

# **PLI, TLI and canine and feline pancreatic disease**

## **INTRODUCTION**

The principle aim of this brief seminar is to provide an update on the sensitive and specific biochemical tests for pancreatic disease and to enable you to optimise their application in practice. In these lecture notes, I have significantly expanded upon the more general aspects of pancreatic disease in both species and included updates from speakers at the recent WSAVA, BSAVA and Atlantic Coast Veterinary Conferences.

## **THE PANCREAS REVISITED**

It is important to recall that the principle functions of the pancreas are endocrine ( $\beta$  islet cell insulin secretion and glucose regulation) and exocrine (acinar cell release of digestive enzymes). Derangements as a result of inflammation or neoplasia of either cell lineage can, therefore, ultimately impact on both functions. For example: Chronic pancreatitis in both dogs and cats can result ultimately in exocrine pancreatic insufficiency (EPI) and a reduction in insulin secretion as a result of fibrosis; pancreatic adenocarcinoma can cause necrosis or blockade of the pancreatic duct with resultant pancreatic inflammation and destruction of the  $\beta$  islet cells.

## **OVERVIEW OF PANCREATIC DISEASE IN DOGS AND CATS**

In both species, pancreatitis is by far the most commonly recognised primary pancreatic disease although there is evidence to suggest that many low-grade chronic canine cases are often dismissed as mild, self-limiting gastro-intestinal disease episodes. Recognition of pancreatitis in cats is improving but necropsy findings suggest that this diagnosis is still frequently missed (67% of feline PM submissions had historic lesions of chronic pancreatitis in one study). Primary EPI or pancreatic acinar atrophy is generally uncommon in dogs although German shepherd dogs and Rough collies are over-represented. Only a handful of feline cases are documented in the international literature. Secondary exocrine and endocrine pancreatic insufficiency resulting from pancreatic fibrosis following long-term chronic pancreatitis is increasingly detected in both species. The incidence of pancreatic adenocarcinoma is significantly lower than pancreatitis but an important consideration in both species.

## **PANCREATITIS**

### **Acute versus Chronic**

The terms acute and chronic pancreatitis refer to the characteristic cellular infiltrates (neutrophilic and mononuclear respectively) detected at histopathological examination. Both forms can be seen in dogs and cats although cats generally suffer far more the chronic type. In practice, chronic cases previously undetected may present acutely i.e. “acute-on-chronic” disease. This is particularly prevalent in our canine patients and consideration must be given to the possibility of underlying chronic disease even following only one episode of “acute” pancreatitis, particularly if there is a history of low-grade GI upset. Cats frequently suffer pancreatitis in concert with inflammatory bowel disease (IBD) and hepatic lipidosis/cholangiohepatitis – so called “Triaditis”. Renal disease may be a sequel in both species.

### **Aetiopathogenesis/risk factors**

Most cases are labelled idiopathic although ‘trigger factors’ appear to exist such as hyperlipidaemia (high fat diets, diabetes mellitus, hypothyroidism, hyperadrenocorticism, obesity) and hypoxia in dogs. Despite these trigger factors, there is plentiful evidence for an underlying genetic predisposition since miniature schnauzers, cocker spaniels, Cavalier King Charles spaniels, collies, terriers and boxers appear over-represented. For example, a terrier binges and develops acute pancreatitis whereas a labrador eating the same meal is unaffected. These genetic predispositions take different forms, however. Miniature schnauzers appear to be predisposed as a result of familial hyperlipidaemia; cocker spaniels have been shown to suffer autoimmune destruction of the pancreas.

Trigger factors in cats appear to include blunt trauma (RTA, surgery), hypoxia and infectious diseases (Toxoplasmosis, FIP, FIV).

