

<https://doi.org/10.17221/145/2018-VETMED>

Hypoglycaemic crisis induced by non-islet cell tumours in two dogs

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Citation: Kim H, Kang JH, Kang BT, Yang MP (2019): Hypoglycaemic crisis induced by non-islet cell tumours in two dogs. *Veterinarni Medicina* 64, 407–416.

Abstract: Two dogs were admitted for the diagnosis and treatment of neoplasia (a hepatic and a mammary tumour, respectively), lethargy, quadriparesis and abnormal mentation with hypoglycaemia. The blood analyses showed severe hypoglycaemia (1.32 and 1.60 mmol/l, respectively). Although prompt treatment, including intravenous administration of dextrose, was initiated, the blood glucose concentrations were not restored to a normal range. After the diagnostic procedures, no aetiology other than the hepatic tumour identified by the abdominal radiography and ultrasonography, and a mammary tumour that might have caused the hypoglycaemia, were identified. Because there was a high suspicion of non-islet cell tumour-induced hypoglycaemia as a paraneoplastic syndrome, the dogs underwent a hepatic lobectomy and total mastectomy with an ovariohysterectomy, respectively. Within 12 hours after surgery, the blood glucose concentrations of both cases had normalised, even without the administration of dextrose. The histopathological examinations identified a hepatocellular adenoma and a mammary carcinoma, respectively. The endocrine analysis of the serum at admission revealed low serum insulin concentrations (< 1.44 pmol/l) and high serum concentrations of insulin-like growth factor 2 in both dogs. Therefore, the diagnosis in both dogs was confirmed to be non-islet cell tumour-induced hypoglycaemia. Both dogs remained alive without the recurrence of hypoglycaemia 24 months later. Previously, the administration of intravenous dextrose has been considered as the initial treatment in dogs with hypoglycaemia; however, this can temporarily ameliorate the clinical signs related to the non-islet cell tumour-induced hypoglycaemia and help the anaesthesia for the surgical tumour resection as an emergency. Therefore, the definitive treatment of non-islet cell tumour-induced hypoglycaemia might be rapid surgical intervention, which can be associated with good prognosis in dogs with severe non-islet cell tumour-induced hypoglycaemia.

Keywords: canine; glucagon; hypoglycaemia; insulin-like growth factor-2; paraneoplastic syndrome

Hypoglycaemia is biochemically defined as a blood glucose concentration of < 3.30 mmol/l (Koenig 2015), although clinical signs often do not occur until it is < 2.75 mmol/l. Various diseases have been identified as causes of hypoglycaemia in dogs, but insulinoma is considered to be the most common aetiology of severe tumour-induced hypoglycaemia. However, non-islet cell tumours that release hormones ectopically can also cause severe hypoglycae-

mia in adult dogs (Leifer et al. 1985). Hypoglycaemia is a relatively common problem encountered in veterinary practice (Idowu and Heading 2018), and if acute and severe it can induce life-threatening neurologic signs and cardiovascular collapse (Leifer et al. 1985), and medical emergencies (Idowu and Heading 2018). Rapid clinical intervention, with immediate correction of the hypoglycaemia, is necessary to alleviate these clinical signs.

Whipple's triad is used to identify hypoglycaemia, which consists of clinical signs consistent with hypoglycaemia, a low blood glucose concentration, and improvement of the clinical signs following correction of the hypoglycaemia (Leifer et al. 1985). To alleviate the clinical signs consistent with hypoglycaemia, the rapid symptomatic treatment and therapy targeting the aetiology are both important (Idowu and Heading 2018). In veterinary medicine, the initial symptomatic treatment of hypoglycaemia usually consists of the administration of intravenous dextrose, glucagon and/or glucocorticoids (Leifer et al. 1985; Loose et al. 2008; Datte et al. 2016). However, there is a paucity of information regarding the efficacy of symptomatic treatment in dogs with non-islet cell tumour-induced hypoglycaemia (NICTH), which has been sporadically reported in veterinary medicine. The mainstay of therapy for NICTH in human medicine is surgical resection if feasible (Bodnar et al. 2014), but descriptions of such interventions are lacking in veterinary literature. Previous reports have suggested that the prognosis is poor, although the rarity of this condition in dogs means that it is hard to draw definitive conclusions (Boari et al. 1995; Zini et al. 2007; Rossi et al. 2010). Thus, the purpose of this case report is to describe the clinical course and the successful treatment of NICTH in two dogs.

