HOKKAIDO UNIVERSITY

Title	Serial measurement of pancreatic lipase immunoreactivity concentration in dogs with immune-mediated disease treated with prednisolone
Author(s)	Ohta, H.; Morita, T.; Yokoyama, N. et al.
Citation	Journal of small animal practice, 58(6), 342-347 https://doi.org/10.1111/jsap.12652
Issue Date	2017-06
Doc URL	https://hdl.handle.net/2115/70667
Rights	This is the peer reviewed version of the following article: H. Ohta, T. Morita, N. Yokoyama, T. Osuga, N. Sasaki, K. Morishita, K. Nakamura, M. Takiguchi, Serial measurement of pancreatic lipase immunoreactivity concentration in dogs with immune-mediated disease treated with prednisolone, Journal of Small Animal Practice, Vol. 58, Issue 6, June 2017, which has been published in final form at https://doi.org/10.1111/jsap.12652. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.
Type	journal article
File Information	JSAP_58(6)_342-347_for_HUSCAP.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Original Article

Serial measurement of pancreatic lipase immunoreactivity concentration in dogs with immune-mediated disease treated with prednisolone

H. Ohta,[*] DVM, PhD; T. Morita,[*] DVM; N. Yokoyama,[*] DVM; T. Osuga,[*] DVM, PhD; N. Sasaki,[*] DVM, PhD; K. Morishita,[†] DVM; K. Nakamura,[†] DVM, PhD and M. Takiguchi,[*] DVM, PhD.

[*]Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.

[†]Veterinary Teaching Hospital, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.

Corresponding author: H. Ohta, DVM, PhD, Laboratory of Veterinary Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9, Sapporo, Hokkaido 060-0818, Japan.

Phone & Fax: +81-11-706-5223, E-mail: h-ohta@vetmed.hokudai.ac.jp

This study was presented in part at the 2015 American College of Veterinary Internal Medicine Forum, Indianapolis, IN, USA.

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 (≤ 200 μg/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 μg/L) in three dogs, and abnormal (≥ 400 μg/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 μg/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

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

112 Each dog was treated with an immunosuppressive dose of prednisolone (2–2.2 mg/kg, q
113 24 h, PO) (Prednisolone, Takeda Pharmaceutical, Osaka, Japan) as the initial treatment for at
114 least 7 days. Subsequently, the dose of prednisolone was reduced by 0.5 mg/kg/day every 2–4
115 weeks until relapse was observed (Reusch 2015).

116 ***Exclusion criteria***

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

125 ***Canine pancreatic lipase immunoreactivity measurement***

126 Dogs were re-evaluated at our hospital at 1–4-week intervals to monitor clinical signs of
127 immune-mediated disease, and clinical sings of iatrogenic HAC or pancreatitis as described
128 by Mawby (2014). A serum sample was obtained from each dog at each visit to monitor the
129 haematological side effects of prednisolone administration (e.g., ALT, AST and ALP) up to a
130 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)

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

196 In this study, we analysed 10 dogs with naturally-occurring immune-mediated disease,
197 with a median baseline cPLI concentration of 82.5 μg/L (range: 43–175). Several dogs
198 already had relatively high cPLI concentrations at presentation compared with the
199 pre-treatment (baseline) serum cPLI concentrations in six healthy dogs reported in a previous
200 study (mean±SD = 35.9±18.2) (Steiner *et al*. 2009), although these concentrations were
201 within the previously mentioned reference interval. Thus, underlying disorders might have
202 affected the baseline cPLI concentrations and the results of serial serum cPLI concentration
203 measurements in these dogs. Three of the dogs in our study had IBD. It has been reported that
204 IBD dogs with elevated cPLI concentrations have a worse clinical outcomes compared with
205 those with normal cPLI concentrations (Kathrani *et al*. 2009). In this report, the authors
206 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 (≥ 400 μg/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 μg/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

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

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

There are several limitations to our study. First, histopathological examination of the pancreas, the most definitive diagnostic tool for pancreatitis, was not performed. Second, 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.