In addition, an exhaustive list of drugs and toxins are implicated but many are the result of extrapolation from human medicine. The more relevant agents appear to be potassium bromide, furosemide, oestrogen, azathioprine, salicylates, sulphonamides, tetracyclines, thiazide diuretics and L-asparaginase, vinca alkaloids zinc toxicosis, organophosphate poisoning,

### **Clinical Presentation – dogs**

Everyone will be familiar with the presentation of the canine acute (or perhaps acute on chronic) pancreatitis case i.e. vomiting, cranial abdominal pain, lethargy and anorexia but appreciably these signs are non-specific. Beware of the telltale signs of systemic involvement i.e. weakness, dehydration, pyrexia, increased heart rate, arrhythmias, coagulation defects and shock.

Diarrhoea is reported with increasing frequency in subsequently confirmed cases of pancreatitis and is an important consideration for chronic pancreatic inflammation. Pain can provide significant stimulus to drink and increase the frequency of urination and pancreatitis should be included on the initial differential list of any PU/PD dog. In addition, consider a history review of diabetic patients for previous GI signs since chronic pancreatic inflammation may be causal.

Some patients with acute disease can present with only low-grade, vague GI signs and patients with marked fibrosis of the pancreas from multiple ‘bouts’ may similarly show lessened abdominal pain. In summary – keep an open mind.

### **Clinical presentation – cats**

Cats can be frustratingly covert and non-specific about their pancreatitis, presenting most commonly with just anorexia/inappetance and lethargy. In addition, the frequent triaditis cases, secondary renal disease and acute respiratory distress syndrome (ARDS) often turn suspicion away from pancreatic disease. Hypothermia, diarrhoea, weight loss and ataxia have been reported but chronic pancreatitis can also frequently be sub-clinical, i.e. a consideration for fussy eaters with mood swings related to pain episodes.

### **Diagnostic laboratory tests and interpretation**

Baseline results:

Lipase                      In dogs, serum lipase has been part of the traditional panel for pancreatitis but important factors contribute to a lack of specificity and sensitivity of this test. Firstly, different forms of lipase exist, secreted

by the pancreas, gastric and duodenal mucosa, and adipose tissue and the traditional lipase test does not differentiate these forms. Secondly, lipase is deactivated by the kidneys and Kupffer cells of the liver. Elevations of lipase may then be associated with reduced renal clearance, hepatic disease and GI disease in addition to pancreatitis and pancreatic neoplasia. Steroidal usage also elicits a lipase increase. To further confound matters, many patients subsequently confirmed as having pancreatitis on histopathology have normal lipase values. This may reflect a relatively short half-life.

**Amylase** Considerations regarding amylase evaluation in dogs are similar to those with lipase. Defective renal and hepatic clearance will elevate results so cautious interpretation and adjunctive tests are required as with lipase. Steroidal influences do not appear to influence amylase activity but the small intestine also secretes amylase, reducing specificity.

Generally, in dogs, amylase and lipase elevations of more than three times the upper reference range are highly suggestive of pancreatitis but can be seen in pancreatic neoplasia (adenocarcinoma). Milder increases are non-specific and confounding influences such as azotaemia and intestinal disease must be assessed. Pancreatitis cannot be ruled out if no elevations in amylase and lipase are detected.

In cats, most studies have detected no significant difference between the values of amylase and lipase in health, pancreatitis and extra-pancreatic disease. Elevations of either enzyme may raise suspicion but normal values in the cat will certainly NOT exclude pancreatitis from the differential list.

**Azotaemia** Azotaemia is a common finding in patients with pancreatitis and may be pre-renal (hypovolaemia) or renal in origin. Urinalysis is required to aid further differentiation of the causes of mild azotaemia or lone urea/creatinine elevations. It must also be noted that upper GIT disease with haemorrhage will elevate urea and creatinine is commonly above reference range in large breed, well-muscled dogs. Acute renal failure can occur as a sequel to pancreatitis in both species. In cats, this typically reflects interstitial nephritis. Appreciably, any reduction in glomerular filtration rate (GFR) confounds interpretation of other supportive results due to elevation of lipase, amylase and TLI.

