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Protease inhibitors, inflammatory markers, and their association with outcome in dogs with naturally occurring acute pancreatitis

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Abstract

Background: Acute pancreatitis (AP) presumably is associated with pancreatic protease activation, protease inhibitor (PI) depletion, and inflammatory mediator secretion.

Objectives: Examine PIs and inflammatory mediator concentrations in dogs with AP and their association with death.

Animals: Thirty-one dogs diagnosed with AP based on clinical signs, ultrasonographic findings, and increased canine pancreatic lipase immunoreactivity (cPLI) and 51 healthy control dogs.

Methods: Antithrombin and α_2-antiplasmin activity (ATA and α_2AP, respectively) and concentrations of α_1-proteinase inhibitor (α_1PI), α_2-macroglobulin (α_2MG), C-reactive protein (CRP), interleukins (ILs)-2,6,8 and tumor necrosis factor-α (TNF-α) were prospectively measured. Severity of AP was assessed by clinical severity scoring systems.

Results: Mortality rate was 19%. Antithrombin activity was lower ($P = .004$) and maximal CRP, IL-6, and TNF-α concentrations higher ($P < .04$) in the AP group compared to the controls, whereas IL-2, IL-8, α_1PI, and α_2AP concentrations did not differ between groups. Serum α_2MG concentration was not reliably detected. Serum cPLI, CRP, and IL-6 concentrations were significantly and positively correlated. The ATA was lower ($P = .04$), and canine acute pancreatitis severity (CAPS) scores higher ($P = .009$) in nonsurvivors compared to survivors. Higher CAPS scores were associated ($P < .05$) with decreased ATA and increased cPLI, CRP, and IL-6 concentrations.

Conclusions and Clinical Importance: Systemic inflammation in dogs with AP is manifested by increased inflammatory mediator concentrations, correlating with cPLI and CRP concentrations. Hypoantithrombinemia is associated with death. Serum

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Abbreviations: AP, acute pancreatitis; APP, acute phase protein; ATA, antithrombin activity; cPLI, canine pancreatic lipase immunoreactivity; CRP, C-reactive protein; CSI, clinical severity index; D-D, D-dimer; DGGR-lipase, 1,2-o-dilauryl-rac-glycero glutaric acid-(6′-methylresorufin) ester lipase; DIC, disseminated intravascular coagulation; ELISA, enzyme linked immunosorbent assay; FDR, false discovery rate; FFP, fresh frozen plasma; iCa, ionized (free) calcium; IL, interleukin; MODS, multiple organ dysfunction syndrome; PI, protease inhibitor; RI, reference interval; SIRS, systemic inflammatory response syndrome; TNF-α, tumor necrosis factor-α; α_1PI, α_1-proteinase inhibitor; α_2AP, α_2-antiplasmin; α_2MG, α_2-macroglobulin; αMGs, α-macroglobulins.

concentrations of α_2AP and α_1PI are less useful prognostic markers. The CAPS score is a useful prognostic marker in dogs with AP.

1 | INTRODUCTION

Acute pancreatitis (AP) is common in dogs and may be severe, with local and systemic complications and mortality rates ranging from 27% to 58%.[1-3] The pathogenesis of AP may involve intra-acinar cellular trypsinogen activation to trypsin, which can activate pancreatic proteases and lipases, resulting in cellular damage, tissue necrosis and inflammatory mediator secretion.[4,5] Recently however this common channel theory has been challenged by studies in mice, showing that intra-acinar cellular trypsin activation is not the sole key factor in the pathogenesis of AP.[6,7]

The inflammatory response in AP is complex, and may be localized or systemic, determining disease severity and course.[4,5,8] Various inflammatory cytokines are secreted, among which tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β, IL-2, and IL-6 increase early,[4,8-15] promoting leukocyte recruitment and inflammation and potentially leading to development of systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS).[4,8-15] In humans with AP, plasma cytokine concentrations are positively correlated with disease severity and prognosis.[12,15] Only 2 studies have characterized the inflammatory response in spontaneous AP in dogs.[16,17] In 1 study, serum TNF-α concentration was not associated with disease severity.[16] The second study showed decreased serum IL-1β concentration when clinical signs had resolved, whereas IL-6, IL-10, IL-18, and TNF-α concentrations did not differ between survivors and non-survivors.[17] The cytokine response patterns and their associations with the etiology, prognosis and response to treatment in dogs with AP are mostly unknown.

Many intra- and extra-pancreatic defense mechanisms are responsible for pancreatic enzyme inhibition.[4,5] These include protease inhibitors (PIs), consisting of α-macroglobulins (αMGs), α_1-proteinase inhibitor (α_1PI, formerly named α_1-antitrypsin), antithrombin, and α_2-antiplasmin (α_2AP).[4,5,18-21] The common channel theory proposes that AP ensues when defense mechanisms are overwhelmed, allowing ongoing enzyme-induced tissue damage and resulting in inflammation.[5] It is therefore to be expected that in severe AP, serum PI concentrations will be decreased, while pancreatic protease and lipase activities will be increased. If so, serum PI concentrations might serve as markers of severity and prognosis of AP,[4,5,18-20] potentially guiding treatment (ie, administering fresh frozen plasma [FFP] to supplement PIs). Nevertheless, although several of these marker candidates showed promising associations with the severity of AP in humans, reported data are conflicting, with differences among studies in optimal sampling time points,

short half-life of some markers (eg, αMGs-trypsin complexes), various tissue protease sources (eg, neutrophil polymorphonuclear elastase) and assay differences.[18,22] In dogs, α_2MG concentration was significantly decreased in spontaneous AP compared to healthy controls, but was a poor disease severity indicator and was only weakly negatively correlated ($r = -.147$) with serum trypsin-like immunoreactivity concentration.[23] In another study of dogs with various diseases, α_1PI concentration was normal, or even increased, in AP cases,[24] in contrast to the hypothesized trend. Conversely, antithrombin activity (ATA) was decreased in dogs with AP, suggesting consumption.[25]

The primary aims of our prospective study were: (1) characterize the concentrations of several PIs (ie, ATA, α_2MG, α_1PI, and α_2AP) and proinflammatory cytokines (ie, IL-2, IL-6, IL-8, and TNF-α) in dogs diagnosed with AP and in healthy control dogs and (2) investigate the associations of these analytes with short-term death in dogs with AP. A secondary aim was to evaluate previously reported clinical severity scoring systems for AP in dogs, including local and systemic complications (ie, SIRS and hemostatic derangements), and their association with concentrations of PIs and inflammatory mediators and death.

2 | MATERIALS AND METHODS

2.1 | Selection of dogs

This prospective study was conducted from April 2015 to April 2016 in a university teaching hospital, which admits both referral and primary cases. It was approved by the Institutional Ethics Committee and included dogs with their owners' informed consent. Dogs diagnosed with AP were included in the study if they had no previous history of AP and presented with ≥2 of the following acute (duration ≤10 days) clinical signs: anorexia, vomiting, diarrhea and abdominal pain. Diagnosis of AP was confirmed by 1,2-o-dilauryl-rac-glycero glutaric acid-(6′-methylresorufin) ester (DGGR) lipase activity >250 U/L (reference interval [RI], 5-107 U/L), or by serum amylase activity ≥4500 U/L (above approximately 3× −fold the amylase upper reference limit), along with ultrasonographic evidence of AP, including ≥2 of the following: pancreatomegaly, pancreatic hypo-echogenicity, peripancreatic hyperechoic mesentery, and presence of peripancreatic fluid.[26] Additionally, canine pancreatic lipase immunoreactivity (cPLI, [canine Spec cPL], Idexx Laboratories, Westbrook, Maine) concentration was measured, and only dogs in which cPLI concentration was ≥400 µg/L (RI, 0-200 µg/L), either at presentation or on the next day, thereby in

agreement with increased DGGR lipase or amylase activity, were included. Dogs that died within 24 hours after presentation, and those for which owners had elected euthanasia for financial reasons were excluded. Finally, dogs with a known chronic underlying disease, or dogs in which AP was considered a minor disease process (eg, AP in a dog with immune-mediated hemolytic anemia) were excluded.

Staff- and breeder-owned dogs were included in the healthy control group. They were deemed healthy based on normal history, physical examination findings, CBC, serum biochemistry, cPLI concentration, and C-reactive protein (CRP) concentration (RI, 0-7 mg/L).

