Clinical findings, neurological manifestations and survival of dogs with insulinoma: 116 cases (2009-2020)

PAPER

Clinical findings, neurological manifestations and survival of dogs with insulinoma: 116 cases (2009-2020)

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Objectives: To review the clinical findings and outcome in dogs diagnosed with insulinoma, and to assess which factors are predictors of overall survival. Additionally, to describe the neurological manifestations of this population and their correlation with survival.

Materials and Methods: Retrospective multicentric study of canine insulinoma cases (2009 to 2020). Signalment, clinical history, neurological examination, diagnostic findings, treatment and outcome were obtained from clinical records. Univariate and multivariate analyses were used to compare the overall survival.

Results: One hundred and sixteen cases were included. Median duration of clinical signs before presentation was 1.5 months. The most common presenting clinical signs were weakness (59.5%), epileptic seizures (33.6%) and changes in consciousness or behaviour (27.6%). Three dogs were suspected to have paroxysmal dyskinesia. Thirty-two dogs had an abnormal neurological examination, most commonly showing obtundation (28.1%), decreased withdrawal reflexes (21.9%) and absent menace response (18.8%). Overall survival for dogs undergoing surgery (20 months) was significantly longer than in medically treated (8 months; adjusted hazard ratio: 0.33; 95% confidence interval: 0.18, 0.59). Presence of metastases was the only other variable associated with prognosis (adjusted hazard ratio 1.72; 95% confidence interval: 1.02, 2.91).

Clinical Significance: Clinical signs of canine insulinoma are vague and non-specific. Weakness, epileptic seizures and changes in mentation or behaviour were the most commonly reported. Obtunded mentation and forebrain neurolocalisation were the main neurological manifestations. Dogs undergoing surgery had a longer overall survival compared to medically treated cases, and dogs with metastasis had a shorter overall survival regardless of treatment modality. Abnormalities in the neurological examination did not correlate with prognosis.

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INTRODUCTION

Insulinoma is a rare tumour of the pancreatic beta cells that secretes insulin and has been most commonly reported in medium to large breed dogs (Nelson 2015). Excess insulin secretion causes hypoglycaemia and a wide range of clinical signs related to neuroglycopenia and to hypoglycaemia-induced catecholamine secretion (Goutal *et al.* 2012). The typical signs reported of this condition are mostly derived from old publications and include generalised epileptic seizures (48%), collapse (40%), generalised weakness (37%), muscle twitching or trembling (20%), ataxia (20%), exercise intolerance (15%), pelvic limb weakness (14%) and disorientation (10%); with the majority of animals presenting with multiple clinical manifestations (Kruth *et al.* 1982, Leifer *et al.* 1986, Dunn *et al.* 1993, Tobin *et al.* 1999, Trifonidou *et al.* 1998, Moore *et al.* 2002, Ettinger *et al.* 2017). Hence, severe neurological signs are currently considered as the most common complaint. There is a wide range in the duration of clinical signs before presentation, with one review by Trifonidou *et al.* (1998) reporting a mean of 21 weeks (range 1 to 183 weeks). However, two recent studies reported a median duration of clinical signs of 3 weeks (range 0 to 52 weeks) and 4.1 weeks (0 to 40 weeks), respectively, suggesting that nowadays dogs are diagnosed earlier (Cleland *et al.* 2020, Del Busto *et al.* 2020).

Diagnosis of this condition can be challenging due to intermittent and non-specific clinical signs, laboratory abnormalities or imaging findings. A presumptive diagnosis can be obtained by documenting hypoglycaemia and serum insulin concentrations within or above reference intervals (Ettinger *et al.* 2017). Ultrasound (US) is reported to have a variable low sensitivity for detecting the tumour (28 to 75%), with contrast enhanced CT having a higher sensitivity of up to 94% (Robben *et al.* 2005, Buishand *et al.* 2018). Final diagnosis is confirmed by histology (Goutal *et al.* 2012). Treatment regimens include both medical and surgical options. Reported overall survival time is variable ranging from 2.4 to 6.5 months for medical management and 12.4 to 65.4 months for surgical management (Tobin *et al.* 1999, Polton *et al.* 2007,Cleland *et al.* 2020, Del Busto *et al.* 2020).

Although there are several publications describing this condition over the years, the clinical presentation is based on old literature (Goutal *et al.* 2012) and recent publications mainly focus on diagnostic techniques (Buishand *et al.* 2018) and surgical outcome (Cleland *et al.* 2020, Del Busto *et al.* 2020). The purposes of this study were to retrospectively review the clinical findings and outcome in a large group of dogs diagnosed with insulinoma and to assess which factors are predictors of overall survival, including treatment modality. Additionally, we aimed to describe the neurological manifestations of this population and their correlation with survival.