References

Behrend, E. N. & Kemppainen, R. J. (1997) Glucocorticoid therapy. Pharmacology, indications, and complications. *Veterinary Clinics of North America. Small Animal Practice* **27**, 187-213

Behrend, E. N., Kooistra, H. S., Nelson, R., *et al*. (2013) Diagnosis of spontaneous canine hyperadrenocorticism: 2012 ACVIM Consensus Statement (Small Animal). *Journal of Veterinary Internal Medicine* **27**, 1292-1304

285 Day, M. J., Bilzer, T., Mansell, J., *et al*. (2008) Histopathological standards for the diagnosis
286 of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report
287 from the World Small Animal Veterinary Association Gastrointestinal Standardization Group.
288 *Journal of Comparative Pathology* **138**, S1-S43
289 Fittschen, C. & Bellamy, J. E. (1984) Prednisone treatment alters the serum amylase and
290 lipase activities in normal dogs without causing pancreatitis. *Canadian Journal of*
291 *Comparative Medicine* **48**, 136-140
292 Goldstein, R. E. (2010) Swollen joints and lameness. In: Textbook of Veterinary Internal
293 Medicine. 7th edn. Eds S. J. Ettinger and E. C. Feldman. W. B. Saunders, St. Louis, MO,
294 USA. pp 130-133
295 Granger, L. A., Hilferty, M., Francis, T. (2015) Variability in the ultrasonographic appearance
296 of the pancreas in healthy dogs compared to dogs with hyperadrenocorticism. *Veterinary*
297 *Radiology & Ultrasound* **56**, 540-548
298 Huth, S. P., Relford, R., Steiner, J. M., *et al*. (2010) Analytical validation of an ELISA for
299 measurement of canine pancreas-specific lipase. *Veterinary Clinical Pathology* **39**, 346-353
300 Kathrani, A., Steiner, J. M., Suchodolski, J., *et al*. (2009) Elevated canine pancreatic lipase
301 immunoreactivity concentration in dogs with inflammatory bowel disease is associated with
302 a negative outcome. *Journal of Small Animal Practice* **50**, 126-132
303 Levine, R. A. & McGuire, R. F. (1988) Corticosteroid-induced pancreatitis: a case report

304 demonstrating recurrence with rechallenge. *American Journal of Gastroenterology* **83**,
305 1161-1164
306 Lowrie, M., Smith, P. M., Garosi, L. (2013) Meningoencephalitis of unknown origin:
307 investigation of prognostic factors and outcome using a standard treatment protocol.
308 *Veterinary Record* **172**, 527
309 Mawby, D. I., Whittemore, J. C., Fecteau, K. A. (2014) Canine pancreatic-specific lipase
310 concentrations in clinically healthy dogs and dogs with naturally occurring
311 hyperadrenocorticism. *Journal of Veterinary Internal Medicine* **28**, 1244-1250
312 McCord, K., Morley, P. S., Armstrong, J., *et al*. (2012) A multi-institutional study evaluating
313 the diagnostic utility of the Spec cPL™ and SNAP® cPL™ in clinical acute pancreatitis in
314 84 dogs. *Journal of Veterinary Internal Medicine* **26**, 888-896
315 Morita, T., Shimada, A., Yashiro, S., *et al*. (2002) Myofiber expression of class I major
316 histocompatibility complex accompanied by CD8+ T-cell-associated myofiber injury in a
317 case of canine polymyositis. *Veterinary Pathology* **39**, 512-515
318 Ohmi, A., Ohno, K., Uchida, K., *et al*. (2012) A retrospective study of inflammatory
319 colorectal polyps in miniature dachshunds. *Journal of Veterinary Medical Science* **74**, 59-64
320 Ohno, K., Yokoyama, Y., Nakashima, K., *et al*. (2006) C-reactive protein concentration in
321 canine idiopathic polyarthritis. *Journal of Veterinary Medical Science* **68**, 1275-1279
322 Ohta, H., Takada, K., Torisu, S., *et al*. (2013) Expression of CD4+ T cell cytokine genes in the

colorectal mucosa of inflammatory colorectal polyps in miniature dachshunds. *Veterinary Immunology and Immunopathology* **155**, 259-263

