J Clin Lab Invest* 2016; 76: 10–16.
33. Adam SS, Key NS and Greenberg CS. D-dimer antigen: current concepts and future prospects. *Blood* 2009; 113: 2878–2887.
34. Yang N, Zhang DL and Hao JY. Coagulopathy and the prognostic potential of D-dimer in hyperlipidemia-induced acute pancreatitis. *Hepatobiliary Pancreat Dis Int* 2015; 14: 633–641.
35. Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis–2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; 62: 102–111.
36. Pedragosa A, Merino J, Aranda JL, et al. Clinical profile of patients with very high hypertriglyceridemia from the Registry of Hypertriglyceridemia of the Spanish atherosclerosis society. *Clin Investig Arterioscler* 2013; 25: 8–15. [in Spanish, English abstract].
37. Huang YX, Jia L, Jiang SM, et al. Incidence and clinical features of hyperlipidemic acute pancreatitis from Guangdong, China: a retrospective multicenter study. *Pancreas* 2014; 43: 548–552.
38. Baranyai T, Terzin V, Vajda A, et al. Acute pancreatitis caused by hypertriglyceridemia. *Orv Hetil* 2010; 151: 1869–1874. [in Hungarian, English abstract].
39. Navarro S, Cubiella J, Feu F, et al. Hypertriglyceridemic acute pancreatitis Is its clinical course different from lithiasic acute pancreatitis? *Med Clin (Barc)* 2004; 123: 567–570. [in Spanish, English abstract].
40. Deng LH, Xue P, Xia Q, et al. Effect of admission hypertriglyceridemia on the episodes of severe acute pancreatitis. *World J Gastroenterol* 2008; 14: 4558–4561.
41. Twilla JD and Mancell J. Hypertriglyceridemia-induced acute pancreatitis treated with insulin and heparin. *Am J Health Syst Pharm* 2012; 69: 213–216.
42. Stefanutti C, Di Giacomo S and Labbadia G. Timing clinical events in the treatment of pancreatitis and hypertriglyceridemia with therapeutic plasmapheresis. *Transfus Apher Sci* 2011; 45: 3–7.
43. Wang HL and Yu KJ. Sequential blood purification therapy for critical patients with hyperlipidemic severe acute pancreatitis. *World J Gastroenterol* 2015; 21: 6304–6309.
44. Valdivielso P, Ramírez-Bueno A and Ewald N. Current knowledge of hypertriglyceridemic pancreatitis. *Eur J Intern Med* 2014; 25: 689–694.
45. Gupta S, Shekhawat VP and Kaushik GG. D-dimer, a potential marker for the prediction of severity of acute pancreatitis. *Clin Lab* 2015; 61: 1187–1195.
46. Levi M and Ten Cate H. Disseminated intravascular coagulation. *N Engl J Med* 1999; 341: 586–592.
47. Rimmer E, Kumar A, Doucette S, et al. Activated protein C and septic shock: a propensity-matched cohort study*. *Crit Care Med* 2012; 40: 2974–2981.
48. Lindenauer PK, Rothberg MB, Nathanson BH, et al. Activated protein C and hospital mortality in septic shock: a propensity-matched analysis. *Crit Care Med* 2010; 38: 1101–1107.
49. Anger KE, Degrado JR, Greenwood BC, et al. Evaluation of recombinant activated protein C for severe sepsis at a tertiary academic medical center. *Ther Clin Risk Manag* 2013; 9: 277–284.
50. Annane D, Timsit JF, Megarbane B, et al. Recombinant human activated protein C for adults with septic shock: a randomized controlled trial. *Am J Respir Crit Care Med* 2013; 187: 1091–1097.
51. Salomone T, Tosi P, Palareti G, et al. Coagulative disorders in human acute pancreatitis: role for the D-dimer. *Pancreas* 2003; 26: 111–116.
52. Kong H, Ding Z, Zhu XC, et al. d-Dimer change in human acute pancreatitis as determined by serumal triglyceride. *Pancreas* 2011; 40: 1103–1106.
53. Ke L, Ni HB, Tong ZH, et al. D-dimer as a marker of severity in patients with severe acute pancreatitis. *J Hepatobiliary Pancreat Sci* 2012; 19: 259–265.
54. Boskovic A, Pasic S, Soldatovic I, et al. The role of D-dimer in prediction of the course and outcome in pediatric acute pancreatitis. *Pancreatology* 2014; 14: 330–334.