



Title	Serial measurement of pancreatic lipase immunoreactivity concentration in dogs with immune-mediated disease treated with prednisolone
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1 **Original Article**

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3 **Serial measurement of pancreatic lipase immunoreactivity concentration in**
4 **dogs with immune-mediated disease treated with prednisolone**
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20 This study was presented in part at the 2015 American College of Veterinary Internal
21 Medicine Forum, Indianapolis, IN, USA.
22
23

Abstract

Objectives: In this pilot study, we performed serial measurements of the serum canine pancreatic lipase immunoreactivity (cPLI) concentration in dogs with various immune-mediated diseases treated with an immunosuppressive dose of prednisolone.

Methods: Ten client-owned dogs with newly-diagnosed immune-mediated disease that had normal cPLI concentrations ($\leq 200 \mu\text{g/L}$) were treated with an immunosuppressive dose of prednisolone (2–2.2 mg/kg/day initially, which was gradually decreased). These 10 dogs were re-evaluated at our hospital at 1–4-week intervals. Serum samples were obtained from each of the dogs prior to treatment (baseline cPLI) and during immunosuppressive treatment for serial measurements of cPLI (Spec cPL™). The highest cPLI concentration detected during immunosuppressive treatment was defined as the peak cPLI.

Results: Peak cPLI concentrations were classified as normal in two dogs, questionable (201–399 $\mu\text{g/L}$) in three dogs, and abnormal ($\geq 400 \mu\text{g/L}$) in five dogs. Peak cPLI concentrations were significantly higher than baseline cPLI concentrations with no evidence of clinical pancreatitis.

Clinical significance: The peak cPLI concentration was higher than 400 $\mu\text{g/L}$ in five out of 10 dogs with immune-mediated disease during treatment with an immunosuppressive dose of prednisolone, without evidence of clinical pancreatitis. It remains unclear whether these dogs had subclinical pancreatitis or whether the observed increase in the serum cPLI concentration was related to the administration of prednisolone.

Keywords: cPLI, Dog, Immune-mediated disease, Prednisolone

INTRODUCTION

Pancreatitis is the most common exocrine pancreatic disease in dogs. Accurate clinical diagnosis of pancreatitis remains challenging because no single noninvasive diagnostic method is completely reliable. At present, combinations of clinical signs, haematological and biochemical laboratory findings, and B-mode ultrasonography, are commonly used to diagnose pancreatitis in dogs (Steiner *et al.* 2003).

Canine pancreatic lipase immunoreactivity (cPLI) is widely used for the diagnosis of canine pancreatitis. Canine pancreatic lipase is reported to be exclusively expressed in pancreatic acinar cells (Steiner *et al.* 2002) and is thought to be a sensitive and specific marker for pancreatitis (Steiner *et al.* 2008, Trivedi *et al.* 2010). At present, cPLI concentration determination is considered the most sensitive and specific assay among various serum tests for the diagnosis of canine pancreatitis, with a reported sensitivity of 71.7%–77.8% and specificity of 80%.5–88.0% (McCord *et al.* 2012). Currently, Spec cPL™, a commercially available cPLI assay, is the most frequently used noninvasive biochemical test for the diagnosis of canine pancreatitis (McCord *et al.* 2012).

The advantages of cPLI assays over traditional lipase activity assays are based on two facts: (1) pancreatic lipase is exclusively of pancreatic origin; and (2) in contrast to the traditional activity assays for lipase, which indiscriminately measure the activity of lipases of multiple organ origins (such as the stomach and liver), the cPLI assay is able to detect the unique three-dimensional structure of pancreatic lipase without interference from other lipases (Steiner *et al.* 2002). Therefore, the cPLI assay has inherent advantages over traditional serum lipase activity assays. Additionally, experimentally induced chronic renal failure (Steiner *et al.* 2001) and 4 weeks of peroral (PO) administration of prednisone (2.2 mg/kg/day) to healthy dogs (Steiner *et al.* 2009) were not found to have any clinically significant effect on the serum cPLI concentration, although traditional lipase activity assays have been reported to be influenced by such conditions (Polzin *et al.* 1983, Fittschen & Bellamy 1984).