MATERIALS AND METHODS

Study design

This was a retrospective multicentric study performed at six referral centres. All patients included were client-owned dogs and all the investigations performed were obtained during clinically indicated procedures. Medical records of dogs with a presumptive or final diagnosis of insulinoma presenting from 2009 to 2020 were examined.

Medical records review

Information obtained from the medical records included signalment, type and duration of clinical signs, physical and neurological examination, complete blood count, serum biochemistry, insulin and fructosamine levels, diagnostic imaging techniques (US or CT), histopathology, treatment and, where available, follow-up information. Inclusion criteria were: (1) paired glucose and insulin measurements, (2) a full neurological examination recorded at presentation and neurolocalisation and (3) imaging suggestive of an insulinoma with low glucose and normal or high serum insulin documented, or histopathological confirmation of an insulinoma. Patients were excluded if they had any other concurrent systemic disease.

Clinical findings

Presenting clinical signs as reported by owners were categorised based on a modified criteria used by Ettinger *et al.* (2017) and Goutal *et al.* (2012). The categories were: epileptic seizures (split into focal or generalised as described by the Epilepsy Task Force consensus report) (Berendt *et al.* 2015); collapse/syncope; weakness (split into generalised, pelvic limb and exercise intolerance); ataxia (split into proprioceptive, vestibular or cerebellar); changes in consciousness and behaviour (defined as disorientation, lethargy, anxiousness, pacing or hiding); polyphagia; polyuria/polydipsia; blindness; anorexia; vomiting; diarrhoea; muscle fasciculations (Lowrie & Garosi 2016); suspected paroxysmal dyskinesia (Lowrie & Garosi 2017) and weight loss.

Neurological examination findings and neurolocalisation were reviewed. All neurological examinations were performed by a board-certified neurologist, internal medicine specialist or by a resident of these two specialities under direct supervision. All dogs with epileptic seizures were localised to the forebrain. After the diagnosis was reached, all cases were classified according to World Health Organisation (WHO) stages. Stage I being local disease confined to the pancreas, stage II presence of locoregional metastasis or stage III distant metastasis (Owen *et al.* 1980). Treatment modalities were divided in medical management and surgery.

Statistical analysis

Numerical variables were presented as median, interquartile range (IQR) and range, and compared between groups with Mann-Whitney U test. Categorical variables were expressed as counts and percentages, and compared between groups using Pearson's chi-squared or Fisher exact test. Dogs that were euthanised within 24 hours of establishing the diagnosis of insulinoma were not included in the survival analysis (n=9). An odds ratio (OR) with 95% confidence interval (CI 95%) was calculated for the association between euthanasia during the first 24 hours of presentation and abnormal neurological examination findings (considered as a yes or no answer). Survival analysis was performed on all dogs allocated in any of the two treatment groups (surgical

versus medical) for which follow-up information was available (n=93). Data on overall survival were considered censored if dogs stayed alive on the last follow-up appointment (n=32). Death of any reason (including euthanasia at presentation, sudden death, euthanasia due to recurrence of the disease, financial reasons and death due to any other cause) was considered an event. Prognostic factors of the overall survival were first identified using the univariable Cox proportional hazard model (dichotomous and continuous variables) or the generalised Mantel-Cox log rank test (ordinal categorical variables with more than two categories). Variables for which the P-value of the between-group comparisons or of the univariable survival analysis was below 0.1 were entered into the multivariable Cox proportional hazard model as potential confounders. The multivariable model aimed to assess the role of the type of treatment as an independent prognostic factor of overall survival in insulinoma. The model was developed according to the backward elimination procedure and was shown to adhere to proportional hazards assumption based on a plot of the scaled Schoenfeld residuals and their lowess smooth *versus* time, natural logarithm of time, Kaplan–Meier estimate, rank of time as well as based on the insignificant results of the score tests. Crude hazard ratios (HR) and adjusted hazard ratios ($HR_{adjusted}$) with CI 95% were presented to describe the results of the univariable and multivariable analysis, respectively. Survival rates for the entire range of times were estimated using the Kaplan–Meier product-limit estimator with CI 95% calculated with log-minus-log method. A significance level (α) of 0.05 was assumed in all statistical tests except for the univariable survival analysis when α was 0.1. The statistical analysis was performed in TIBCO Statistica 13.3.0 (TIBCO Software Inc., Palo Alto, CA) and IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY).

RESULTS

Signalment

One hundred and twenty-four dogs with insulinoma were identified. Seven dogs were excluded due to incomplete records (absent imaging, neurological examination, or insulin levels) and one dog was excluded due to concurrent systemic disease (immune-mediated haemolytic anaemia). A total of 116 dogs met the inclusion criteria and were included in the study. Most common represented breeds included Crossbreed (32), West Highland White Terrier (12), Boxer (12), Springer spaniel (9) and German shepherd dog (5). See Data S1 for full list of breeds. Age at presentation ranged from 4 to 18 years with the median (IQR) of 9 (8 to 11) years, and bodyweight ranged from 2.3 to 44.6 kg with the median (IQR) of 21 (12 to 30) kg. Forty-eight (41.4%) dogs were male (24; 50% neutered and 24; 50% entire) and 68 (58.6%) dogs were female (51; 75% neutered and 17; 25% entire). Duration of clinical signs before presentation ranged from 1 day to 24 months with the median (IQR) of 1.5 (1 to 3) months.