Case description

CASE 1

A 14-year-old, intact female mixed breed dog, weighing 8.5 kg, was referred for further investigation of its lethargy and hepatic tumour, which was identified by the referring veterinarian. The dog had a history of intermittent depression and dull mental status, which had been observed for one week. The owner reported that the dog had been well before the development of these clinical signs. There was no history of exposure to oral hypoglycaemic drugs or toxins such as xylitol.

On physical examination, the dog showed lethargy, abdominal distension, quadriparesis, and abnormal mentation, but no other neurologic signs were evident. The heart rate, respiratory rate, rectal temperature, and systolic blood pressure were within the normal range. No heart murmur or lung adventitious sounds were detected on auscultation. The

mucous membranes were pink, with a capillary refill time of < 1 sec. A discrete and non-painful intra-abdominal mass was palpated. Before conducting other diagnostic tests, the blood glucose concentration was measured using a handheld glucometer (AlphaTrak®2, Zoetis Inc., NJ, USA), which showed that it was below the detection limit (< 0.83 mmol/l). Therefore, a hypoglycaemic crisis was suspected and a 0.5 g/kg bolus of 50% dextrose diluted 1 : 1 in normal saline was immediately administered, followed by a constant rate infusion (CRI) of 5% dextrose. Then, because the hypoglycaemia persisted and the dog remained depressed, a 50 ng/kg of glucagon (Garcon inj., Dalim BioTeck Inc, Seoul, Republic of Korea) was also administered intravenously. After this, the blood glucose concentration temporarily increased to 15.68 mmol/l and the dog became more active, but 3 h later, its blood glucose concentration once again returned to a level that was too low to measure with the handheld glucometer.

Alongside the symptomatic treatment, diagnostic tests were performed to identify the aetiology of the hypoglycaemia. Hepatic dysfunction or failure induced by liver pathology related to the massive tumour infiltration was initially suspected on the basis of the diagnosis of a hepatic tumour, but this was later thought to be unlikely because the serum bile acid concentration was within the normal range and no coagulopathy was detected. Abnormalities in the biochemical screen included high serum alanine aminotransferase activity (552 IU/l; reference range, 21–102 IU/l) and alkaline phosphatase activity (297 IU/l; reference range, 29–97 IU/l). There were no abnormalities in the complete blood count (CBC), electrolytes, or basal cortisol concentration, implying that sepsis and hypoadrenocorticism were also unlikely to be the cause of the hypoglycaemia. In addition, the urinalysis results were unremarkable and the SNAP cPL (IDEXX Reference laboratory Inc., Seongnam-si, Republic of Korea) test was negative. A low serum insulin concentration (< 1.44 pmol/l; reference range, 14.35–150.68 pmol/l) was identified with a low blood glucose concentration (1.32 mmol/l; reference range, 3.58–6.49 mmol/l), thus ruling out insulinoma. The abdominal radiographs revealed an egg-shaped mass in the upper abdomen, and the abdominal ultrasonographic examination showed the presence of a solitary hyperechoic hepatic mass of 29 mm in diameter with an amorphous margin (Figure 1A).

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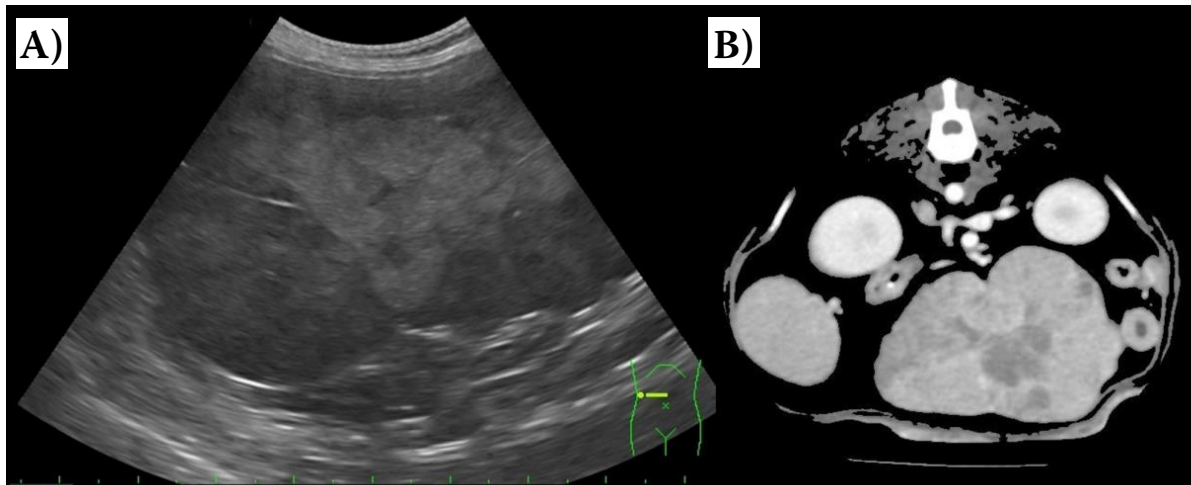


