UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

THE EFFECT OF PREDNISOLONE THERAPY ON CANINE SERUM LEVELS OF 1,2-O-DILAURYL-RAC-GLYCERO GLUTARIC ACID-(6′-METHYLRESORUFIN) ESTER (DGGR) LIPASE

BEATRIZ COSTA GAGO MENDOZA

ORIENTADOR:

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BEATRIZ COSTA GAGO MENDOZA

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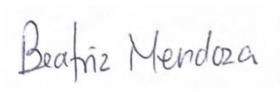

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O EFEITO DA PREDNISOLONA NO DOSEAMENTO DA 1,2-O-DILAURYL-RAC--GLYCERO GLUTARIC ACID-(6′-METHYLRESORUFIN) ESTER (DGGR) LIPASE EM CÃES

Resumo

1,2-o-dilauryl-rac-glycero glutaric acid-(6'-methylresorufin) ester (DGGR) lipase é um biomarcador recentemente disponível, que tem vindo a ser cada vez mais utilizado na exploração clínica de pancreatite em cães, sobretudo pelo seu custo acessível face à lipase pancreática específica *(cPLI)*. Foi demonstrada uma boa concordância entre a cPLI e a DGGR lipase. Estudos prévios avaliaram a influência da corticoterapia no doseamento de cPLI. Contudo, a influência na DGGR lipase ainda não é conhecida. Este estudo visa avaliar o efeito da prednisolona nos níveis sérios de DGGR lipase em cães.

Foi efetuado um estudo prospetivo de coorte, que incluiu a medição da DGGR lipase em dois grupos: o grupo de estudo (GE) constituído por cães aos quais foi administrada prednisolona por via oral com justificação médica na dose inicial de 0.5-1.7mg/kg/dia durante pelo menos 3 semanas e o grupo controlo (GC) composto por cães saudáveis sem tratamento concomitante. Como critério de inclusão consideraram-se cães com valores de DGGR lipase abaixo do valor de referência (<80 U/L). A DGGR lipase foi quantificada em três pontos temporais (Dia 0 (T0), Dia 7-10 (T1) e Dia 21-30 (T2)). A análise foi efetuada com recurso a um *kit* previamente validado (Randox® DGGR lipase). Foram incluídos 34 cães (17 cães em cada grupo, emparelhados relativamente ao género e idade). Em T0 não se observou diferença estatisticamente significativa entre grupos (p=0.868). A dose inicial média de prednisolona foi de 0.94 (±0.85) mg/kg/dia, tendo decrescido para 0.45 (±0.05) mg/kg/dia após T1. A concentração mediana de DGGR lipase no GE em cada ponto temporal (T0, T1 e T2) foi: 24.74 (14.45-31.48) U/L, 36.82 (23.8-80.16) U/L e 29.52 (15.91-48.48) U/L, respetivamente. Observou-se um efeito estatisticamente significativo da prednisolona nos valores de DGGR lipase ao longo de T0, T1 e T2 (p=0.007). Foi verificada uma baixa correlação entre as variações de DGGR lipase e a dose de prednisolona correspondente em T0-T1 e T1-T2 (r_s=0.371 e r_s=0.121, respetivamente). Em relação ao GC não se observaram diferenças estatisticamente significativas ao longo de T0, T1 e T2 (p=0.926).

Sugere-se que a DGGR lipase seja afetada pela administração oral de prednisolona por justificação médica. No entanto, como os valores permanecem abaixo do limite máximo considerado (160 U/L), esta variação não aparenta ser clinicamente relevante.

Palavras-chave: cão; lipase; pancreatite; corticosteróide; diagnóstico de laboratório

THE EFFECT OF PREDNISOLONE THERAPY ON CANINE SERUM LEVELS OF 1,2-O-DILAURYL-RAC-GLYCERO GLUTARIC ACID-(6′-METHYLRESORUFIN) ESTER (DGGR) LIPASE

Abstract

1,2-o-dilauryl-rac-glycero glutaric acid-(6'-methylresorufin) ester (DGGR) lipase is a widely available biomarker, increasingly used in the investigation of canine pancreatitis mainly due to its low cost compared to pancreatic lipase immunoreactivity (cPLI). A previous study showed a good agreement between cPLI and DGGR lipase concentration. While the effect of corticotherapy on cPLI quantification has been studied, its influence on DGGR lipase is unknown. This study aims to evaluate the effect of prednisolone therapy in canine DGGR lipase serum levels.

A prospective cohort study was conducted, including the measurement of DGGR lipase in two groups: the study group (SG) composed of dogs treated with oral prednisolone for a medical reason, at the initial dosage of 0.5-1.7 mg/kg/day for at least 3 weeks, and the control group (CG) composed of healthy untreated dogs. As an inclusion criterion, animals had basal DGGR lipase within the reference range (<80 U/L). DGGR lipase was measured at three time points (Day 0(T0), Day 7-10(T1), and Day 21-30(T2)) in both groups. The analysis was performed using a previously validated kit (Randox® DGGR lipase). Thirty-four dogs were included (17 dogs for each group, which were age and sex-matched). At T0, there was no significant difference in DGGR lipase concentrations between groups (p=0.868). Mean starting dosage of prednisolone was 0.94 (±0.85) mg/kg/day, decreasing to 0.45 (±0.05) mg/kg/day after T1.

The median DGGR lipase concentration in SG at each time point (T0, T1, and T2) was: 24.74 (14.45-31.48) U/L, 36.82 (23.8-80.16) U/L and 29.52 (15.91-48.48) U/L, respectively. There was a statistically significant effect of prednisolone on DGGR lipase values (p=0.007) over T0, T1, and T2. A poor correlation was verified between the variations of DGGR lipase and the correspondent prednisolone dosage of T0-T1 and T1-T2 (r_s=0.371 e r_s=0.121, respectively). In CG, DGGR lipase did not significantly change over the three time points (p=0.926).

This study suggests that DGGR lipase levels are affected by oral prednisolone therapy in dogs treated for a medical reason. However, as values remained below the considered significant upper limit (160 U/L), this variation does not seem to be clinically relevant.

Key-words: dog; lipase; pancreatitis; corticosteroid; laboratory diagnosis

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List of abbreviations and symbols

% - percentage

µg - microgram

1,2 DiG - 1,2 diglyceride

AI - activity index

ALT - alanine aminotransferase

A.m. - ante meridiem

CEIE - Comissão de Ética para a Investigação e Ensino

CG - control group

CI - chronicity index

cPL - canine pancreatic lipase

cPLI - canine pancreatic lipase immunoreactivity

CT - computed tomography

DGGR - 1,2-o-dilauryl-rac-glycero glutaric acid-(6′-methylresorufin) ester

ELISA - enzyme-linked immunosorbent assay

ENS - enteric nervous system

EPI - exocrine pancreatic insufficiency

FFAs - free fatty-acids

FMV-UL - Faculty of Veterinary Medicine - University of Lisbon

HAC - hyperadrenocorticism

ICC - intraclass correlation coefficient

IDIU - Infectious Diseases Isolation Unit

IF – intrinsic factor

IQR - interquartile range

Kcat - catalytic constant

kDa – kilodaltons

Km - Michelis constant

L - litter

MCS - mean cumulative severity

mg - milligram

min. - minutes

mRNA - messenger ribonucleic acid

nm - nanometre

p.m. - post meridiem

RIA - radioimmunoassay

rcf – relative centrifugal force

r_s - Spearman’s rank correlation coefficient

SD - standard deviation

SG - study group

Spec cPL - specific canine pancreatic lipase

Spec fPL - specific feline pancreatic lipase

TAP - trypsinogen activation peptide

TLI - trypsin-like immunoreactivity

U - units

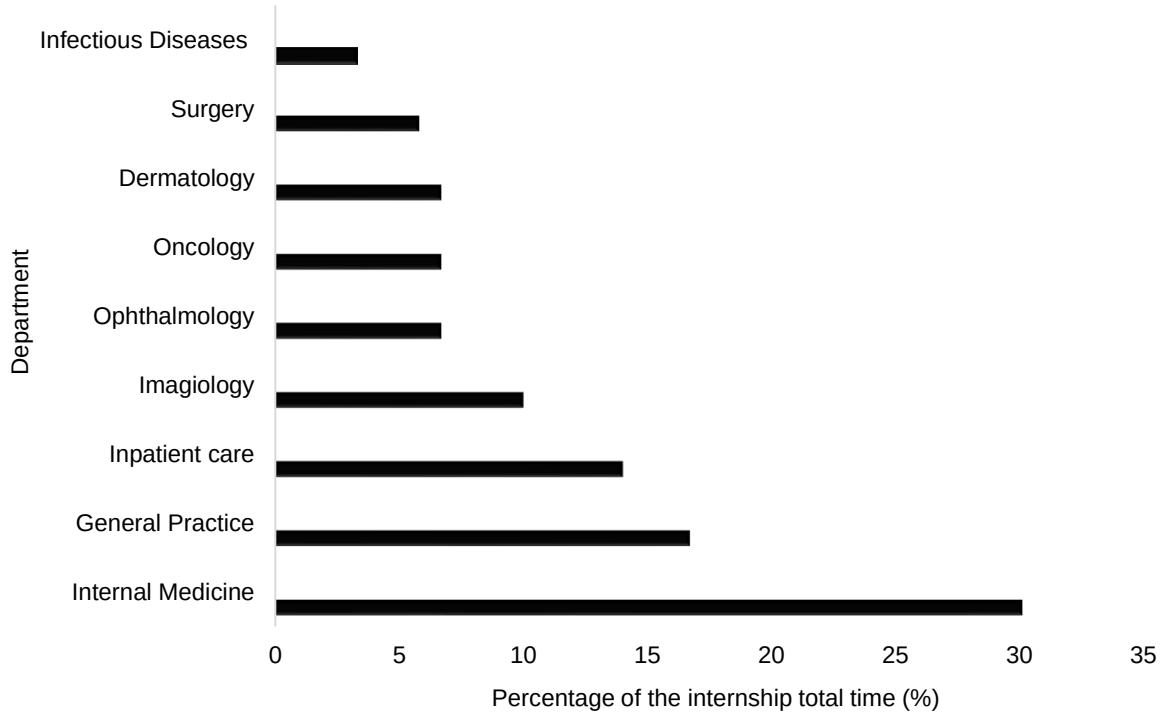
κ - Cohen's kappa coefficient

1. TRAINEESHIP REPORT

This report describes the work done during the curricular internship that took place from the 2nd September 2019 to the 28th February 2020 at the Veterinary Hospital of the Faculty of Veterinary Medicine - University of Lisbon. The activities were performed in a rotation basis across multiple departments such as internal medicine, ophthalmology, imagiology, surgery, dermatology, inpatient care, oncology, general practice, and infectious diseases under the supervision of the Veterinary Surgeon or the Veterinary Nurse on duty. During this period, there was an opportunity to participate in weekly journal clubs and staff seminars.

The time spent in each department varied in terms of the period of the day (day or night) as well as duration (from 8 to 12 hours a day). The internship had a duration of 6 months, totalling 1198 hours. The time spent in each department is depicted in graph 1.

Graph 1. Percentage of total time spent in each department.



Internal Medicine

Of all rotations, the one in internal medicine was the longest. This rotation was under the supervision of Professor Doctor Rodolfo Leal, the responsible for the department and board-certified specialist, who also supervised the project. This rotation was essential for the accomplishment of the current work since it allowed the recruitment of most animals enrolled in the study.

The daily routine started with medical rounds which usually took place around 8.30 a.m. focusing on the inpatient care animals in the presence of staff from several departments. At this stage, a detailed presentation of each animal was made by the night-shift clinician and the differential diagnosis was discussed. The entire group participated in the discussion of the complementary exams, surgeries, and treatments that needed to be performed during the day.

During the morning period, first-opinion, re-evaluation, referral, and second-opinion small-animal consultations were attended. Clinical cases were mostly from endocrinological, gastroenterological, nephrological, and respiratory diseases. Apart from Professor Rodolfo, once a week it was possible to attend consultations with the responsible intern of the internal medicine team (Dr. Joana Dias, Dr. Telmo Casimiro, or Dr. Sara Prata). Under supervision, it was possible to participate in the collection of prior clinical history and anamnesis of the patients as well as to perform a thorough physical examination. There was also the opportunity to participate in medical procedures such as blood sample collection, blood pressure measurements, and cystocentesis. Moreover, there was a chance to discuss the different possible approaches, differential diagnosis, and treatment options, which took place mainly in the afternoon. During this period, the results from the performed complementary exams were discussed and communicated to the owners. Also, it was possible to develop the skills required for the elaboration of a medical report, by actively taking place in this process. Complementary exams such as upper and lower gastrointestinal endoscopy, rhinoscopy, bronchoscopy with bronchoalveolar lavage, and bone marrow aspiration were performed about once a week.

Ophthalmology

The time spent with the ophthalmology team allowed the attendance of consultations and surgeries. The consultations were attended in both morning and afternoon periods over the entire week, while surgeries were attended during the morning periods over three defined days in each week.

During this rotation, the student had the opportunity to attend first-opinion, re-evaluation, referral, and second-opinion consultations of small animals, horses, and exotic animals as well. Moreover, it was possible to perform exams such as fundoscopic exam, Schirmer’s test, and fluorescein test. Also, peripheral intravenous catheterisations and blood collections were performed under supervision.

Additionally, several ophthalmological surgeries were attended, such as cataract surgery and enucleation.

Imagiology

The time spent in this rotation was divided into two periods: a week attending radiography and computed tomography (CT) exams and 2 weeks participating in ultrasonography exams. During this period, it was possible to improve skills related to exam positioning and image interpretation. The shifts had a duration of 8 hours and started at 9 a.m. or 2 p.m.

During the first week, which involved the attendance of radiography and CT exams, the student also assisted in various procedures such as myelography and contrast-CT. Moreover, all steps from anaesthetic induction to post-exam care were performed, under close supervision.

During the second period of 2 weeks, there was the opportunity to attend several ultrasonography exams as well as ultrasound guided percutaneous needle biopsies, cardiac and abdominal ultrasounds, and echo guided pericardiocentesis. Also, it was possible to perform ultrasound-guided cystocentesis and to train abdominal ultrasound under supervision.

General Practice

This was the second longest rotation of the internship. The shifts had a duration of 8 hours, starting at 8 a.m. or 1 p.m., depending on the week. During the morning shifts, it was possible to attend the medical round of the inpatient care animals. In this service, there was mainly the possibility of attending first-opinion and preventive-medicine consultations, which provided an excellent environment to improve communication skills and to perform medical procedures under supervision. During this period, it was possible to collect the prior clinical history, anamnesis, and to perform a good physical exam in most animals. During all the clinical cases there was the chance to discuss the different possible approaches with the several clinicians on duty, as well as the differential diagnosis, and treatment options. Some emergency procedures such as cardiorespiratory resuscitation were also assisted.

Infectious Diseases

During this rotation, the activities were developed in the Infectious Diseases Isolation Unit (IDIU) with afternoon shifts of 8 hours during a week. All the activities took place under the supervision of the responsible clinician on duty. The activities included clinical monitoring, medication preparation and administration, and the correct hygienization of contaminated infectious material. Moreover, it was possible to attend some infectious disease consultations and to participate in the clinical discussion of the differential diagnosis and treatment options.

Surgery

During this rotation, the scheduled surgeries took place from Monday to Friday during an 8 hour shift and emergency surgery took place whenever needed.

At the beginning of the shifts, which started at 8 a.m., the animals were admitted by the student, and a pre-surgery checklist was filled by asking some questions to the owners. During the surgery service, there was the opportunity to perform procedures such as peripheral venous catheterisation, pre-anaesthetic drugs preparation and administration, trichotomy and surgical asepsis, anaesthetic induction, animal intubation, animal transference onto the surgical table, monitoring, assistance in the surgical procedures, and post-surgical monitoring. These were performed under the supervision of the responsible surgeon or nurse.

