Acute pancreatitis, azotaemia, cholestasis and haemolytic anaemia in a dog: a case report

M. Guadarrama-Olhovich, L.E. Garcia Ortuno, J.A. Ruiz Remolina, C. Lopez Buitrago, J. Ramirez Lezama, J. Bouda

Faculty of Veterinary Medicine and Husbandry, National Autonomous University of Mexico, Mexico City, Mexico

ABSTRACT: We here report acute pancreatitis with multiorganic complications in a female Cocker Spaniel. The most important alterations in clinical pathology were renal azotaemia, hyperbilirubinaemia with a prevalence of conjugated bilirrubin and increased alkaline phosphatase by cholestasis; hyperamylasaemia due to pancreatitis; dehydration, hyponatraemia, hypochloraemia and hypokalaemia related to vomiting; metabolic acidosis and respiratory alkalosis corresponding to mixed acid-base disorder; markedly regenerative anaemia with spherocytes and agglutination due to immune-mediated haemolytic anaemia and intravascular haemolysis; leukocytosis with a left shift. Proteinuria, glucosuria, bilirubinuria and haemoglobinuria were detected in the urine. Severe suppurative pancreatitis with peripancreatic necrosis and suppurative esteatitis, tubulorrhectic nephrosis, severe hepatitis and intrahepatic cholestasis corresponded with alterations described by clinical pathology. In order to diagnose acute pancreatitis in dogs with multiple complications, it is very important to integrate the results of clinical pathology with the anamnesis and physical examination of the animal. It is especially important to note that the serum activity of amylase correlates with time after the pancreatic attack.

Keywords: exocrine pancreas; multiorganic complications; clinical pathology; necropsy; dog

Exocrine pancreas diseases occur more often in dogs older than seven years of age, and acute pancreatitis is a relatively frequent disease. The factors involved in its development are diverse; they include obesity, excessive consumption of fatty food, bacterial infections of the ascending gastrointestinal tract, biliary tract disease, ischaemia, hyperlipidaemia, drugs, duodenal or bile reflux, abdominal trauma, pancreatic duct obstruction and hypercalcaemia, among others (Steiner 2003; Chan 2006; Shukla 2010). It seems that most cases of pancreatitis remain undiagnosed (Steiner 2003), especially those with multiorganic complications.

Case description

A female Cocker Spaniel dog, eight years old that ate bones and had not defecated for two days, was referred with abdominal pain and was treated at the private clinic with omeprazole, metoclopramide and ademetionine. Nine days after the first experience of abdominal pain, the dog presented with oliguria, vomiting, anorexia, coughing, difficulty in breathing and nasal discharge. She was then hospitalised at the Veterinary Hospital (VH), Faculty of Veterinary Medicine and Husbandry (FVMH) of the National Autonomous University of Mexico. On admission at the VH, the dog was depressed, icteric and in normal body condition (3/5). Respiratory rate was 36/min, heart rate 120/min and rectal temperature 38.3 °C. Physical examination revealed tenderness in the middle and cranial abdomen, 6% dehydration, jaundiced mucous membranes, enlarged popliteal lymph nodes and positive cough reflex. Samples were taken for haemogram, clinical biochemistry and urinalysis, which were referred to the Clinical Pathology Laboratory of the same faculty. Subsequently, a 0.9% NaCl solution, ampicillin, ranitidine and metoclopramide were given.

Table 1. Haemogram of a dog with acute pancreatitis, azotaemia, cholestasis and haemolytic anaemia

Analyte		Reference range
PCV (l/l)	0.18	0.37–0.55
Haemoglobin (g/l)	53	120–180
RBC ($\times 10^{12}$/l)	1.8	5.5–8.5
MCV (f/l)	100	60–77
MCHC (g/l)	294	320–360
Reticulocytes ($\times 10^9$/l)	151	< 60
Platelets ($\times 10^9$/l)	136	200–600
Total proteins (g/l)	65	60–75
WBC ($\times 10^9$/l)	38.9	6.0–17.0
Segmented neutrophils ($\times 10^9$/l)	30.4	3.0–11.5
Band neutrophils ($\times 10^9$/l)	2.7	0–0.3
Lymphocytes ($\times 10^9$/l)	2.3	1.0–4.8
Monocytes ($\times 10^9$/l)	2.7	0.1–1.4
Eosinophils ($\times 10^9$/l)	0.8	0.1–0.9

Anisocytosis 1+, central pallor, polychromasia, spherocytes, agglutination, giant platelets, many metarubricytes, icteric plasma 3+

Table 2. Clinical biochemistry of dog with acute pancreatitis, azotaemia, cholestasis and haemolytic anaemia