In veterinary medicine, prednisone/prednisolone is one of the most frequently used drugs, especially in inflammatory and immune-mediated disorders. Previously, glucocorticoids were assumed to cause pancreatitis in small animals. In fact, sporadic cases of pancreatitis in dogs treated with glucocorticoids were reported (Behrend & Kemppainen 1997). However, the administration of dexamethasone to healthy dogs at various doses for up to 3 weeks did not cause pancreatitis (Parent *et al.* 1982). In addition, as mentioned previously, 4 weeks of prednisone administration to healthy dogs did not have any significant effect on the serum cPLI concentration (Steiner *et al.* 2009). In fact, glucocorticoids are no longer included in the list of drugs suspected of being associated with pancreatitis (Xenoulis & Steiner 2013). However, it is unknown whether glucocorticoids are a contributory factor for drug-induced pancreatitis in sick animals.

Thus, in this pilot study we serially measured the serum cPLI concentration in dogs with various immune-mediated diseases treated with an immunosuppressive dose of prednisolone.

MATERIALS AND METHODS

Inclusion criteria

Client-owned dogs with immune-mediated disease that were presented to our hospital between November 2012 and November 2014 were prospectively recruited for this study. Dogs were diagnosed with immune-mediated diseases such as meningoencephalitis of unknown aetiology (MUE), immune-mediated polyarthritis (IMPA), idiopathic inflammatory bowel disease (IBD), immune-mediated polymyositis (IMPM) or inflammatory colorectal polyps (ICRP) at our teaching hospital according to previously published diagnostic criteria. Meningoencephalitis of unknown aetiology (MUE) was suspected based on the evidence of

74 single, multiple or diffuse intracranial lesions by MRI, a cerebrospinal fluid (CSF) pleocytosis
75 (total nucleated cell count: >5 nucleated cells/ μ l with >50% mononuclear cells), and negative
76 results for CSF bacterial culture and the canine distemper virus antibody test (Lowrie *et al.*
77 2013). Immune-mediated polyarthritis (IMPA) was diagnosed based on non-erosive
78 polyarthritis that was confirmed by radiographic assessment of the joints and the results of
79 joint fluid analysis, with no evidence of visceral abnormalities on thoracic or abdominal
80 radiographs, and an abdominal ultrasound. The diagnosis of non-infectious polyarthritis was
81 confirmed by the absence of bacteria and an increased number of non-degenerative
82 neutrophils as determined by cytologic evaluation, along with negative bacterial culture
83 results from the joint fluid (Ohno *et al.* 2006, Goldstein 2010). In addition, serologic assays
84 including the detection of antibodies against *Borrelia burgdorferi*, *Anaplasma*
85 *phagocytophilum* and *Ehrlichia canis*, as well as the antigen of *Dirofilaria immitis* were
86 performed in an attempt to rule out secondary IMPA. The criteria for the diagnosis of
87 idiopathic inflammatory bowel disease (IBD) were as follows: (1) a history of chronic
88 diarrhoea with/without vomiting of at least 3 weeks duration; (2) exclusion of identifiable
89 underlying disorders by a complete blood count, serum biochemistry, serum trypsin-like
90 immunoreactivity, urinalysis, faecal examination for evidence of endoparasites, and
91 abdominal ultrasonography; (3) and the presence of lymphoplasmacytic inflammation on
92 histopathological review of duodenal biopsies (Day *et al.* 2008, Washabau *et al.* 2010,

93 Schmitz *et al.* 2012). Food-responsive diarrhoea was ruled out when complete remission in
94 clinical signs was not observed after adherence to a 2-week elimination diet.
95 Antibiotic-responsive diarrhoea was ruled out in the absence of a complete response to
96 2-week therapeutic trials of metronidazole (10–15 mg/kg q 12 h, PO) or tylosin (20 mg/kg q
97 12 h, PO). Immune-mediated polymyositis (IMPM) was diagnosed based on an increased
98 concentration of creatine kinase on a blood test and histopathological evidence of myofiber
99 necrosis and infiltrations of lymphocytes, macrophages and plasma cells in the temporal,
100 masseter, and biceps femoris muscles (Morita *et al.* 2002). Miniature dachshunds with clinical
101 signs of large bowel diarrhoea were diagnosed with inflammatory colorectal polyps (ICRP)
102 based on clinical and histological findings in a previous report (Ohmi *et al.* 2012). In brief,
103 ICRP were characterised by the formation of multiple small polyps around the large bowel
104 mucosa, almost invariably restricted to the descending colon and rectum, sometimes
105 accompanied by space-occupying solitary large polyp formation. Histopathological findings
106 associated with ICRP were increased goblet cells with dilated crypts, infiltration of
107 inflammatory cells (predominantly neutrophils and macrophages), and proliferation of
108 granulation tissue (Ohmi *et al.* 2012). Based on the existence of idiopathic inflammation and
109 the effectiveness of immunosuppressive therapy, ICRP have been proposed to be a novel form
110 of canine IBD (Ohta *et al.* 2013). Written informed consent was provided by all dog owners
111 of dogs enrolled in the study.