**Liver enzyme elevations** Adjacent inflammatory toxins and inappropriately released digestive enzymes plus hypoxia may appreciably cause hepatocellular damage evoking an increase in ALT. Intra-hepatic and post-hepatic cholestasis is typically minor but resultant mild to moderate elevations of ALKP and GGT are also common. This finding frequently lags hepatocellular damage by 24 hours. Bile duct blockade can be seen which will significantly increase cholestasis and hence markedly elevate ALKP and GGT. This is more common in cats since they lack the accessory bile duct of most dogs but can be seen in severe disease in dogs and pancreatic adenocarcinoma of both species. Consider pancreatitis and

triaditis as a major differential for any feline presenting with liver enzyme elevations.

Total bilirubin	This can naturally ensue from intrahepatic and any extrahepatic cholestasis mentioned above. This is again, an important lead to pancreatic disease in cats.
Hyperlipidaemia	Many causal factors are proffered for this finding and there is a notable 'chicken and egg' argument i.e. Is the hyperlipidaemia commonly accompanying pancreatitis cause or effect? Cholestasis and mobilisation of peri-pancreatic abdominal fat by inappropriate lipase release are known sequelae to pancreatitis but the possible aetiology of hyperlipidaemia should not be negated, particularly in Miniature Schnauzers, obese patients, Cushing's sufferers and diabetics.
Hypocalcaemia	This must be interpreted with caution since any hypoalbuminaemia will result in total calcium appearing suppressed. Peri-pancreatic fat necrosis and saponification are implicated in genuine hypocalcaemia. This finding has been correlated with more severe pancreatitis with accompanying evidence for systemic inflammation (leukogram) and tends therefore to be associated with a poorer prognosis. Hypocalcaemia should trigger aggressive therapy.
Hyperglycaemia	Stress hyperglycaemia is highly likely given the pain levels frequently detected in pancreatitis, however, as mentioned above, monitoring and consideration of $\beta$ -cell destruction from chronic pancreatitis in both species is necessary.
Inflammatory Leukogram	In both species, acute disease is occasionally associated with a leukogram denoting active inflammation i.e. a neutrophilia with increased numbers of immature band neutrophils present. Neutrophils may show signs of toxic change if acute pancreatic necrosis exists. White blood cell populations may change rapidly and in a response to a wide variety of disease processes. These findings are therefore neither sensitive nor specific but support systemic influences from an inflammatory focus. Lymphopaenia is frequently seen commensurate with a stress response.
Anaemia/ Haemoconcentration	Mild normochromic, normocytic, non-regenerative anaemia of chronic disease is most commonly detected. Haemoconcentration is frequently found in acute cases. More severe anaemias may accompany DIC and any bleeding tendencies should be investigated further with clotting times and D-dimers.

The above are only to be considered as indicators and are by no way sensitive or specific pointers toward pancreatitis. In particular in cats and even in some dogs, many of these parameters may be within reference range although pancreatitis is latently confirmed. These findings are listed in order to help you to establish a pattern of abnormalities, which might, when considered in context with other clinical findings, obviate more specific tests to rule pancreatic inflammation in or out.

Specific tests:

#### Pancreatic lipase immunoreactivity (PLI)

This is the most sensitive and specific test to date for the diagnosis of pancreatitis in both cats and dogs, boasting 82% sensitivity. The test assays the mass of circulating lipase specifically of pancreatic origin. This negates the potential for false positives from gastrointestinal disease and in addition, this assay appears unaffected by steroidal influences, both endogenous and exogenous. Because it is not the enzymatic activity of the lipase that is under test but simply the circulating mass of enzyme, renal deactivation does not influence the value: Traditional lipase assays measure the enzymatic activity of the lipase but as previously mentioned, this may be lipase activity from a variety of sources. The kidneys usually attenuate this enzymatic activity but when GFR is reduced, high active levels remain in circulation. Because the specific pancreatic lipase immunoreactivity assay measures specifically the total pancreatic lipase and not the enzymatic activity, this is unaffected by changes in GFR. For dogs, this test is now available as a quick and stable ELISA but for cats, sera must still be sent to the Texas A&M University for feline specific PLI.