Analyte		Reference range
Glucose (mmol/l)	3.0	3.38–6.88
Urea (mmol/l)	93.2	2.1–7.9
Creatinine (μmol/l)	719	60–132
Cholesterol (mmol/l)	4.35	2.85–7.76
Triglycerids (mmol/l)	2.25	0.6–1.2
Total bilirubin (μmol/l)	853	1.7–5.16
Conjugated bilirubin (μmol/l)	492	0–4.2
Unconjugated bilirubin (μmol/l)	361	0–2.5
ALT (μkat/l)	2.75	< 1.70
AST (μkat/l)	3.59	< 0.92
ALP (μkat/l)	14.36	< 1.78
Creatine kinase (μkat/l)	3.85	< 3.55
Total protein (g/l)	54	56–75
Albumin (g/l)	20	29–40
Globulin (g/l)	34	23–39
Ca (mmol/l)	1.86	2.17–2.94
P (mmol/l)	7.5	0.8–1.80
K (mmol/l)	2.69	3.8–5.4
Na (mmol/l)	121	141–152
Cl (mmol/l)	79	108–117
Total CO_2 (mmol/l)	9	17–25
Anion gap (mmol/l)	36	12–24
SID (mmol/l)	42	30–40
Osmolality (mmol/kg)	330	280–305
Amylase (μkat/l)	47.67	< 18.53

Icteric serum 3+; SID = strong ion difference (Na – Cl)
Anion gap [(Na + K) – (Cl + HCO_3)]

The haemogram showed severe macrocytic hypochromic regenerative anaemia (reticulocytosis) in the presence of spherocytes and a positive agglutination test due to the haemolytic process; mild thrombocytopaenia due to consumption or destruction, neutrophilic leukocytosis with a left shift and monocytosis by inflammation and nonspecific lymphocytosis by antigenic stimulation (Table 1).

Blood serum biochemistry (Table 2) showed renal azotaemia with hyperphosphoremia and hyperamylasaemia related to decreased renal excretion. However, the hyperamylasaemia was also influenced by pancreatitis. Hyperbilirubinaemia was found predominantly with conjugated bilirubin and increased alkaline phosphatase (AP) due to cholestasis. The marked increase in unconjugated bilirubin was associated with a haemolytic process. Both the increases in ALT and AST were due to hepatocellular degeneration; however, part of the increase in AST and CK were associated with muscle activity and catabolism. Hypoproteinaemia due to hypoalbuminaemia was caused by decreased intake and chronic inflammation. Hyponatraemia, hypochloraemia and hypokalaemia were associated with losses due to vomiting. The acid-base status analysed in serum corresponded to a mixed disorder due to metabolic acidosis (low total CO_2) caused by an accumulation of acids (increased anion gap) and hypochloraemic metabolic alkalosis due to vomiting. With the acid-base analyser, metabolic acidosis and respiratory alkalosis were found in venous blood, (pH = 7.36, pCO_2 = 18 mmHg, HCO_3 = 10.4 mmol/l), corresponding also to mixed acid-base disorder.

The urinalysis revealed aciduria associated with metabolic acidosis, decreased urine density and glucosuria related to kidney damage, haemoglobinuria due to intravascular haemolysis with associated proteinuria and bilirubinuria caused by hyperbilirubinaemia (Table 3).

Table 3. Urinalysis of dog with acute pancreatitis, azotaemia, cholestasis and haemolytic anaemia

Analyte	
Transparency: cloudy	
Colour: dark yellow	Urine sediment
pH: 5.0	
Density: 1.015	RBCs/hpf: 0–2
Glucose: 2.8 mmol/l (1+)	WBCs/hpf: 0–1
Protein: 0.3 g/l (1+)	Epithelial cells/hpf: clumps transitional
Bilirubin: 2+	Casts/lpf: 0–1
Blood: negative	Crystals: 0
Haemoglobin: 3+	Bacteria: Coccus 1+

Direct observations in dark field and plate microagglutination tests were performed for diagnosis of *Leptospira* spp. and both were negative. On the third day after admission at the VH, FVMH (12 days after the first abdominal pain) the dog had a cardiopulmonary arrest and died. A postmortem study was then carried out. Diffuse suppurative pancreatitis accompanied by peripancreatic severe suppurative steatonecrosis with multifocal calcification were found in the interstitium; diffuse congestion with moderate renal haemoglobinuric nephrosis and deposits of pigment were seen in the cytoplasm and tubular lumen along with degeneration and slight tubular necrosis; severe hepatitis with periportal fibrosis, mild and diffuse hepatic cord dissociation and moderate and diffuse intrahepatic cholestasis were also observed.