Each dog was treated with an immunosuppressive dose of prednisolone (2–2.2 mg/kg, q 24 h, PO) (Prednisolone, Takeda Pharmaceutical, Osaka, Japan) as the initial treatment for at least 7 days. Subsequently, the dose of prednisolone was reduced by 0.5 mg/kg/day every 2–4 weeks until relapse was observed (Reusch 2015).

Exclusion criteria

Dogs were excluded from the study if they had received glucocorticoids (≥ 0.5 mg/kg/day) or other immune-modulating medications (e.g., azathioprine, cyclosporine) within the previous month (Rhoades *et al.* 2016). Dogs were excluded from the study if they received immune-modulating medications other than prednisolone following diagnosis. Dogs with clinical signs of hyperadrenocorticism (HAC) (as described in the ACVIM Consensus Statement regarding HAC; Behrend *et al.* 2013) were excluded from the study. Dogs with clinical signs of pancreatitis (e.g., abdominal pain, vomiting, diarrhoea or anorexia) were excluded from the study if the serum cPLI concentration was ≥ 200 $\mu\text{g/L}$ at the initial visit.

Canine pancreatic lipase immunoreactivity measurement

Dogs were re-evaluated at our hospital at 1–4-week intervals to monitor clinical signs of immune-mediated disease, and clinical signs of iatrogenic HAC or pancreatitis as described by Mawby (2014). A serum sample was obtained from each dog at each visit to monitor the haematological side effects of prednisolone administration (e.g., ALT, AST and ALP) up to a maximum of 12 weeks. Aliquots of serum samples were stored at -20°C , and used for serial

measurement of cPLI. Serum cPLI concentrations were measured in all samples within 2 months of collection using a commercial ELISA (Spec cPL™ test, IDEXX Laboratories, Westbrook, ME, USA) that has been analytically validated for use in dogs (Huth *et al.* 2010). The reference interval of the assay was 0–200 µg/L. A concentration ≥ 400 µg/L was considered diagnostic for pancreatitis, while a concentration between 201 µg/L and 399 µg/L was considered to be in the gray zone. The pre-treatment cPLI concentration was defined as the baseline cPLI. The highest cPLI concentration during immunosuppressive treatment was defined as the peak cPLI. Serial serum cPLI concentration measurements were terminated if any of the following three conditions were met: monitoring period reached 12 weeks, the dog died due to an underlying disorder, or a follow-up was not completed by the owner.

Statistical analysis

The normality distribution of the baseline cPLI concentrations and the peak cPLI concentrations was assessed by the Shapiro-Wilk test. Comparison of the baseline cPLI concentrations and the peak cPLI concentrations was performed using the Wilcoxon single-rank sum test. Statistical analyses were performed using JMP 8 statistical software (SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as $P < 0.05$.

RESULTS

Study population

During the study period, 10 dogs fulfilled the inclusion criteria. Of these, seven were

female (six neutered) and three were male (two neutered). The median age was 9 years (range: 3–10 years). Breeds included Miniature Dachshund (four dogs), Toy Poodle (two dogs), and one each of Shih Tzu, Boston Terrier, Labrador Retriever and Welsh Corgi. These dogs were diagnosed with IBD (n = 3), IMPA (n = 2), ICRP (n = 2), MUE (n = 2) or IMPM (n = 1).

Pancreatic-specific lipase assay

The number of analysis points of cPLI before and during treatment for the 10 dogs yielded a median of five (range: 2–6) (Fig. 1). The reasons for terminating serial cPLI concentration measurements were that in seven dogs a follow-up was not completed, the monitoring period reached 12 weeks in two dogs, and one dog (Dog 4) with IMPM died due to poorly controlled dysphagia. The results of serial cPLI monitoring are presented in Fig. 1. In addition, the prednisolone dosages administered each week are presented in Fig. 2. In one dog (Dog 5), the prednisolone dosage was reduced from 2 mg/kg/day to 1 mg/kg/day at week 3 because severe clinical signs of iatrogenic HAC were observed. The median value for the baseline serum cPLI concentration was 82.5 µg/L (range: 43–175). As shown in Fig. 1, serum cPLI concentrations increased above 400 µg/L in five out of 10 dogs (Dogs 1–5) after prednisolone administration. The remaining five dogs (Dogs 6–10) showed no significant increase in the cPLI concentration (< 400 µg/L). Peak cPLI concentrations were classified as normal in two dogs (1 ICRP and 1 MUE), questionable in three dogs (3 IBD), and abnormal

in five dogs (2 IMPA, 1 ICRP, 1 MUE and 1 IMPM). The median value for the peak cPLI concentrations was 390.5 µg/L (range: 85–1000). The peak cPLI concentrations were significantly higher than the baseline cPLI concentrations ($P = 0.001$) (Fig. 3). In total, there were eight time points in which the serum cPLI concentration increased to more than 400 µg/L (Fig. 1). None of the dogs showed clinical signs of pancreatitis (e.g., abdominal pain, vomiting, diarrhoea or anorexia) during the serial cPLI concentration measurements at any of the time points.