Figure 1. The sagittal plane ultrasonography of the liver, showing a solitary hyperechoic hepatic mass, 29 mm in diameter, with an amorphous margin (A), and a representative post-contrast computed tomography image (B) of Case 1

During the diagnostic procedures and treatments of the hypoglycaemia, the blood glucose concentrations ranged from < 0.83 to 2.53 mmol/l, accompanied by abnormal mentation and quadriparesis. Therefore, the concentration of the dextrose CRI was increased to 10% and intravenous dexamethasone sodium phosphate (Dexamethasone inj., Je Il Pharm Co, Daegu, Republic of Korea) was administered at a dose of 0.1 mg/kg every 12 hours. Following this, the clinical signs improved, but the hypoglycaemia (blood glucose 1.43–2.97 mmol/l) persisted all day.

The following morning, the dog still had a low blood glucose concentration, but did not display any clinical signs. However, when the concentration or rate of dextrose CRI was reduced, the lethargy recurred, and no other aetiology other than the hepatic tumour that might have caused the hypoglycaemia had been identified. Therefore, paraneoplastic syndrome secondary to the hepatic tumour was suspected and computed tomography (CT) was performed to determine whether surgical resection of the hepatic tumour might be feasible. The CT scan showed a solitary tumour located in

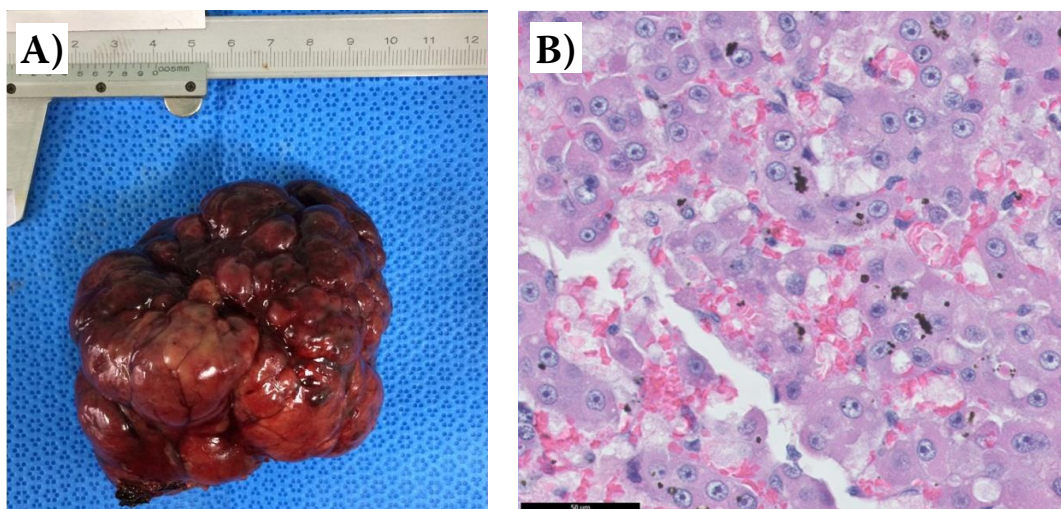


Figure 2. A photograph of the resected right medial liver lobe containing a mass (A) and the histologic section of this mass (B) from Case 1. The histopathology revealed the mass to be a non-encapsulated, expansile, densely cellular neoplasm composed of polygonal neoplastic cells arranged in 2–5 cell-thick aggregates, with 2–8 cell-thick trabeculae and irregular sinusoids. The neoplastic cells contained abundant eosinophilic cytoplasm and cytoplasmic vacuoles. The nuclei were round with coarsely stippled chromatin and 1–2 distinct nucleoli. Mild anisocytosis and anisokaryosis were also apparent. Multiple areas of haemorrhage and inflammation were present. H&E, bar = 50 μ m

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the right medial liver lobe (Figure 1B) and no metastasis. Subsequently, an exploratory laparotomy was performed with the owner's consent. Before the surgery, a bolus of 50 ng/kg of glucagon was intravenously administered to prevent a further decrease in the blood glucose concentration. The solitary hepatic mass was removed by a right medial liver lobectomy (Figure 2A) and no metastatic lesions were identified in the adjacent organs. Soon after the liver lobectomy, the blood glucose concentration was normal even without the administration of dextrose CRI, glucagon, or dexamethasone.