Parent, J. (1982) Effects of dexamethasone on pancreatic tissue and on serum amylase and lipase activities in dogs. *Journal of the American Veterinary Medical Association* **180**, 743-746

Polzin, D. J., Osbome, C. A., Stevens, J. B., *et al*. (1983) Serum amylase and lipase activities in dogs with chronic primary renal failure. *American Journal of Veterinary Research* **44**, 404-410

Reusch, C. E. (2015) Glucocorticoid Therapy. In: Canine & Feline Endocrinology, 4th edn. Eds E. C. Feldman, R. W. Nelson, C. E. Reusch. Elsevier, St. Louis, MO, USA. pp 555-577

Rhoades, A. C., Vernau, W., Kass, P. H., *et al.* (2016) Comparison of the efficacy of prednisone and cyclosporine for treatment of dogs with primary immune-mediated polyarthritis. *Journal of the American Veterinary Medical Association* **248**, 395-404

Schmitz, S., Garden, O. A., Werling, D., *et al*. (2012) Gene expression of selected signature cytokines of T cell subsets in duodenal tissues of dogs with and without inflammatory bowel disease. *Veterinary Immunology and Immunopathology* **146**, 87-91

Steiner, J. M., Finco, D. R., Gumminger, S. R., *et al*. (2001) Serum canine pancreatic lipase immunoreactivity (cPLI) in dogs with experimentally induced chronic renal failure Proceedings of the American College of Veterinary Internal Medicine Forum. May 23 to 26,

342 Denver, CO, USA. pp 311

343 Steiner, J. M., Berridge, B. R., Wojcieszn, J., *et al*. (2002) Cellular immunolocalization of
344 gastric and pancreatic lipase in various tissues obtained from dogs. *American Journal*
345 *Veterinary Research* **63**, 722-727

346 Steiner, J. M. (2003) Diagnosis of pancreatitis. *Veterinary Clinics of North America. Small*
347 *Animal Practice* **33**, 1181-1195

348 Steiner, J. M., Newman, S., Xenoulis, P., *et al*. (2008) Sensitivity of serum markers for
349 pancreatitis in dogs with macroscopic evidence of pancreatitis. *Veterinary Therapeutics* **9**,
350 263-273

351 Steiner, J. M., Teague, S. R., Lees, G. E., *et al*. (2009) Stability of canine pancreatic lipase
352 immunoreactivity concentration in serum samples and effects of long-term administration of
353 prednisone to dogs on serum canine pancreatic lipase immunoreactivity concentrations.
354 *American Journal of Veterinary Research* **70**, 1001-1005

355 Topazian, M. & Pandol, S. J. (2009) Acute pancreatitis. In: Textbook of Gastroenterology, 5th
356 edn. Ed T. Yamada. Wiley-Blackwell, West Sussex, UK. pp 1761-1810

357 Trivedi, S., Marks, S. L., Kass, P. H., *et al*. (2011) Sensitivity and specificity of canine
358 pancreas-specific lipase (cPL) and other markers for pancreatitis in 70 dogs with and without
359 histopathologic evidence of pancreatitis. *Journal of Veterinary Internal Medicine* **25**,
360 1241-1247

Washabau, R. J., Day, M. J., Willard, M. D., *et al*. (2010) Endoscopic, biopsy, and histpathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. *Journal of Veterinary Internal Medicine* **24**, 10-26

Xenoulis, P. G. & Steiner, J. M. (2013) Necrosis and inflammation: canine. In: Canine and Feline Gastroenterology. Eds R. J. Washabau and M. J. Day. Elsevier, St. Louis, MO, USA. pp 812-821

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**

during immunosuppressive treatment) cPLI concentrations for the 10 dogs

Conflict of Interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.