In some cases, direct involvement during the surgery was not possible. Nonetheless, it was still feasible to observe and discuss with the surgeons the surgical techniques and other aspects regarding pre- and post-operative care. During this period, the attended surgeries included ovariohysterectomies, orchiectomies, caesarean sections with the respective neonatal reanimation, correction of brachycephalic syndrome, cystotomies, removal of mammary chains, among others.

Oncology

This rotation lasted two weeks, with shifts of 8 hours starting at 8 a.m. or 2 p.m. These weeks were focused on oncology consultations and chemotherapy sessions. Additionally, it was possible to attend first-opinion, re-evaluation, referral, and second-opinion small-animal consultations. During this period, prior clinical history and anamnesis were collected and the physical exam technique was improved. Under supervision, there was the opportunity of performing blood sample collections and some other procedures.

Dermatology

The period of 2 weeks spent in the dermatology department was mostly focused on attending first-opinion, re-evaluation, and second-opinion small-animal consultations. In addition to consultations, it was also possible to attend dermatology lectures. The 8 hour shift varied between morning and afternoon periods.

During this rotation, there was the opportunity to discuss the different possible approaches, differential diagnosis, and treatment options with the clinician on duty, performing some procedures such as blood collection, anamnesis, and physical exams. Also, skin biopsies and video otoscopies were attended.

Inpatient care

The time spent in this rotation was organised over the 6 month period including weekends with 12 hour day and night shifts. In this service, the shift started with a medical round followed by animal related activities.

During this period, there was the chance to follow the progress of the different clinical cases, participating in the animals' clinical monitorization, drug preparation, and administration according to the indications of the clinician and nurse on duty.

The evolution of the clinical condition was discussed with the clinician as well as the discharge plan for each animal. Concerning medical procedures and similarly to the remaining services, there was the possibility of performing blood sample collection, cystocentesis, and peripheral venous catheterisations. Some of the cases offered the opportunity to attend some emergency procedures such as cardiorespiratory resuscitation.

Scientific communications

An abstract with the preliminary results was submitted and presented as an oral communication at *Congresso Internacional Hospital Veterinário Montenegro,* in February 2020 (Annexe 1).

A poster with the results will be presented at the European College of Veterinary Internal Medicine – Companion Animals Congress, in September 2020 (Annexe 2).

A paper with the results has been accepted to the Journal of Veterinary Internal Medicine.

Apart from this study, a clinical case followed during the internal medicine rotation and concerning Shar-Pei fever was presented as a poster at *Congresso Internacional Hospital Veterinário Montenegro*, in February 2020 (Annexe 3).

2. LITERATURE REVIEW

2.1. Pancreas

The pancreas is located in the cranial abdomen, caudally to the stomach. It is composed of a left lobe positioned between the transverse colon and the greater curvature of the stomach, a right lobe adjacent to the proximal duodenum, and, at last, the body which is between the two lobes (Watson 2014, Evans 1993). It is composed of two different functional types of glandular tissue – the endocrine and exocrine. The endocrine portion is organised into discrete islets called islets of Langerhans, within the parenchyma, and makes up 2% (Evans 1993; Jubb and Stent 2015) of the pancreatic tissue. The exocrine portion comprises 98% of

the pancreatic tissue and its detailed anatomy and physiology are discussed in the following section (Evans 1993; Herdt and Sayegh 2013).

2.1.1. Exocrine pancreas

The pancreas is mostly composed of acinar tissue lacking a conventional capsule (Jubb and Stent 2015). This structure is characteristic of a typical acinar gland in which the acini are connected by a system of ducts (Herdt and Sayegh 2013). In most dogs, the smaller ducts of the pancreatic lobules coalesce in a structure to form two larger pancreatic ducts existing in the duodenum (Watson 2015). The exocrine pancreas is responsible for digestive secretions, bicarbonate, and intrinsic factor (IF) which are delivered into the intestinal lumen. The acinar cells produce the digestive enzymes, whereas centroacinar cells and duct cells secrete electrolyte solution rich in sodium bicarbonate (Herdt and Sayegh 2013) and intrinsic factor (Vaillant et al. 1990).

Some digestive enzymes can induce lesions to the pancreatic cells, and for this reason, they are synthesised as inactive precursors called zymogens (Herdt and Sayegh 2013). The zymogens are only activated in the intestinal lumen and are separated intracellularly from lysosomes (Herdt and Sayegh 2013). The zymogens of digestive enzymes are trypsinogen, chymotrypsinogen, proelastase, prophospholipase, kallikreinogen, and procarboxypeptidase (Steiner 2008). By contrast, other enzymes such as lipase, amylase, carboxylesterase, desoxyribonuclease, and ribonuclease are secreted in their active form (Steiner 2008).

Pancreatic secretion is, mainly, triggered by the presence of fat and proteins in the small intestinal lumen and involves the release of acetylcholine and hormones such as secretin and cholecystokinin (Watson 2014). There are nerve fibres from the enteric nervous system (ENS) ending close to the pancreatic acinar glands (Herdt and Sayegh 2013). These neurons will release acetylcholine by impulses arriving from other neurons of the ENS or by parasympathetic fibres from the vagus nerve (Herdt and Sayegh 2013). Cholecystokinin is the primary hormonal stimulus for acinar cells (Herdt and Sayegh 2013) and is synthesised by I cells located in the duodenum and jejunum (Rehfeld 2004). On the other hand, secretin is the primary hormonal stimulus for centroacinar and duct cells and is synthesised by S cells mostly located in the duodenum (Afroze et al. 2013). When these hormones interact with binding sites on the surfaces of pancreatic cells, secretion is stimulated (Herdt and Sayegh 2013).

All the digestive enzymes produced by the exocrine portion of the pancreas are crucial for the digestion of most nutrients. Although there is a complex structural and functional interrelationship between both endocrine and exocrine tissues, there is also an important interdependence between them (Jubb and Stent 2015).

The exocrine pancreas is considered a labile organ. It synthesises a large amount of protein and consumes the corresponding amount of precursor substrate (Jubb and Stent 2015). However, the homeostatic regulation of the pancreatic tissue continues to be poorly understood (Jubb and Stent 2015). It is known that there is evidence of trophic hormones from islets and that the response of exocrine pancreas to changes in nutrients intake is rapid and produces remarkable alterations in the composition of pancreatic secretion (Jubb and Stent 2015).

2.2. Pancreatitis and lipase

The pancreas is considered the only significant source of lipase (Watson 2014) and it was shown by Brobst et al. (1970)[1] (cited by Hoffmann 2008) that experimentally induced pancreatitis in dogs increases the serum activity of this enzyme (Hoffmann 2008). Thus, serum lipase has been a subject of discussion since it is considered a powerful tool to evaluate pancreatic lesions (Dröes and Tappin 2017). Considering pancreatitis as the most common exocrine pancreatic disease in both dogs and cats (Steiner 2008), it has become relevant to study its underlying pathophysiology and which risk factors should be considered relevant in the context of possible pancreatitis. Furthermore, this information integrated with the previous clinical history will help deciding the need of measuring a serum pancreatic biomarker such as lipase, which is secreted in its active form (Steiner 2008).

Trypsin is the major protease secreted by the pancreas. It is secreted as the zymogen trypsinogen and is central to the pathogenesis of pancreatitis. Inappropriate and prematurely intracellular activation of trypsinogen to trypsin, leads to activation of other zymogens such as proelastase, prophospholipase A2, and chymotrypsinogen, resulting in autodigestion and inflammation (Dröes and Tappin 2017). Trypsin activation within the acinar cells appears to be caused by blockage of the acinar cell apex, oxidative stress, or hypotension (Spillmann 2017).

Thus, under normal circumstances, self-defence mechanisms are in place to stop premature activation of trypsin (Jubb and Stent 2015; Watson 2015; Spillmann 2017). Therefore, the disruption of these mechanisms might underlie the causes of pancreatitis (Watson 2015). However, the causes are usually unknown, and several risk factors have been proposed (Spillmann 2017). It is documented that factors such as hypoxia, hypercalcemia, hyperlipidaemia, and other cellular injuries could affect trypsin activation control, making them

[1] Brobst D, Ferguson AB, Carter JM. 1970. Evaluation of serum amylase and lipase activity in experimentally induced pancreatitis in the dog. J Am Vet Med Assoc. 157(11):1697–702. url: https://pubmed.ncbi.nlm.nih.gov/5530372/

risk factors for pancreatitis (Jubb and Stent 2015). Other factors such as dietary indiscretion and overweight (Lem et al. 2008), zinc toxicosis (Mikszewski et al. 2003; Blundell and Adam 2012), and drugs are also documented. Some drugs have been reported to be associated with pancreatitis such as azathioprine (Moriello et al. 1987), potassium bromide with phenobarbitone (Gaskill and Cribb 2000), clomipramine (Kook et al. 2009) and sulfonamides (Trepanier et al. 2003), among others. Additionally, it is described that some breeds have a higher risk of developing pancreatitis. Cook et al. (1993)[1] and Hess et al. (1999)[2] (cited by Lem et al. 2008) identified miniature schnauzers, Yorkshires terriers, and other terriers as a group and Watson et al. (2007) documented Cavalier King Charles spaniels, cocker spaniels, collies, and boxers. Also, Pápa et al. (2011) reported that pancreatitis is common in poodles, Dachshunds, laika, and Alaskan malamute. Moreover, dogs with concurrent endocrine diseases such as diabetes mellitus or hypothyroidism may be at increased risk of acute pancreatic necrosis (Jubb and Stent 2015). On the other hand, it is discussable whether hyperadrenocorticism (HAC) is a predisposing factor for pancreatitis (Behrend 2015). At last, other factors such as acute enteritis or gastroenteritis (Rallis et al. 1996), abdominal trauma, infections such as *Babesia*, and any condition that causes hypotension such as anaesthesia have also been identified as potential risk factors for pancreatitis in dogs (Xenoulis and Steiner 2013b). However, it is important to note that some of the associations considered above were not based on studies using histopathology, the gold standard for pancreatitis diagnosis (Xenoulis 2015).

"Functional and structural changes in the normal pancreas are not self-initiated, but rather occur in response to systemic metabolic activity, although in practice the relationship between cause and effect is often obscure" (Jubb and Stent 2015, p.353). Therefore, the aetiology of acute and chronic pancreatitis remains idiopathic (Spillmann 2017), but the risk factors aforementioned together with a compatible clinical investigation should raise the suspicion of pancreatitis.

Despite the fact that the pancreas is the main significant source of lipase, it is nowadays recognised that its production is not limited to this organ. For instance, Simpson et al. (1991) evaluated the effect of pancreatectomy on plasma lipase activity and other biomarkers

[1] Cook AK, Breitschwerdt EB, Levine JF, Bunch SE, Linn LO. 1993. Risk factors associated with acute pancreatitis in dogs: 101 cases (1985-1990). J Am Vet Med Assoc. 203(5):673–9. url: https://pubmed.ncbi.nlm.nih.gov/8407536/

[2] Hess RS, Kass PH, Shofer FS, Winkle TJV, Washabau RJ. 1999. Evaluation of risk factors for fatal acute pancreatitis in dogs. J Am Vet Med Assoc. 214(1):46–51. url: https://pubmed.ncbi.nlm.nih.gov/9887939/

(Simpson et al. 1991). The authors observed that lipase values decreased after pancreatectomy, but these values were not significantly different from the pre-operative ones. Therefore, this study reinforced the fact that the pancreas is not the only source of serum lipase activity. Also, Steiner et al. (2006) observed no significant differences in the serum lipase activity between dogs with and without exocrine pancreatic insufficiency (EPI), further supporting the hypothesis that there is a significant portion of lipase from non-pancreatic origin. Apart from the pancreatic lipase, many other lipases have been described, such as gastric, hepatic, lipoprotein, hormone-sensitive, and other less prominent lipases (Steiner et al. 2002). Nonetheless, pancreatic lipase is still considered a useful biomarker, being the research focused on directing the measurement exclusively to a pancreatic origin.

2.3. Pancreatic biomarkers: state of the art

As pancreatic tissue is the source of several enzymes, their measurement is a logical approach to assess pancreatic disease (Simpson et al. 1991). These tests are based on the principle that different lesions will affect the levels of pancreatic enzymes leaking into the bloodstream. While acute lesions cause an increase in the levels of pancreatic enzymes, atrophy, which is associated with a chronic process, causes its decrease (Simpson et al. 1991). As pancreatitis can be present in both scenarios, it is difficult to distinguish in between these two possibilities by using this approach. Thus, it is important to note that such a distinction is only possible using histopathology (Xenoulis 2015). Acute pancreatitis is defined as a neutrophilic inflammation without fibrosis and exocrine atrophy (Newman et al. 2006). By contrast, the permanent histopathological changes such as fibrosis and acinar atrophy with mononuclear, often lymphocytic, inflammation are suggestive of chronic pancreatitis (Newman et al. 2004; Watson et al. 2007; Bostrom et al. 2012). The decreased leakage of pancreatic enzymes into the bloodstream is reflected by the lower correlation between some pancreatic serological markers and histopathologic features of chronic pancreatitis compared with histopathologic features of acute pancreatitis (Trivedi et al. 2011). However, the presence of atrophy, fibrosis, and hyperplastic nodules with acute lesions such as neutrophilic infiltration, pancreatic, and peripancreatic fat necrosis were also described. This suggests that some dogs presenting acute pancreatitis likely had previous episodes of pancreatitis or ongoing chronic pancreatitis (Neilson-Carley et al. 2011). Also, the presence of active pancreatic disease, such as pancreatic and peripancreatic fat necrosis, possibly contributes to the occurrence of clinical signs in chronic pancreatitis. On the other hand, the absence of active disease is less likely to develop clinical signs and might escape clinical diagnosis (Bostrom et al. 2012). While it has been traditionally accepted that acute pancreatitis is the most common manifestation in dogs, some results support that chronic pancreatitis is also common (Watson and Herrtage 2006;

Watson et al. 2007) and clinically significant in a referral population (Watson and Herrtage 2006), suggesting that it may be under-recognised or subclinical in some dogs (Xenoulis et al. 2008).

Despite the fact that there are no studies describing the pathophysiology of naturally occurring acute or chronic pancreatitis in dogs and cats, some authors discussed it based on findings from human and experimental animal work (Watson 2015). The relationship between acute and chronic pancreatitis is important since some chronic cases result from acute disease. However, it is unclear how many cases progress from acute to chronic disease or how many started due to chronic inflammation. This is the case in autoimmune chronic pancreatitis, which is caused by an unclear trigger and the role of an acute episode is still a hypothesis (Watson 2015).

The gold standard for the confirmation of pancreatitis and its definition as an acute or chronic disease is pancreatic histopathology (Xenoulis and Steiner 2013a). Nevertheless, it is still not routinely used since the sampling procedure presents several medical risks that should not be neglected (Dröes and Tappin 2017). Moreover, a single biopsy can be insufficient because the inflammation is usually non-uniform (Newman et al. 2004). For those reasons, results such as sensitivity and specificity should be interpreted with caution since they rely upon an imperfect gold standard (Xenoulis 2015). Therefore, in cases where there is not a reasonable reason to do a biopsy, other less invasive diagnostic tests may be considered. The digestive enzymes are the most commonly used biomarkers of pancreatic disease, namely pancreatitis (Ruaux 2003). However, several factors should be taken into consideration when considering the performance of these tests, since it relies upon different criteria used for diagnosis, type of pancreatitis and/or used cut-off values among studies (Xenoulis 2015). Table 1 provides an overview of selected studies evaluating the sensitivity and/or specificity of different laboratory tests for the diagnosis of pancreatitis in dogs, considering the different gold standards and the pancreatitis classifications.

Considering the pancreatic secretion and the pathophysiology of pancreatitis, trypsinogen, amylase, and lipase had been historically evoked as potential indicators of pancreatic inflammation. Based on the studies of Steiner et al. (2008), Watson et al. (2010), Trivedi et al. (2011) and McCord et al. (2012), the specificity and sensitivity of these tests are summarised in Table 1. As a result of the influence of histopathological lesions in enzyme leakage, it becomes important to refer to how these lesions have been classified in each study. The histopathologic characterisation, when considered, is based on a grading scheme for exocrine pancreatic lesions (Newman et al. 2006). This grading scheme comprises the

quantification and severity of lesions through the mean cumulative severity (MCS), which then allows calculating the activity index (AI) and the chronicity index (CI). The AI comprises processes involving necrosis or inflammation and the CI comprises processes more indicative of a chronic ongoing or previously resolved pancreatic lesion (Newman et al. 2006).