On the histopathologic examination, a hepatocellular adenoma was diagnosed (Figure 2B). In addition, the serum insulin-like growth factor (IGF)-2 concentration was measured using an enzyme-linked immunosorbent assay, which showed a higher serum IGF-2 concentration compared to that of healthy dogs at presentation (Table 1), but this gradually decreased after the tumour resection. On the basis of these findings, the dog was

diagnosed to have NICTH resulting from hepatocellular adenoma. Five days later, the blood glucose concentration was still within the normal range and the dog displayed no clinical signs, and the dog was, thus, discharged. The dog remained alive without recurrence of the hypoglycaemia 24 months later.

CASE 2

A 13-year-old, intact female Shih-Tzu, weighing 4.5 kg, was referred because of collapsing. The dog had no previous history of ill-health except that the dog had undergone a left caudal lumpectomy to remove a mammary tumour, which was performed one year before presentation. The owner reported the development of left subcutaneous inguinal masses during the preceding two months. On the day before presentation, a seizure-like episode had occurred.

On physical examination, the dog showed lethargy and abnormal mentation. The vital signs were

Table 1. The laboratory analysis of the serial serum samples obtained from two dogs with non-islet cell tumour-induced hypoglycaemia

	Before surgery		After surgery				Reference intervals	Healthy dogs (<i>n</i> = 14)
	at presenta- tion	after glucose and glucagon infusion	day 1	day 3	day 7	day 240		
Case 1								
*Glucose (mmol/l)	1.32	3.47	8.42	7.43	6.93	6.66	3.58–6.49	
**Insulin (pmol/l)	< 1.44	ND	ND	ND	ND	ND	14.35–150.68	
†IGF-2 (ng/ml)	94.0	96.9	64.9	61.4	59.7	42.2	NA	††47.4–57.6
Case 2								
*Glucose (mmol/l)	1.60	1.05	5.23	4.90	5.23	4.79	3.58–6.49	
**Insulin (pmol/l)	< 1.44	ND	ND	ND	ND	ND	14.35–150.68	
†IGF-2 (ng/ml)	192.8	174.8	72.6	52.3	49.4	39.2	NA	††47.4–57.6

IGF = insulin-like growth factor; NA = not applicable; ND = not determined

*The blood glucose levels were determined using an automated analyser (Hitachi 7020, Hitachi High-Technologies Co., Tokyo, Japan)

**The insulin levels were determined using a chemiluminescent immunoassay-based autoanalyser (Immulite 1000, DPC, Los Angeles, CA)

†The serum IGF-2 concentrations were determined using a sandwich enzyme-linked immunosorbent assay using a commercial kit (MyBioSource Inc., San Diego, CA) designed to measure canine IGF-2 levels. The assays were performed according to the manufacturer's protocol. All the samples, standards, and controls were assayed in duplicate. The optimal density at 450 nm was determined using an automated microplate reader (ELx808, Bio-Tek Instruments Inc., Winooski, VT). The lower and upper detection limits were 10 and 250 ng/ml, respectively. The intra-assay and inter-assay coefficients of variation were both < 10%

††The range of the serum IGF-2 concentration in the age (± 1) matched-control dogs (*n* = 14)

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normal and the mucous membranes were pale pink and displayed a normal capillary refill time (< 2 sec). No heart murmur or lung adventitious sounds were detected and a brief neurologic examination was unremarkable. Two firm subcutaneous masses were identified in the left inguinal lesion (Figure 3A); these measured 5.6×5.8 cm and 6.7×5.0 cm in diameter. No lymph node enlargement was detected.

During the physical examination, the dog showed generalised tonic-clonic seizures and emergency treatments with a 0.5 mg/kg of intravenous diazepam (Diazepam inj., Myungin Pharm., Seoul, Republic of Korea) was initiated. While the anti-convulsive therapy was being administered, a low blood glucose concentration (1.16 mmol/l) was identified using a handheld glucometer (AlphaTrak[®]2, Zoetis Inc., NJ, USA). Therefore, a bolus of 0.5 g/kg of 50% dextrose diluted 1 : 1 in normal saline was administered, followed by a 5% dextrose CRI. Following this, the seizures stopped, but the dog's blood glucose concentration remained low (2.09 mmol/l). Sepsis was considered unlikely to be the cause of the hypoglycaemia because a fever or hypothermia had not been detected and other vital signs were