Clinical signs

Forty-five (38.8%) dogs presented with a single clinical sign, and 71 (61.2%) dogs presented with a combination of clinical signs. Overall, the most common clinical presentation was weakness (69/116, 59.5%), which was further split into generalised weakness (54/116, 46.6%), pelvic limb weakness (15/116, 12.9%) and exercise intolerance (7/116, 6%), which accompanied generalised or pelvic limb weakness in seven dogs. For dogs presenting with a combination of clinical signs, it was most common to present with two signs (34 cases, 29.3%), with weakness and episodes of collapse being the most common combination (9/34 cases). See Table 1 for a summary of all presenting clinical signs. Three dogs were suspected to have paroxysmal dyskinesia on presentation, based on the owner's descriptions and the videos provided examined by board certified neurologists. See Table 2 for a detailed description of these cases.

Only eight (6.9%) dogs had abnormal physical examination findings, which included a systolic heart murmur (6/8), bilateral pelvic limb muscle atrophy (1/8) and a right shoulder subcutaneous mass (1/8). Thirty-two (27.6%) dogs had an abnormal neurological examination at presentation. The most common findings observed were: obtundation (9/32, 28.1%), decreased withdrawal reflexes (7/32, 21.9%) and absent menace response (6/32, 18.8%; four bilaterally and two unilaterally). See Table 3 for a full summary of the neurological examination findings. In 42 (36.2%) of the 116 dogs, neurological signs were localised in the forebrain (diffuse brain in 36 and left forebrain in six, based on the neurological examination and/or history of generalised epileptic seizures). In seven (6%) of 116, neurological signs were localised in the peripheral nervous system, in four (3.5%) of 116, they were classified as multifocal (all localising in forebrain due to epileptic seizures, and in T3-L3 spinal cord segments) and one of 116 in each of the following locations: brainstem, T3-L3 spinal cord segments and L4-S3 spinal cord segments.

Diagnostic findings

Complete blood count and serum biochemistry results were available for 98 (84.5%) of 116 cases and were largely unremarkable. See Data S2. Glucose at presentation varied between 1.1 and

Table 1. Most common clinical signs reported in 116 dogs diagnosed with insulinoma

Clinical sign	Number of dogs (percentage)
Weakness	69 (59.5)
• Generalised	• 54 (46.6)
• Pelvic limb	• 15 (12.9)
• Exercise intolerance	• 7 (6)
Epileptic seizures	39 (33.6)
• Generalised seizures	• 36 (31)
• Focal seizures	• 3 (2.6)
Change in consciousness or behaviour	32 (27.6)
Collapse/syncope	24 (20.7)
Muscle fasciculations	24 (20.7)
Proprioceptive ataxia	21 (18.1)
Polyphagia	4 (3.4)
Polyuria polydipsia	4 (3.4)
Vomiting	4 (3.4)
Diarrhoea	4 (3.4)
Suspected paroxysmal dyskinesia	3 (2.6)
Anorexia	1 (0.9)
Blindness	1 (0.9)
Weight loss	1 (0.9)

Table 2. Clinical information of the three cases of suspected paroxysmal dyskinesia on presentation

Signalment	Duration of clinical signs before presentation	Frequency	Description of the episodes	Mentation during the episodes	Duration	Pre-ictal or post-ictal signs	Autonomic signs	Glucose at presentation (mmol/L)	Diagnostic tests	Progression and treatment before insulinoma diagnosis	Diagnosis of Insulinoma	Outcome
9-year-old male entire standard poodle	2-month history	Weekly	Pacing episodes overnight with persistent flexion of one limb (occurred in both thoracic and pelvic)	Alert	10 minutes to 4 hours	No	No	4.5	Full CBC and serum biochemistry (unremarkable) Brain MRI (unremarkable) Cerebrospinal fluid analysis (unremarkable)	Hypoallergenic and gluten free diet trial for 4 weeks Clinical signs stable the first 2 months, and later increased in frequency to daily	3 months after the initial presentation CBC and serum biochemistry were repeated and documented hypoglycaemia (1.8 mmol/L) and serum insulin 10.6 µIU/ml; further investigations revealed an insulinoma WHO stage III	Surgical treatment, followed by prednisolone, diazoxide, levetiracetam and diabetic prescription diet Overall survival and follow-up 18 months Episodes resolved after surgery
7-year-old male entire Jack Russell Terrier	4-day history	3-5 episodes a day	Sporadic episodes of persistent flexion of one limb (either thoracic, pelvic or both) and generalised tremors	Alert	30 seconds to 30 minutes	No	No	4.3	Full CBC and serum biochemistry (unremarkable) Brain MRI (unremarkable) Cerebrospinal fluid analysis (unremarkable)	Hypoallergenic and gluten free diet trial for 4 weeks, and episodes decreased to one every month. Three months later episodes increased again to weekly. CBC and serum biochemistry were performed (unremarkable). Drug trials included acetazolamide, phenobarbital and potassium bromide with no to minimal response	12 months after initial presentation, deterioration from once episode a week to three to five episodes a day. Serum biochemistry documented hypoglycaemia (1.7 mmol/L) and serum insulin 73 µIU/mL; further investigations revealed an insulinoma WHO stage I	Surgical treatment, followed by prednisolone, diazoxide and diabetic prescription diet Overall survival and follow-up 22 months Episodes resolved after surgery