2.2 | PIs, proinflammatory cytokines, hemostatic tests, and CRP measurement

Blood samples were collected at presentation (day 0) and 24 hours post-presentation (day 1). Complete blood count (Advia 2120i, Siemens Medical Solutions Diagnostics GmbH, Erlangen, Germany), routine serum biochemistry and DGGR-lipase activity (Cobas 6000, Roche, Mannheim, Germany, at 37°C) were measured at presentation in all dogs. To investigate whether ATA and α_2AP activity changed because of their anticoagulant or PI role or both, hemostatic tests were performed, including prothrombin time (PT), activated partial thromboplastin time (aPTT; ACL-9000, Instrumentation Laboratory, Milano, Italy) and D-dimer (D-D) concentration (Cobas 6000, Roche, Mannheim,

Germany, at 37°C; Reagent, Tina-quant D-Dimer Gen.2, Roche, Mannheim, Germany). If all other hemostatic tests were within RI, major antithrombin and α_2AP consumption because of their anticoagulant activity was considered less likely. Serum and plasma sample aliquots from days 0 and 1 were stored for up to 1 year at $-80°$C, pending measurement of cPLI, CRP (Tridelta Development Ltd., Maynooth, Ireland), ATA and α_2AP activities (ACL-9000, Instrumentation Laboratory, Milano, Italy), α_2MG (Human α_2MG DuoSet ELISA R&D Systems, DY1938, Minneapolis, MN), α_1PI (in-house ELISA, Gastrointestinal Laboratory, Texas A&M University, College Station, Texas), and IL-2, IL-6, IL-8, and TNF-α (Canine Proinflammatory Panel 3, Meso Scale Discovery, Meso Scale Diagnostics, Rockville, Maryland; Analyzer, MSD Quickplex SQ 120, Meso Scale Diagnostics, Rockville, Maryland; Analysis software, DISCOVERY WORKBENCH 4.0, Meso Scale Diagnostics, Rockville, Maryland). Samples requiring analysis in a different laboratory (CRP, cPLI, α_1PI, IL-2, IL-6, IL-8, and TNF-α) were shipped on dry ice at the end of the study. Treatments were implemented at the discretion of the attending clinicians. Clinicians were blinded to the PI and cytokine concentrations. Demographic, historical, clinical, laboratory, imaging, and outcome (including duration of hospitalization) data were collected from the medical records.

Serum IL-2, IL-6, IL-8, and TNF-α concentrations were measured using a multiplex electrochemiluminescence immunoassay previously used in dogs according to the manufacturers' instructions.[27-29] The minimal and maximal detection limits reported by the manufacturer for IL-2,

TABLE 1 Protease inhibitor concentrations in 31 dogs with acute pancreatitis and in 51 healthy control dogs

Analyte		Controls[a] (n = 51)	Acute pancreatitis (n = 31)	P value	Adjusted P value[b]
Antithrombin activity$_{min}$ (%)[c]	n (%) Median (range)	25 (49) 160 (116-200)	25 (81) 119 (41-193)	<.001	.004
Antithrombin activity$_0$ (%)[c]	n (%) Median (range)		25 (81) 149 (41-193)	.03	.09
Antithrombin activity$_1$ (%)[c]	n (%) Median (range)		25 (81) 134 (63-194)	.008	.03
α2-Antiplasmin activity$_{min}$ (%)	n (%) Median (range)	28 (55) 100 (90-186)	25 (81) 99.0 (88-106)	.44	
α2-Antiplasmin activity$_0$ (%)	n (%) Median (range)		25 (81) 102 (88-108)	.42	
α2-Antiplasmin activity$_1$ (%)	n (%) Median (range)		25 (81) 100 (91-106)	.64	
α1-Proteinase inhibitor$_{min}$ (mg/L)[d]	n (%) Median (range)	28 (55) 1801 (1337-2366)	31 (100) 1701 (1390-3251)	.44	
α1-Proteinase inhibitor$_{max}$ (mg/L)[d]	n (%) Median (range)		31 (100) 1974 (1475-3286)	.05	.11
α1-Proteinase inhibitor$_0$ (mg/L)	n (%) Median (range)		31 (100) 2037 (1390-3286)	.12	
α1-Proteinase inhibitor$_1$ (mg/L)	n (%) Median (range)		31 (100) 1788 (1400-3251)	.98	

Abbreviation: n, number of cases.

[a]Each analyte was measured once in the control group and twice in dogs with acute pancreatitis. Subscripts 0, 1, min and max refer to concentrations on day 0 (presentation), day 1 (1 day post-presentation) and minimal or maximal concentrations during days 0 and 1, respectively.

[b]Adjusted P value, significant raw P values were adjusted using the Benjamini-Hochberg false discovery rate procedure.

[c]Antithrombin activity reference interval is 87%<.

[d]α1-Proteinase inhibitor reference interval is 732-1802 mg/L.

IL-6, IL-8, and TNF-α were 7.6 and 20 000, 2.4 and 10 000, 1.3 and 10 000, and 0.17 and 5000 pg/mL, respectively. The α_2MG concentration was measured using a human-specific enzyme linked immunosorbent assay (ELISA),[30] with a dynamic range of 0.625 to 40.0 ng/mL, and intra-assay variance coefficient of <5%. The use of this assay had not been reported previously in dogs.

2.3 | Clinical scoring indexes for AP and AP-associated local and systemic complications

Clinical disease severity scores were assigned to each dog with AP using 3 previously published canine AP clinical scoring indexes (CSIs),[31,32] including a recently validated organ score CSI,[31,33] the canine acute pancreatitis severity (CAPS) and the simplified CAPS (sCAPS[32]; Appendix S1).

Local abdominal complications included peripancreatic or diffuse effusion, cholestasis, and intestinal ileus.[31,33] Systemic complications included: (1) acute kidney injury (AKI), diagnosed based on the International Renal Interest Society grading system[34]; (2) disseminated intravascular coagulation (DIC), defined as presence of anomalies in each of the following categories: coagulation factor and platelet consumption (≥2 of the following: thrombocytopenia [platelet count, $<143 \times 10^9$/L], prolonged aPTT [aPTT, >20 s; RI, 17.4-11.0 s] and PT [PT, >10 s; RI, 8.4-6.0 s]), coagulation factor inhibitor consumption (hypoantithrombinemia [ATA <87%; RI, 87-140%]) and increased fibrinolysis (D-D concentration > 500 ng/mL; RI, <250 ng/mL)[35]; (3) Hypercoagulability (suspected when PT, aPTT and platelet count were all within RI, while D-D concentration was increased, or with presence of clinical or imaging evidence of thrombosis [ie, absent femoral pulse in absence of hypotension, Doppler-ultrasonography evidence of a thrombus interrupting blood flow])[36]; and (4) SIRS

TABLE 2 Serum C-reactive protein and cytokine concentrations in 31 dogs with acute pancreatitis and in 51 healthy control dogs

Analyte		Controls[a] (n = 51)	Acute pancreatitis (n = 31)	P value	Adjusted P value[b]
C-reactive protein$_0$[c] (mg/L)	n (%) Median (range)	28 (55) 0.0 (0.0-9.0)	29 (93.5) 100.8 (10.1-614.4)	<.001	.004
C-reactive protein$_1$ (mg/L)	n (%) Median (range)		27 (87) 96.2 (7.2-352.0)	<.001	.004
C-reactive protein$_{max}$ (mg/L)	n (%) Median (range)		31 (100) 103.3 (10.1-614.4)	<.001	.004
IL-2$_0$ (pg/mL)	n (%) Median (range)	28 (55) 37.1 (7.7-119.4)	29 (93.5) 27.4 (3.3-375.3)	.43	
IL-2$_{Day 1}$ (pg/mL)	n (%) Median (range)		27 (87) 39.2 (4.1-314.7)	.82	
IL-2$_{max}$ (pg/mL)	n (%) Median (range)		31 (100) 41.4 (5.6-375.3)	.41	
IL-6$_0$ (pg/mL)	n (%) Median (range)	28 (55) 26.6 (10.3-104.0)	29 (93.5) 106.4 (14.7-1884.9)	<.001	.004
IL-6$_1$ (pg/mL)	n (%) Median (range)		27 (87) 90.3 (10.4-946.3)	<.001	.004
IL-6$_{max}$ (pg/mL)	n (%) Median (range)		31 (100) 119.4 (14.7-1884.9)	<.001	.004
IL-8$_0$ (pg/mL)	n (%) Median (range)	28 (55) 3166 (709-7114)	29 (93.5) 3086.9 (289-11 934.9)	.88	
IL-8$_1$ (pg/mL)	n (%) Median (range)		27 (87) 3260.6 (475.4-11 644.4)	.37	
IL-8$_{max}$ (pg/mL)	n (%) Median (range)		31 (100) 5309 (475-11 935)	.08	
TNF-α$_0$ (pg/mL)	n (%) Median (range)	28 (55) 5.7 (1.8-43.6)	29 (93.5) 6.90 (0.56-233.00)	.22	
TNF-α$_1$ (pg/mL)	n (%) Median (range)		27 (87) 5.8 (0.8-51.4)	.23	
TNF-α$_{max}$ (pg/mL)	n (%) Median (range)		31 (100) 10.4 (0.8-233.0)	.008	.03