normal at presentation. However, neutrophilia ($37\,800/\mu\text{l}$; reference range, $5050\text{--}16\,760/\mu\text{l}$) with a left shift and mild toxic changes and monocytosis ($1830/\mu\text{l}$; reference range, $160\text{--}1120/\mu\text{l}$) were identified, and 30 mg/kg of prophylactic cefotaxime (Cefotaxime inj., Dong-A ST, Seoul, Republic of Korea) was administered intravenously. The CBC also revealed mild normocytic normochromic anaemia (haematocrit = 31.2%; reference range, 37.3–61.7%), and the abnormalities detected in the biochemical screen included a high serum alkaline phosphatase activity (358 IU/l; reference range, 29–97 IU/l). The electrolytes, urine analysis, and basal cortisol concentration were normal; therefore, it was considered that hypoadrenocorticism was also unlikely to be the cause of the hypoglycaemia. The findings of the low serum insulin concentration (< 1.44 pmol/l; reference range, 14.35–150.68 pmol/l) with low blood glucose (1.60 mmol/l; reference range, 3.58–6.49 mmol/l) excluded insulinoma as a diagnosis. There were no abnormalities on the abdominal or thoracic radiographs, or on the abdominal ultrasonographic examination.

During the diagnostic procedures, a CRI of 10 ng/kg/min of glucagon in 5% dextrose-contain-

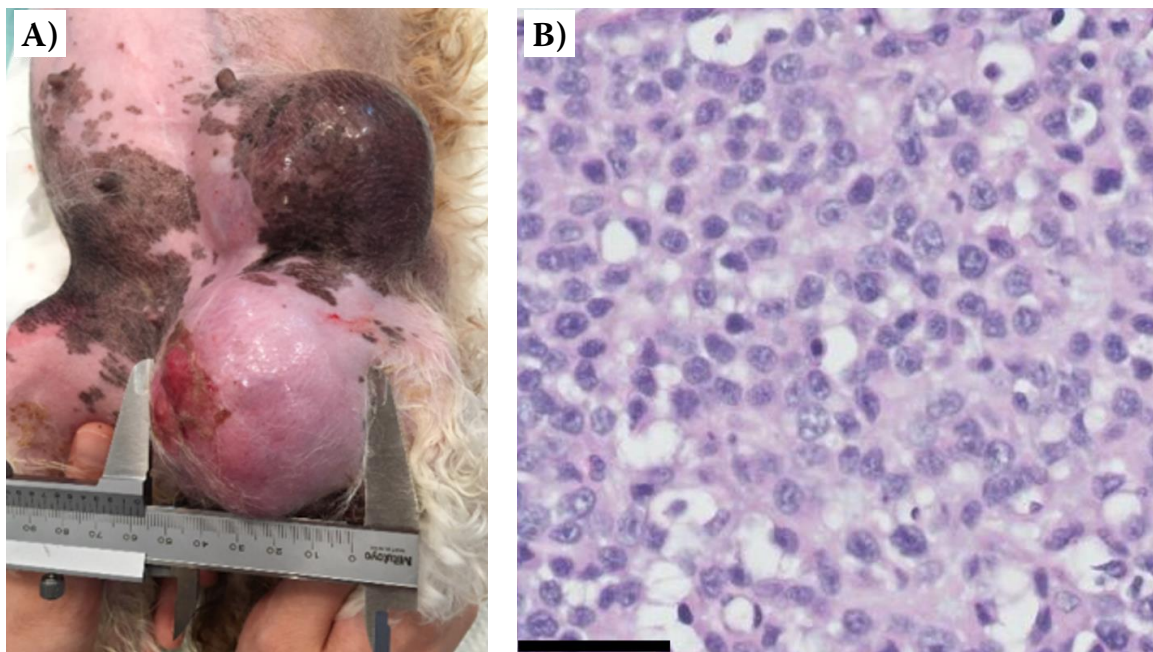


Figure 3. The gross appearance of the mammary tumours (A) and a histologic section of a tumour (B) from Case 2. The histopathology revealed that they were infiltrative, multinodular, densely cellular neoplasms composed of polygonal to pleomorphic cells arranged in packets, with rare, solid glandular structures. The neoplastic cells had moderately eosinophilic cytoplasm and round-to-oval nuclei. Their nuclei had finely to coarsely stippled chromatin and 1–2 large distinct nucleoli. Mild anisocytosis and anisokaryosis were apparent. There were multifocal areas of neoplastic myoepithelial differentiation and multiple large areas of necrosis also. H&E, bar = 50 μm

ing fluid was administered due to the persistent hypoglycaemia, and the dose was gradually increased to 50 ng/kg/min (alongside the blood glucose monitoring). However, the blood glucose concentration remained low, ranging from < 0.83 to 1.16 mmol/l despite using the maximum previously reported rate (50 ng/kg/min) of glucagon (Datte et al. 2016). Although this treatment ameliorated the clinical signs consistent with the hypoglycaemia, the hypoglycaemia persisted; thus, the definitive treatment of the underlying cause was necessary. The diagnostic procedures had not identified a likely cause for the hypoglycaemia, other than the rapidly growing inguinal tumours. Therefore, because NICTH associated with the inguinal tumours was suspected, a tumour resection was recommended to ameliorate the hypoglycaemia, but the owner declined the surgery.