Regarding sensitivity, the results from a study with dogs that presented macroscopic evidence of pancreatitis documented a histologically acute mild to moderate pancreatitis in all the animals (Steiner et al. 2008). It is possible that the sensitivity would increase if there was a histological characterisation of more severe cases of pancreatitis. In another study, Watson et al. (2010) included dogs with histologically confirmed chronic pancreatitis, with severe and mild to moderate lesions associated, and the sensitivity for some serum markers was evaluated. Trivedi et al. (2011) encountered a group of dogs presenting, on the majority, histopathologic features of acute and chronic pancreatitis, concurrently. Therefore, the groups of acute and chronic pancreatitis were combined. The two formed groups were based on the MCS, and thus, the mild and moderate to severe pancreatitis groups were considered. The sensitivity and specificity were evaluated for several serum markers in dogs. At last, McCord et al. (2012) characterised a study group for acute pancreatitis not based on histopathology, but considering the medical record, laboratory findings, and ultrasound interpretation. However, a more recent analysis method has been used to evaluate unbiased parameters and calculate specificity and sensitivity.

2.3.1. Trypsin-like immunoreactivity (TLI)

TLI is an immunoassay that measures trypsinogen and, to a lesser degree, trypsin concentration in serum (Xenoulis 2015). Since serum TLI concentration in dogs increases after the experimental induction of pancreatitis but decreases more rapidly than amylase and lipase activities and present low sensitivity, it is considered a poor indicator of pancreatitis (Simpson et al. 1989; Mansfield and Jones 2000; Xenoulis 2015). The blood half-life of TLI has not been reported; however, it is likely short due to the rapid action of endopeptidases, whose main role is to inactivate pancreatic enzymes (Hoffmann and Solter 2008). Steiner et al. (2008) described a low sensitivity of 36.4% (Table 1) and no correlation with the AI for acute mild to moderate pancreatitis. On the other hand, Watson et al. (2010) described a sensitivity of 17% for chronic pancreatitis (Table 1). Trivedi et al. (2011) documented a sensitivity of 30% and 29%, for mild and moderate to severe pancreatitis, respectively (Table 1). In the same study, the reported specificity was 100%. However, this could be explained by the relatively low number of dogs without histopathologic evidence of pancreatitis.

Table 1. Comparison between the sensitivity and specificity of TLI, Amylase, 1,2 DiG lipase, cPLI, and specific canine pancreatic lipase (Spec cPL) reported in previous studies

Gold standard and pancreatitis classification	TLI		Amylase		1,2 DiG lipase		cPLI	Spec cPL				
	Sens. (%)	Spec. (%)	Sens. (%)	Spec. (%)	Sens. (%)	Spec. (%)	Sens. (%)	Sens. (%)		Spec. (%)		
								≥400 µg/l				
Clinical criteria Acute pancreatitis	-	-	-	-	-	-	-	70		77		**Haworth et al. (2014)**
								>200 µg/L	≥400 µg/L	>200 µg/L	≥400 µg/L	
Histopathology Pancreatitis	-	-	-	-	-	-	-	58	33	80	90	**Mansfield et al. (2012)**
Clinical criteria			>1240 U/L		>750 U/L			>200 µg/L	>400 µg/L	>200 µg/L	>400 µg/L	**McCord et al. (2012)**
Acute pancreatitis	-	-	52.7	79.1	47.2	91.4	-	86.5-93.6	71.7-77.8	66.3-77.0	80.5-88.0	
Histopathology										>200 µg/L	>400 µg/L	**Neilson-Carley et al. (2011)**
	-	-	-	-	-	-	-	-		95.0	97.5	
Histopathology	>35 µg/L		>1240 U/L		>750 U/L			>200 µg/L	>400 µg/L	> 200 µg/L	>400 µg/L	
Mild pancreatitis	30.0	100	7.0	100	54.0	43.0	-	43.0	21.0	86.0	100	**Trivedi et al. (2011)**
Moderate to severe pancreatitis	29.0		14.0		71.0		-	71.0	71.0			
Histopathology Chronic pancreatitis	>35 µg/l		>1126 U/L	>3378 U/L	>250 U/L	>750U/L		>102 µg/L	>200 µg/L			**Watson et al. (2010)**
	17	-	67	14 -	44	28 -		58	26	-	-	
Histopathology Acute mild-moderate pancreatitis	>35 µg/L		>1380 U/L		>691 U/L			>102 µg/L	>200 µg/L	>200 µg/L	>400 µg/L	**Steiner et al. (2008)**
	36.4	-	18.2	-	13.6	-		77.3	63.6	72.7	63.6	

Legend: 1,2 DiG, 1,2 diglyceride; cPLI, canine pancreatic lipase immunoreactivity; Sens., sensitivity; Spec., specificity; Spec cPL, specific canine pancreatic lipase; TLI, Trypsin-like immunoreactivity.

Nonetheless, it is used as a specific indicator of pancreatic exocrine insufficiency, as it is accepted that TLI is exclusive of pancreatic origin (Batt 1993). In agreement with these results, also Simpson et al. (1991) demonstrated that pancreatectomised dogs have markedly lower TLI concentrations. Thus, 100% of both sensitivity and specificity have been documented for EPI diagnosis (Williams and Batt 1988). The low sensitivity and the longer turnaround time for TLI compared to serum lipase and amylase have become TLI of little interest for pancreatitis evaluation in dogs (Hoffmann and Solter 2008).

Also, some investigators evaluated an enzyme linked immunosorbent assay for trypsinogen activation peptide (TAP). However, healthy dogs presented an unexpectedly broad range of TAP concentration detectable in the urine. Furthermore, plasma and urinary TAP concentrations were significantly increased only in dogs with severe and necrotizing pancreatitis (Mansfield and Jones 2000).

2.3.2. Amylase

Amylase is not exclusively of pancreatic origin, as it is also produced in the small intestine and liver (Hoffmann and Solter 2008). These findings are in agreement with previous studies conducted in pancreatectomised dogs from Simpson et al. (1991), where serum amylase activity results were consistent with non-pancreatic sources for amylase. Steiner et al. (2008) described a sensitivity of 18.2% (Steiner et al. 2008) (Table 1) and observed no correlation between AI and serum amylase activity. Concerning chronic pancreatitis, Watson et al. (2010) documented a sensitivity of 67% considering the reference interval of 167-1126 U/L, while a higher cut-off of 3378 U/L resulted in a sensitivity of 14% (Table 1). Trivedi et al. (2011) documented serum amylase as the least sensitive serological assay for the diagnosis of pancreatitis with a sensitivity of 7% for mild pancreatitis and 14% for moderate to severe pancreatitis (Table 1). The specificity described was 100% (Trivedi et al. 2011), considering the low number of dogs without histopathologic evidence of pancreatitis in this study (Table 1). McCord et al. (2012) documented 52.7% for sensitivity and 79.1% for specificity, emphasising the low performance of amylase (Table 1). Moreover, an older study reported that an increase in serum amylase activity could support a diagnosis of pancreatitis, but this was a poor indicator without the knowledge of lipase levels (Strombeck et al. 1981). Furthermore, it is described that amylase has the lowest sensitivity for mild or moderate to severe pancreatitis among pancreatic markers (Trivedi et al. 2011). For all reasons stated above, it should not be the first choice as a screening test (Kook 2017).

2.3.3. Lipase

As previously stated, lipase is mainly produced in the pancreas and its function is centred on triglycerides hydrolysis. Its sensitivity and specificity have also been described in the studies of Steiner et al. (2008), Watson et al. (2010), Trivedi et al. (2011), and McCord et al. (2012). These studies are all detailed in the following section. For a long time, amylase and lipase serum activities have been important tools for the diagnosis of pancreatitis.

Despite all extra-pancreatic sources, there is a specific pancreatic lipase that is of interest in the diagnosis of pancreatic disease. This molecular protein has 42 kDa and hydrolyses triglycerides at positions 1 and 3, releasing a monoglyceride. This lipase binds at the lipid-water interface emulsified in the presence of bile salts, calcium, and colipase (Hoffmann and Solter 2008).

Therefore, many studies have focused on developing more selective and accessible methods for the detection of lipase from a pancreatic origin. These assays are based on serum lipase activity and serum lipase immunoreactivity.

2.3.3.1. Serum lipase activity

Determination of serum lipase activity has been used for the diagnosis of pancreatitis in dogs for several decades (Steiner et al. 2006). Lipases hydrolyse lipids, such as triglycerides, which are apolar. As these lipids are important for long-term energy storage, they are converted into polar products, allowing their transport in and out of cells. As a result, there are many different lipases in the organism responsible for hydrolysing apolar triglycerides, which share functional and structural features (Steiner 2017).

Although the variety of existing methodologies rely on the detection of different substrates, many of these present low specificities. Thus, other enzymes from non-pancreatic origin may contribute to the detected lipolytic activity (Ruaux 2003).

Several methods have been developed for the measurement of lipolytic activity such as the titrimetric (Tietz and Fiereck 1966), turbidimetric (Burlina and Galzigna 1973; Orda et al. 1984), nephelometric (Wagner and Macy 1982) and colorimetric (Fossati et al. 1992) methods. The titrimetric method measures the neutralised free fatty-acids (FFAs), while the colorimetric method evaluates the coloured end product after hydrolysis. The nephelometric and turbidimetric methods measure, respectively, the light scattered and transmitted (Hasan et al. 2009; Encyclopaedia Britannica 2017). However, titrimetric, turbidimetric, and nephelometric methods presented enough limitations to be later replaced by other methods

(Fossati et al. 1992). Therefore, the colorimetric method is the current most utilised serum lipase activity quantification technique.

2.3.1.1.1. Colorimetric method

Around thirty years ago, a rapid and easy colorimetric method, based on the principle of Imamura et al. (1989)[1] (cited by Fossati et al. 1992), was validated for assaying lipase activity in human serum.

In veterinary medicine, concerning canine serum lipase assessment, a modification of the colorimetric method for automated analysers was compared to titrimetric method (Walter et al. 1992). The authors have shown that this is a reliable indicator of serum lipase activity in dogs, making it an attractive alternative to the titrimetric procedure (Walter et al. 1992). The determined reference range was 90-527 U/L (Walter et al. 1992). A few years later, Mackenzie et al. (1996) achieved similar results using a different automated analyser and determined a similar reference range (0-561 U/L). The results supported the utility of the colorimetric method as a reliable indicator of serum lipase activity in dogs. This methodology uses the natural long-chain fatty acid 1,2-diglyceride (1,2 DiG), a clear substrate that is hydrolysed into 2-monoglyceride by lipase in the presence of colipase, deoxycholate, and calcium ions (Figure 1) (Fossati et al. 1992; Walter et al. 1992). A sequence of enzymatic reactions with the 2-monoglyceride lipase, glycerol kinase, glycerol phosphate oxidase, and peroxidase, produce a violet quinone monoimine dye (Figure 1) with peak absorption at 550 nm (Fossati et al. 1992; Walter et al. 1992). The rate of increase in light absorbance is directly proportional to the lipase activity in the serum (Walter et al. 1992). The bile salts (deoxycholate) and colipase are responsible for preventing the rapid and irreversible inactivation of lipase. The calcium ions are added to increase the interaction of lipase and the substrate surface and to stabilize the enzyme's active site (Fossati et al. 1992).

[1] Imamura S, Hirayama T, Arai T, et al. 1989. An Enzymatic Method Using 1,2-Diglyceride for Pancreatic Lipase in Serum. Clin Chem. 35(6): 1126,1989.

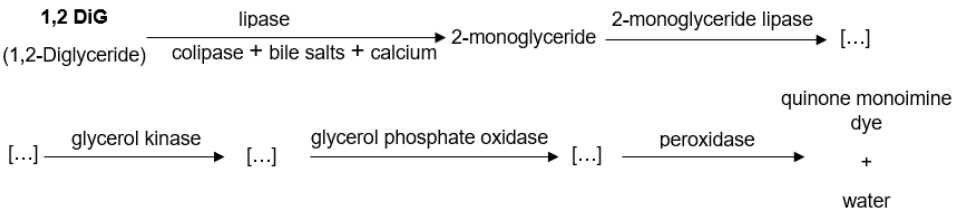


Figure 1. Reaction sequence of 1,2 DiG lipase method (Adapted from Fossati et al. 1992).

Legend: […], chemical reaction steps not included in the figure.

The specificity of the colorimetric assay for pancreatic lipase in human serum was attributed to 1,2 DiG and both colipase and deoxycholate, which initiate the conversion of the diglyceride to monoglyceride while inhibiting the hepatic and lipoprotein lipases (Fossati et al. 1992; Mackenzie et al. 1996).

Several studies have evaluated the specificity and sensitivity of these assays (Table 1 and 2). In studies where the clinical criteria were considered the gold standard, specificity achieved the highest values ranging from 73% to 91.4% (Graca et al. 2005; McCord et al. 2012), while Trivedi et al. (2011) who considered histopathology as the gold standard, reported a specificity of 43% (Table 1).

Table 2. Comparison between the sensitivity and specificity of Lipase, 1,2 DiG, DGGR lipase, and Spec cPL reported in previous studies

Gold standard and pancreatitis classification	1,2 DiG lipase				DGGR lipase				Spec cPL		
	Sens. (%)		Spec. (%)		Sens. (%)		Spec. (%)		Sens. (%)	Spec. (%)	
					> 216 U/L				≥400 µg/L		
Clinical criteria Acute pancreatitis	-		-		85.7 – 90.9		64.0 – 74.3		81.0-90.9	74.1-81.1	**Cridge et al. (2018)**
					> 245 U/L				>400 µg/L		
Histopathology Chronic pancreatitis	-		-		57				42		
							100			100	**Goodband et al. (2018)**
Acute pancreatitis					0				33		
	>500 U/L	>699 U/L	>500 U/L	>699 U/L	>120 U/L	>180 U/L	>120 U/L	>180 U/L			
Clinical criteria Acute pancreatitis	73.3	60.0	73.0	73.0	93.3	73.3	53.3	66.6	-	-	**Graca et al. (2005)**

Legend: 1,2 DiG, 1,2 diglyceride; DGGR lipase, 1,2-o-dilauryl-rac-glycero glutaric acid-(6′-methylresorufin) ester lipase; Sens., sensitivity; Spec., specificity; Spec cPL, specific canine pancreatic lipase.

Concerning lipase sensitivity, the lowest values were documented in studies using animals with histopathologic chronic pancreatitis (Watson et al. 2010) and mild to moderate pancreatitis diagnosed (Steiner et al. 2008) (Table 1). Watson et al. (2010) documented a sensitivity of 44% and 28%, considering the different cut-offs of 250 U/L and 750 U/L, respectively (Table 1). Steiner et al. (2008) described a lower sensitivity of 13.6% but described a significant correlation between AI and serum lipase activity (Table 1).

Graca et al. (2005), who diagnosed pancreatitis based on clinical criteria, reported different sensitivities depending on the cut-off used, which was determined to be 73.3% for 500 U/L and 60% for 699 U/L (Table 1). Trivedi et al. (2011) found that 1,2 DiG lipase had the highest sensitivity when using a cut-off of 750 U/L, followed by Spec cPL (Kook 2017) (Table 1). However, the high sensitivity range of 54-71% should be interpreted with caution due to the nature of the study and the presence of dogs with extra-pancreatic disease. On the other hand, McCord et al. (2012), which used the clinical criteria as the gold standard, estimated a lower sensitivity of 47.2%.