Abbreviations: IL, interleukin; n, number of cases; TNF-α, tumor necrosis factor-α.
[a]Each analyte was measured once in the control group and twice in dogs with acute pancreatitis; Subscripts 0, 1 and max refer to concentrations on day 0 (presentation), day 1 (1 day post-presentation) and maximal concentrations during days 0 and 1, respectively.
[b]Adjusted P value, significant raw P values were adjusted using the Benjamini-Hochberg false discovery rate procedure.
[c]C-reactive protein reference interval is 0-7 (mg/L).

diagnosed based on criteria used in the CAPS scoring system[32] (Appendix S1). Diabetic ketoacidosis (DKA) was diagnosed based on presence of persistent fasting hyperglycemia (blood glucose concentration >250 mg/dL), acidosis (venous blood pH <7.34) and hyperketonemia (serum β-hydroxybutyric acid [BHBA; Cobas 6000, Roche, Mannheim, Germany] concentration ≥0.8 mmol/L; RI, 0.0-0.7 mmol/L).[37]

Survivors were defined as dogs alive at 30 days post-presentation, whereas nonsurvivors consisted of dogs that died or were euthanized because of AP-related clinical deterioration within 30 days of presentation. All deaths were AP-related.

2.4 | Statistical analysis

The distribution pattern of continuous variables was examined using the Shapiro-Wilk's test. Pearson's or Spearman's correlation tests, based on data distribution pattern, were used to examine

TABLE 3 Antithrombin activity, pancreatic lipase immunoreactivity concentration and lipase activity, and their association with the outcome in 31 dogs with acute pancreatitis

Analyte		Survivors (n = 25)	Nonsurvivors (n = 6)	P value	Adjusted P value[a]
ATA_0 (%)	n (%)	21 (84)	5 (83)	.015	.04
	Median (range)	154 (41-193)	91 (75-140)		
ATA_1 (%)	n (%)	21 (84)	5 (83)	.002	.007
	Median (range)	131 (41-193)	82 (63-134)		
ATA_{min} (%)	n (%)	21 (84)	5 (83)	.003	.009
	Median (range)	131 (41-193)	77 (63-111)		
$cPLI_0$ (µg/L)	n (%)	24 (96)	6 (100)	.16	
	Median (range)	2326.0 (536-51 968)	4316 (1256-13 896)		
$cPLI_1$ (µg/L)	n (%)	22 (88)	6 (100)	.09	
	Median (range)	1530 (123-30 576)	4331 (1107-26 352)		
$cPLI_{max}$ (µg/L)	n (%)	25 (100)	6 (100)	.11	
	Median (range)	2552 (536-51 968)	4466 (1915-26 352)		
Lipase activity$_0$ (U/L)	n (%)	19 (76)	5 (83)	.09	
	Median (range)	1232 (294-13 085)	3000 (610-7876)		

Notes: cPLI reference interval is 0-200 (µg/L); Lipase, 1,2-o-dilauryl-rac-glycero glutaric acid-(6′-methylresorufin) ester, (DGGR) lipase activity, DGGR lipase reference interval is 5-107 (U/L); Subscripts 0, 1, min and max refer to concentrations on day 0 (presentation), day 1 (1 day post-presentation) and minimal and maximal concentrations during days 0 and 1, respectively.
Abbreviations: ATA, antithrombin activity; cPLI, canine pancreatic-like immunoreactivity; n, number of cases.
[a]Adjusted P value, significant raw P values were adjusted using the Benjamini-Hochberg false rate discovery procedure.

TABLE 4 Serum C-reactive protein, interleukin-6, and tumor necrosis factor-α concentrations and their association with the outcome in 31 dogs with acute pancreatitis

Analyte		Survivors (n = 25)	Nonsurvivors (n = 6)	P value[a]
CRP_0 (mg/L)	n (%)	24 (96)	6 (100)	.68
	Median (range)	100 (10-471)	122 (13-614)	
CRP_1 (mg/L)	n (%)	22 (88)	6 (100)	.08
	Median (range)	65 (7-230)	155 (25-352)	
CRP_{max} (mg/L)	n (%)	25 (100)	6 (100)	.35
	Median (range)	101 (10-471)	174 (25-614)	
$IL-6_0$ (pg/mL)	n (%)	24 (96)	6 (100)	.12
	Median (range)	104 (15-521)	209 (42-1885)	
$IL-6_1$ (pg/mL)	n (%)	22 (88)	6 (100)	.16
	Median (range)	69 (10-946)	133 (61-905)	
$IL-6_{max}$ (pg/mL)	n (%)	25 (100)	6 (100)	.21
	Median (range)	115 (14.7-946)	209 (65.3-1885)	
$TNF-\alpha_{max}$ (pg/mL)	n (%)	25 (100)	6 (100)	.92
	Median (range)	8.05 (0.80-233)	11.6 (3.6-44.0)	

Notes: Subscripts 0, 1 and max refer to concentrations on day 0 (presentation), day 1 (1 day post- presentation) and maximal levels during days 0 and 1, respectively.
Abbreviations: CRP, C-reactive protein; IL, interleukin; n, number of cases; TNF, tumor necrosis factor.
[a]Raw P values; Because all comparisons depicted in this table were insignificant, all P values presented above are raw values, unadjusted for false discovery rate.

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TABLE 5 Significant correlations ($r > .5$) between pancreatic lipases, protease inhibitors, hemostatic analytes, C-reactive protein, and cytokines in 31 dogs with acute pancreatitis

Analyte	Correlated with	r^a	P value
$cPLI_0$	$IL\text{-}6_1$	.75	0
	CRP_0	.52	.005
$cPLI_1$	$IL\text{-}6_0$	.55	.003
	$IL\text{-}6_1$	.68	0
	$IL\text{-}6_{max}$	.57	.001
	Ionized calcium	−.62	.009
CRP_0	$IL\text{-}6_1$	.58	.001
	cholesterol	.68	0
	TG	.52	.023
CRP_1	$IL\text{-}6_1$	.65	0
	$IL\text{-}6_{max}$	.51	.006
	Ionized calcium	−.67	.003
CRP_{max}	$IL\text{-}6_1$	.58	.001
	$IL\text{-}6_{max}$	.62	0
	Ionized calcium	−.58	.009
	Cholesterol	.58	.001
$IL\text{-}2_0$	$IL\text{-}6_0$	.65	0
	$IL\text{-}6_{max}$	.56	.001
	$IL\text{-}8_0$	.92	0
	$IL\text{-}8_1$	.60	.001
	$IL\text{-}8_{max}$	.74	0
	$TNF\alpha_1$	.53	.004
$IL\text{-}2_1$	$IL\text{-}8_1$	.89	.000
	$IL\text{-}8_{max}$	.84	.000
	$TNF\alpha_0$	.504	.007
	$TNF\alpha_1$	.80	0
	$TNF\alpha_{max}$	.84	0
	Hemoglobin	−.52	.007
	Hematocrit	−.53	.005
$IL\text{-}2_{max}$	$IL\text{-}6_1$	.61	.001
	$IL\text{-}8_0$	.67	0
	$IL\text{-}8_1$	.89	0
	$IL\text{-}8_{max}$	.82	0
	$TNF\alpha_1$	.839	0
	Hematocrit	−.53	.003
$IL\text{-}6_1$	Ionized calcium	−.73	.001
	Amylase	.62	.001

TABLE 5 (Continued)

Analyte	Correlated with	r^a	P value
$IL\text{-}8_0$	$TNF\alpha_1$	.60	.001
$IL\text{-}8_1$	$TNF\alpha_0$	.59	.001
	$TNF\alpha_1$	.86	.000
	$TNF\alpha_{max}$	.93	0
α_1PI_0	Duration of clinical signs	−.50	.002
$\alpha_2antiplasmin_0$	Duration of clinical signs	−.52	.007

Notes: Subscripts 0, 1 max and min refer to concentrations on day 0 (presentation), day 1 (1 day post-presentation), maximal and minimal concentrations during days 0 and 1, respectively.