Reference ranges for dogs are 0-200 $\mu$ g/L with values greater than 400 $\mu$ g/L consistent with pancreatic inflammatory disease. Reference ranges for cats are less than 12.9 $\mu$ g/L with values greater than 18.1 $\mu$ g/L consistent with pancreatic inflammatory disease. Appreciably this leaves an equivocal void in between the upper reference range and the values consistent with pancreatitis. Patients with results falling into this zone warrant continued monitoring and close correlation of the timing of samples with historic and clinical evidence for pancreatic disease. It is helpful to try to establish the pattern of a rising or falling PLI and the relation of this to the clinical presentation as chronic pancreatitis cases and cats with triaditis can frequently fall into this zone. These equivocal results must be considered non-specific pending further study. Pancreatic adenocarcinoma can occasionally lead to necrosis and pancreatic inflammation and therefore can variably elevate PLI. It should be noted that atrophy of functional acinar cells resulting EPI and therefore reduced PLI values can also ensue. In summary, an elevated PLI value simply represents pancreatic inflammation and adenocarcinoma cannot be excluded as causal.

#### Trypsin-like immunoreactivity (TLI)

This assay is covered in more depth in the section on EPI, however, it is important to mention here since high values of TLI above 50.0 $\mu$ g/L in dogs or 100.0 $\mu$ g/L in cats in the absence of azotaemia and in the presence of compatible clinical signs should increase suspicion of pancreatitis and provoke further investigation and PLI measurement. Aside from patients with reduced GFR, malnourished dogs and cats with pancreatic nodular hyperplasia or small intestinal disease also show elevations of TLI. In addition, TLI half-life is very short and therefore only 30% of pancreatitis cases are detected on TLI measurement making PLI the test of choice.

#### Trypsin activation peptides (TAP)

Premature activation of trypsinogen can lead to an increase in TAP detectable in both urine and serum. Urine TAP is highly specific but very insensitive and very labile. Serum TAP lacks specificity. This test is therefore not considered of value.

## **PANCREATIC ADENOCARCINOMA**

### **Clinical presentation – dogs and cats**

Pancreatic adenocarcinoma occurs relatively infrequently in dogs and is more widely reported in cats but remains markedly under diagnosed in both species. At best, it is detected late in development. This is largely due to a very non-specific presentation in dogs and cats although obstruction of the pancreatic duct can result in secondary atrophy and signs of EPI. Alternatively, tumour necrosis can elicit pancreatic inflammation and more classic pancreatitis signs. Lameness, bone pain and dyspnoea related to metastasis may be observed.

### **Diagnosis**

An inflammatory leukogram and anaemia of chronic disease plus liver enzyme elevations and bilirubinaemia are the most common findings – appreciably non-specific. Azotaemia and hypokalaemia are also frequently reported. Hyperglycaemia is commonplace and frequently resultant from destruction of  $\beta$  islet cells by the tumour mass. A high index of suspicion is warranted in dogs with excessively high lipase and cPLI values that are refractory to supportive therapy. PLI may also be strongly suppressed due to development of EPI. Many cases have unremarkable haematology and biochemistry.

Both radiographic and ultrasonographic findings tend to show marked crossover with those in pancreatitis. The cellular exfoliation from pancreatic adenocarcinomas is often poor, reducing the diagnostic yield from cytology of peritoneal fluid or direct pancreatic aspiration to around 25%. In many cases, the diagnosis must be made at laparotomy. It should be noted that benign adenomas and nodular hyperplasia are also seen in both species necessitating histopathological differentiation of any pancreatic masses discovered at laparotomy. Canine insulinoma (and rarely feline) is a further differential for discovery of a pancreatic mass but the presentation is generally somewhat characteristic.