However, it is recognised that human and canine patients can have increased serum lipase activity due to extra-pancreatic illness. On the other hand, this parameter can be normal in dogs that do have pancreatitis (Steiner 2003). Despite the fact that many assays have relied on the measurement of serum lipase activity, it is believed that “the 1,2-diglyceride assay is not useful for diagnosing pancreatitis in dogs and that usage of this assay most likely has contributed to the generally poor perception of traditional catalytic lipase assays” (Kook et al. 2014, p.867).

1,2 DiG was the most used substrate, at least until 2005, when a new and stable assay based on the use of 1,2-o-dilauryl-rac-glycero glutaric acid-(6′-methylresorufin) ester (DGGR) lipase was described (Graca et al. 2005). The findings from this work suggest that the DGGR lipase method is more specific than the 1,2 DiG method for the detection of pancreatic lipase activity in dogs (Graca et al. 2005). So, over the last 15 years, this substrate has been used.

More recently, a point of care colorimetric assay has been described, the FUJI DRI-CHEM SLIDE LIP-P. It uses triolein as a substrate and negatively charged detergent as an auxiliary agent (Ishioka et al. 2011). Currently, there are different available substrates for lipase assays, but 1,2 DiG and DGGR are the most utilised ones (Dröes and Tappin 2017).

2.3.3.2. Serum lipase immunoreactivity

The lipases in circulation share the same substrate specificity, but it is known that the lipase produced in the pancreas is antigenically and structurally distinct from the others (Ruaux

2003). In dogs, a single gene encoding for a pancreatic lipase has been identified (Mickel et al. 1989[1] cited by Hoffmann and Solter 2008). Apart from this, only one protein of 50.7 kDa showed homology with classical pancreatic lipases from other species, which was recovered from dog pancreas by affinity purification (Steiner and Williams 2002). Also, it was shown through immunolocalization that canine pancreatic lipase (cPL) is exclusively expressed in pancreatic acinar cells, suggesting that cPL is a specific marker for pancreatic acinar cells (Steiner et al. 2002).

A radioimmunoassay (RIA) was developed for measuring canine pancreatic lipase immunoreactivity (cPLI) (Steiner and Williams 2003). RIA allows the measurement of the concentration of a specific analyte, while lipase activity measures the function of an analyte. Thus, theoretically, cPLI should only be increased during pancreatic inflammation. This method is well suited for this purpose but presented some disadvantages for clinical use (Steiner et al. 2003). Therefore, it was replaced by a quantitative enzyme-linked immunosorbent assay (ELISA) for the measurement of cPLI, and a reference interval from 2.2 to 102.1 µg/L was established in clinically healthy dogs (Steiner et al. 2003). Steiner et al. (2006) showed that cPLI is derived only from the exocrine pancreas. Using dual monoclonal antibodies for capture and detection, the cPLI assay was then developed into specific canine pancreatic lipase (Spec cPL) assay, which is suitable for commercial application and provides rapid results (Huth et al. 2010). Spec cPL values below 200 µg/L are considered normal, while values above 400 µg/L are considered highly suggestive of pancreatitis. The range from 200 to 400 µg/L is considered a *grey zone* (Steiner et al. 2008; Dröes and Tappin 2017).

The sensitivity of cPLI in dogs with macroscopic evidence of pancreatitis was documented as 77.3% for values higher than the upper limit of the reference range (102 µg/L) and 63.6% for the suggested cut-off of pancreatitis (200 µg/L) (Steiner et al. 2008) (Table 1). Concerning the Spec cPL, the sensitivity for values above the upper limit of the reference range (200 µg/L) was 72.7% and 63.6% for the suggested cut-off of pancreatitis (400 µg/L) (Table 1). However, in this study, most of the considered animals had mild pancreatitis as judged by histologic AI scores. Concerning the influence of chronic histopathological features, Watson et al. (2010) also described the sensitivity for cPLI in dogs, which ranged from 58% (>102 µg/L) to 26% (>200 µg/L) (Table 1). The higher correlation with the AI score described by Steiner et

[1] Mickel FS, Weidenbach F, Swarovsky B, LaForge KS, Scheele GA. 1989. Structure of the canine pancreatic lipase gene. J Biological Chem. 264(22):12895–901. url: https://pubmed.ncbi.nlm.nih.gov/2502543/

al. (2008) combined with these findings from Watson et al. (2010) suggests that the Spec cPL may be more useful for the identification of dogs with acute pancreatitis than with chronic pancreatitis (Trivedi et al. 2011).

According to Trivedi et al. (2011), Spec cPL presented a good overall performance as a pancreatic biomarker. The sensitivity for mild to severe pancreatitis ranged from 43.0-71.0% (>200 µg/L) to 21.0-71.0% (>400 µg/L) (Table 1) and the specificity ranged from 86.0% (>200 µg/L) to 100% (>400 µg/L) (Table 1). Still, the specificity in this study might be partially attributable to a small population of healthy animals (n=7). However, these findings are in agreement with the results from Neilson-Carley et al. (2011) where the documented specificity was 97.5% using a cut-off of 400 µg/L (Table 1). This high specificity documented in both studies suggests that this test has a low rate of false positives (Trivedi et al. 2011).

Mansfield et al. (2012) also described the specificity and sensitivity of the Spec cPL assay in a sample population of sick dogs but using histopathology as the gold standard for diagnosis. Although the nature of the study has preselected dogs with severe disease, the sensitivity was low and ranged from 33% (≥400 µg/l) to 58% (>200 µg/l), which is less than the other studies (Table 1). However, this aspect could also be due to the very small number of animals with true disease. The specificity identified was similar to previous studies with histopathologic based diagnosis (Neilson-Carley et al. 2011) (Table 1).

With the objective of developing an in-clinic rapid test, a point-of-care semiquantitative assay (SNAP® cPL) was developed using the same antibodies for pancreatic lipase (Beall et al. 2011). A negative result corresponds to Spec cPL below 200 µg/L, while a positive result corresponds to Spec cPL above 200 µg/L. The results from this test are well correlated to Spec cPL results (Beall et al. 2011). The colour intensity of a spot is determined by the observer and compared to a reference spot, being the results lighter than (200 µg/L), equal to (200-400 µg/L) or darker than (400 µg/L) the reference spot (Dröes and Tappin 2017). McCord et al. (2012) reported a sensitivity of 91.5-94.1% and a specificity of 71.1-77.5% for SNAP® cPL (Table 3). Regarding the Spec cPL, the sensitivity was 86.5-93.6% (>200 µg/L) and 71.7-77.8% (>400 µg/L), while the specificity was 66.3-77.0% (>200 µg/L) and 80.5-88.0% (>400 µg/L) (McCord et al. 2012). In another study, lower specificities were described, probably due to an exclusive population of sick dogs with similar signals and without a histologic diagnosis (Haworth et al. 2014) (Table 3). However, the sensitivity of 70% for Spec cPL was consistent with other studies (Table 1) and SNAP® presented a sensitivity of 82% (Haworth et al. 2014). Recently, Cridge et al. (2018) documented sensitivity and specificity for the SNAP® cPL of 73.9-100% and 71.1-

77.8%, respectively (Table 3). Concerning the Spec cPL, it registered a sensitivity of 81.0-90.9% and a specificity of 74.1-81.1% (Cridge et al. 2018) (Table 3).

Table 3. Comparison between the sensitivity and specificity of SNAP® cPL and Spec cPL, reported in previous studies

Gold standard and pancreatitis classification	SNAP® cPL		Spec cPL				
	Sens. (%)	Spec. (%)	Sens. (%)		Spec. (%)		
			≥400 µg/L				
Clinical criteria Acute pancreatitis	73.9-100	71.1-77.8	81.0-90.9		74.1-81.1		**Cridge et al. (2018)**
Clinical criteria Acute pancreatitis	82.0	59.0	>400 µg/L				**Haworth et al. (2014)**
			70.0		77.0		
Clinical criteria			>200 µg/L	>400 µg/L	>200 µg/L	>400 µg/L	**McCord et al. (2012)**
Acute pancreatitis	91.5-94.1	71.1-77.5	86.5-93.6	71.7-77.8	66.3-77.0	80.5-88.0	

Legend: SNAP® cPL, SNAP® canine Pancreatic Lipase; Sens., sensitivity; Spec., specificity; Spec cPL, specific canine pancreatic lipase.

Therefore, the studies mentioned above are in agreement regarding the performance of the SNAP® cPL tests (McCord et al. 2012; Haworth et al. 2014; Cridge et al. 2018). Also, it is known that SNAP® and Spec cPL have a higher sensitivity than measurement of total lipase for the diagnosis of clinical acute pancreatitis (McCord et al. 2012). Although there is a good agreement between SNAP® cPL and Spec cPL (Haworth et al. 2014; Cridge et al. 2018), there is also some degree of discordance in dogs without clinical acute pancreatitis presenting positive SNAP® results with Spec cPL lower than 200 µg/L (McCord et al. 2012; Haworth et al. 2014; Cridge et al. 2018).

A negative SNAP® cPL result is useful to rule out pancreatitis when there are suggestive clinical signs, while a positive result is highly suggestive of pancreatitis (Steiner 2017). Still, the collection of a serum sample to quantitatively measure the cPL is recommended in order the allow monitoring disease progression (Steiner 2017).

Recently, an in-house semiquantitaive assay, the VetScan cPL Rapid Test, was developed, giving a rapid point-of-care numerical result rather than a binary one (Cridge et al. 2018). However, this test failed basic analytical validation when performed in a research laboratory under non-clinical circumstances (Steiner and Lidbury 2018; Steiner et al. 2019). Moreover, lack of linearity, precision, and reproducibility was reported for another in-house

quantitative pancreatic lipase assay denominated Vcheck cPL (Steiner et al. 2018). These tests were compared and the results were unsatisfactory under clinical circumstances, suggesting that additional improvements are needed before clinical utilisation (Cridge, Mackin, et al. 2020).

Despite the fact that cPLI exhibits increased specificity compared to other tests, its technical complexity compared to serum lipase activity quantification complicates its adoption as a standard diagnostic tool (Ruaux 2003; Graca et al. 2005).

2.4. DGGR lipase

The DGGR lipase method uses DGGR as the substrate for the chromogenic substrate technology. A human assay based on DGGR as substrate for pancreatic lipase activity was proposed (Panteghini et al. 2001). This method is based on the principle that cleavage of DGGR, a glycerol-derived compound, results in the formation of dicarbonic acid ester, that due to its instability is hydrolysed under alkaline pH to yield glutaric acid and methylresorufin (Figure 2). The methylresorufin is a bluish-purple chromophore with peak absorption at 580 nm, and its formation rate is directly proportional to the lipase activity (Panteghini et al. 2001). As in the 1,2 DiG method, the colipase, bile salts, and calcium aim to provide optimal reactivity and specificity (Figure 2).

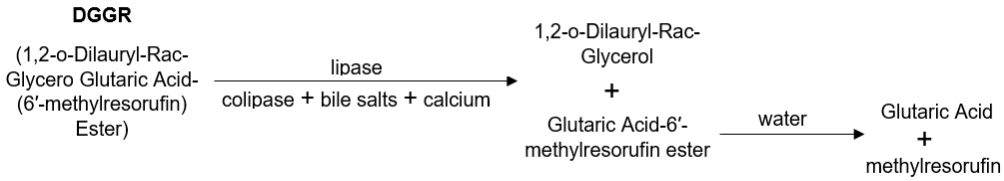


Figure 2. Reaction sequence of DGGR lipase method (Adapted from Panteghini et al. 2001).

This method was validated for use in dogs using an automatic analyser, when a reference interval of 8-120 U/L was also defined, and the performance evaluated (Graca et al. 2005). Later, it was also validated for use in cats (Oppliger et al. 2013). An advantage of this method is the suggested selectivity of the substrate (Graca et al. 2005), which provides a more specific methodology compared to 1,2 DiG for the detection of pancreatic lipase activity in dogs (Graca et al. 2005). Also, a study of dogs and cats comparing 1,2 DiG assay with the DGGR lipase assay reported significantly lower values of canine lipase measured by DGGR lipase than by 1,2 DiG (Papasouliotis et al. 2008)**.** The characteristics responsible for the higher selectivity are the higher catalytic constant (Kcat) and the lower Michelis constant (Km) compared to the 1,2 DiG technique (Graca 2005; Nelson and Cox 2008). The ratio (Kcat/Km),

usually referred to as the specificity constant, suggests that the enzyme and substrate interactions are more selective (Nelson and Cox 2008).

Depending on the cut-off, the sensitivities of 93.3% (>120 U/L) and 73.3% (>180 U/L) and specificities of 53.3% (>120 U/L) and 66.6% (>180 U/L) were obtained (Graca et al. 2005), (Table 2). However, the diagnosis was based on history, physical examination, and ultrasound findings (Graca et al. 2005). The surprising low specificity of 53.3% could be due to the influence of the chosen gold standard for diagnostic, which was not histopathology but clinical criteria and may have been insufficient to confirm or rule out acute pancreatitis. Moreover, it is possible that extra-pancreatic lipases and esterases may have reacted with the substrate or that the small sample influences the results. Nonetheless, the study had an acceptable correlation coefficient (Spearman's rank correlation (r_s)= 0.84) for the two methods (DGRR and 1,2 DiG) and DGGR presented a higher sensitivity compared to the 1,2 DiG method, despite the unexpected low specificity. Therefore, it was indicated as a reliable method for lipase determination in dogs, as the higher sensitivity makes it preferable as a screening test and raises the possibility of adopting a single pancreatic test routinely accessible (Graca et al. 2005).

2.4.1. DGGR lipase and Spec cPL: What do we know?

Despite the continuous progress of clinical research towards the development of a better pancreatic biomarker, currently there is no blood test 100% accurate for the diagnosis of pancreatitis (Goodband et al. 2018). However, at present, Spec cPL is considered the most sensitive and specific test for diagnosing pancreatitis (Neilson-Carley et al. 2011; Trivedi et al. 2011; McCord et al. 2012). A recent study conducted by Kook et al. (2014) showed a high agreement (Cohen's kappa coefficient (κ)= 0.803) of the DGGR lipase assay and Spec cPL. Also, in the same study, it was considered, for the first time, a twofold DGGR lipase *grey zone*, supported by the analogously high intraindividual variabilities described for Spec cPL (Carney et al. 2011; Kook et al. 2014). Regarding its correlation with abdominal ultrasound, Kook et al. (2014) also showed a slight to fair agreement between pancreatic ultrasonography results and DGGR lipase results in dogs.

To clarify and to explore the specificity of this method, the DGGR lipase was measured in dogs with EPI and compared to healthy dogs (Steiner et al. 2015). Despite the fact that dogs with EPI had a significantly lower serum lipase activity, 69% had serum lipase activities within the considered reference range (31-174 U/L). These findings suggest that DGGR is not exclusively hydrolysed by pancreatic lipase, making DGGR assays not specific for this enzyme. Also, recently, Lim et al. (2020) showed the influence of DGGR lipase activity by

hepatic and/or lipoprotein lipases and concluded that DGGR lipase assay is not specific for feline and canine pancreatic lipases. Conversely, it was shown that Spec cPL and specific feline pancreatic lipase (Spec fPL) concentrations are analytically specific for pancreatic lipase (Lim et al. 2020).

The previous study concerning DGGR lipase specificity has lacked a histological diagnosis for pancreatitis in dogs (Graca et al. 2015). However, in a recent small and preliminary study, DGGR lipase was evaluated and validated based on histopathology as the gold standard and a reference interval of 23-245 U/L was considered (Goodband et al. 2018). In this study, also DGGR lipase and Spec cPL showed good agreement with a κ of 0.679. The three cases of acute pancreatitis enrolled in the study presented DGGR lipase within the reference interval, leading to zero sensitivity, while Spec cPL (>400 µg/L) presented a sensitivity of 33% (Table 2). Regarding the eight cases of chronic pancreatitis, the sensitivity was 57% and 42% for DGGR lipase and Spec cPL, respectively (Table 2). The specificity of the study was 100% for both. Despite the small number of cases and all the related limitations, this study showed that DGGR and Spec cPL have similar sensitivity and specificity for the diagnosis of acute and chronic pancreatitis. Even considering that DGGR is not exclusively hydrolysed by pancreatic lipase (Steiner et al. 2015), this study still suggests that it is more specific than 1,2 DiG assay (Goodband et al. 2018). Also, Cridge et al. (2018) reported a good agreement (ICC (intraclass correlation coefficient) = 0.89) between the DGGR lipase and Spec cPL. Values under 140 U/L were considered to be within the reference limits, while values within the interval 141-216 U/L were considered to be equivocal, and values above 216 U/L supportive of a pancreatitis diagnosis. The poor specificity of 64.0-74.3% supports the idea that this test is not specific for pancreatic lipase. Moreover, the similar sensitivity compared to Spec cPL (Table 2) suggests again that it may be more appropriate as a screening assay (Cridge et al. 2018), emphasising that positive results must be confirmed with a more specific test such as cPLI (Graca et al. 2005).