DISCUSSION AND CONCLUSIONS

According to the necropsy report, acute pancreatitis could be the origin of the renal, hepatobiliary and haemolytic pathology in this dog. Pancreatitis develops when proteolytic enzymes are activated within the pancreas, resulting in a pancreatic self-digestion (Klimes et al. 2000). Conversion of trypsinogen to trypsin is the beginning of this process and promotes the activation of other zymogens, particularly proelastase and phospholipase, which amplify pancreatic damage (Wang et al. 2009). The progressive activation of high amounts of protease and phospholipase inside the pancreas has been associated with the gradual transformation of oedematous to haemorrhagic or necrotic pancreatitis, producing a systemic process and consumption of plasma protease inhibitors, which are vital for protection against other effects of proteolytic enzymes in the vascular space. Once inhibitors are no longer present, animals can die quickly due to the systemic complications that are triggered such as disseminated intravascular coagulation (DIC) and shock by free proteases that activate the clotting, fibrinolysis, complement and kinin cascades (Williams and Steiner 2005; Zhang and Li 2009).

In acute pancreatitis, the release of enzymes such as amylase and lipase from the cytoplasm of damaged acinar cells may increase and be reflected by their increased serum activity. Nonetheless, based on these findings, the diagnosis of pancreatitis can be extremely difficult because these tests are highly sensitive but not specific. The half-life of serum amylase in healthy dogs is one to five hours, but in dogs with pancreatitis may last one to three days and result in an increase in enzyme concentration up to 29 times above the reference value, returning to normal between the third and fifth day after reaching the highest level (Hoffmann and Solter 2008). With regard to the clinical pathological findings in this animal, the increase in amylase was rather modest to justify a diagnosis of pancreatitis given the parameters of amylase used as an indicator of pancreatic inflammation. However, this value can be explained by the clinical picture which began nine days before the laboratory studies, and which reflected the decreased activity of amylase due to its degradation.

Due to the half-life of the enzyme, when diagnosing acute pancreatitis by serum amylase, levels should be determined after the first signs of abdominal pain (Steiner 2003), and its increase should be four to five times above the reference value to allow an accurate diagnosis of pancreatitis based

solely on this enzyme (Hoffmann and Solter 2008). The best marker identified so far is immunoreactive lipase which besides being species-specific, is also specifically pancreatic in origin and therefore indicative of pancreatitis; moreover, its concentration does not change with renal impairment or administration of steroids, which makes it a highly specific and sensitive marker (Xenolius et al. 2008).

Regardless of the cause of pancreatitis, the reported systemic consequences are numerous, and include cholestasis, acute renal failure and altered haemodynamic processes (Williams and Steiner 2005).

Severe peripancreatic inflammation and pancreatic abscesses are causes of complete or partial extrahepatic biliary obstruction (Williams and Steiner 2005). Pancreatitis can cause intrahepatic biliary obstruction due to the activation and movement of pancreatic enzymes through the portal system involving the portal or the microscopic bile duct region and commonly causes cholestasis (Williams and Steiner 2005; Center 2009). The postmortem study of the case described here confirmed chronic hepatobiliary damage due to mild periportal liver fibrosis with hepatic cord dissociation. Hyperbilirubinaemia was also present with a predominance of the conjugated fraction and increase in AP, both associated with the process of intrahepatic cholestasis, probably owing to periportal liver fibrosis, reported in the histopathological study of the necropsy (Williams and Steiner 2005).

Another known complication of acute pancreatitis in humans is the development of acute renal failure (Wang et al. 2009). The occurrence of this complication is influenced by factors such as hypovolaemia and renal endothelial damage caused by a severe systemic inflammatory response. Furthermore, dehydration in a patient with acute pancreatitis is a predisposing factor for the development of hypovolaemia, primarily by vomiting that leads to a significant loss of chloride and thus to hypochloraemic metabolic alkalosis. When hypovolaemia is exacerbated, a decrease in the glomerular filtration rate occurs; subsequently, ischaemia leads to acute kidney injury and metabolic acidosis is triggered by the accumulation of phosphate and sulphate. Moreover, a severe inflammatory response, mainly triggered by the action of tumour necrosis factor α (TNFα) and interleukin 1 (IL1), leads to widespread vascular damage of the renal endothelium, which provokes an acute inflammatory process with variable intensity and lesion distribution, and consequently, tubulointerstitial damage. Ischaemia, followed by irreversible cell damage, exacerbates acute renal failure. Kidney damage was evident in this animal, since laboratory tests clearly indicated renal azotaemia, hyperphosphoremia, glucosuria, and decreased renal concentrating capacity. At necropsy, this was reflected in tubulorrhectic nephrosis with haemoglobin crystals in the lumen of renal tubules along with pigment deposition in the cytoplasm and tubular lumen, injuries that alter renal function (Newman et al. 2007). Furthermore, a mixed acid-base disorder was present, i.e., metabolic acidosis due to the accumulation of acids and hypochloraemic metabolic alkalosis (Table 2), which probably exacerbated the renal damage.