Abbreviations: α_1PI, α_1-proteinase inhibitor concentration; clinical signs duration, lag of time from onset of clinical signs to presentation to the hospital; cPLI, canine pancreatic-like immunoreactivity; CRP, C-reactive protein; IL, interleukin; $TNF\alpha$, tumor necrosis factor α.

[a]Spearman's correlation tests were done when ≥1 of the tested variables had non-normal distribution, whereas Pearson's correlation tests were done when both tested variables were distributed normally.

relationships between continuous variable pairs. Results of most variables were distributed non-normally, and with the limited study group sizes, group continuous variables were compared using Mann-Whitney *U*-tests. Associations between categorical variable pairs were examined using Fisher's exact tests. Because the control group's PI and inflammatory mediator concentrations were measured only once, but measured twice in the AP group, several comparisons were made. The lowest ATA and α_2AP activity, the highest CRP and cytokine concentrations and both lowest and highest α_1PI concentrations of day 0 and day 1 measurements of dogs with AP were used for study group comparisons, in addition to comparisons of the control group's single measurements with the study group's day 0 and day 1 measurements. Only analytes that differed significantly between dogs with AP and healthy control dogs were further tested for associations with outcome in dogs with AP. All tests were 2-tailed. Raw *P* values ≤.05 were adjusted for false discovery rate (FDR) using the Benjamini-Hochberg procedure.[38] In such cases, both the unadjusted and adjusted *P* values are presented in the tables. Throughout the text, adjusted *P* values are presented, unless otherwise specified. Statistical analyses were performed using a statistical software package (SPSS 25.0 for Windows, IBM, Armonk, NY).

3 | RESULTS

Our study initially included 33 dogs with AP and 51 healthy control dogs. Two dogs in which serum DGGR lipase activity was increased (>2000 U/L in both), but day 0 and day 1 serum cPLI concentrations were <400 μg/L, were excluded. Mortality rate in the remaining 31 dogs with AP was 19% (6 nonsurvivors). Nonsurvivor dogs died within 3-14 days from presentation (median, 5 days). The age of dogs with AP (median, 9 years; range, 3-15 years) was higher (*P* = .02) compared to that of the control group (median, 4.5 years; range, 0.75-13). The AP group included 13 females (42%; neutered, 12) and 18 males (58%; neutered, 11). The control group included 32 females (63%; neutered, 20), and 19 males (37%; neutered, 13). Most dogs in the AP and control groups were of mixed breed (18; 58% and 20; 39%, respectively). No sex difference was found between groups (unadjusted *P* = .11).

3.1 | PIs and proinflammatory cytokines in the AP and control groups

Day 1 and minimal ATA measured during the observation period were significantly ($P = .03$ and $P = .004$, respectively) lower in dogs with AP compared to controls (Table 1). Serum α_2AP activity did not differ between groups. Peak α_1PI concentration was higher (unadjusted $P = .05$) in the AP group, but insignificantly so after adjusting for FDR ($P = .1$; Table 1). Day 0, day 1, and maximal serum CRP and IL-6 concentrations were significantly ($P = .004$ for all) higher in dogs with AP compared to the control group. Maximal TNF-α concentration was higher in the AP group ($P = .03$) compared to the control group, whereas IL-8 and IL-2 concentrations did not differ between these 2 groups (Table 2).

Day 0 and day 1 α_2MG concentrations in dogs with AP (day 0 median, 1.6 ng/mL; range, 0-7.9; day 1 median, 1.5 ng/mL; range, 0-7.7) as well as in healthy control dogs (median, 1.15 ng/mL; range, 0-7.5) were extremely low, or undetectable, and were not different between groups.

3.2 | Pancreatic lipase, PIs, proinflammatory cytokines, hemostatic tests, and outcome

The DGGR lipase activity and cPLI concentration at presentation were moderately, positively correlated ($r = .662$; $P = .001$). Day 0 DGGR-lipase activity and day 1 cPLI concentration were higher, albeit insignificantly, among nonsurvivors compared to survivors (unadjusted

$P = .09$ for both, Table 3). Hypoantithrombinemia on days 0 and 1 was significantly ($P = .04$ and $P = .007$, respectively) associated with death (Table 3), whereas serum concentrations of IL-6, TNF-α, and CRP were not associated with death (Table 4).

Day 0 cPLI concentration was strongly and positively correlated with day 1 serum IL-6 and day 0 serum CRP concentrations (Table 5). Day 0 serum CRP concentration was positively and moderately correlated with day 1 serum IL-6 and day 0 serum cholesterol and triglyceride concentrations (Table 5). Day 1 serum cPLI and CRP concentrations were inversely and moderately correlated with serum ionized calcium (iCa) concentration (Table 5). Day 0 and day 1 serum IL-2, IL-6, and IL-8 concentrations were positively and strongly correlated (Table 5). The results of all hemostatic analytes were not associated with death, and all hemostatic test results, in both survivors and nonsurvivors of AP, were within RI (Table 6).

3.3 | CSI score associations with PI and proinflammatory cytokine concentrations and with outcome

Among the 3 applied AP CSIs, only the CAPS results were significantly ($P = .009$) associated with outcome (Table 7). The CAPS and sCAPS scores were strongly correlated ($r = .89$; $P < .001$), whereas the CAPS and organ score CSI scores were weakly correlated ($r = .45$; $P = .01$). The CAPS score associations with cPLI concentration and with PIs and inflammatory mediator concentrations that were significantly

TABLE 6 Hemostatic test results and the outcome of 31 dogs with acute pancreatitis

Analyte	Reference interval		Survivors (n = 25)	Nonsurvivors (n = 6)	P value	Adjusted P value[a]
Prothrombin time$_0$ (s)	6.0-8.4 (s)	n (%)	20 (80)	5 (83)	.22	
		Median (range)	7.1 (6.9-10.5)	7.2 (7.0-8.7)		
Prothrombin time$_1$ (s)		n (%)	21 (84)	5 (83)	.04	.09
		Median (range)	7.1 (6.8-8.3)	7.4 (7.0-9.4)		
Prothrombin time$_{max}$ (s)		n (%)	21 (84)	5 (83)	.09	
		Median (range)	7.1 (6.9-10.5)	7.5 (7.0-9.4)		
aPTT$_0$ (s)	11.0-17.4 (s)	n (%)	20 (80)	5 (83)	.37	
		Median (range)	13.2 (8.7-27.2)	11.9 (10.4-75.0)		
aPTT$_1$ (s)		n (%)	21 (84)	5 (83)	.53	
		Median (range)	12.7 (7.5-25.5)	12.9 (10.4-34.2)		
aPTT$_{max}$ (s)		n (%)	21 (84)	5 (83)	.95	
		Median (range)	13.2 (8.7-27.2)	12.9 (10.4-75.0)		
D-dimer$_0$ (ng/mL)	0-250 (ng/mL)	n (%)	20 (80)	4 (67)	.79	
		Median (range)	192 (14-4298)	157 (108-875)		
D-dimer$_1$ (ng/mL)		n (%)	21 (84)	5 (83)	.75	
		Median (range)	201 (88-4034)	329 (69-2223)		
D-dimer$_{max}$ (ng/mL)		n (%)	21 (80)	5 (83)	.70	
		Median (range)	208 (88-4298)	329 (108-2223)		

Notes: Subscripts 0, 1 max, and min refer to levels on day 0 (presentation), day 1 (1 day post presentation), and maximal and minimal concentrations during days 0 and 1, respectively.

Abbreviations: aPTT, activated partial thromboplastin time; n, number of cases; PT, prothrombin time.

[a]Adjusted P value, significant raw P values were adjusted using the Benjamini-Hochberg false discovery rate procedure.