In sum, the DGGR lipase presents advantages such as the lower cost and a shorter protocol duration, estimated in one hour (Kook et al. 2014), being a reliable alternative to Spec cPL (Goodband et al. 2018).

2.5. Pancreatitis diagnosis: the importance of biomarkers

The accepted definitive diagnosis of pancreatitis remains histopathologic, but in the absence of practical and relevant reasons to obtain biopsies, a clinical diagnosis is established (Graca et al. 2005; McCord et al. 2012; Haworth 2014; Cridge et al. 2018). This is possible using the new serum diagnostic assays in association with data from clinicopathologic

abnormalities and pancreatic ultrasound. It is also important to note that even in the presence of clinical signs, the abdominal ultrasound performance without a concurrent quantitative pancreatic lipase assay is considered anecdotal by some authors (Cridge, Sullivant, et al. 2020).

Regarding abdominal ultrasound, Steiner et al. (2008) have reported a sensitivity of 66.7%. However, the AI index was higher for dogs where abdominal ultrasonography was performed, suggesting that it is more sensitive in dogs with more severe pancreatic pathology. Recently, Cridge, Sullivant, et al. (2020) described the association between abdominal ultrasound findings, the Spec cPL, clinical severity indices, and clinical diagnosis in dogs with pancreatitis. These authors reported that ultrasonographic evidence of pancreatitis has a weak correlation with Spec cPL, and a moderate correlation with the clinical diagnosis of pancreatitis. These results indicate that ultrasonographic evidence of pancreatitis is neither indicative of pancreatic lipase levels above the reference interval, nor a clinical diagnosis of pancreatitis. Thus, these authors recommend the use of a quantitative pancreatic lipase assay. Also, no significant association between the ultrasonographic assessment of severity of pancreatitis and modified clinical severity indices was observed, suggesting that abdominal ultrasound is a poor indicator of the severity of pancreatitis. The sensitivity ranged from 42% to 89% and specificity ranged from 43% to 92%, depending on the number of the ultrasonographic features considered. These results indicate that while it does not provide a definitive diagnosis, it can assist in the classification of equivocal cases.

The clinical diagnosis supported by the serum lipase assays has become a focus of interest, considering the challenging and definitively antemortem diagnosis of pancreatitis.

2.6. Disease interference on lipase - clinical relevance

The use of a diagnostic test involves several assumptions to support the interpretation. In the context of lipase, it is assumed to be pancreatic specific, to have a constant rate of synthesis and clearance, and no significant re-absorption from other sites in the gastrointestinal tract (Ruaux 2003). Since lipase secretion is not exclusively from pancreatic origin (Simpson et al. 1989), diseases of other organs must be considered when high serum lipase activity is detected. Lipase clearance is mainly renal, meaning that glomerular function must be considered. Moreover, despite the fact that gastrointestinal mucosa is relatively impermeable to digestive enzymes, chronic gastroenteritis seems to contribute to changes in the enzyme's circulation (Ruaux 2003).

Strombeck et al. (1981) described that the reduced clearance of extra-pancreatic lipase could interfere with the results. Since then, higher cut-offs have been considered to support

pancreatitis diagnosis, in order to increase lipase activity specificity (Xenoulis 2015). However, it has been shown that even those increases can result from non-pancreatic disorders (Mansfield and Jones 2000).

In dogs, it is known that serum pancreatic lipase is removed from circulation by glomerular filtration. Studies are, however, non-consensual. Indeed, it was previously suggested that naturally occurring kidney disease could induce an elevated lipase activity (Polzin et al. 1983[1] cited by Watson 2010). However, in a more recent study, experimentally induced chronic renal disease did not increase 1,2 DiG lipase nor clinically relevant cPLI (Steiner et al. 2010; Xenoulis and Steiner 2012). Also, the experimental acute kidney injury did not seem to affect the 1,2 DiG lipase or Spec cPL (Hulsebosch et al. 2016). So, while some studies reported an influence of renal disease on lipase activity (Strombeck et al. 1981; Mansfield and Jones 2000), others did not (Steiner et al. 2010; Hulsebosch et al. 2016). Concerning the Spec cPL in dogs, there are studies considering experimental kidney injury (Steiner et al. 2010; Hulsebosch et al. 2016), but it has not been verified yet in dogs with naturally occurring renal disease (Haworth 2014).

Hepatic disease has also been attributed as a cause of an increase in serum lipase activity (Strombeck 1981; Quigley et al. 2001). However, pancreatic lipase is specific for long-chain fatty esters of glycerol, while hepatic lipase is more specific for short-chain fatty acids. For this reason, the use of 1,2 DiG is supposed to limit the amount of hepatic lipase detected, although the detection of other enzymes is still possible (Quigley et al. 2001). Also, positive cPLI results have been reported in dogs with hepatopathies (Mansfield et al. 2012; McCord et al. 2012).

Other conditions such as gastroenteritis have also been attributed as a cause for increased serum lipase activity (Rallis et al. 1996). This can be due to the production of lipase by non-pancreatic organs, or due to duodenal reflux causing subclinical pancreatitis (Haworth 2014). In yet another study, using a group of 36 dogs with upper gastrointestinal foreign bodies, 13 had Spec cPL above 400 µg/L (Trehy et al. 2014), suggesting that it could be derived from a secondary pancreatopathy or an increased leakage of immunoassay positive lipase (Cridge et al. 2018).

[1] Polzin DJ, Osborne CA, Stevens JB, Hayden DW. 1983. Serum amylase and lipase activities in dogs with chronic primary renal failure. Am J Vet Res. 44(3):404–10. url: https://pubmed.ncbi.nlm.nih.gov/6188392/

Dogs with the naturally occurring canine HAC showed Spec cPL concentrations with significantly higher values (>400 µg/L) and more positive SNAP® results than healthy dogs (Mawby et al. 2014). This association could reflect vacuolar hepatopathy secondary to HAC. However, a standardized diagnostic evaluation for pancreatitis was not used and as such, the predisposition of chronic steroid excess from HAC to subclinical pancreatitis remains unclear (Behrend 2015). Also, even if Hess (1999)[1] (cited by Mawby et al. 2014) has considered HAC as a risk factor to pancreatitis, half of the dogs used in this study received medical management for HAC before developing pancreatitis.

Regarding the influence of endogenous hypercortisolemia on DGGR lipase, it has also been observed that the DGGR lipase can be higher in approximately half of the dogs with hyperadrenocorticism (Bennaim et al. 2018). With the aim of evaluating this observation, Linari et al. (2019) reported DGGR lipase concentrations above the reference interval (10-130 U/L) in 63.2% of the dogs with hypercortisolism. In this study, dogs with hypercortisolism were included only if they had an absence of clinical and ultrasonographic signs suggestive of acute pancreatitis.

All these different conditions should be considered when interpreting the lipase results since they interfere with the assumptions of the basis of a diagnostic test.

2.7. Corticotherapy in veterinary medicine

Glucocorticoids are among the most frequently used drugs in veterinary medicine. The therapeutic application is based on the goal to control the process that is activated in response to a disease. For this purpose, the clinician should identify the intended action before starting therapy (Reusch 2015). Glucocorticoids can be used as a physiological replacement, suppression of inflammation or immune system, and other actions such as in neurological or neoplastic disorders. A given dosage may be only approximated to the guideline since glucocorticoid sensitivity differs between individuals (Reusch 2015).

Prednisolone is currently one of the most prescribed glucocorticoids in veterinary clinician practice. Being an intermediate-acting glucocorticoid, oral prednisolone is the treatment of choice for replacement, not emergent cases use of anti-inflammatory and immunosuppressive therapies (Reusch 2015). Its structure reveals an increase of anti-inflammatory effect and a reduction of mineralocorticoid activity compared with cortisone and cortisol (Reusch 2015). Regarding glucocorticoid reduction protocols, prednisolone is the

[1] Hess RS, Kass PH, Shofer FS, Winkle TJV, Washabau RJ. 1999. Evaluation of risk factors for fatal acute pancreatitis in dogs. J Am Vet Med Assoc. 214(1):46–51. url:https://pubmed.ncbi.nlm.nih.gov/9887939/

preferred one for alternate-day therapy. Therefore, it enables accurate dose titration and the possibility of removal in case of adverse effect (Reusch 2015).

2.7.1. Corticotherapy and pancreatic biomarkers: Is there any interference?

In a study in which animals did not present any histological pancreatic damage, Parent (1982)[1] (cited by Steiner and Williams 2003) attributed the increase in serum lipase activity to administration of prednisone or dexamethasone (Reusch 2015).

Regarding cPLI, a study using oral prednisone (2.2 mg/kg/day) for 4 weeks in dogs with stable X-linked hereditary nephritis, showed no influence on cPLI concentrations (Steiner et al. 2009). The mean serum cPLI concentration did not differ significantly over time, between pre-treatment or at the end of the four week treatment. However, the duration of steroid administration could have been insufficient to result in altered cPLI concentrations (Mawby et al. 2014). Conversely, when there is HAC, because the disease develops insidiously, it is likely that dogs had been experiencing steroids longer than one month (Mawby et al. 2014). On the other hand, when dogs with immune-mediated disease were treated with 2.0-2.2 mg/kg/day oral prednisolone as initial treatment with a subsequent reduction, different results were obtained (Ohta et al. 2017). From the ten dogs used in the study, three had borderline results (201.0-399.0 µg/L) and five abnormal concentrations (≥400 µg/L). Possible reasons for the increased cPLI included: underlying disorder, different bioavailability related to prednisone/prednisolone, diversity in the non-standardised dosage, and duration of treatment. Furthermore, neither a histopathological examination nor an abdominal ultrasound of the pancreas was performed after prednisolone administration.

Regarding the effects of immunosuppressive subcutaneous prednisolone (4 mg/kg twice per day) for two or three weeks on cPLI in healthy dogs, a significant increase in the serum cPLI concentration was observed (Ohta et al. 2018). However, most observed increases remained under 200 µg/L and only one dog, from the six in the study, registered an abnormal value (≥400 µg/L). In this study, pancreatic histological analysis performed before and after prednisolone administration did not reveal abnormalities. Also, expression of the gene encoding for pancreatic lipase, before and after prednisolone administration, was not detected in the liver (Ohta et al. 2018). In addition, the serum lipase activity was measured using FUJI

[1] Parent J. 1982. Effects of dexamethasone on pancreatic tissue and on serum amylase and lipase activities in dogs. J Am Vet Med Assoc. 180(7):743–6. url:https://pubmed.ncbi.nlm.nih.gov/6177674/

DRI-CHEM system and the results showed an increase in lipase activity, but these were not statistically significant.

More studies are definitely needed in order to understand how the corticosteroids affect lipase production and to clarify which lipase quantifications methods are influenced by corticotherapy.

3. THE EFFECT OF PREDNISOLONE THERAPY ON CANINE SERUM LEVELS OF DGGR LIPASE

3.1. Introduction and objectives

DGGR lipase has become an interesting option for small animal clinicians due to its attractive practical characteristics and the reported reliability (Graca et al. 2005; Goodband et al. 2018). Furthermore, the agreement with the known most specific pancreatic lipase assay makes it a potential test, widely available, and less expensive that can support a clinical diagnosis of pancreatitis (Kook et al. 2014). For the above mentioned reasons, DGGR lipase has been increasingly used in Europe. However, it is important to understand whether this parameter is affected by concurrent conditions. Considering corticotherapy as a common day-to-day treatment, the possibility of this treatment influencing DGGR lipase remains unclear. While prednisolone and prednisone therapy does not seem to affect, at least above 400 µg/L, serum cPLI quantification in healthy dogs (Ohta et al. 2018) or with X-linked hereditary nephritis (Steiner et al. 2009), dogs with immune-mediated disease presented values above 400 µg/L (Ohta et al. 2017). The influence of prednisolone on DGGR lipase is currently unknown. This study aimed to evaluate the influence of prednisolone on DGGR lipase. It is hypothesized that the effect of prednisolone (0.5-2.0 mg/kg/day) on DGGR lipase would not be clinically relevant.

3.2. Material and methods

A prospective cohort study was conducted including two groups: a study group (SG) and a control group (CG). This study was approved by the local welfare and ethical committee known as *Comissão de Ética para a Investigação e Ensino* (CEIE) and authorised by all owners (Annexes 4 and 5).

3.2.1. Sample population

3.2.1.1. Study Group

The SG consisted of dogs submitted to prednisolone therapy for at least 3-4 weeks. From August 2019 to February 2020, dogs presented to consultation at the Veterinary Hospital of FMV-UL (Faculty of Veterinary Medicine - University of Lisbon) and that required prednisolone therapy for a medical reason were recruited.

As inclusion criteria, the animals must have had the basal DGGR lipase value within the considered reference range (< 80 U/L) (Lucibello 2017) and been diagnosed with a disease that justified the onset of oral prednisolone at the initial dosage of 0.5-2.0 mg/kg/day over, at least, 21 days. Dogs were excluded if DGGR lipase was above the considered reference range (80 U/L) (Lucibello 2017), if they received steroids over the previous 4 weeks or if they were in poor clinical condition or in which vital prognosis was engaged.

3.2.1.2. Control Group

The control group was composed of healthy dogs from private owners selected from September 2019 to February 2020 at the Veterinary Hospital of FMV-UL. Only healthy dogs without concurrent diseases were included in the study. As inclusion criteria, the animals must have had the basal DGGR lipase value within the considered reference range (<80 U/L) (Lucibello 2017) and no medications (namely steroids) were allowed over the previous 4 weeks. The CG and SG were age and sex-matched.

3.2.2. Measurement of DGGR lipase

Serum samples in the SG were collected at the time of diagnosis (day 0/ T0) before the onset of prednisolone therapy. The following collections were taken at day 7-10 (referred to as T1), and day 21-30 (referred to as T2), corresponding to 7-10 and 21-30 days after the beginning of prednisolone therapy. From the CG, the collection was performed at the same time points, starting at T0 (according to the owner's availability), T1 (7-10 days later), and T2 (21-30 days later). Serum samples were obtained by centrifugation at 559 rcf for 10 min and stored at 4ºC when analysed on the same day, or at -20ºC when analysed within more than 24 hours.

Overall, DGGR lipase was measured at T0, T1, and T2 in both groups (Figure 3). The analysis was performed using a previously validated kit for use in dogs (Randox® DGGR lipase, LI 3837) (Lucibello 2017). It was performed by trained clinical pathologists who performed the DGGR lipase measurement in accordance with the manufacturer's guidelines at the *Laboratório Prof. Doutor M. Braço Forte* at FMV-UL.