Three days later, the dog was readmitted, having collapsed, and was minimally responsive following a series of seizures. The blood glucose concentration was below the detection limit of the handheld glucometer (< 0.83 mmol/l). A bolus of 0.5 g/kg of 50% dextrose was administered, followed by a 5% dextrose CRI. The dog continued to show intermittent seizures; therefore, diazepam, phenobarbital (Phenotal Inj., Daehan New Pharm Co., Ltd., Hwasung-si, Republic of Korea), and mannitol (Mannitol inj., JW Pharmaceutical, Seoul, Republic of Korea) were administered intravenously. However, the blood glucose concentrations remained low (< 0.83–1.54 mmol/l); therefore, a bolus of glucagon (50 ng/kg) was administered intravenously and then a glucagon CRI (50 ng/kg/min) was initiated. The dog's mental status gradually improved, but the intermittent tonic-clonic seizures, which were alleviated by intravenous diazepam administration, continued. The rate of the glucagon CRI was gradually increased to 5 µg/kg/min, which is 100 times more than the previously reported maximum rate (Datte et al. 2016), after which there were no more seizures, although the low blood glucose concentration persisted.

To treat the hypoglycaemia, we decided that the surgical resection of the inguinal tumours was essential. Prior to surgery, a fine-needle aspiration of the inguinal masses was performed, which revealed numerous epithelial cell clusters comprising cells containing small quantities of cytoplasm and large round nuclei with coarse chromatin. On the basis of the location and the malignant features, mammary

carcinoma was suspected. Therefore, a total mastectomy and ovariohysterectomy were performed. Immediately after the surgery, the dog's blood glucose concentration gradually increased, even when the glucagon CRI was withdrawn. Hyperglycaemia (11.99 mmol/l) was identified 5 h after surgery, at which time the dextrose CRI was also discontinued.

By the following day, the dog's blood glucose concentration was within a normal range and the dog had regained normal activity; therefore, the dog was discharged at the owner's request. The histopathologic examination of the resected mass revealed it to be a complex type of mammary gland carcinoma with an intermediate degree (grade II) of malignancy (Figure 3B). An enzyme-linked immunosorbent assay showed a higher serum IGF-2 concentration than that of healthy dogs at presentation, which gradually decreased after surgery. These results were consistent with the diagnosis of NICTH secondary to the mammary carcinoma. The dog remained alive, without recurrence of the tumour or hypoglycaemia, 24 months later.

DISCUSSION AND CONCLUSIONS

This report describes NICTH in two dogs. The condition was not improved by general symptomatic treatments such as glucagon and dextrose administration, which have been considered as the initial treatment under hypoglycaemic crisis. After surgical resection of the tumours, the dog's blood glucose levels were dramatically normalised and the recurrence of their hypoglycaemia was not observed over a 2-year period, implying that canine NICTH is amenable to the definitive treatment by tumour resection. NICTH is a paraneoplastic syndrome that can cause severe hypoglycaemia in dogs (Zini et al. 2007), but information regarding the optimum treatment for NICTH has been lacking in veterinary literature, despite the poor associated prognosis. The cases reported herein firstly provide evidence that the rapid surgical resection of the tumour as a definitive treatment is likely to improve the chances of a successful outcome for dogs with NICTH.

The mainstay of the treatment for NICTH in human patients is surgical resection (Dutta et al. 2013), but evidence for the success of this approach has been lacking to date in veterinary literature. Despite being treated with both dextrose and glucagon, the

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dogs reported here continued to suffer from hypoglycaemia, in contrast to the situation reported in many human patients, in whom intravenous dextrose-containing fluids administration suffices to prevent further hypoglycaemia (Dutta et al. 2013). However, as described for case 1, 3 h after a single bolus intravenous glucagon injection of 50 ng/kg, which was the maximum dose required to normalise the glycaemia in a previous study (Datte et al. 2016), the blood glucose concentration remained low. It is likely that the 3-h duration of the effect of a glucagon bolus was insufficient to ameliorate the hypoglycaemia. Furthermore, the continuous administration of 5% dextrose-containing fluids was also insufficient to manage the NICTH in dogs.

