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Short Communication

Serum α_1-proteinase inhibitor concentrations in dogs with exocrine pancreatic disease, chronic hepatitis or proteinuric chronic kidney disease

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ABSTRACT

Serum canine α_1-proteinase inhibitor ($c\alpha_1$-PI) concentrations were evaluated in dogs with pancreatitis ($n = 24$), exocrine pancreatic insufficiency (EPI; $n = 29$), chronic hepatitis (CH; $n = 11$) or proteinuric chronic kidney disease (CKD-P; $n = 61$) to determine whether systemic proteinase/proteinase-inhibitor balance is altered in these conditions. Dogs with CKD-P had significantly lower $c\alpha_1$-PI concentrations than dogs with pancreatitis, EPI or CH; 16% of dogs with CKD-P had serum $c\alpha_1$-PI concentrations below the reference interval. Serum and urine $c\alpha_1$-PI concentrations were inversely correlated in dogs with CKD-P, but not in dogs with CH. This suggests that renal loss of $c\alpha_1$-PI contributes to decreased serum concentrations in dogs with CKD-P, while hepatic $c\alpha_1$-PI synthesis with CH either is not compromised or is counterbalanced by extrahepatic production.

α_1-Proteinase inhibitor (α_1-PI), previously also known as α_1-antitrypsin, is a serine proteinase inhibitor produced predominantly by hepatocytes, but which can also be synthesized by macrophages, pneumocytes and intestinal epithelial cells (Carlson, 1984; Janciauskiene et al., 2011). Decreased serum and increased fecal canine α_1-PI ($c\alpha_1$-PI) concentrations have been reported in dogs with inflammatory bowel disease (IBD), protein-losing enteropathy (PLE) and/or hypocobalaminemia (Grützner et al., 2013; Equilino et al., 2015; Heilmann et al., 2016). Serum $c\alpha_1$-PI concentrations are also decreased in dogs with systemic inflammation or sepsis, suggesting that $c\alpha_1$-PI has a role as a negative type II acute-phase protein (Heilmann et al., 2017).

Decreased serum $c\alpha_1$-PI concentrations might also be found in dogs with compromised hepatic production due to chronic hepatitis (CH) or with renal protein loss due to overload of renal tubular reabsorption or tubular damage (i.e. proteinuric chronic kidney disease, CKD-P), potentially altering the systemic proteinase/proteinase-inhibitor balance in affected animals. In addition, the proteinase/proteinase-inhibitor balance could be disturbed by other conditions, such as pancreatitis or exocrine pancreatic insufficiency (EPI). The aim of this study was to evaluate serum $c\alpha$1-PI concentrations in dogs with pancreatitis, EPI, CH and CKD-P. We hypothesized that serum $c\alpha_1$-PI concentrations are decreased in dogs with pancreatitis, CH and CKD-P, in support of a role for $c\alpha_1$-PI deficiency states in the progression or pathophysiology of these diseases.

Serum samples, comprising surplus material from other studies or laboratory archives that were stored for 2–87 months (median 26 months) at −20 °C or −80 °C, were used from dogs with pancreatitis ($n = 24$), EPI ($n = 29$), CH ($n = 11$) or CKD-P ($n = 61$). Urine samples were available for measurement of $c\alpha_1$-PI concentrations from 6/11 dogs with CH and 30/61 dogs with CKD-P. The diagnosis of each disease was confirmed on the basis of clinical

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Table 1
Characteristics of dogs included in this study ($n = 125$).

Parameter	Pancreatitis	EPI	CH	CKD-P	*P*
n	24	29	11	61	
Age (years): median [range][a]	9.0 (1.0–13.0)^{A}	5.8 (1.0–14.0)^{B}	11.0 (4.0–17.0)^{A}	7.0 (0.3–14.1)^{B}	<0.001
Sex (females:males)	14:10	14:15	6:5	34:27	0.81
Disease-specific parameters	Spec cPL: median 930 (range 413–1001) μg/L	Serum cTLI: median 0.8 (range 0.1–2.3) μg/L	Idiopathic CH ($n = 8$)[b] Copper-associated CH ($n = 1$) CH with bridging fibrosis ($n = 1$) CH with cirrhosis ($n = 1$)	Immune complex-mediated glomerulonephritis ($n = 27$) Glomerulosclerosis ($n = 16$) Amyloidosis ($n = 6$) Other renal disease ($n = 12$)	
Serum $c\alpha_1$-PI concentration: mean ± SD	1716 ± 366 mg/L^{A}	1941 ± 507 mg/L^{A}	1606 ± 367 mg/L^{A}	1034 ± 406 mg/L^{B}	<0.001
Serum $c\alpha_1$-PI concentration below RI: *n* (%)	0 (0%)	0 (0%)	0 (0%)	10 (16%)	<0.001
Serum $c\alpha_1$-PI concentration above RI: *n* (%)	10 (42%)	16 (55%)	4 (36%)	3 (5%)	<0.001

CH, chronic hepatitis; CKD-P, proteinuric chronic kidney disease; EPI, exocrine pancreatic insufficiency; RI, reference interval (732–1802 mg/L; Heilmann et al., 2013), SD, standard deviation; Spec cPL, canine pancreas-specific lipase; cTLI, canine trypsin-like immunoreactivity.
[a] Medians (or means) not sharing the same superscript are significantly different at $P < 0.05$.
[b] One dog had clinicopathologic and ultrasonographic evidence of CH/cirrhosis without hepatic biopsy being performed; two dogs also had a gall bladder mucocoele.

signs, clinicopathologic evaluation, diagnostic imaging and disease-specific investigations.