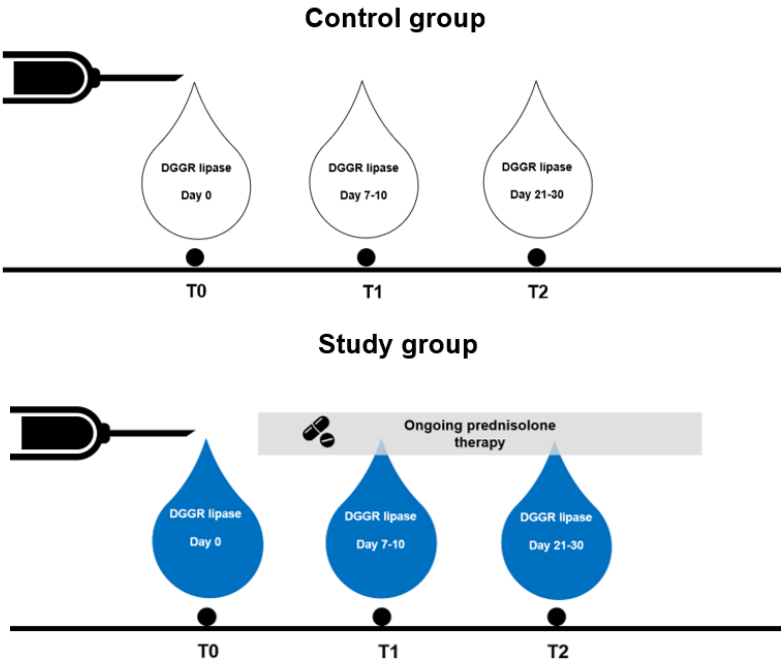


Figure 3. Study design scheme.

3.2.3. Reference interval and *grey zone*

A reference interval under 80 U/L, previously validated in dogs (Lucibello 2017) was considered. Recognising a possible intraindividual variability in serum lipase activity in healthy dogs (which leads to values outside the reference range) as well as the impossibility to rule out transient mild pancreatitis in clinically healthy dogs, following Kook et al. (2014), a twofold *grey zone* (80-160 U/L) was established (Kook et al. 2014; Kook 2017).

3.2.4. Prednisolone treatment

Dogs from the SG were treated with oral prednisolone at the initial dosage of 0.5-2.0 mg/kg/day followed by the clinically justified reduction protocol over the subsequent 21-30 days (Reusch 2015). On T1, the DGGR lipase concentration corresponded to the ongoing initial dosage and on T2 to the ongoing reduced dosage.

3.2.5. Abdominal Ultrasound

In both SG and CG, an abdominal ultrasound was considered in dogs showing an increase of DGGR lipase over 160 U/L during the study.

3.2.6. Statistical analysis

All the collected data were recorded, descriptive statistics calculated, and figures created using Microsoft Office Excel 2016. The statistical tests were implemented using the

commercial statistical software IBM® SPSS® Statistics version 26. A p-value<0.05 was considered significant for all the tests. The data were tested for normality using the Shapiro-Wilk test.

Statistical differences concerning sex and age between SG and CG were determined. The continuous variable (age) was compared by Student's t-test. The nominal variable (sex) was compared using the Pearson's Chi-squared test.

The values of DGGR lipase concentration over T0, T1, and T2 were analysed for both SG and CG. Mean serum DGGR lipase concentrations for the three time points were compared using a repeated-measures ANOVA, which considered the Mauchly's Test for Sphericity and the Greenhouse-Geisser correction. Post-hoc analysis considered Bonferroni post hoc test.

The values of DGGR lipase concentration over T0, T1, and T2 were compared between groups using the Mann–Whitney U-test.

To determine the correlation between DGGR lipase and prednisolone dosage variations, the Spearman's rank correlation coefficient (r_s) was used.

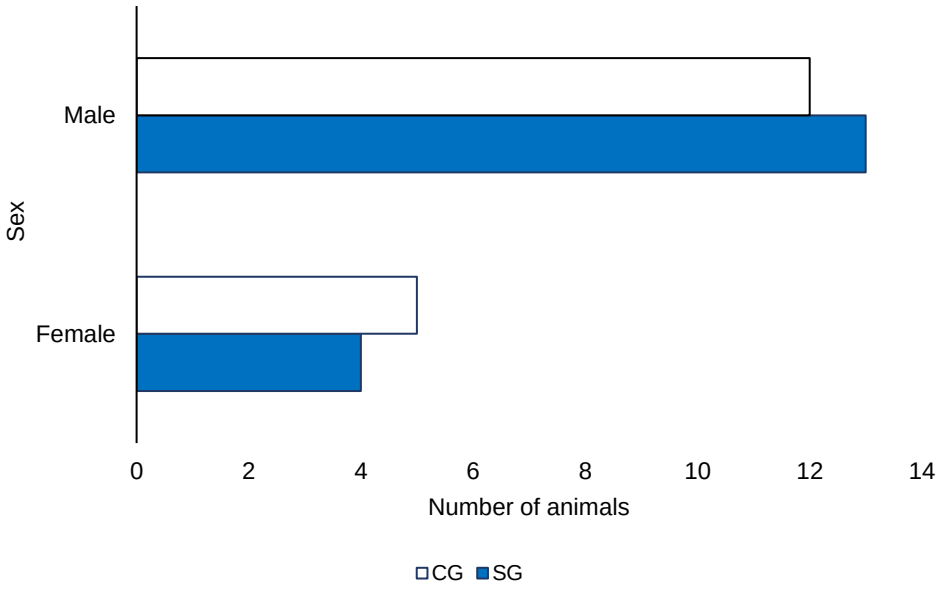
3.3. Results

3.3.1. Sample characterisation

3.3.1.1. SG

From August 2019 to February 2020, at the Veterinary Hospital of FMV-UL, 17 dogs from private owners, were included in the study group. The SG group consisted of 4 (24%) females, between 6 and 10 years old, and 13 (76%) males, between 1 and 13 years old (Graph 2). The age of the study group presented a mean (± standard deviation (SD)) age of 6.71 (± 3.86) years (Graph 3). The breed characterisation is described in Table 4 and the disease characterisation which justified the onset of prednisolone is described in Table 5.

Graph 2. Gender characterisation of the SG and CG



Graph 3. Ages of the SG and CG

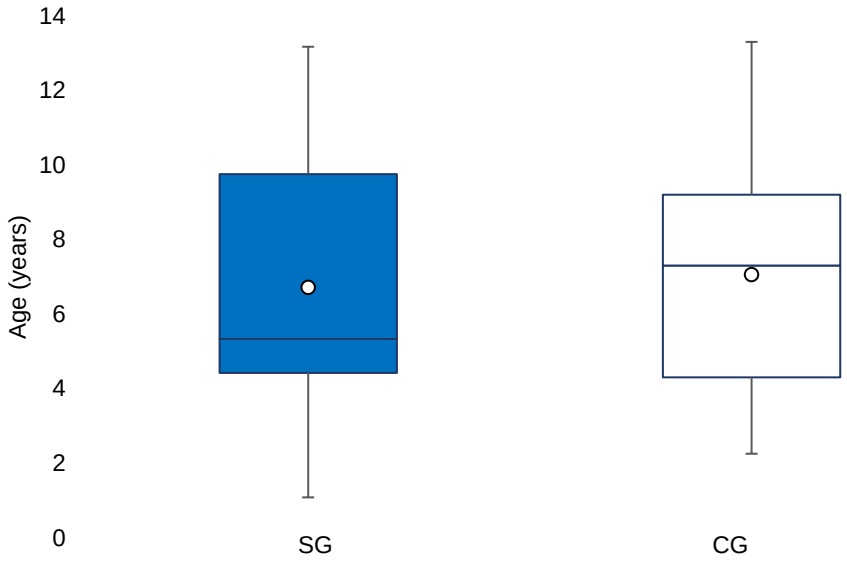


Table 4. Breed characterisation of the SG

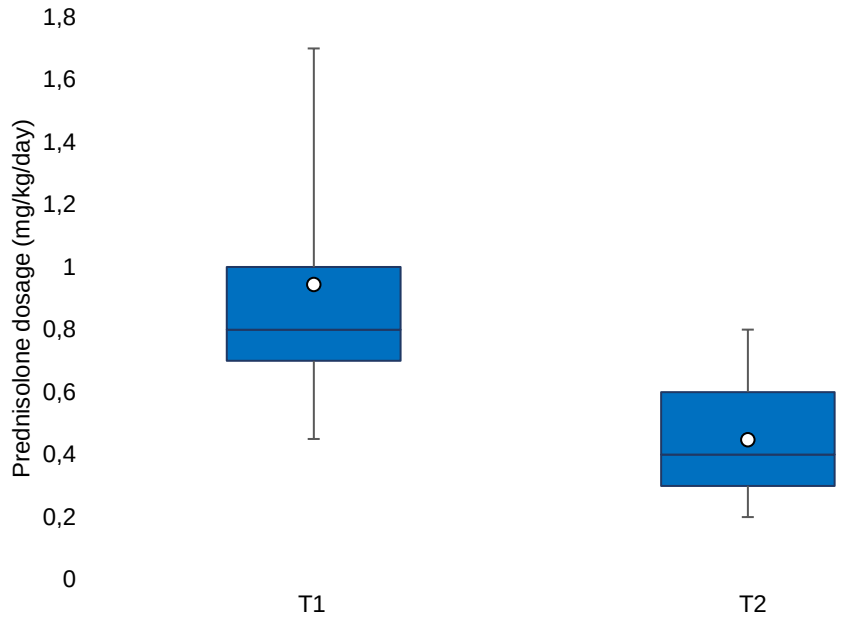
Breed	n (%)
Yorkshire terrier	3 (17.6%)
Labrador retriever	2 (11.8%)
Golden retriever	1 (5.9%)
Pit bull terrier	1 (5.9%)
Whippet	1 (5.9%)
Parson terrier	1 (5.9%)
Akita Inu	1 (5.9%)
Mixed breed	1 (5.9%)
Chihuahua	1 (5.9%)
Poodle	1 (5.9%)
Spinone Italiano	1 (5.9%)
German spitz	1 (5.9%)
Boxer	1 (5.9%)
Miniature pinscher	1 (5.9%)

Table 5. Disease characterisation of the SG

Disease	n (%)
Chronic tracheobronchitis	8 (47.1%)
Steroid-responsive enteropathy	5 (29.4%)
Ophthalmological disease	2 (11.8%)
Immune-mediated polyarthritis	1 (5.9%)
Intervertebral disc disease	1 (5.9%)

The mean dosage of prednisolone was 0.94 (±0.85) mg/kg/day, decreasing to a mean of 0.45 (±0.05) mg/kg/day after T1 (Graph 4).

Graph 4. Prednisolone dosage on T1 and T2 of the SG



3.3.1.2. CG

The control group was composed of 17 healthy dogs from the medical staff and students of the Veterinary Hospital FMV-UL, selected from August 2019 to February 2020. This group consisted of 5 (29%) females, between 7 and 10 years old, and 12 (71%) males, between 2 and 13 years old (Graph 2 and 3). The age presented a mean of 7.05 (± 3.18) years old (Graph 3). The breed characterisation is described in Table 6. There were no statistical differences between the age (p=0.781) and sex (p=0.697) of SG and CG, making the two groups age and sex-matched.

Table 6. Breed characterisation of the CG

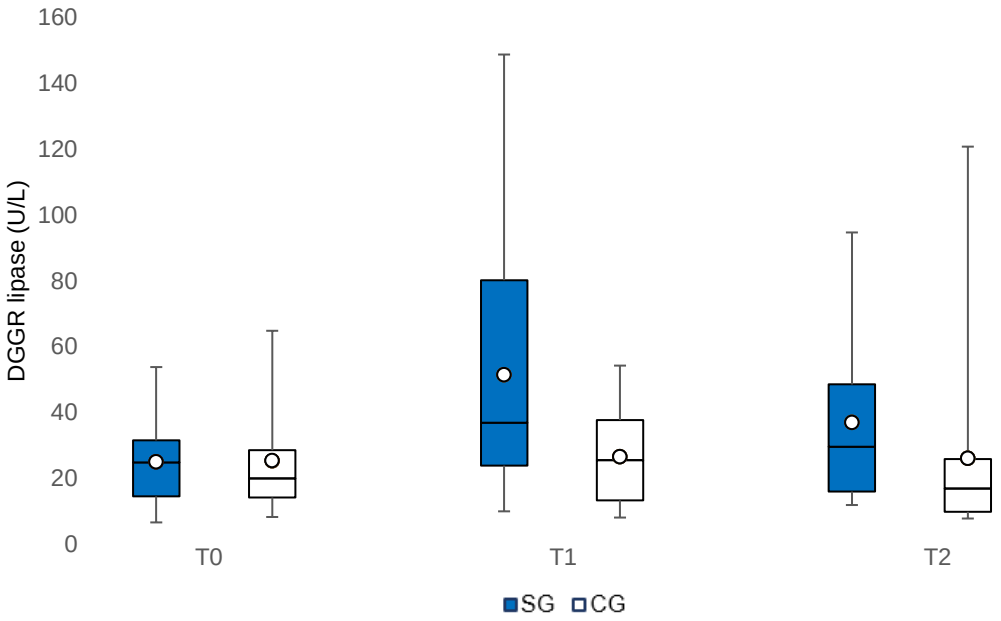
Breed	n (%)
Mixed breed	7 (41.2%)
Dachshund	3 (17.6%)
Yorkshire terrier	2 (11.8%)
Shetland sheepdog	1 (5.9%)
Jack Russel terrier	1 (5.9%)
Great Dane	1 (5.9%)
West Highland white terrier	1 (5.9%)
Labrador retriever	1 (5.9%)

3.3.2. DGGR lipase over time

The median (IQR (interquartile range)) value of DGGR lipase in the SG at each time point (T0, T1, and T2) was: 24.74 (14.45-31.48) U/L, 36.82 (23.8-80.16) U/L and 29.52 (15.91-48.48) U/L, respectively (Graph 5). There was a statistically significant effect of prednisolone on DGGR lipase values (F(1.119,17.901)= 8.903, p=0.007). The median value of DGGR lipase increased from T0 to T1 and decreased from T1 to T2 with statistically significant difference (p=0.022 and p=0.019, respectively). From T0 to T2, the median value increased, but the difference was not statistically different (p=0.068).

For the CG, the median DGGR lipase at each time point (T0, T1, and T2) was: 19.91 (14.12-28.5) U/L, 25.46 (13.25-37.64) U/L and 16.84 (9.78-25.8) U/L, respectively (Graph 5). The mean serum DGGR lipase concentrations did not differ significantly over time (F(1.311,20.980)= 0.025, p=0.926).

Graph 5. DGGR lipase over T0, T1 and T2 of the CG and SG



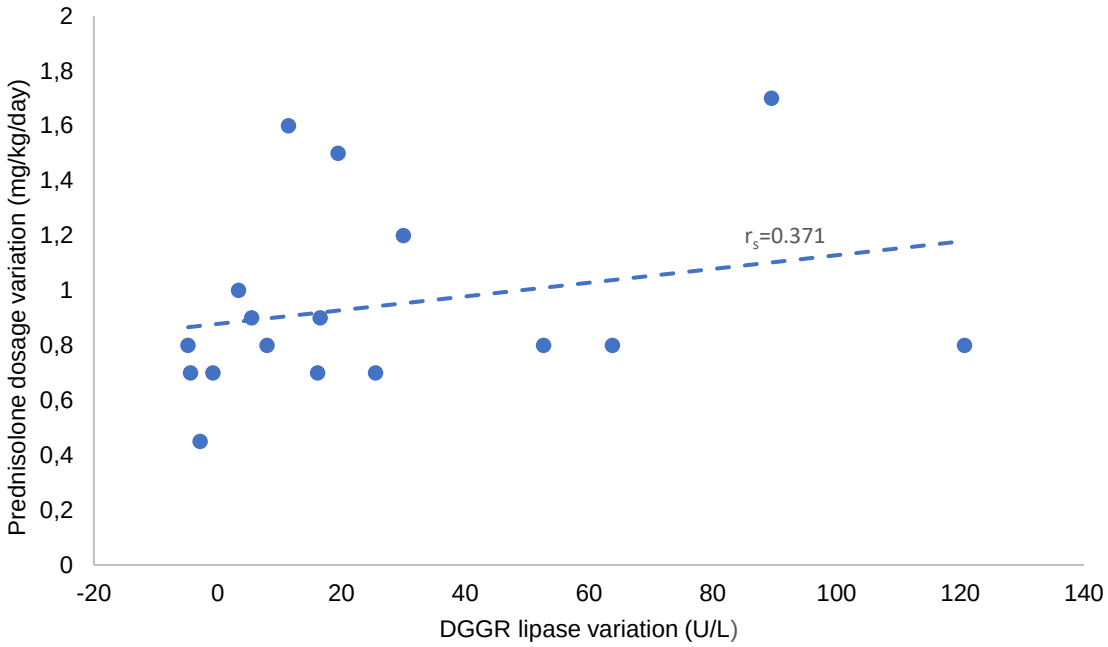
3.3.3. DGGR lipase comparison between SG and CG

At T0 and T1, the values of DGGR lipase in the SG were not significantly different from the CG (p=0.708 and p=0.099, respectively). Conversely, at T2, the values of DGGR lipase in the SG were significantly different from the CG (p=0.045).