Glucagon has been recommended for the treatment of hypoglycaemia in humans (Dutta et al. 2013), but there has only been one case report and one retrospective study published of dogs with hypoglycaemia (Fisher et al. 2000; Datte et al. 2016). A maximum rate of 50 ng/kg/min for a CRI of glucagon might be appropriate because glucagon is the most important stimulus for hepatic glucose production under conditions of insulin-induced hypoglycaemia (Rivera et al. 2010). It is also recommended that glucagon be administered as a CRI rather than by regular injection, based on the pharmacokinetic data (Zeugswetter et al. 2012). However, as described for case 2, the recommended dose of glucagon CRI was insufficient to normalise the glycaemia. This may be accounted for by the case-specific differences: the previously described infusion rate may be more appropriate for hyperinsulinemic hypoglycaemic dogs (Datte et al. 2016). Furthermore, the blood glucagon concentration is often within the normal range in human patients with NICTH, which may be because the hypoglycaemia is, in part, induced by the inhibitory effect of IGF-2 on the glucagon action (de Groot et al. 2012). Therefore, the 50 ng/kg/min rate of glucagon CRI previously reported in veterinary literature (Datte et al. 2016) may not be sufficient to treat canine NICTH if it occurs because of the inhibitory effects of IGF-2 on the glucagon action. In this situation, an immediate surgical resection might be necessary to for a successful outcome, rather than medical treatment.

In some countries, the intravenous administration of glucagon is not available. Instead, glucose solutions of various concentrations are used. In addition, the serum insulin is not always checked

in dogs with hypoglycaemia and a diagnosis of NICTH might not be readily available in general practice. Therefore, medical treatments may be initiated prior to surgical tumour resection to ameliorate the clinical signs related to hypoglycaemia and to help anaesthesia in an emergency. Depending on the blood glucose levels, glucose is administered in the form of intravenous dextrose or as solutions of varying concentrations (Idowu and Heading 2018). However, this can be counterproductive because elevated glucose concentrations induce hyperinsulinaemia via a normal homeostatic mechanism in dogs with insulinomas or NICTH (Idowu and Heading 2018). This can cause rebound hypoglycaemia. Furthermore, the sole glucose administration might be insufficient in dogs with NICTH, as described in the present cases. Diazoxide and somatostatin analogues such as octreotide can be considered, but treatment with diazoxide might be ineffective in NICTH cases showing normal or decreased blood insulin concentrations (Finotello et al. 2016). Furthermore, the use of octreotide intended for use in human clinical practice could worsen the clinical signs because it inhibits growth hormone synthesis (Finotello et al. 2016). Alternatively, glucocorticoids and a growth hormone to counteract the insulin-like effects has been proposed in human medicine (Bourcigaux et al. 2005). In case 1, 10% dextrose CRI and intravenous dexamethasone sodium phosphate (0.1 mg/kg every 12 h) improved the clinical signs, but the hypoglycaemia persisted. It may be possible to administer a growth hormone to increase the blood glucose concentrations in NICTH dogs treated with glucocorticoids and intravenous glucose. However, the use of glucocorticoids and a growth hormone has not been investigated in veterinary medicine. Therefore, slow glucose infusion to prevent rebound hypoglycaemia, along with other treatment options such as glucocorticoids, can be attempted with caution as an emergency treatment before a surgical tumour resection; however, further information will be necessary to elucidate the value and safety of this medical approach.

NICTH has been reported in dogs with various tumours (Beaudry et al. 1995; Boari et al. 1995; Battaglia et al. 2005; Snead 2005; Zini et al. 2007; Rossi et al. 2010), but only three cases of IGF-2-related NICTH have been reported in veterinary literature (Boari et al. 1995; Zini et al. 2007; Rossi et al. 2010). Furthermore, the NICTH associated

with a hepatocellular adenoma and high IGF-2 concentrations, as described for case 1, has not been reported in dogs. IGF-2, a 7.4 kDa single chain protein, is mainly synthesised by hepatocytes (Zini et al. 2007; Fischer et al. 2000). The structural and functional similarities of IGF-2 with insulin suggest that IGF-2 may induce hypoglycaemia by binding to the insulin receptors (Finotello et al. 2016). In a study of human patients aimed at differentiating malignant from benign liver lesions, all the hepatocellular adenomas demonstrated a cytoplasmic IGF-2 expression, with strong immunohistochemical staining being observed in 70% of these tumours (Lai et al. 2014). The mechanism responsible for IGF-2 overproduction in NICTH has not been well described, but an allele containing a 19-nucleotide deletion within exon 9 of the IGF-2 sequence and different promoter usage for IGF-2 was identified in a human patient with metastatic hemangiopericytoma, suggesting that the IGF-2 overproduction could have been associated with a loss of the IGF-2 gene imprinting and the use of a different promoter (Lawson et al. 2009). Thus, NICTH could be the result of IGF-2 overproduction associated with the activation of foetal promoters and loss of imprinting in the hepatocellular adenoma, but further studies will be necessary to confirm this mechanism and to determine whether it is widespread.