TABLE 7 Association between results of clinical severity scoring systems for acute pancreatitis in dogs[a] and the outcome of 31 dogs with acute pancreatitis

Scoring system	Severe[b] acute pancreatitis		Mild to moderate[c] acute pancreatitis		P value	Adjusted P value[d]
	Survivors (n = 25) n (%)	Nonsurvivors (n = 6) n (%)	Survivors (n = 25) n (%)	Nonsurvivors (n = 6) n (%)		
CAPS[e]	7 (28)	6 (100)	18 (72)	0 (0)	.002	.009
sCAPS[f]	7 (28)	5 (83)	18 (72)	1 (5)	.02	.06
Organ score	14 (56)	6 (100)	11 (44)	0 (0)	.05	.12

Abbreviation: n, number of cases.

[a](1) Based on Ruaux CG and Atwell RB. A severity score for spontaneous canine acute pancreatitis. Aust Vet J 1998;76:804-808; (2) Based on Fabres V, Dossin O, Reif C, et al. Development and validation of a novel clinical scoring system for short-term prediction of death in dogs with acute pancreatitis. J Vet Intern Med 2019;33:499-507; (3) Based on Kuzi S, Mazor R, Segev G, et al. Prognostic markers and assessment of a previously published clinical severity index in 109 hospitalized dogs with acute presentation of pancreatitis. Vet Rec. 2019 Oct 29. pii: vetrec-2019-105 364. doi:10.1136/vr.105364. [Epub ahead of print].

[b]Severe disease was assigned to dogs with CAPS score ≥ 11, sCAPS score ≥ 6, and an organ score ≥ 5.

[c]Mild to moderate disease was assigned to dogs with a CAPS score ≤ 10, a sCAPS score ≤ 5, and an organ score ≤ 4.

[d]Significant "raw" P values were adjusted using the Benjamini-Hochberg procedure for false discovery rate.

[e]Canine acute pancreatitis severity scoring, based on Fabres V, Dossin O, Reif C, et al. Development and validation of a novel clinical scoring system for short-term prediction of death in dogs with acute pancreatitis. J Vet Intern Med. 2019;33:499-507.

[f]Simplified canine acute pancreatitis severity scoring, based on Fabres V, Dossin O, Reif C, et al. Development and validation of a novel clinical scoring system for short-term prediction of death in dogs with acute pancreatitis. J Vet Intern Med. 2019;33:499-507.

higher in dogs with AP compared to the control group and also associated with outcome of AP were examined. Higher (≥11) CAPS scores were significantly associated with decreased day 1 and minimal ATA, as well as with higher day 0 cPLI and day 1 CRP and IL-6 concentrations ($P = .01$, $P = .03$, $P = .03$, $P = .05$, and $P = .04$, respectively).

3.4 | Associations of AP-related complications and duration of clinical signs with PI and proinflammatory cytokine concentrations and with outcome

Several systemic complications recorded in the AP group were not associated with death. Disseminated intravascular coagulation was diagnosed in 1 nonsurvivor. Severe thrombocytopenia (platelets <63 000/μL) occurred in 2 dogs (1 nonsurvivor with DIC and 1 survivor). Hypercoagulability was suspected in 9 dogs (2 nonsurvivors). Acute kidney injury was diagnosed in 8 dogs (3 nonsurvivors). Systemic inflammatory response syndrome was noted in 24 dogs (77%; including all 6 nonsurvivors). Diabetic ketoacidosis was present in 5 dogs (1 nonsurvivor). Local abdominal complications were noted in 9 dogs (2 nonsurvivors). All of these specific complications were not associated with PI and proinflammatory cytokine concentrations.

The time lag from onset of clinical signs to presentation (median, 3 days; range, 0.5-10 days) was not associated with death ($P = .17$). Hospitalization duration was shorter ($P = .04$) when dogs were presented earlier (duration of clinical signs ≤2 days; median, 4 days, range 2-14) compared to dogs presented later (median, 5 days; range, 2-17 days). The $α_1$PI concentration was moderately and inversely correlated with the time lag from onset of clinical signs to presentation (Table 5) and was significantly ($P = .008$) higher in dogs presented

early (median, 2208 mg/L; range, 1765-3287) compared to those presented later (median, 1735 mg/L; range, 1390-2641 mg/L).

4 | DISCUSSION

The severity of AP presumably is determined by the extent of pancreatic protease and inflammatory mediator release into the systemic circulation, leading to both local and systemic complications.[4,5,11,31,32] The latter include SIRS and MODS, and their manifestations contribute to the CSI scores, which have been associated with the prognosis of AP in both humans and dogs.[4,5,11,31-33] In our study, concentrations of pancreatic lipase (cPLI and DGGR lipase) and inflammatory cytokines did not differ between survivors and nonsurvivors of AP. The former finding is in disagreement with previous studies of AP in dogs, in which markedly increased cPLI concentrations[39] or serum lipase activities[40] were more frequent in nonsurvivors. This discrepancy possibly resulted from differences in duration of clinical signs before presentation, reflected in higher (albeit insignificantly so) day 1 cPLI concentrations among nonsurvivors in our study. In addition, in our study, higher CAPS scores (≥11), reflective of more severe AP, were associated with cPLI concentration, supporting results of the aforementioned studies.[39,40] In our study, serum cPLI concentration was significantly and positively correlated with serum inflammatory marker concentrations, including IL-6 and CRP, linking increased cPLI concentration with the inflammatory response. The lack of association between inflammatory cytokine concentrations and outcome in our study is consistent with previous findings, suggesting that inflammatory cytokines are not useful prognostic markers in dogs with AP.[17] Nevertheless, the utility of serial measurements in clinical decision making warrants future evaluation.

In agreement with previous findings,[25,41] ATA was significantly lower in nonsurvivors, decreasing early during hospitalization. The activity of α_2AP was examined in dogs with AP for the first time in our study. Its activity in the AP and control groups was similar, suggesting a limited role of α_2AP in dogs with AP.

In contrast to antithrombin, which is depleted in dogs with AP, α_1PI concentration neither differed between controls and dogs with AP nor between survivors and nonsurvivors of AP. Alpha-1 proteinase inhibitor is considered the main plasma pancreatic protease inhibitor,[21,42] and therefore is expected to be consumed, and possibly depleted during AP. However, contrary to this hypothesis, α_1PI is not depleted, but rather is increased in humans with AP,[22,43,44] supporting a positive acute phase protein (APP) role, beyond its PI function.[22,43-45] The APP role of α_1PI in dogs is controversial. Most studies demonstrate increased serum α_1PI concentration in dogs with inflammatory diseases,[24,46,47] although in 1 study of dogs with SIRS or sepsis, it was decreased, thereby suggesting it is a negative APP.[48] Nevertheless, other causes for this discrepancy, such as hepatopathy or protein loss could not be ruled out.[48] In our study, α_1PI concentration was higher, albeit insignificantly so, after adjustment for multiple comparisons, in dogs with AP compared to controls, in agreement with studies of humans, warranting future studies in dogs.

Interestingly, in humans, α_2AP and antithrombin inhibit pancreatic trypsin much faster (200-fold) than does α_1PI.[21,49] Although antithrombin depletion in AP might be attributed to its antithrombotic role, abnormal clotting times or thrombocytopenia were rare in our study. Thus, decreased ATA in AP more likely reflects its consumption as a PI, although viscoelastic tests were not performed, limiting our ability to assess antithrombin consumption in hypercoagulable states unaccompanied by increased D-D concentration. Possibly, when severe pancreatic protease leakage overwhelms plasma antithrombin, α_1PI might play a secondary PI role.[21] This assumption is supported in our study by the negative correlation between α_1PI concentration and the duration of historical clinical signs, suggesting that some α_1PI consumption does occur in more prolonged AP. As a possible second line defense PI,[21] α_1PI seems a less useful early prognostic marker in dogs with AP, accounting for our observed lack of association between its concentration and death.

The αMGs are responsible for inhibition of trypsin and other serine proteases, and are depleted in humans and dogs with AP.[18,23] Nevertheless, the prognostic utility of serum α_2MG concentration in humans with AP is disappointing, with sensitivity and specificity of 52%-83% and 59%-83%, respectively.[18] The utility of αMGs in most clinical settings is limited by rapid αMG-trypsin complex clearance.[50,51] Additionally, in dogs with AP, α_2MG concentration is not associated with outcome,[23] suggesting limited utility as surrogate severity marker.[16,42] The α_2MG concentrations, as measured using a commercial assay designed for humans, in our study were 100-fold lower compared to previous results in dogs and humans,[23,52] likely indicating failure of this particular assay to accurately detect the canine protein, precluding investigation of group differences.