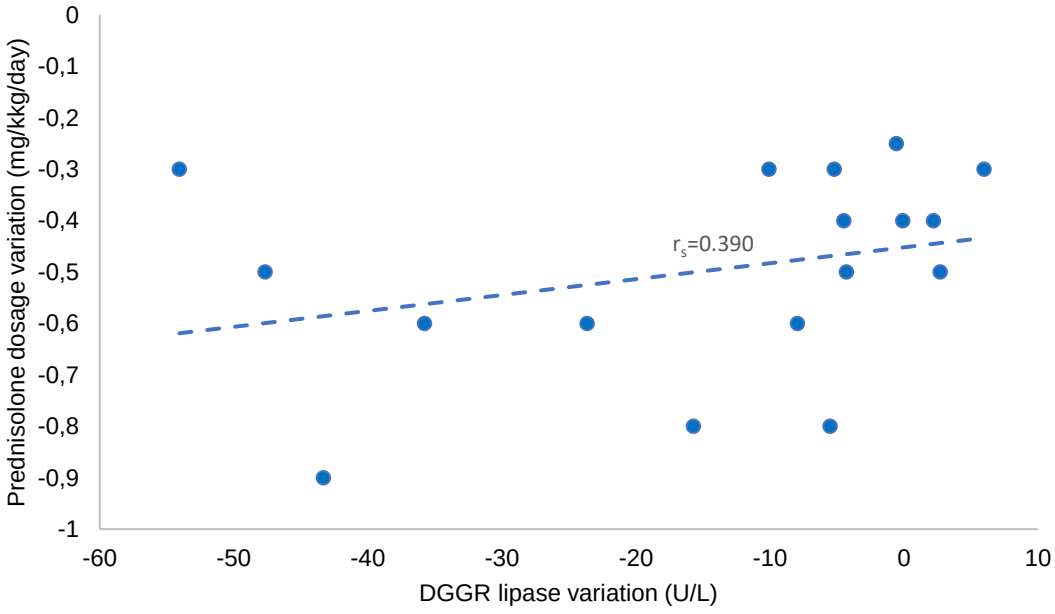
3.3.4. Correlation between DGGR lipase and prednisolone dosage variations

The variation in lipase concentration was not significantly correlated with the variation in prednisolone dosage at T0-T1 (r_s=0.371, p=0.143) and T1-T2 (r_s=0.390, p=0.121) (Graphs 6 and 7, respectively).

Graph 6. Correlation of DGGR lipase and prednisolone variation on T0-T1



Graph 7. Correlation of DGGR lipase and prednisolone variation on T1-T2



3.3.5 Abdominal ultrasound

Because there were no values above 160 U/L, the upper limit of the considered *grey zone*, abdominal ultrasound was not performed in any of these dogs.

3.4. Discussion

This study shows that prednisolone therapy can induce DGGR lipase changes, but that these effects are not significant for clinical practice. Therefore, it clarifies the usefulness of DGGR lipase in a dog under prednisolone therapy.

Canine pancreatitis is a serious disease in which the definitive diagnosis is still challenging. In fact, clinical signs are non-specific, and the historic lipase and amylase enzyme assays present a poor performance, reason why they are not routinely recommended (Steiner et al. 2008; Watson et al. 2010; Trivedi et al. 2011; McCord et al. 2012). Therefore, the clinical dilemma concerning pancreatic biomarkers has been answered by the DGGR lipase and cPLI.

DGGR lipase has a good agreement with cPLI (Kook et al. 2014) and presents the advantages of being less expensive, faster, and easier to perform (Graca et al. 2005). Also, since its validation for use in dogs, DGGR lipase is considered acceptable for inclusion in routine chemistry panels as a screening biomarker for pancreatitis in dogs (Graca et al. 2005). Even though it is a widely available parameter with increasing use in Europe, little is known about the potential influence of concurrent diseases and medical treatments on DGGR lipase levels.

Regarding endogenous corticosteroids, studies had shown that dogs with HAC can have DGGR lipase values above the reference interval (Bennaim et al. 2018; Linari et al. 2019). However, studies evaluating the influence of exogenous corticosteroids in this parameter were lacking. Therefore, recognising that steroid therapy is commonly prescribed in veterinary medicine (Reusch 2015), to date, it was unclear if DGGR lipase values were affected by this kind of treatment. Consequently, the use of this biomarker in dogs previously treated with steroids is, nowadays, limited by the incertitude and validity of this result. Therefore, these diagnosis limitations which the clinicians must deal with in clinical practice reflect the usefulness and relevance of this study.

The characteristics of the study population were not standardised. However, it is believed that the various diseases and the respective prednisolone dosages of the SG clearly reflect the clinical practice in a university teaching hospital. The CG and SG were age and sex-matched to reduce variable differences apart from the prednisolone treatment, between groups. Therefore, it was possible to analyse, over time, the differences between DGGR lipase

values of a non-healthy group under prednisolone therapy and a healthy group without any ongoing treatment. Additionally, the study design also allowed to assess the differences between DGGR lipase values before (T0) and after (T1 and T2) corticotherapy in a non-healthy group of dogs starting treatment – the SG.

Concerning the DGGR lipase over time with concurrent oral prednisolone administration (SG), it was shown a statistically significant difference from T0 to T1 and from T1 to T2. However, the difference between the T0 and T2 was statistically insignificant. Therefore, it is suggested that the potential effect of prednisolone on DGGR lipase levels is reduced when the dosage is decreased.

This study indicates that there is a variation of the DGGR lipase values, characterized by an initial increase (from T0 to T1) and subsequent decrease (from T1 to T2). Simultaneously, the prednisolone was introduced from T0 to T1 and decreased from T1 to T2. Therefore, the variation over T0-T1 and T1-T2 was concomitant to the prednisolone dosage variation, and because of that, the correlation between both was calculated but found poor. Despite the influence of prednisolone on DGGR lipase values, this shows that there is a poor dose-effect correlation. This is in disagreement with other studies which indicate that the dose of prednisolone might be important in determining its effect on pancreatic tissue and cPLI (Ohta et al. 2018). Based on the lower bioavailability of prednisolone when prednisone was administered than when prednisolone was administered (Colburn et al. 1976), the dosage as an important effect factor is suggested. A study using oral prednisone 2.2.mg/kg/day showed no influence on cPLI (Steiner et al. 2009). Conversely, oral prednisolone 2.0-2.2 mg/kg/day showed different results (Ohta et al. 2017).

Otherwise, the CG did not show statistically significant differences over time. In fact, there is some intraindividual variability, reinforcing that a higher sample would be needed to reduce it and increase the statistical power of the study.

Regarding the comparison of DGGR lipase values between SG and CG, no statistically significant difference in DGGR lipase concentration was observed on the time point before starting corticotherapy (T0). Then, analysing DGGR lipase variation from that point showed that SG and CG evolved differently.

Despite the observed variation in the SG, the median values did not exceed the upper limit of the considered reference interval (80 U/L). Also, the maximum registered was 148.7 U/L which is not above the upper limit of the considered *grey zone* (80-160 U/L). As previously mentioned, this twofold *grey zone* was based and emphasised by Kook et al. (2014) and Kook

(2017) to consider a possible intraindividual variability in serum lipase activity in healthy dogs as well as the impossibility to rule out transient mild pancreatitis in clinically healthy dogs.

From a physiopathological perspective, it is unclear the mechanism behind the serum DGGR lipase increase over the prednisolone treatment. On one hand, the question is if even subclinical, pancreatitis could have been developed during the time of the study or there are other reasons for the DGGR lipase increase.

Concerning the possibility of pancreatitis, an abdominal ultrasound was planned to be performed in dogs showing an increase above 160 U/L. However, none of the dogs showed it. The correlation between abdominal ultrasound and clinical diagnosis of pancreatitis is poor (Cridge, Sullivant, et al. 2020). Moreover, the agreement between the ultrasonographic diagnosis of pancreatitis and DGGR lipase is also discussable (Kook et al. 2014). Although an abdominal ultrasound would have been helpful to evaluate pancreatic silhouette, it was judged unnecessary because none of the dogs showed any clinical signs of clinical pancreatitis such as vomiting, diarrhoea or abdominal pain (Xenoulis 2015). From the 17 dogs of SG, 5 were diagnosed with steroid-responsive enteropathy and, naturally, presented the history of diarrhoea which is compatible with pancreatic disease clinical signs. However, the inclusion criterion, such as these animals presenting DGGR lipase under 80 U/L, allowed to achieve the objective of the study, which was to assess DGGR lipase variation in dogs under prednisolone therapy. Furthermore, during the clinical investigation, an abdominal ultrasound was performed to 4 of the 5 animals diagnosed with steroid-responsive enteropathy and pancreas did not show echographic alterations. Also, the gastrointestinal signs make these 5 animals an interesting group to observe DGGR lipase variation in dogs with gastrointestinal disease under prednisolone therapy.

Several drugs have been reported to induce pancreatitis (Whitney et al. 1987; Gaskill et al. 2000; Trepainer et al. 2003; Kook et al. 2009). Among them, also glucocorticoids were historically evoked to cause pancreatitis in small animals (Reusch 2015). However, these were based on sporadic cases with other associated risk factors. Despite this controversy, nowadays, corticosteroids are not considered a risk factor for pancreatitis (Reusch 2015). Also, Ohta et al. (2018) in their study, where documented significant increase in the serum cPLI concentration over prednisolone treatment, did not reveal pancreatic histological abnormalities before and after prednisolone administration. However, among others, some limitations of this previous study were identified, such as the non-complete histological examination, the only three weeks of monitoring, and the small sample. Nowadays, in dogs and cats, that possibility has now largely been dismissed (Reusch 2015).

Apart from occult pancreatitis, other reasons can be considered for DGGR lipase increase. Ohta et al. (2018) have suggested the increased synthesis of pancreatic lipase, increased cellular permeability to this enzyme, or a decreased rate of its renal clearance. Although some studies report an influence of renal disease on lipase activity concentration (Strombeck et al. 1981; Mansfield and Jones 2000), other authors disagree with it (Steiner et al. 2010; Hulsebosch et al. 2016). In the present study, the animals of SG did not have a history of renal disease and did not present related clinical signs over the study period. Therefore, it seems unlikely to be explained by decreased renal function. The lipase production from extra-pancreatic tissues is also a possibility. In contrast to Ohta et al. (2018), in this study, pancreatic lipase messenger ribonucleic acid (mRNA) expression was not studied in any tissue to clarify that. Furthermore, it is known that DGGR lipase is not specific to the pancreas (Steiner et al. 2015; Lim et al. 2020), so the possibility of non-pancreatic lipase cannot also be ruled out based on this study. In addition, it is also known that glucocorticoids have a role in lipid metabolism through the stimulation of hormone-sensitive lipase and the inhibition of lipoprotein lipase (Elliot 2001). Hormone-sensitive lipase promotes adipocyte lipolysis, while lipoprotein lipase promotes fat uptake into adipocytes (Herdt and Sayegh 2013). Also, the hypertriglyceridemia associated with hyperadrenocorticism is suggested to be due to stimulation of hormone sensitive lipase (Elliot 2001). Therefore, the stimulation of hormone-sensitive lipase activity supports the possibility of a non-pancreatic origin for lipase increase over corticotherapy.

The study had some limitations. Firstly, the absence of histopathological pancreatic examination impaired a correct assessment of concurrent tissue changes that may occur with prednisolone therapy. As this is the gold standard for the diagnosis of pancreatitis it would have been interesting to investigate potential changes. As mentioned, it is nowadays discussable due to its invasiveness and the possibility of insufficient results related to the non-uniform distribution of lesions (Newman et al. 2004). However, recently, its relevance in the diagnostic evaluation of pancreatic diseases was confirmed (Aupperle-Lellbach et al. 2020). Therefore, considering its limitations, many recent studies have used the clinical diagnosis as a gold standard for pancreatitis (Cridge, Sullivant, et al. 2020). Thus, the possibility of pancreatitis cannot be completely ruled out. However, even if it had been performed, it would not be very sensitive, due to the possibility of occult inflammation associated with highly localised histologic lesions (Newman et al. 2004). Another possible limitation to consider is a comparison with cPLI. This was not performed due to financial reasons, but also because the good and excellent agreements between cPLI and DGGR lipase had already been scrutinized by Kook et al. (2014) and Cridge et al. (2018), respectively.

Secondly, as a colorimetric method, the absence of haemolysis or lipemic serum was not assured in all the samples from both groups. One of the reasons why the lipemic serum control was not possible was because the fasting was not possible to standardise. Although it was advised, that was not possible to be sure of it as it depends on the owner's compliance. However, no sample had gross lipemia or haemolysis. Even though, Graca et al. (2005) have shown minimal changes in the presence of high concentrations of lipid and haemoglobin for lipase activity with the DGGR assay. Additionally, in another study, haemolysis did not result in significant changes to the DGGR lipase activity and it was not significantly different after 12 months of storage at −80°C (Goodband 2018).

Third, a parallel pancreatitis risk factor to the beginning of corticotherapy was identified in a post-operatory period from a dog submitted to ophthalmologic surgery from the SG on T1. Studies in humans have shown high sensitivity of the pancreas to changes in its microcirculation (Cuthbertson and Christophi 2006). Therefore, associated anaesthesia, which leads to hypotension, is considered a risk factor in canine pancreatitis (Xenoulis and Steiner 2013b). In fact, in this animal was observed an increase from 32.32 U/L to 96,14 U/L. However, once again, the DGGR lipase on T1 was not above 160 U/L and no clinical signs associated with the pancreatic disease were observed. Since some studies reported an influence of renal disease (Strombeck et al. 1981; Mansfield and Jones 2000) and hepatic disease (Strombeck 1981; Quigley et al. 2001) on lipase activity, it would also be important to better clarify hepatic and renal function. A complete chemistry panel would be important to perform in all the animals. Nevertheless, 9 of the 17 dogs in the SG, had biochemical results up to two months before and presented normal liver enzymes - alanine aminotransferase (ALT) -, and renal parameters – urea and creatinine.

Fourth, the oral administration of prednisolone was owner dependent. The compliance was, therefore, a strong constraint factor, and since it was not possible to assure, it became a possible limitation of this study. Nonetheless, as dogs improved from the medical conditions that justified prednisolone therapy, it is unlikely that the therapeutic schedule was dismissed.

Fifth, the dosage range considered in this study was also a limitation because it was case-dependent and not possible to standardise or to have a narrower range. Furthermore, and due to medical reasons and progressive prednisolone reduction, the dosage range was not maintained over the time of the study. However, this was a clinical study and the dosage was applied to each animal depending on the underlying disease and the individual glucocorticoid sensitivity (Reusch 2015). Following the first treatment, the dosage was reduced

to the lowest necessary concentration after the respective control of clinical signs (Reusch 2015).

Lastly, the number of dogs used in this study was small and more dogs would be needed to increase the statistical power of the study. Regardless pancreatitis has been developed or not, the underlying disorder itself, diversity in the dosage, age, and sex could have contributed to the diversity of DGGR results among all the dogs because were not possible to standardise them.

3.5. Conclusion

DGGR lipase is recently used by small animal practitioners. However, the interest related to its advantages has led to the need for validation in several common clinical situations. Therefore, this research clarified the effect of prednisolone on DGGR lipase values.