The various haematologic abnormalities reflected in patients with acute pancreatitis can be reticulocytosis, haemolytic anaemia (HA) coagulation abnormalities, thrombocytopaenia, and leukocytosis. In this case there was as much intravascular as immune-mediated HA. The latter has been described in chronic inflammatory conditions, resulting from immune system disorders (Brockus and Andreasen 2005) and in this case it was associated with severe systemic inflammation caused by pancreatitis. On the other hand, intravascular haemolysis occurred in this case report provoking a diffuse deposition of haemoglobin in the cortex and medulla of renal tubules, causing severe haemoglobinuria and kidney damage (Newman et al. 2007).

When the complications in this animal suffering the effects of acute pancreatitis are listed, i.e., acute renal failure, haemolytic anaemia and thrombocytopaenia, it may be possible that it was developing one of the complications that occur in humans with acute pancreatitis but which is scarcely reported in veterinary medicine, haemolytic uraemic syndrome (HUS) (Sinha and Rai 2005). Rare cases have been reported in horses, dogs and cattle, but of gastroenteric origin (Chantrey et al. 2002).

HUS develops in the following two to three days after pancreatitis onset and disappears when the disease is resolved. One possible mechanism is that endothelial damage itself triggers it. The inflammatory response to acute pancreatitis can trigger the onset of HUS in an average of three days. The clinical course suggests that acute pancreatitis precedes the development of HUS and is not the cause of it. The systemic inflammatory response in pancreatitis mediated by IL6, IL8, TNFα and other cytokines may contribute to the development of an acute episode of HUS. In addition, pancreatitis

is associated with endothelial cell damage, which may contribute to the development of the syndrome (Sinha and Rai 2005; Swisher et al. 2007).

Renal disease predominates in HUS. It is rare during acute pancreatitis, although if it occurs, it has a sudden onset and clinical manifestations may be heterogeneous, even leading to death without diagnosis and treatment. HUS is characterised by thrombotic microangiopathy, injury to the endothelium of small arterioles and capillaries leading to the formation of platelet clusters and thrombosis. Thrombocytopaenia and haemolytic anaemia result from the consumption of platelets and fragmentation of erythrocytes in the affected vessels, respectively (Chantrey et al. 2002; Dell'Orco et al. 2005). Meanwhile, the severe and diffuse deposit of haemoglobin in the renal cortex and medulla, secondary to haemoglobin from the acute intravascular haemolytic crisis, causes tubulorrhectic nephrosis and exacerbates renal injury (Newman et al. 2007).

Clotting pathogenesis is also related to the release of trypsin from pancreatic inflammation. Some complications consistent with disseminated intravascular coagulation (DIC), such as the elevation of fibrin degradation products (FDP), hypofibrinogenemia and increase of clotting times, have been reported in this disease, which may be due to endothelium damage that triggers a hypercoagulable state. In this syndrome, it is unlikely that widespread DIC is triggered localized DIC cannot be ruled out (Hess et al. 1998; Saif 2005; Sinha and Rai 2005; Williams and Steiner 2005).

HUS in humans has been associated with bacterial and viral infections, neoplasms, antiphospholipid syndrome, pregnancy, chemotherapy, immunosuppressive drugs and rarely to pancreatitis (Sinha and Rai 2005).

In this case, it is difficult to determine what the trigger for the systemic alterations was since the dog was initially treated in private practice and samples for laboratory analysis were taken 9 days after the clinical onset. However, it is likely that the systemic response to pancreatitis provoked the hepatobiliary damage, mainly by metabolism of released pancreatic enzymes; the endothelial damage may have been caused by the action of pancreatic enzymes that produce intravascular haemolysis and by the deposition of haemoglobin which would implicate the renal tubules as the cause of renal failure.

In conclusion, for the diagnosis of acute pancreatitis in dogs with multiorganic complications, it is very important to integrate the results of haematology, clinical biochemistry and urinalysis with the anamnesis and physical examination of the animal. Moreover, special attention should be paid to the time of blood sampling for laboratory analysis and serum amylase activities must be correlated with the date of the pancreatic attack.

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Corresponding Author:

Jan Bouda, National Autonomous University of Mexico, Faculty of Veterinary Medicine and Husbandry, Pathology Department, Mexico City, Circuito Exterior, Ciudad Universitaria, Delegacion Coyoacan, Mexico, D.F. C.P. 04510
Tel. + 52 562 887 888, E-mail: bouda@unam.mx