Pancreatic acinar injury leads to release of nuclear proteins (eg, histones), heat shock proteins and nuclear and mitochondrial DNA,

inducing sterile inflammation through common immune sensors and inflammatory cytokines.[8,53,54] This inflammatory storm results in SIRS, potentially damaging the lungs and kidneys, and resulting in MODS.[4,55] In a murine AP model, TNF-α mRNA increased first, its concentrations positively associated with severity of inflammation.[56] Tumor necrosis factor-alpha has complex pro- and anti-inflammatory activities in AP, depending on its binding, either to TNF-α receptor 1 or 2.[56,57] Type-1 receptor binding leads to programmed cell death, whereas type-2 receptor binding induces cell survival and inflammation.[56,57] Both receptors also are shed as soluble forms, allowing circulating TNF-α inactivation.[58] With these complex binding and activity patterns, accurate measurement of TNF-α using commercial assays may be difficult, because some only measure free TNFα, which has a short half-life.[59,60] In our study, serum TNFα concentration was higher in dogs with AP compared to healthy controls, in contrast to previous findings,[17] possibly because of assay differences. Nevertheless, its concentration was not associated with death or CAPS score, supporting a limited role for TNFα in prognostication of AP in dogs.

Interleukin-6, a proinflammatory cytokine, contributes to lung injury and pancreatic necrosis in AP in humans.[61,62] It induces hepatic APP synthesis, including CRP.[63] It increases within 24 hours of presentation, and is an early and sensitive prognostic marker of AP in humans.[15,63] In experimental AP in beagles, neutrophil-derived IL-6 mRNA peaks within 3 hours, in association with increased serum lipase activity and edematous changes in the pancreas.[64] In another experimental AP study in dogs, IL-6 administration prevented bacterial translocation,[65] in the face of intestinal barrier function impairment, which occurs in dogs with AP.[66] In our study, in agreement with previous findings,[17] serum IL-6 concentration was higher in the AP group compared to the control group, but was not associated with death, in contrast to findings in humans with AP.[15,63] Serum IL-6 and CRP concentrations were positively correlated, supporting the role of IL-6 in inducing hepatic APP synthesis.[63] In agreement with an experimental pancreatitis model in dogs,[64] day 0 serum IL-6 concentration was correlated with higher day 1 serum cPLI concentration, linking lipase-induced tissue damage and pancreatic lesions with inflammation in dogs with AP.[4]

Interleukin-8 stimulates neutrophil activity.[10] Its concentration is inconsistently associated with the severity of AP in humans, especially in later disease stages, peaking within 3 days from disease onset.[10,67] Serum IL-8 concentration has not been reported previously in dogs with naturally occurring AP. Its day 0 and day 1 concentrations in our study did not differ between the AP and control groups. These findings however were likely influenced by the timing of sample collection, early during the disease course, because IL-8 concentration possibly is associated with later complications.[14]

Interleukin-2 is a marker of systemic lymphocyte activation in humans with AP.[68,69] Its soluble receptor (sIL-2R) concentration peaks 1 day after onset of symptoms, remains high for at least 7 days, and is associated with pulmonary, renal and septic complications, and overall severity of AP in humans.[10,69] Preventing IL-2-mediated lymphocytic activation in mice with experimentally-induced AP decreased systemic complications.[70] Serum IL-2 concentration in dogs with spontaneous

AP has not been reported previously. In our study, its concentrations did not differ between AP and control groups, possibly suggesting a lesser role of IL-2 in dogs with AP, although the results also might have been influenced by the assay used, which measures IL-2 and not sIL-2R.

C-reactive protein has been used as a prognostic marker in humans with AP,[13,71,72] but because serum CRP concentration peaks 3 days after onset of symptoms, its clinical usefulness early during AP is limited.[10] Similar to humans, previous studies showed that serum CRP concentration at presentation in dogs with AP increases, but only its concentration on days 3 or 4 significantly differs between survivors and nonsurvivors.[2,39] In our study, serum CRP concentration was measured relatively early during the course of AP, perhaps accounting for its lack of association with outcome. Interestingly, serum CRP concentration was positively and moderately correlated with both day 0 serum triglycerides and cholesterol concentrations. Hyperlipidemia in AP is associated with prolonged hospitalization, frequent MODS and increased fatality in humans.[73,74] Hypertriglyceridemia is also a therapeutic target in humans with AP, in whom heparin and insulin are used to lower serum triglyceride concentrations.[75] Necrotic peripancreatic fat tissue may become an important source of inflammatory mediators, as manifested by increased concentrations of leptin, resistin, visfatin, and IL-6,[17] in support of our findings.

Although our findings might have prognostic utility, their clinical ramifications, mainly whether supplementation of depleted PIs is warranted, remain unclear. Although antithrombin treatment prevents development of AP and attenuates AP-induced AKI in experimental AP models in mice and rats,[76,77] FFP administration was associated with death in an uncontrolled retrospective study of dogs with AP.[78] In humans with AP, FFP administration did not affect outcome compared to negative controls,[79] and FFP administered as a source of extrinsic antiproteases in severe AP failed to prevent a decrease in α_2MG, or to increase patients' serum trypsin inhibition capacity.[44] Nevertheless, a more precise approach, particularly relying on ATA, might better guide FFP treatment in dogs with AP, warranting prospective studies.

Our study had several limitations. First, its cohort size was limited, rendering it somewhat statistically underpowered. Indeed, several results that were borderline significant (ie, higher cPLI concentrations in nonsurvivors) might become significant in larger studies. Nevertheless, several present findings (eg, lack of association between cytokine concentrations and death, associations of ATA and CAPS score with death) are consistent with previous results,[25,32,33] strengthening our conclusions. Second, the dynamic nature of inflammation in naturally occurring AP largely depends on the duration of clinical signs, as evident in differences between early and late presentations. This situation possibly affected our results, emphasizing the inherent limitations of clinical studies of naturally occurring diseases, where sampling times vary during the disease course. Third, with their similar clinical presentations, differentiating first AP episodes from acute flare-ups of chronic, subclinical pancreatitis is not feasible clinically. Therefore, the AP group should more accurately be regarded as including dogs presenting with acute signs of pancreatitis, rather than dogs with AP. Fourth, a potential limitation may be caused by cPLI, PI, and

cytokine decay during storage. Data from dogs and humans however indicate these analytes are stable for several years,[80-82] suggesting a negligible or minimal storage duration effect on these deeply frozen samples. Lastly, analytes were measured only twice, within the first 2 days of hospitalization, precluding assessment of later changes, which may serve as future therapeutic targets. Nevertheless, early findings during the disease course, which were assessed in our study, are more relevant for the diagnosis and early prognostication of AP.

In conclusion, antithrombin is an important PI in dogs with AP. Its depletion is an important negative prognostic sign in such dogs, warranting prospective studies targeting its supplementation (ie, using FFP), which might improve survival. Although α_2AP activity was lower in non-survivors, the differences between the outcome groups were small, and its activity did not differ between AP and control groups, suggesting that it is a less important PI and prognostic marker in dogs with AP. Conversely, α_1PI seems to behave as a positive APP, possibly a second line PI, decreasing later during AP. Although inflammatory cytokines do increase in dogs with AP, supporting ongoing systemic inflammation, their concentrations were not associated with death, in agreement with previous findings.[17] Finally, the CAPS scoring system has been prospectively validated, and higher CAPS scores were significantly associated with decreased ATA and increased inflammatory marker (CRP and IL-6) and cPLI concentrations. Higher scores, alongside decreased ATA, are indicative of severe AP and a less favorable prognosis.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

This study was approved by the Veterinary Teaching Hospital and Koret School of Veterinary Medicine, Hebrew University, Institutional Ethics Committee (Reference number KSVM-VTH/11_2014).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Mansfield C. Acute pancreatitis in dogs: advances in understanding, diagnostics, and treatment. *Top Companion Anim Med*. 2012;27:123-132.