This study suggests that DGGR lipase is affected in dogs treated with oral prednisolone therapy. However, as values remained below the significant upper limit (160 U/L), this variation does not seem to be clinically relevant over the 21-30 days of treatment. Additionally, the documented correlation between the variations of DGGR lipase and the correspondent prednisolone dosage was poor, stressing that its effect does not seem to be dosage-dependent.

Regardless of the dose limitations, this clinical study reflects a day-to-day clinical practice dilemma, and conclusions can be widely applied by clinicians.

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5. ANNEXES

Annexe 1. Abstract submitted and presented as an oral communication at *Congresso Internacional Hospital Veterinário Montenegro*

A INFLUÊNCIA DA CORTICOTERAPIA NO DOSEAMENTO DA DGGR-LIPASE EM CÃES – ESTUDO PROSPETIVO

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O diagnóstico de pancreatite mantém-se atualmente desafiante, em parte por não existir ainda um biomarcador completamente fiável. O diagnóstico incide sobretudo nos sinais clínicos, ecográficos e na quantificação de biomarcadores como a cPLI (canine Pancreatic Lipase Immunoreactivity) e, mais recentemente, da DGGR Lipase (*1,2-o-Dilauryl-Rac-Glycero Glutaric Acid-(6'-methylresorufin) Ester*). Apesar destes biomarcadores apresentarem uma boa correlação entre si (Kook et al, 2014), a DGGR-Lipase é fidedigna, rápida e menos dispendiosa, tendo tido um uso crescente na Europa. Um estudo comprovou que a corticoterapia não influencia o doseamento da cPLI (Steiner et al, 2009). Contudo, desconhece-se o seu efeito sobre a DGGR-Lipase.

Este estudo visa avaliar a influência da corticoterapia na quantificação da DGGR-Lipase em cães. Foi efetuado um estudo de Coorte prospetivo que incluiu cães sem sinais de doença digestiva e com valores de DGGR-Lipase dentro dos valores de referência (<80 U/L) (Lucibello et al, 2017). Estes cães apresentavam um diagnóstico clínico (doença respiratória, dermatológica ou neurológica) que justificou a instituição de corticoterapia (prednisolona) durante um mínimo de três semanas, numa dose inicial de 0.5-2mg/kg/dia. A DGGR-Lipase foi quantificada em três pontos temporais (T0 referente ao Dia 0; T1 e T2 referentes aos dias 7-10 e 21-30 após o início do tratamento, respetivamente). O T0 foi considerado o controlo individual de cada animal. Para esta análise, foi utilizado o kit DGGR-Lipase da Randox®, previamente validado para doseamento em canídeos (Lucibello et al, 2017). O estudo foi

aprovado pela respetiva Comissão de Ética e Bem Estar local. A análise estatística foi efetuada com recurso a testes não paramétricos. Dada a reconhecida variabilidade intra-individual dos biomarcadores de pancreatite, apenas foram considerados clinicamente significativos os valores de DGGR-Lipase acima de 160 U/L (2x o limite superior de referência) (Kook, 2017).

Oito cães foram incluídos no estudo. Todos os animais apresentavam uma DGGR-Lipase dentro dos valores de referência em T0, com uma mediana de 27,72U/L (IQR=22,73-31,69). Em T1, os valores subiram para 59,82U/L (IQR=30,78-100,67), sendo este aumento estatisticamente significativo (p=0,017). De T1 para T2, verificou-se uma redução do valor de DGGR-Lipase para 43,04 U/L (IQR=27,91-54,1), a qual também foi estatisticamente significativa (p=0,025). Em T1 a mediana da dose de prednisolona em curso foi de 0,8mg/kg (IQR=0,8-1,08)) e em T2 de 0,35mg/kg (IQR=0,28-0,58)). Foi verificada uma baixa correlação entre a variação da DGGR-Lipase no intervalo T0-T1 e a correspondente variação da dose de prednisolona (r_s=0,342), assim como no intervalo T1-T2 (r_s=0,096).

A baixa correlação entre a DGGR-Lipase e a correspondente dose de prednisolona em curso pode advir do reduzido número da amostra. Apesar das flutuações observadas, em nenhum dos pontos foi registada uma subida acima de 160 U/L.

Estes resultados sugerem que a corticoterapia pode induzir flutuações nos valores de DGGR-Lipase. Apesar de estatisticamente significativa, esta variação não aparenta ser clinicamente relevante dado que os valores permanecem abaixo de 160 U/L. Ainda assim, a interpretação da DGGR-Lipase deve ser criteriosa em animais com suspeita de pancreatite, sobretudo se já tiverem recebido tratamento prévio com corticosteróides.

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Annexe 2. Abstract accepted for a poster at the European College of Veterinary Internal Medicine – Companion Animals Congress

The effect of oral prednisolone therapy in canine 1,2-o-dilauryl-rac-glycero glutaric acid-(6'-methylresorufin)) ester (DGGR) lipase serum levels

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1,2-o-dilauryl-rac-glycero glutaric acid-(6'-methylresorufin) ester (DGGR) lipase is an inexpensive and widely available biomarker, increasingly used in the investigation of canine pancreatitis. A previous study showed a good agreement between canine pancreatic lipase immunoreactivity (cPLI) and DGGR lipase dosage. While prednisolone therapy does not seem to affect serum cPLI quantification, its influence on DGGR lipase is unclear. This study aims to evaluate the effect of prednisolone therapy in canine DGGR lipase serum levels.

A prospective cohort study was conducted, including two groups: a study group (SG) consisted of dogs treated with oral prednisolone for a medical reason, at the initial dosage of 0.5-1.5mg/kg/day, for at least 3 weeks and a control group (CG) composed by healthy untreated dogs. As an inclusion criterion, animals had basal DGGR lipase within the reference range (<80 U/L). DGGR lipase was measured at three time points (Day 0(T0), Day 7-10(T1) and Day 21-30(T2)) in both groups. The analysis was performed using a previously validated kit (Randox® DGGR lipase). Recognising an intraindividual variability, a twofold “gray zone” was

set (80-160 U/L). The study was approved by the local Ethical Committee. Data was expressed as median ± inter-quartile range (IQR) and compared using nonparametric statistical tests ($p<0.05$).

Thirty-four dogs were included (17 dogs for each group, which were age and sex matched). At T0, there were no significant difference on DGGR lipase concentrations between groups ($p=0.868$). Dogs from SG (n=17) received prednisolone due to: immune-mediated polyarthritis (1/17), intervertebral disc disease (1/17), post-surgical and immune-mediated uveitis (2/17), steroid-responsive enteropathy (5/17) and chronic tracheobronchitis (8/17). Median starting dosage of prednisolone was 0.8mg/kg/day (0.7-1), decreasing to 0.4mg/kg/day (0.3-0.6) after T1. The median DGGR lipase concentration at each time point (T0, T1 and T2) was: 24.74 U/L (14.45-31.48), 36.82 U/L (23.8-80.16) and 29.52 U/L (15.91-48.48), respectively. There was a significant increase on DGGR lipase concentration from T0 to T1 ($p=0.002$) and a subsequent decrease from T1 to T2 ($p=0.004$). A poor correlation was verified between the variations of DGGR lipase and the correspondent prednisolone dosage of T0-T1 and T1-T2 ($r_s=0.254$ and $r_s=0.3$, respectively). In CG, DGGR lipase did not significantly change over the three time points ($p=0.887$ when T1 compared to T0; and $p=0.076$ when T2 compared to T1).

This study suggests that DGGR lipase is affected by oral prednisolone therapy in dogs treated for a medical reason. However, as values remained below the significant upper limit (160 U/L), this variation does not seem to be clinically relevant.

Annexe 3. Poster presented at *Congresso Internacional Hospital Veterinário Montenegro*

INOVAÇÃO & DESENVOLVIMENTO SEM SEGREDOS
ST. MARIA DA FEIRA · 20 A 22 FEVEREIRO 2020

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Doença auto-inflamatória do Shar-Pei: Apenas em cães de raça? A propósito de um caso clínico

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INTRODUÇÃO

Os cães de raça Shar-Pei são predispostos para um quadro inflamatório sistémico caracterizado por febres altas episódicas, associado a artrites, dermatites, otites e deposição de substância amilóide em vários órgãos (Olsson, 2013). Apesar de controverso, acredita-se que estas alterações advêm de mutações genéticas, destacando-se uma duplicação a montante da região codificadora da enzima Acido-Hialurónico-Sintetase (HAS2), (Mia Olsson, 2011) e outra a nível da codificação da proteína Mdm2, Transformed 3 T3 cell double minute 2, p53 Binding Protein (MTBP) (Julia Metzger, 2017).

Apesar de mais frequente em cães de raça pura, dada a complexidade da base genética desta síndrome, é possível que a DAISP ocorra em cães cruzados. Contudo, os relatos desta síndrome em cães não Shar-Pei são escassos.

SEGUIMENTO

1 mês após o Início do tratamento:

Verificou-se uma melhoria substancial dos sinais clínicos e do estado geral do animal. A colchicina foi bem tolerada e não induziu sinais digestivos, tendo sido mantida durante 3 meses até novo doseamento da SAA. A dose de prednisolona foi novamente reduzida até 0,15 mg/kg duas vezes por semana.

4 meses após o Início do tratamento:

Nesta reavaliação verificou-se uma ligeira distensão dos tarsos, o que levantou a suspeita de um controlo sub-normal da doença. Deste modo, foi recomendado um aumento da dose de prednisolona para 0,15mg/kg três vezes por semana. A SAA foi novamente doseada tendo-se observado a redução para 4.95 µg/ml. Tendo ocorrido uma resposta positiva à colchicina, sem evidências de efeitos secundários, o tratamento foi continuado.

1 mês com prednisolona (0,3mg/kg)

3 meses com prednisolona (0,15mg/kg)

CASO CLÍNICO

♀ Shar-Pei x ♂ Serra d'Aires

Um cão macho castrado de 6 anos, fenotipicamente similar a um exemplar da raça Serra de Aires, foi apresentado à consulta por edema dos tarsos (desde há 3 semanas). A mãe também apresentava edema crónico dos tarsos.

Anamnese

Os sinais apresentados eram episódicos desde a idade jovem e melhoravam aquando da administração pontual de prednisolona.
Já tinham sido anteriormente despistadas doenças vetoriais (Leishmania, Ehrlichia, Dirofilaria e Anaplasma), as quais se revelaram negativas.

Exame Físico

Apenas se detetou tumefação articular e febre (39.3ºC).

Investigação clínica

Nesta fase, a DAISP foi o principal diagnóstico diferencial considerado. No entanto, uma origem imuno-mediada (poliartrite) também foi evocada, não podendo esta ser excluída. Assim, a realização de citopunções articulares foi adiada devido ao historial familiar e ao facto das articulações afectadas serem persistentemente os tarsos. De facto, enquanto que uma poliartrite se exprime sobretudo a nível dos tarsos e carpos, a DAISP traduz-se numa tumefação preferencial dos tarsos onde há uma maior quantidade de ácido hialurónico.

Foram realizadas análises gerais (hemograma, bioquímicas, urina II e rácio proteína-creatinina urinário) e uma ecografia abdominal, as quais não revelaram alterações.

Foi doseada a proteína amiloide sérica (SAA), biomarcador de inflamação sistémica e amiloidose em cães, a qual estava marcadamente elevada (SAA 47.5 µg/ml; Ref. < 4.6 µg/ml), confortando a suspeita clínica de DAISP.

Assim sendo, foi iniciado tratamento médico com prednisolona (1mg/kg q24h PO com redução progressiva até 0,3mg/kg) e colchicina (0.03mg/kg q24h PO).

CONCLUSÃO

Os achados encontrados na anamnese, resultados analíticos e a resposta ao tratamento instituído suportam o nosso principal diagnóstico diferencial: DAISP.

Este caso **documenta a ocorrência de DAISP em cães fenotipicamente distintos da raça Shar-Pei**, sensibilizando a comunidade médico-veterinária e reforçando a necessidade de mais estudos para uma melhor compreensão desta doença.

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Annexe 4. Study group authorisation

Autorização para Participação em Estudo Clínico

Titulo do projecto: O efeito dos corticosteróides exógenos nos niveis séricos de DGGR-Lipase em cães

GRUPO: Cães submetidos a tratamento com corticosteroides

Eu abaixo assinado_____ (nome do proprietário), portador do CC _____ proprietário do canídeo_____ Raça _____ Sexo _____ Idade_____

DECLARO

- que fui informado e me foi solicitada a minha autorização para participação do meu animal num estudo designado: **O efeito dos corticosteróides exógenos nos niveis séricos de DGGR-Lipase em cães**

- que a participação do meu animal no estudo é totalmente voluntária

- que a participação do meu animal no estudo pode ser revogada em qualquer momento, sem dar qualquer explicação;

- que o meu cão apresenta uma doença que justifica o inicio de corticosteróides por via oral

- que fui informado de forma clara e compreensível, com especial atenção ao tipo das avaliações de diagnóstico que o meu cão beneficia bem como os riscos e benefícios genéricos e específicos do tratamento com corticosteróides

- que fui informado em detalhes sobre as colheitas de sangue às quais o meu animal deve ser submetido

Foi-me também referido que:

- A informação final obtida no estudo poderá ser consultada após pedido ao investigador responsável, se assim o desejar.

- A privacidade dos meus dados e dos dados do meu animal será respeitada no caso dos resultados serem utilizados em comunicações escritas ou orais, ou em publicações, que possam resultador do estudo.

A informação incluída nesta declaração foi-me também descrita oralmente pelo médico veterinário **Rodolfo Oliveira Leal (OMV 4408).**

Deste modo, dou fé de:

- ter lido a informação que me foi entregue

- ter podido fazer as perguntas que entendi por necessárias sobre o estudo

- ter recebido informação suficiente sobre o estudo

- ter compreendido que posso retirar o meu animal do estudo quando quiser e sem dar explicações, sem que isso se repercuta nos cuidados médicos a prestar ao meu animal.

Assim, autorizo a divulgação da informação recolhida no estudo para o propósito do mesmo, com a salvaguarda do respeito pela privacidade dos meus dados e do meu animal, concedendo livremente a minha autorização para participação do meu animal.

Data:_____

Assinatura_____

Annexe 5. Control group authorisation

Eu, _____ (nome do proprietário), portador do CC _____ proprietário do canídeo_____ Raça _____ Sexo _____ Idade_____

Autorização para Participação em Estudo Clínico

Titulo do projecto: O efeito dos corticosteróides exógenos nos niveis séricos de DGGR-Lipase em cães

GRUPO: Cães saudáveis

DECLARO

- que fui informado e me foi solicitada a minha autorização para participação do meu animal num estudo designado: O efeito dos corticosteróides exógenos nos niveis séricos de DGGR-Lipase em cães

- que a participação do meu animal no estudo é totalmente voluntária

- que a participação do meu animal no estudo pode ser revogada em qualquer momento, sem dar qualquer explicação;

- que fui informado de forma clara e compreensível, com especial atenção ao tipo das avaliações de diagnóstico que o meu cão beneficia

- que fui informado em detalhes sobre as colheitas de sangue às quais o meu animal deve ser submetido

Foi-me também referido que:

- A informação final obtida no estudo poderá ser consultada após pedido ao investigador responsável, se assim o desejar.

- A privacidade dos meus dados e dos dados do meu animal será respeitada no caso dos resultados serem utilizados em comunicações escritas ou orais, ou em publicações, que possam resultador do estudo.

A informação incluída nesta declaração foi-me também descrita oralmente pelo médico veterinário Rodolfo Oliveira Leal (OMV 4408).

Deste modo, dou fé de:

- ter podido fazer as perguntas que entendi por necessárias sobre o estudo

- ter recebido informação suficiente sobre o estudo

- ter compreendido que posso retirar o meu animal do estudo quando quiser e sem dar explicações, sem que isso se repercuta nos cuidados médicos a prestar ao meu animal.

Assim, autorizo a divulgação da informação recolhida no estudo para o propósito do mesmo, com a salvaguarda do respeito pela privacidade dos meus dados e do meu animal, concedendo livremente a minha autorização para participação do meu animal.