DISCUSSION

In this pilot study, five of the 10 dogs with immune-mediated disease displayed increased serum cPLI concentrations during the administration of an immunosuppressive dose of prednisolone.

Currently, the effect of corticosteroid administration on the pancreas is poorly understood, and there is controversy regarding corticosteroid-induced pancreatitis. In humans, there is only one report in which recurrence of pancreatitis was shown after re-challenge with corticosteroid administration (Levine & McGuire 1988). Currently, corticosteroid administration is not considered a risk factor for pancreatitis, and corticosteroids are considered to be only a “possible/questionable” cause of drug-induced pancreatitis in humans (Topazian & Pandol 2009). In veterinary medicine, it has been shown that the serum cPLI concentration remained unaltered after 4 weeks of oral prednisone (2.2 mg/kg/day)

administration to six healthy young adult female heterozygous dogs with X-linked hereditary nephritis (Steiner *et al.* 2009). The present study demonstrated that five out of 10 dogs with immune-mediated disease had abnormal cPLI concentrations during treatment with an immunosuppressive dose of prednisolone without clinical pancreatitis. However, the reason for this increase in cPLI concentration remains unknown because of the small number of cases investigated. Possible reasons for this increased cPLI concentration may be the underlying disorder itself, diversity in the dosage and duration of prednisolone administration, differences between prednisone and prednisolone, or differences in the age of the dogs.

In this study, we analysed 10 dogs with naturally-occurring immune-mediated disease, with a median baseline cPLI concentration of 82.5 µg/L (range: 43–175). Several dogs already had relatively high cPLI concentrations at presentation compared with the pre-treatment (baseline) serum cPLI concentrations in six healthy dogs reported in a previous study (mean±SD = 35.9±18.2) (Steiner *et al.* 2009), although these concentrations were within the previously mentioned reference interval. Thus, underlying disorders might have affected the baseline cPLI concentrations and the results of serial serum cPLI concentration measurements in these dogs. Three of the dogs in our study had IBD. It has been reported that IBD dogs with elevated cPLI concentrations have a worse clinical outcomes compared with those with normal cPLI concentrations (Kathrani *et al.* 2009). In this report, the authors speculated that high cPLI concentrations might be associated with concurrent pancreatic

207 inflammation similar to the case of IBD-associated chronic pancreatitis in humans (Kathrani
208 *et al.* 2009). Thus, it is possible that concurrent pancreatitis might have affected the peak cPLI
209 concentrations in the three IBD dogs analysed in this study. However, none of these three IBD
210 dogs had abnormal cPLI concentrations ($\geq 400 \mu\text{g/L}$) after prednisolone administration.
211 Furthermore, four out of the five dogs with immune-mediated disease that had abnormal peak
212 cPLI concentrations were diagnosed with non-gastrointestinal disease (2 IMPA, 1 MUE and 1
213 IMPM). Thus, it seemed unlikely that pre-existing gastrointestinal inflammation was the sole
214 cause of increased cPLI concentration. In addition, the five dogs with abnormal peak cPLI
215 concentrations were diagnosed with IMPA (two dogs), MUE (one dog), ICRP (one dog) and
216 IMPM (one dog). However, diagnoses of MUE and ICRP were also given to five dogs with
217 normal to questionable peak cPLI concentrations. Thus, the relationship between the results of
218 serial cPLI concentration measurements and disease category could not be determined in this
219 study because of the small number of cases examined.

220 In this study, the dosage and duration of prednisolone administration could not be
221 standardised. In addition, we focused on the peak cPLI concentration, but not the mean or
222 median cPLI concentration, to examine whether the serum cPLI concentration increased
223 above $400 \mu\text{g/L}$ at any time point during prednisolone administration. Thus, it is not possible
224 to make a definitive conclusion about the relationship between prednisolone administration
225 and the increase in serum cPLI concentration. In fact, the timing at which the peak cPLI

concentration was reached varied among the dogs. In addition, point-to-point variations in the serum cPLI concentration were observed among the 10 dogs. From the results of the present study, the possibility of day-to-day variations in the serum cPLI concentration that are not associated with prednisolone administration could not be excluded.