Table 2. Continued.

Signalment	Duration of clinical signs before presentation	Frequency	Description of the episodes	Mentation during the episodes	Duration	Pre-ictal or post-ictal signs	Autonomic signs	Glucose at presentation (mmol/L)	Diagnostic tests	Progression and treatment before insulinoma diagnosis	Diagnosis of Insulinoma	Outcome
9-year-old male entire boxer	3-month history	First monthly and increased frequency to twice weekly	Episodes of sudden generalised wobbliness that progressed to inability to walk, due to persistent flexion and contraction of both thoracic and pelvic limbs (either side)	Alert	10 to 15 minutes	No	No	3	Investigations for the mild hypoglycaemia confirmed the presence of an insulinoma WHO stage I	NA	At presentation serum insulin levels 40 µIU/ml	Medical treatment consisting of prednisolone (0.125 to 0.25 mg/kg/day) Overall survival and follow-up 16 months Episodes resolved when medical management was started

CBC Complete blood count, MRI Magnetic resonance imaging, WHO World Health Organisation, NA Not applicable

Table 3. Neurological examination abnormalities detected in 32 dogs diagnosed with insulinoma

Neurological Examination	Finding – number (% of 32 cases)
Mentation	Obtunded – 9 (28.1)
Behaviour	Abnormal behaviour (disorientation, lethargy, anxiousness) – 6 (18.8)
Posture	Generalised tremors – 2 (6.3)
Gait	Proprioceptive ataxia of all four limbs – 3 (9.4) Non ambulatory tetraparesis – 2 (6.3) Circling to one side – 1 (3.1) Compulsive pacing – 1 (3.1) Pelvic limb proprioceptive ataxia – 1 (3.1)
Cranial nerves	Decreased/absent menace response – 6 (18.8) • Bilateral – 4 • Unilateral – 2 Unilaterally reduced nasal sensation – 1 (3.1)
Segmental reflexes and muscle tone	Reduced withdrawal reflexes – 7 (21.9) • All limbs – 5 • Pelvic limbs – 2 Reduced patellar reflex bilaterally – 1 (3.1) Flaccid limb tone – 2 (6.4)
Proprioception	Delayed proprioception in all limbs – 4 (12.5) Delayed proprioception on one side – 2 (6.3) Delayed proprioception in both pelvic limbs – 1 (3.1) Delayed proprioception on one limb – 1 (3.1)
Hyperaesthesia	Not noted in any patient

6.5 mmol/L with the median (IQR) of 2.0 (1.7 to 2.3) mmol/L. Using a value of 3.5 mmol/L or less to define hypoglycaemia, 94% (109/116) of cases were hypoglycaemic at initial presentation with the median (IQR) of 1.9 (1.7 to 2.2) mmol/L. The remaining 6% (7/116) had normal glucose levels with the median (IQR) of 4.3 (3.9 to 4.9) mmol/L, two of which presented with suspected paroxysmal dyskinesia. Paired insulin concentration ranged from 3 to 290 µIU/mL with the median (IQR) of 36 (25 to 50) µIU/mL. Fructosamine was measured in 32 of 116 cases (27.6%), with a median value of 238.5 µmol/L (77 to 324 µmol/L). All animals had imaging performed. Fifty-nine (51%) had US, 43 (37%) had CT and 14 (12%) had both US and CT. In two cases that had only CT, and one case that had only US, a pancreatic lesion was not identified but was found at subsequent exploratory laparotomy and confirmed on histopathology. Of the 14 animals having both US and CT, four tumours were not seen on US but seen on CT, and one tumour was not identified on CT but found on US. Sixty (51.7%) cases had evidence of metastasis at the time of diagnosis. Fifty-two (86.7%) were identified at imaging (US or CT), and eight (13.3%) during the surgical procedure without being identified on imaging before. Fifty-six (48.3%) cases were classified as WHO stage I (pancreatic lesion only), 41 (35.3%) stage II (local lymph nodes) and 19 (16.4%) stage III (distant spread).