A diagnosis of pancreatitis required a serum canine pancreas-specific lipase (Spec cPL) concentration >400 μg/L (reference interval, RI, 0–200 μg/L), along with compatible clinical signs, diagnostic imaging findings and the exclusion of other differential diagnoses. EPI was diagnosed on the basis of a serum canine trypsin-like immunoreactivity (cTLI) concentration ≤ 2.5 μg/L (RI 5.7–45.2 μg/L) combined with marked hypocobalaminemia (serum cobalamin concentration < 150 ng/L, i.e., below the detection limit of the assay; RI 251–908 ng/L) and a serum Spec cPL concentration within the RI.

A diagnosis of CH required consistent findings on histopathological evaluation of a liver biopsy (Rothuizen et al., 2006), assessment of hepatic copper (quantification or semiquantitative histological evaluation) and negative culture of hepatic biopsy specimens (Bexfield, 2017), with the exception of one dog that had clinicopathologic and ultrasonographic evidence of liver insufficiency and CH/cirrhosis, but no hepatic biopsy. In addition, dogs were assigned a clinical disease severity index graded from 0 (normal) to 3 (severely abnormal) using a scoring system derived from the canine IBD activity index (Jergens et al., 2003), including activity level, hepatic encephalopathy, body condition, icterus, ascites, and gastrointestinal signs (range of possible cumulative scores 0–18).

Dogs in the CKD-P group had persistent proteinuria, i.e. a urine protein:creatinine ratio (UPC) ≥ 1.0 for 0.4–48 months (median 7.5 months) based on clinical history, with ($n = 8$) or without azotemia ($n = 14$), and/or chronic lesions on routine histopathological, immunofluorescence and/or electron microscopic assessment of a renal biopsy (i.e. interstitial fibrosis, glomerulosclerosis and/or amyloidosis) (IRIS Canine GN Study Group Diagnosis Subgroup et al., 2013). Ten dogs in the CKD-P group also had abdominal ultrasound findings consistent with a chronic nephropathy.

Serum and urine $c\alpha_1$-PI concentrations were measured by radioimmunoassay (Heilmann et al., 2013). Data were evaluated for normality and equal variances using a Shapiro–Wilk test and a Brown–Forsythe test, respectively, and are presented as mean ± standard deviation (SD) or median (range). Serum $c\alpha_1$-PI concentrations were compared amongst the four groups of dogs by analysis of variance (ANOVA) with Tukey's post hoc test. Associations between parameters were tested using the Spearman rank sum correlation coefficient ρ, with the 95% confidence interval (CI) or a likelihood ratio test. Statistical significance was set at a $P < 0.05$. Prism v7.0 (GraphPad Software) was used for all data analyses.

Serum $c\alpha_1$-PI concentrations and characteristics of dogs ($n = 125$) included in this study are summarized in Table 1. Dogs with CKD-P had significantly lower serum $c\alpha_1$-PI concentrations than dogs with pancreatitis, EPI or CH ($P < 0.001$; Table 1; Fig. 1), whereas serum $c\alpha_1$-PI concentrations did not differ significantly amongst dogs with pancreatitis, EPI or CH. Mean serum $c\alpha_1$-PI concentrations were close to the upper limit of the RI in dogs with pancreatitis or CH, and were above the upper limit of the RI in dogs with EPI.

All dogs with biopsy-confirmed CH had histopathological evidence of chronic inflammation. The severity of inflammation was graded as 1–4 (median 3), fibrosis as 1–4 (median 3) and vacuolar change as 0–3 (median 2). There was no association between serum $c\alpha_1$-PI concentrations and hepatic inflammation scores ($P = 0.41$), fibrosis scores ($P = 0.46$) or vacuolation scores ($P = 0.72$). The possibility of an association between hepatic copper accumulation and serum $c\alpha_1$-PI concentrations could not be evaluated in this study, since quantitative copper analysis was not

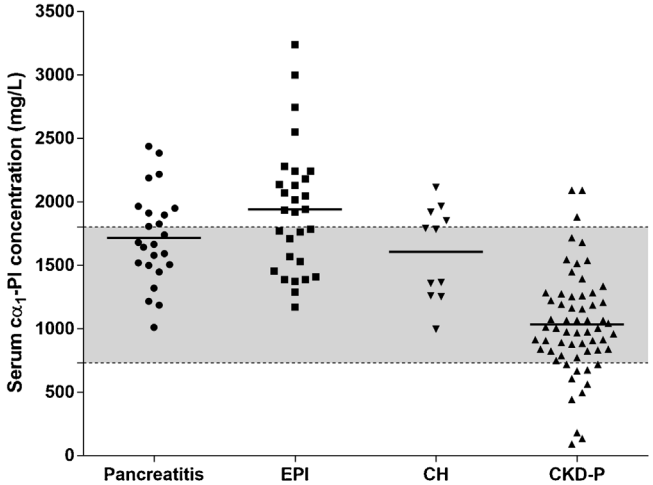

Fig. 1. Serum canine α_1-proteinase inhibitor ($c\alpha_1$-PI) concentrations in dogs with pancreatitis, exocrine pancreatic insufficiency (EPI), chronic hepatitis (CH) or chronic proteinuric kidney disease (CKD-P). Solid black lines indicate means for each group; the gray-shaded area between the dotted lines represents the reference interval for serum $c\alpha_1$-PI concentration (732–1802 mg/L; Heilmann et al., 2013).