A possible explanation for the differences between the results of previous experimental study and the present study is the different formulation of prednisone and prednisolone. Prednisone is an inactive compound (or prodrug) until metabolised in the liver by the enzyme 11- β hydroxysteroid dehydrogenase type 1 (Reusch 2015). In dogs, oral administration of prednisone may not result in the systemic prednisolone concentrations achieved using oral prednisolone. It has been reported that the relative bioavailability of prednisolone was only 65% when prednisone was administered compared with prednisolone (Reusch 2015). Thus, the oral bioavailability of prednisolone used in this study (2–2.2 mg/kg/day) could be higher than that of prednisone used in the previous study (2.2 mg/kg/day, PO) (Steiner *et al.* 2009). The difference in prednisolone and prednisone oral bioavailability might be one of the reasons for the differences in the results observed between the two studies.

The median age of the dogs used in this study was 9 years (range: 3–10). By contrast, in the previous experimental report, young adult dogs were employed (Steiner *et al.* 2009). Thus, differences in the age of the dogs used might also contribute to the differences between independent studies. However, the median age of the five dogs with abnormal serum cPLI

245 concentrations (9 years) did not differ from that of the five dogs with normal to questionable
246 serum cPLI concentrations (9 years) in the current study. In addition, the number of dogs used
247 in the current study was small. Thus, further studies with larger numbers of dogs are
248 warranted to determine whether the age of dogs has an influence on serial cPLI concentrations
249 during prednisolone treatment.

250 It is widely accepted that pancreatic inflammation is relatively common, but its exact
251 incidence in dogs is unknown because many dogs display subclinical or mild disease.
252 Recently, dogs with HAC were found to have elevated serum cPLI concentrations compared
253 with healthy dogs (Mawby *et al.* 2014). In addition, the number of dogs with pancreatic
254 hyperechogenicity was significantly higher in dogs with HAC compared with healthy dogs
255 (Granger *et al.* 2015). Thus, it is possible that excess endogenous steroids caused subclinical
256 pancreatitis and may have resulted in pancreatic hyperechogenicity in dogs with HAC. The
257 underlying mechanism responsible for the increased serum cPLI concentration observed in
258 five dogs with immune-mediated disease could not be determined in this study. However, it is
259 possible that the five dogs with increased serum cPLI concentrations may have been
260 harbouring subclinical pancreatitis.

261 There are several limitations to our study. First, histopathological examination of the
262 pancreas, the most definitive diagnostic tool for pancreatitis, was not performed. Second,
263 abdominal ultrasound examination of the pancreas to look for subclinical pancreatitis was not

performed after prednisolone administration. Third, dogs with abnormal serum cPLI concentrations at presentation were excluded from this study. Thus, the potential diversity of serum cPLI concentrations among dogs with immune-mediated disease was not investigated. Also, it remains unknown whether dogs with abnormal serum cPLI concentrations would show an improvement in the serum cPLI concentration after prednisolone administration. Finally, the number of dogs used in this study was small. An additional study using a higher number of cases, along with standardised treatment and monitoring protocols, is therefore needed.

In conclusion, five of 10 dogs with immune-mediated disease showed an increased serum cPLI concentration after administration of an immunosuppressive dose of prednisolone without clinical pancreatitis. However, it remains to be determined whether this increase in serum cPLI concentration was associated with subclinical pancreatitis or whether it was affected by the administration of prednisolone.

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Figure legends

Fig. 1—Graph showing the serial serum cPLI concentrations in 10 dogs

Each colour (symbol and solid line) represents the results from one dog (for dog 1 to dog 10). Square symbols indicate the peak cPLI concentration for each of the 10 dogs. Black dotted line represents the cPLI concentration = 400 µg/L. Grey dotted line represents the cPLI concentration = 200 µg/L.

Fig. 2—Graph showing the prednisolone dosages administered each week in the 10 dogs

Each colour (bar) represents the results from one dog (for dog 1 to dog 10). Dosages in each bar represent the initial prednisolone dosage (mg/kg/day) in each of the 10 dogs.

Fig. 3—Comparison of the baseline (pre-treatment) and peak (highest concentration

380 **during immunosuppressive treatment) cPLI concentrations for the 10 dogs**

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382 Conflict of Interest

383 None of the authors of this article has a financial or personal relationship with other people or

384 organisations that could inappropriately influence or bias the content of the paper.

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