2. Mansfield CS, James FE, Robertson ID. Development of a clinical severity index for dogs with acute pancreatitis. *J Am Vet Med Assoc*. 2008;233:936-944.

3. Hess RS, Kass PH, Shofer FS, van Winkle T, Washabau RJ. Evaluation of risk factors for fatal acute pancreatitis in dogs. *J Am Vet Med Assoc*. 1999;214:46-51.

4. Mansfield C. Pathophysiology of acute pancreatitis: potential application from experimental models and human medicine to dogs. *J Vet Intern Med*. 2012;26:875-887.

5. Watson P. Pancreatitis in dogs and cats: definitions and pathophysiology. *J Small Anim Pract*. 2015;56:3-12.

6. Talukdar R, Sareen A, Zhu H, et al. Release of cathepsin B in cytosol causes cell death in acute pancreatitis. *Gastroenterology*. 2016;151: 747-758 e745.

7. Dixit AK, Sarver AE, Yuan Z, et al. Comprehensive analysis of microRNA signature of mouse pancreatic acini: overexpression of miR-21-3p in acute pancreatitis. *Am J Physiol Gastrointest Liver Physiol*. 2016;311:G974-G980.

8. Kylanpaa L, Rakonczay Z Jr, O'Reilly DA. The clinical course of acute pancreatitis and the inflammatory mediators that drive it. *Int J Inflam*. 2012;2012:360685.

9. Makhija R, Kingsnorth AN. Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreat Surg*. 2002;9:401-410.

10. Mayer J, Rau B, Gansauge F, Beger HG. Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications. *Gut*. 2000;47:546-552.

11. Norman J. The role of cytokines in the pathogenesis of acute pancreatitis. *Am J Surg*. 1998;175:76-83.

12. Chen CC, Wang SS, Lee FY, Chang FY, Lee SD. Proinflammatory cytokines in early assessment of the prognosis of acute pancreatitis. *Am J Gastroenterol*. 1999;94:213-218.

13. Pezzilli R, Billi P, Miniero R, et al. Serum interleukin-6, interleukin-8, and beta 2-microglobulin in early assessment of severity of acute pancreatitis. Comparison with serum C-reactive protein. *Dig Dis Sci*. 1995;40:2341-2348.

14. de Beaux AC, Goldie AS, Ross JA, Carter DC, Fearon KCH. Serum concentrations of inflammatory mediators related to organ failure in patients with acute pancreatitis. *Br J Surg*. 1996;83:349-353.

15. Frossard JL, Hadengue A, Pastor CM. New serum markers for the detection of severe acute pancreatitis in humans. *Am J Respir Crit Care Med*. 2001;164:162-170.

16. Ruaux CG, Pennington HL, Worrall S, Atwell RB. Tumor necrosis factor-alpha at presentation in 60 cases of spontaneous canine acute pancreatitis. *Vet Immunol Immunopathol*. 1999;72:369-376.

17. Paek J, Kang JH, Kim HS, Lee I, Seo KW, Yang MP. Serum adipokine concentrations in dogs with acute pancreatitis. *J Vet Intern Med*. 2014;28:1760-1769.

18. Satyanarayan ST, Petrov MS, Phillips A, et al. Protease-related predictors of acute pancreatitis severity: a systematic review of the literature. *J Pancreas*. 2016;17:592-606.

19. Steer ML. Etiology and pathophysiology of acute pancreatitis. In: Go VLWDE, Gardner JD, eds. *The Pancreas*. 2nd ed. New York, NY: Raven Press; 1993:581.

20. Steiner JM. Canine pancreatic disease. In: Ettinger SJ, Feldman EC, eds. *Textbook of Veterinary Internal Medicine*. 7th ed. St Louis, MO: Saunders-Elsevier; 2010:1296-1309.

21. Nobar SM, Guy-Crotte O, Rabaud M, Bieth JG. Inhibition of human pancreatic proteinases by human plasma alpha2-antiplasmin and antithrombin. *Biol Chem*. 2004;385:423-427.

22. Karsidag T, Tuzun S, Kemik AS, Purisa S, Unlu A. Alpha-1 protease inhibitor and antichymotrypsin levels in acute pancreatitis. *Ulus Travma Acil Cerrahi Derg*. 2012;18:195-199.

23. Ruaux CG, Atwell RB. Levels of total alpha-macroglobulin and trypsin-like immunoreactivity are poor indicators of clinical severity in spontaneous canine acute pancreatitis. *Res Vet Sci*. 1999;67:83-87.

24. Heilmann RM, Grutzner N, Hokamp JA, et al. Serum alpha1-proteinase inhibitor concentrations in dogs with exocrine pancreatic disease, chronic hepatitis or proteinuric chronic kidney disease. *Vet J*. 2018;236:68-71.

25. Kuzi S, Segev G, Haruvi E, Aroch I. Plasma antithrombin activity as a diagnostic and prognostic indicator in dogs: a retrospective study of 149 dogs. *J Vet Intern Med*. 2010;24:587-596.

26. Hecht S, Henry G. Sonographic evaluation of the normal and abnormal pancreas. *Clin Tech Small Anim Pract*. 2007;22:115-121.

27. Meso Scale Discovery Multi-Spot Assay System. Canine Proinflammatory Panel 3 Assay Ultra-Sensitive Kit. https://www.mesoscale.com/~/media/files/product%20inserts/canine%20proinflam%20panel%203.pdf. Accessed June, 2019.

28. Buono AMT, Minamoto Y, Lidbury JA, et al. Serum IL-2, IL-6, IL-8, and TNF-α concentrations in dogs with chronic enteropathies. In: ACVIM, Seattle, Washington. 2018:2144-2309.

29. Titmarsh HF, Gow AG, Kilpatrick S, et al. Low vitamin d status is associated with systemic and gastrointestinal inflammation in dogs with a chronic enteropathy. *PLoS One*. 2015;10:e0137377.

30. Human Alpha 2-Macroglobulin DuoSet ELISA. http://resources.rndsystems.com/pdfs/datasheets/dy1938.pdf. Accessed June, 2019.

31. Ruaux CG, Atwell RB. A severity score for spontaneous canine acute pancreatitis. *Aust Vet J*. 1998;76:804-808.

32. Fabres V, Dossin O, Reif C, et al. Development and validation of a novel clinical scoring system for short-term prediction of death in dogs with acute pancreatitis. *J Vet Intern Med*. 2019;33:499-507.

33. Kuzi S, Mazor R, Segev G, et al. Prognostic markers and assessment of a previously published clinical severity index in 109 hospitalised dogs with acute presentation of pancreatitis. *Vet Rec*. 2019;187: 1-7.

34. Cowgill L. International Renal Interest Society. IRIS Grading of Acute Kidney Injury. 2016. http://www.iris-kidney.com/pdf/4_ldc-revised-grading-of-acute-kidney-injury.pdf. Accessed June, 2019.

35. Susan G, Hackner AR. Bleeding disorders. In: Siverstein DC, Hopper K, eds. *Small Animal Critical Care Medicine*. 2nd ed. St Louis, MO: Elsevier Saunders; 2015:554-567.

36. Alan G, Ralph BMB. Hypercoagulable states. In: Siverstein DC, Hopper K, eds. *Small Animal Critical Care Medicine*. 2nd ed. St Louis, MO: Elsevier Saunders; 2015.

37. O'Neill S, Drobatz K, Satyaraj E, Hess R. Evaluation of cytokines and hormones in dogs before and after treatment of diabetic ketoacidosis and in uncomplicated diabetes mellitus. *Vet Immunol Immunopathol*. 2012;148:276-283.

38. Li AFBR. Multiple testing with the structure-adaptive Benjamini-Hochberg algorithm. *J Royal Statist Soc Ser B*. 2018;81:45-74.

39. Sato T, Ohno K, Tamamoto T, et al. Assessment of severity and changes in C-reactive protein concentration and various biomarkers in dogs with pancreatitis. *J Vet Med Sci*. 2017;79:35-40.

40. Mansfield CS, Jones BR, Spillman T. Assessing the severity of canine pancreatitis. *Res Vet Sci*. 2003;74:137-144.

41. Badhal SS, Sharma S, Saraya A, et al. Prognostic significance of D-dimer, natural anticoagulants and routine coagulation parameters in acute pancreatitis. *Trop Gastroenterol*. 2012;33:193-199.