Seven animals with a history of epileptic seizures and two with suspected paroxysmal dyskinesia underwent magnetic resonance imaging of the brain, three of which had a cerebrospinal fluid sample taken at the same point, all of which showed no significant findings. One dog, presenting with non-ambulatory tetraparesis, reduced withdrawal and reduced tone in all limbs, underwent an electromyography, which showed fibrillation potentials and positive sharp waves, and a motor nerve conduction study consistent with an axonopathy.

A diagnosis of insulinoma was confirmed based on histopathology in 70 cases (60.3%), fine needle aspirate in 6 cases (5.2%) and was presumed based on the combination of imaging and paired glucose and insulin results on the remaining 40 cases (34.5%). Seven cases did not have histopathology performed after surgery; reasons for this included financial constraints of owners (four), incomplete clinical records (two) and euthanasia after surgery (one). Two cases (1.7%) had a post-mortem examination histopathology, which confirmed a neuroendocrine carcinoma.

Treatment

Thirty-nine cases (33.6%) were treated medically, and 77 (66.4%) were treated surgically. In the medically treated group, seven of 39 had an abnormal neurological examination, 25 of 39 were classified as WHO stage I, eight of 39 WHO stage II and six of 39 WHO stage III. As treatment modalities, 25 of 39 received glucocorticoids, 18 of 39 special dietary recommendations (consisting of reduced and complex carbohydrate food and frequent small meals), six of 39 diazoxide, three of 39 levetiracetam and one of 39 carboplatin. In the surgical group, 16 of 77 had an abnormal neurological examination, 31 of 77 were classified as WHO stage I, 33 of 77 WHO stage II and 13 of 77 WHO stage III. After the surgical procedure, 36 of 77 received glucocorticoids, 19 of 77 special dietary recommendations, 11 of 77 diazoxide and 2 of 77 toceranib. Furthermore, 16 of 77 dogs required insulin (one developed diabetic ketoacidosis) and seven of 77 developed signs of acute pancreatitis.

Survival analysis

Follow-up was available for 102 (87.9%) dogs. Of these, nine (8.8%) were euthanised immediately (within 24 hours of diagnosis) before assignment to any of the two treatment groups and therefore they were dropped from survival analysis. Abnormal neurological examination (considered as a yes or no answer) did not prove to be a significant risk factor of immediate euthanasia (OR 3.8; CI 95%: 0.90, 15.4; P=0.110). Of 93 dogs assigned to treatment groups, date of death of 61 dogs (65.6%) was known. The remaining 32 dogs (34.4%) were still alive at the time of data collection and were considered as censored observations.

Characteristics of the two treatment groups included in the survival analysis are presented in Table 4 and Data S3. In the univariate analysis, 25 characteristics were included. The median overall survival differed significantly between types of treatment (HR 0.36; CI 95%: 0.20, 0.64; P=0.001) – it was only 8 months (IQR from 3 to 16 months) for dogs treated medically and 20 months (IQR from 9 to 43 months) for dogs that had undergone surgery. The only other factor significantly linked to overall survival in the univariable analysis was castration with the median (IQR) overall survival of 22 (9 to 43) months for castrated dogs and 9 (3 to 24) months for entire dogs regardless of their sex (HR 0.54; CI 95%: 0.32, 0.91; P=0.020) (Data S4). Except for the type of treatment, eight potential confounders were subsequently included in the multivariable analysis in order to exclude accidental relationships, these were: age at presentation, castration, epileptic seizures at presentation, glucose concentration at presentation, WHO stage II or III (i.e. presence of metastases), using corticosteroids, diazoxide and special diet. The surgical treatment was an independent factor significantly and consistently reducing the risk of death ($HR_{adjusted}$ 0.34; CI 95%: 0.19, 0.61; P < 0.001), when controlled for various combinations of the aforementioned potential confounders (Data S5). The patients that had undergone surgery were three times less likely to die of any reason compared to the patients treated medically (Table 5). The proportion of patients who survived 1 year was two times higher among those than had undergone surgery, and the proportion of patients who survived 2 years was sixfold higher among those that had undergone surgery compared to those medically treated. Only dogs treated with surgery had any chance to stay alive for as long as 3 or even 4 years (Table 6,

Table 4. Demographic and medical characteristics of the study population (93 dogs) included in the survival analysis with regards to type of treatment presented and count and percentage unless otherwise stated