NICTH has also been reported in a dog with mammary carcinoma (Rossbodnari et al. 2010). Although the majority of IGF-2 is secreted by the hepatocytes, it can be synthesised by several cell types, including fibroblasts and epithelial cells (Finotello et al. 2016). Indeed, the IGF-2 expression has previously been demonstrated in the epithelial cells of a canine mammary carcinoma (Rossi et al. 2010). The inverse relationship between the serum IGF-2 and blood glucose concentration observed in case 2 suggests that NICTH might have resulted from the IGF-2 overproduction from the dog's mammary carcinoma.

Case 2 had large, rapidly growing mammary tumours, which could have been the result of the autocrine and paracrine cell growth-promoting effects of IGF-2, independent of somatotropin (Zini et al. 2007). In human medicine, it is generally acknowledged that such tumours must be quite large before hypoglycaemia develops (Dynkevich et al. 2013). In the cases reported here, the blood glucose was normalised soon after surgery, but the circulating IGF-2 levels decreased more gradually,

suggesting that a high concentration of IGF-2 or another factor was required for hypoglycaemia in dogs with NICTH. Thus, the mechanism of NICTH in dogs could involve an effect of the IGF-2, glucose utilisation by the tumours itself, or both (Boari et al. 1995; Zini et al. 2007). Non-islet cell tumours may induce hypoglycaemia by using a large quantity of glucose themselves, especially in the case of large masses (Rossi et al. 2010), perhaps involving the upregulation of their hexokinases and glucose transporters, as described for cancer in human patients (Smith 1999).

It is also possible that the enzyme-linked immunosorbent assay only detected 150 kDa ternary complexes of IGF-2 because they have a longer half-life (12–16 h) than the 50 kDa binary complexes (20–30 minutes) and free IGF-2 (10–12 minutes) (Dynkevich et al. 2013). Normally, about 80% of IGF-2 is in 150 kDa ternary complexes with the IGF binding protein-3, but the 50 kDa binary complexes are the main circulating form in canine NICTH (Boari et al. 1995). Furthermore, only the free and binary complexes of IGF-2 can cross the capillary wall and induce a hypoglycaemic effect; therefore, in the future, it will be necessary to differentiate the concentrations of the free IGF and the complexes to clarify the role of IGF-2 in the pathophysiology of canine NICTH, and the lack of this information is a limitation of the present report.

NICTH is a rare cause of hypoglycaemia in dogs, and the majority of dogs with tumours associated with NICTH do not generally develop hypoglycaemia. In humans, the Endocrine Society guidelines recommend investigation in patients with characteristics of NICTH, such as a known malignancy or identification of a large new mass (Cryer et al. 2009). However, a benign tumour can also cause NICTH, as demonstrated by the hepatic adenoma in Case 1. Therefore, the clinician should be aware that rapidly growing tumours, whether malignant or not, can cause severe hypoglycaemia in dogs.

In conclusion, the cases reported here demonstrate that the successful treatment of NICTH is possible in dogs. Although few case reports of canine NICTH have been previously reported, these cases had a poor prognosis (Beaudry et al. 1995; Boari et al. 1995; Battaglia et al. 2005; Snead 2005; Zini et al. 2007; Rossi et al. 2010). In this report, some of the clinical signs improved after the symptomatic treatment with a dextrose and glucagon infusion, but the biochemical hypoglycaemia was

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not normalised by these treatments. Furthermore, after stopping these treatments, the clinical signs related to hypoglycaemia recurred, indicating that the symptomatic treatment alone might not be sufficient for dogs with NICTH; instead, successful treatments of NICTH might not be possible without tumour resection. This contention is supported by the fact that both dogs described in the present report remained alive without the recurrence of hypoglycaemia over 24 months after the tumour resection. Thus, we contend that a rapid diagnosis of NICTH is necessary in hypoglycaemic dogs with a rapid growing tumour and that surgical resection of the tumour can increase the chances of survival in canine NICTH.

Acknowledgement

The authors would like to thank the owners of the dogs included in this report.

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<https://doi.org/10.17221/145/2018-VETMED>

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Received: October 17, 2018

Accepted after corrections: August 6, 2019