## **EXOCRINE PANCREATIC INSUFFICIENCY**

### **Aetiopathogenesis**

EPI is much less commonly encountered than pancreatitis in practice but recognition of the underlying aetiopathogenesis and accurate diagnosis in dogs and cats is important, not least of all since the response to treatment can dramatically improve the quality of life for sufferers. The exocrine pancreas is an organ of immense functional reserve and 90% of the secretory capacity must be lost before signs of maldigestion become prevalent.

Pancreatic acinar atrophy (PAA) is uncommon in the general canine population although certain breeds such as Collies and German shepherd dogs are over-represented, swelling case number sufficiently to make this the main cause of EPI in dogs. This is believed to be autoimmune and due to the huge functional reserve of the pancreas, can present later in life than one would anticipate. Only a handful of feline EPI cases have been documented. Chronic pancreatitis leading to progressive fibrosis of the pancreas and ultimate EPI is the second most common cause in dogs but the most prevalent type seen in cats. This will appreciably often be accompanied by diabetes mellitus. As mentioned above, obstruction of the pancreatic ducts due to pancreatic adenocarcinoma may evoke atrophy of the acinar cells and resultant EPI.

Pancreatic endocrine and exocrine hypoplasia is very rare and only reported in young dogs.

### **Clinical presentation**

Dogs often pass characteristic pale, greasy, voluminous faeces. Occasionally, watery diarrhoea is seen. Weight loss, borborygmi and flatulence are commonly reported, as is coprophagia. Cats often pass loose malodorous faeces but weight loss is not a major feature as in dogs although body condition in general tends to be poor. The coat tends to be greasy and unkempt and frequently, polyphagia is noted. This will be contributed to by malassimilation but 50% of feline EPI patients are diabetic, due to the pathogenesis of underlying chronic pancreatitis. Appreciably many of these signs can also be seen in small intestinal disease.

### **Diagnosis**

Baseline serum haematology and biochemistry are insufficient to conclusively demonstrate EPI. Mild hypocholesterolaemia may be seen in dogs and mild liver enzyme elevations may be seen, in particular ALT. Amylase and lipase are of no value due to the significant production by organs other than the pancreas, as mentioned above. Low B12 and normal to elevated folate is commonly seen but not diagnostic since this pattern represents intestinal bacterial overgrowth. B12 values are important to established however, since B12 deficiency can severely impair the effectiveness of digestive enzyme replacement.

#### **Serum TLI**

This is the most commonly used pancreatic function test to diagnose EPI in dogs and cats. TLI measures only trypsinogen and trypsin that have entered the bloodstream from the pancreas and is therefore highly specific. Species-specific tests exist and reference ranges differ. Canine reference ranges are 5.7-45.2 $\mu$ g/L, feline ranges are 12.0-82.0 $\mu$ g/L. In dogs, values less than 2.5 $\mu$ g/L and in cats, values less than 8.0 $\mu$ g/L are considered diagnostic for EPI. Values within the intermediate zones are rarely associated with signs of EPI but can frequently reflect sub-total acinar cell destruction and continued monitoring is therefore advised to chart progression. Samples must be fasted since a post-prandial elevation of serum trypsinogen can occur. Trypsinogen is eliminated by glomerular filtration, hence, accurate interpretation of TLI is aided by knowledge of urea, creatinine and urine specific gravity. False negatives may therefore occur if there is concurrent reduction in GFR although the increases tend to be mild.

#### **Serum PLI**

Whilst PLI is highly specific for exocrine pancreatic function, there is a small degree of overlap in serum values between normal dogs and those with EPI making TLI the test of choice for EPI.

#### **Canine faecal elastase**

This test is expensive and tends toward many false positive results.