Variable	Type of treatment		P-value
	Surgery (n=65)	Medical (n=28)	
Sex			0.425
Male	29 (44.6)	10 (35.7)	
Female	36 (55.4)	18 (64.3)	
Castration	48 (73.9)	16 (57.1)	0.111
Age (years) (median, IQR and range)	9, 8 to 11 (4 to 13)	9, 7 to 10 (4 to 18)	0.476
Bodyweight (kg) (median IQR and range)	21.3, 12.1 to 29.8 (2.3 to 42.7)	18.3, 11.4 to 30.3 (2.5 to 42.3)	0.544
Time from clinical signs to diagnosis (months) (median IQR and range)†	1.5, 1 to 3 (0.03 to 12)	1.5, 1 to 4 (0.1 to 12)	0.618
Weakness at presentation	39 (60.0)	15 (53.6)	0.564
Seizures at presentation	18 (27.7)	13 (46.4)	0.079
Change in consciousness at presentation	18 (27.7)	11 (39.3)	0.268
Collapse/syncope at presentation	13 (20.0)	5 (17.9)	0.810
Abnormality in neurological examination	16 (24.6)	7 (25.0)	0.969
Glucose at presentation (mmol/L) (median IQR and range)	2.2, 1.9 to 2.5 (1.2 to 6.5)	1.8, 1.4 to 2.2 (1.1 to 4.3)	0.005
WHO staging			0.173
I	25 (38.5)	16 (57.1)	
II	29 (44.6)	7 (25.0)	
III	11 (16.9)	5 (17.9)	

†Data missing for seven dogs replaced by the median of the remaining 91 cases equal to 1.5

Table 5. The Cox proportional hazard model showing the effect of two variables on the risk of death of insulinoma

Variable	Coefficient (SE)	Wald chi-square	P-value	$HR_{adjusted}$ (CI 95%)
Surgical treatment	−1.12 (0.30)	13.92	<0.001	0.33 (0.18, 0.59)
WHO stage II or III	0.54 (0.27)	4.14	0.042	1.72 (1.02, 2.91)

Fig 1). The only other variable found to be significantly linked to overall survival in the multivariate analysis was the presence of metastases ($HR_{adjusted}$ 1.72; CI 95%: 1.02, 2.91; P=0.042) – the patient with WHO stage II or III was approximately 1.5- to two-fold more likely to die regardless of the type of treatment (Table 5 and Data S5).

Due to concerns over the impact of the high proportion of censored cases during the first 6 months in the medical group (8/28 dogs; 29%), the analysis of overall survival was performed repeatedly replacing records for all dogs, which had been censored in the medical group with 24-month overall survival but without changing the overall survival of dogs in the surgical group. Results still suggested that dogs treated surgically had a significant longer overall survival ($HR_{adjusted}$ 0.45; CI 95%: 0.27, 0.75; P=0.002).

DISCUSSION

This study reviewed the clinical findings, neurological manifestations and survival in a large population of dogs with insulinoma over the last decade across referral centres of the United Kingdom and Spain.

Weakness (59.5% cases) was the most common presenting clinical complaint, followed by generalised epileptic seizures (31% cases) and changes in consciousness or behaviour (27.6% cases). Hence, based on our results and a review of the literature, the clinical signs of canine insulinoma seem to be quite variable (Trifonidou *et al.* 1998, Ettinger *et al.* 2017). The predisposition for older, medium to large breed dogs is consistent with previously published values (Dunn *et al.* 1993, Trifonidou *et al.* 1998). Interestingly, the West Highland White Terrier was observed to be the second most common breed in our population (12/116 cases, 10.3%), which has also been observed in two recent publications with a similar incidence (Cleland *et al.* 2020, Del Busto *et al.* 2020). The average duration of clinical signs before presentation (1.5 months) is in line to recent publications reporting 3 to 4 weeks since signs onset (Cleland *et al.* 2020, Del Busto *et al.* 2020). This is, however, shorter than historically reported (3 to 5 months) (Dunn *et al.* 1993, Trifonidou *et al.* 1998). Possible causes for this could be that owners are now more alert to subtle changes in their pets and there is increased awareness of this condition and availability of imaging techniques, which could have resulted in dogs presenting with more vague clinical signs and are diagnosed earlier.

Interestingly, three dogs were suspected to have paroxysmal dyskinesia on presentation, which has not been associated with canine insulinoma before. Two of these dogs had normal glucose levels at the initial presentation, normal brain MRI and cerebrospinal fluid analysis. The presumptive diagnosis was based on the owner's descriptions and videos provided examined by board-certified neurologists. Despite an encephalogram not being performed in any case to fully exclude focal seizures and the fact that we cannot rule out this were two separate conditions occurring at the same time, in all 3 cases clinical signs resolved after treatment (surgery in two cases and prednisolone in one case). Furthermore, an association between paroxysmal dyskinesia and insulinoma has been reported in human literature (Shaw *et al.* 1996,Gupta *et al.* 2011, Deleo *et al.* 2014, Pozzi *et al.* 2015). The pathophysiology is unknown, but it is suspected to be secondary to hypoglycaemia causing dysfunction of the *corpus striatum*. The *corpus striatum* is a brain structure formed by several basal nuclei (caudate, accumbens, putamen, pallidum and claustrum) and the adjacent white matter, which is involved in motor activity (De Lahunta *et al.* 2015). Dysfunction of the *corpus striatum* could cause lack of inhibition to the thalamus leading to hyperkinesias (Shaw *et al.* 1996). Some of the human patients reported did also experience epileptic seizures and neuronal motor axonopathy concurrently (Gupta *et al.* 2011, Deleo *et al.* 2014, Pozzi *et al.* 2015). Movement disorders constitute a novel and constantly evolving group of conditions that could be misdiagnosed as epileptic seizures (Lowrie & Garosi 2017). These cases highlight the importance of considering and excluding systemic causes in dogs presenting with suspected movement disorders.