42. Hedstrom J, Sainio V, Kemppainen E, et al. Serum complex of trypsin 2 and alpha 1 antitrypsin as diagnostic and prognostic marker of acute pancreatitis: clinical study in consecutive patients. *BMJ*. 1996;313: 333-337.

43. Viedma JA, Perez-Mateo M, Agullo J, Dominguez JE, Carballo F. Inflammatory response in the early prediction of severity in human acute pancreatitis. *Gut*. 1994;35:822-827.

44. Goodman AJ, Bird NC, Johnson AG. Antiprotease capacity in acute pancreatitis. *Br J Surg*. 1986;73:796-798.

45. Janciauskiene SM, Bals R, Koczulla R, Vogelmeier C, Köhnlein T, Welte T. The discovery of alpha1-antitrypsin and its role in health and disease. *Respir Med*. 2011;105:1129-1139.

46. Ganrot K. Plasma protein response in experimental inflammation in the dog. *Res Exp Med (Berl)*. 1973;161:251-261.

47. Conner JG, Eckersall PD, Ferguson J, et al. Acute phase response in the dog following surgical trauma. *Res Vet Sci*. 1988;45:107-110.

48. Heilmann RM, Grutzner N, Thames BE, et al. Serum alpha1 -proteinase inhibitor concentrations in dogs with systemic inflammatory response syndrome or sepsis. *J Vet Emerg Crit Care*. 2017;27:674-683.

49. Lasson A, Ohlsson K. Consumptive coagulopathy, fibrinolysis and protease-antiprotease interactions during acute human pancreatitis. *Thromb Res*. 1986;41:167-183.

50. Buchler M, Malfertheiner P, Schoetensack C, et al. Sensitivity of antiproteases, complement factors and C-reactive protein in detecting pancreatic necrosis. Results of a prospective clinical study. *Int J Pancreatol*. 1986;1:227-235.

51. McMahon MJ, Bowen M, Mayer AD, et al. Relation of alpha 2-macroglobulin and other antiproteases to the clinical features of acute pancreatitis. *Am J Surg*. 1984;147:164-170.

52. Banks RE, Evans SW, Alexander D, van Leuven F, Whicher JT, McMahon MJ. Alpha 2 macroglobulin state in acute pancreatitis. Raised values of alpha 2 macroglobulin-protease complexes in severe and mild attacks. *Gut*. 1991;32:430-434.

53. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol*. 2010;10:826-837.

54. Hoque R, Malik AF, Gorelick F, Mehal WZ. Sterile inflammatory response in acute pancreatitis. *Pancreas*. 2012;41:353-357.

55. Oiva J, Mustonen H, Kylanpaa ML, et al. Patients with acute pancreatitis complicated by organ dysfunction show abnormal peripheral blood polymorphonuclear leukocyte signaling. *Pancreatology*. 2013;13:118-124.

56. Norman JG, Fink GW, Denham W, et al. Tissue-specific cytokine production during experimental acute pancreatitis. A probable mechanism for distant organ dysfunction. *Dig Dis Sci*. 1997;42:1783-1788.

57. Brenner D, Blaser H, Mak TW. Regulation of tumour necrosis factor signalling: live or let die. *Nat Rev Immunol*. 2015;15:362-374.

58. Porteu F, Hieblot C. Tumor necrosis factor induces a selective shedding of its p75 receptor from human neutrophils. *J Biol Chem*. 1994; 269:2834-2840.

59. Ramudo L, Manso MA, Sevillano S, de Dios I. Kinetic study of TNF-alpha production and its regulatory mechanisms in acinar cells during acute pancreatitis induced by bile-pancreatic duct obstruction. *J Pathol*. 2005;206:9-16.

60. Granell S, Pereda J, Gomez-Cambronero L, et al. Circulating TNF-alpha and its soluble receptors during experimental acute pancreatitis. *Cytokine*. 2004;25:187-191.

61. Scheller J, Garbers C, Rose-John S. Interleukin-6: from basic biology to selective blockade of pro-inflammatory activities. *Semin Immunol*. 2014;26:2-12.

62. Zhang H, Neuhofer P, Song L, et al. IL-6 trans-signaling promotes pancreatitis-associated lung injury and lethality. *J Clin Invest*. 2013; 123:1019-1031.

63. Heath DI, Cruickshank A, Gudgeon M, Jehanli A, Shenkin A, Imrie CW. Role of interleukin-6 in mediating the acute phase protein response and potential as an early means of severity assessment in acute pancreatitis. *Gut*. 1993;34:41-45.

64. Song R, Yu D, Park J. Changes in gene expression of tumor necrosis factor alpha and interleukin 6 in a canine model of caerulein-induced pancreatitis. *Can J Vet Res*. 2016;80:236-241.

65. Liu Q, Djuricin G, Nathan C, Gattuso P, Weinstein RA, Prinz RA. The effect of interleukin-6 on bacterial translocation in acute canine pancreatitis. *Int J Pancreatol*. 2000;27:157-165.

66. Qin HL, Su ZD, Gao Q, Lin QT. Early intrajejunal nutrition: bacterial translocation and gut barrier function of severe acute pancreatitis in dogs. *Hepatobiliary Pancreat Dis Int*. 2002;1:150-154.

67. Rau B, Steinbach G, Gansauge F, Mayer JM, Grünert A, Beger HG. The potential role of procalcitonin and interleukin 8 in the prediction of infected necrosis in acute pancreatitis. *Gut*. 1997;41:832-840.

68. Widdison AL, Cunningham S. Immune function early in acute pancreatitis. *Br J Surg*. 1996;83:633-636.

69. Pezzilli R, Billi P, Gullo L, et al. Behavior of serum soluble interleukin-2 receptor, soluble CD8 and soluble CD4 in the early phases of acute pancreatitis. *Digestion*. 1994;55:268-273.

70. Mayer JM, Laine VJ, Gezgin A, et al. Single doses of FK506 and OKT3 reduce severity in early experimental acute pancreatitis. *Eur J Surg*. 2000;166:734-741.

71. Mayer AD, McMahon MJ, Bowen M, et al. C reactive protein: an aid to assessment and monitoring of acute pancreatitis. *J Clin Pathol*. 1984;37:207-211.

72. Wilson C, Heads A, Shenkin A, Imrie CW. C-reactive protein, antiproteases and complement factors as objective markers of severity in acute pancreatitis. *Br J Surg*. 1989;76:177-181.

73. Frossard JL, Steer ML, Pastor CM. Acute pancreatitis. *Lancet*. 2008; 371:143-152.

74. Huang YX, Jia L, Jiang SM, Wang SB, Li MX, Yang BH. Incidence and clinical features of hyperlipidemic acute pancreatitis from Guangdong, China: a retrospective multicenter study. *Pancreas*. 2014;43:548-552.

75. Twilla JD, Mancell J. Hypertriglyceridemia-induced acute pancreatitis treated with insulin and heparin. *Am J Health Syst Pharm*. 2012;69: 213-216.

76. Hagiwara S, Iwasaka H, Shingu C, Matsumoto S, Uchida T, Noguchi T. Antithrombin III prevents cerulein-induced acute pancreatitis in rats. *Pancreas*. 2009;38:746-751.

77. Kong Y, Yin J, Cheng D, et al. Antithrombin III attenuates AKI following acute severe pancreatitis. *Shock*. 2018;49:572-579.

78. Weatherton LK, Streeter EM. Evaluation of fresh frozen plasma administration in dogs with pancreatitis: 77 cases (1995-2005). *J Vet Emerg Crit Care*. 2009;19:617-622.

79. Leese T, Holliday M, Watkins M, et al. A multicentre controlled clinical trial of high-volume fresh frozen plasma therapy in prognostically severe acute pancreatitis. *Ann R Coll Surg Engl*. 1991;73:207-214.

80. Huth SP, Relford R, Steiner JM, Strong-Townsend MI, Williams DA. Analytical validation of an ELISA for measurement of canine pancreas-specific lipase. *Vet Clin Pathol*. 2010;39:346-353.

81. Woodhams B, Girardot O, Blanco MJ, et al. Stability of coagulation proteins in frozen plasma. *Blood Coagul Fibrinolysis*. 2001;12:229-236.

82. de Jager W, Bourcier K, Rijkers GT, Prakken BJ, Seyfert-Margolis V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol*. 2009;10:52.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.