A large proportion of dogs presented with clinical signs considered to be neurological in origin; however, only a 27.6% had an abnormal neurological examination. The most common and least surprising abnormality was an obtunded mentation in nine of 32 cases but is important to underline other prevalent alterations, such as reduced withdrawal reflexes, altered menace response, changes in behaviour or delayed proprioception. The most frequent neurolocalisation was diffuse forebrain. Surprisingly, six dogs presented with focal forebrain signs, all of which had a normal brain MRI. The lateralisation of the neurological signs could be secondary to postictal changes in cases with epileptic seizures (even if bilateral post-ictal signs are more common) or secondary to a vascular event not observed in the MRI. In addition, four dogs presenting with epileptic seizures were classified as multifocal, localising in the forebrain but also in T3-L3 spinal cord segments. The suspected myelopathy was not investigated in any case as work-up for the epileptic seizures was prioritised. Considering the age of the patients, these findings were considered most likely incidental and secondary to chronic degenerative intervertebral disc disease, but other causes of myelopathy were not fully excluded. The second most common neurolocalisation was diffuse peripheral nervous system. There are few reports of subclinical and clinical polyneuropathy in dogs with concurrent insulinoma. They all documented similar electrophysiological findings to the one dog from our study, which presented with non-ambulatory tetraparesis and reduced limb tone (Van Ham *et al.* 1997). The pathophysiology of this polyneuropathy is still unknown, but it is believed that the peripheral nervous system is quite resistant to hypoglycaemia, and therefore an immune

Table 6. Summary of the survival rate expressed as Kaplan–Meier estimates for the surgical and medical group

Time (months)	Surgical group (n=65)				Medical group (n=28)			
	Survival rate (CI 95%)	Numbers at risk	Numbers censored	Number of deaths	Survival rate (CI 95%)	Numbers at risk	Numbers censored	Number of deaths
1	89.2 (78.7, 94.7)	60	0	5	82.0 (62.0, 92.1)	22	1	5
6	77.7 (65.1, 86.1)	46	6	8	53.8 (31.6, 71.7)	9	7	6
12	70.3 (57.0, 80.2)	40	3	3	33.6 (13.6, 55.2)	6	1	2
24	41.4 (27.9, 54.4)	21	5	14	6.7 (0.5, 26.0)	2	0	4
36	31.7 (19.1, 45.1)	10	5	6	0	1	0	1
48	8.1 (1.6, 21.8)	4	2	4	0	-	-	-

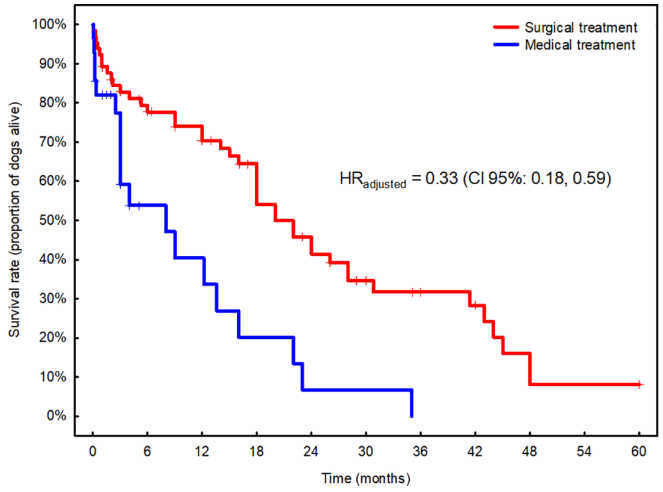

FIG 1. Kaplan–Meier plot indicating survival for dogs that received medical therapy (median 8 months) compared with dogs that had surgery (median 20 months)

mediated paraneoplastic syndrome is considered to be the most likely cause of the clinical signs (Jaspan *et al.* 1982). Overall, the combination of clinical signs and neurological manifestations in this cohort further highlights how vague, non-specific and possibly misleading signs appear to be in dogs presenting with insulinoma.

With regards to diagnostic test results, 94% (109/116) cases were hypoglycaemic at initial presentation. This emphasises the fact that a normal serum glucose concentration at presentation, even if uncommon, does not completely exclude insulinoma and glucose monitoring should be considered in cases presenting with compatible signs. In this study, imaging diagnostic performance was similar to previous reports, with CT being more sensitive than US (Robben *et al.* 2005). Nevertheless, there were still cases that were missed by either one or both modalities, showing no imaging modality is 100% sensitive.

The overall survival of medically treated dogs in our study, 8 months, was higher than previous reports of 2.4 to 6.5 months (Tobin *et al.* 1999, Polton *et al.* 2007). This group had a high proportion (57%) of WHO stage I cases, which could have influenced the overall survival. Nevertheless, this still constitutes a valuable reminder of this treatment modality. The surgical group achieved significantly longer overall survival (20 months), which correlates with previous reports comparing medical and surgical cases (Goutal *et al.* 2012,Cleland *et al.* 2020, Del Busto *et al.* 2020). Survival analysis suggested that the proportions of dogs treated surgically were two- and six- fold more likely to live 1 and 2 years, respectively, compared to medically treated dogs. In addition, the presence of metastases at diagnosis was the only other variable that affected overall survival as previously reported (Cleland *et al.* 2020, Del Busto *et al.* 2020).

The decision on treatment modality can be affected by many factors: severity of clinical signs, presence of metastasis, clinicians' recommendations, surgeons' expertise, financial limitations, etc. In this study, and despite its retrospective nature, there were no differences found between the measured demographics of either group. Interestingly, and opposite to what could be expected, the surgical group had a higher proportion of dogs with metastasis than the medical group and still achieved a longer overall survival. This could be due to some metastasis being detected during exploratory surgery, or to owner's perception of the disease influencing decision on a treatment modality. However, further prospective studies controlling all the above variables would be ultimately required to confirm if surgery is the gold standard treatment as well as what influences treatment decision.

Finally, dogs with an abnormal neurological examination did not show a difference of overall survival regardless of the treatment type, nor were they more likely to be euthanised on presentation. As the neurological examination findings could have a subjective interpretation between clinicians, only the presence of abnormalities in the neurological examination (yes or no answer) was considered for survival analysis. These results were unexpected and might suggest that the severity of the neurological examination is not correlated with the severity of the disease, but to hypoglycaemia that can be successfully managed with treatment. Therefore, it appears that abnormal neurological examination at presentation is not correlated with prognosis.

This study has some limitations. Firstly, its retrospective nature across multiple centres resulted in no standardisation of anamnesis, neurological examination records, confirmed histopathological diagnosis, treatment or follow-up for the dogs included. This could have had an impact on the accuracy of the description of the clinical signs, as well as the clinical record completeness. In addition, we cannot fully exclude concurrent conditions contributing to the clinical signs or survival analysis. Although statistical significance was achieved in survival analysis, incomplete follow-up survival data could limit the accuracy of this. Postoperative hypoglycaemia has been described as predictor for relapse and survival in dogs surgically treated, which was not assessed or recorded consistently in all cases (Cleland *et al.* 2020, Del Busto

et al. 2020). Finally, the dogs included in this study were from a referral population, which might not be fully representative of all cases of canine insulinoma.

In conclusion, canine insulinoma appears to be diagnosed earlier than historically reported and vague, non-specific clinical signs are common. Weakness, epileptic seizures and changes in mentation or behaviour were the most common clinical findings. The most frequent neurological manifestation was an obtunded mentation and the most common neurolocalisation was diffuse forebrain. Dogs undergoing surgery had a longer overall survival compared to medically treated cases, and dogs with metastasis had a shorter overall survival regardless of treatment modality. Abnormalities in the neurological examination did not correlate with prognosis.

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Portions of this study were accepted at the 2020 Annual Symposium of BSAVA but due to the COVID-19 pandemic will be presented as an oral communication at the 2021 Annual Symposium of BSAVA; Manchester, 25 to 28 April 2021.

Conflict of interest

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

Ethical statement

Ethical approval was obtained from the ethical review committee of each institution.

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Supporting Information

The following supporting information is available for this article:

Data S1. Breeds of the 116 dogs included in the study

Data S2. Complete blood count and serum biochemistry results for dogs with abnormal findings only. Values within reference ranges are not included

Data S3. (A) Demographic characteristics of the study population presented as n and percentage in parenthesis (categorical variables) or median, interquartile range (IQR) and range in parentheses (numerical variables). (B) Medical characteristics of the study population presented as n and percentage in parenthesis (categorical variables) or median, interquartile range (IQR) and range in parentheses (numerical variables)

Data S4. (A) The univariable analysis of the relationships between numerical variables (demographic and medical characteristics of the patients) and their overall survival time (OS). (B) The univariable analysis of the relationships between categorical variables (demographic and medical characteristics of the patients) and their overall survival time (OS)

Data S5. Variables included in subsequent steps of the process of developing Cox proportional hazard model according to the backward elimination procedure