ORIGINAL ARTICLE

Prognostic factors and long-term outcome in dogs diagnosed with primary and secondary immune thrombocytopenia in Ireland

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Objectives: The objectives of this study were to describe the outcome and prognostic factors in dogs diagnosed with primary and secondary immune thrombocytopenia (ITP).

Materials and Methods: Medical records of dogs diagnosed with primary and secondary ITP in a referral hospital in Ireland were retrospectively evaluated. Short- and long-term survivals were analysed using Cox proportional-hazards regression models.

Results: Medical records from 49 dogs were included. Primary and secondary ITP were diagnosed in 81.25% and 18.75% of dogs, respectively. The survival rate was 69% at 2 weeks (95% confidence interval [CI]: 0.54 to 0.80), 63% at 3 months (95% CI: 0.48 to 0.75) and 51% at 2 years (95% CI: 0.37 to 0.65). The overall median survival time was 985 days (primary ITP only: 1084 days; secondary ITP only: 225 days). Dogs surviving 30 days post-diagnosis had a median long-term survival time of 10 years. A lower haematocrit was negatively associated with survival [hazard ratio (HR) 0.96, 95% CI: 0.92 to 0.99]. Neutrophilia (HR: 0.44, 95% CI: 0.20 to 0.96) was associated with a 66% decreased risk of death. Band neutrophilia was associated with shorter hospitalisation (regression coefficient −3.56, 95% CI: −5.70 to -1.42). Presence of petechiae and ecchymoses (regression coefficient 2.41, 95% CI: 0.41 to 4.42), and the use of a second-line immunosuppressive agent (SLI) (regression coefficient 2.11, 95% CI: 0.11 to 4.12) were significantly associated with longer hospitalisation but not with survival.

Clinical Significance: A lower haematocrit was the only variable associated with a worse prognosis in dogs diagnosed with ITP. Dogs with confirmed secondary ITP had an overall shorter median survival time. Dogs surviving over 30 days had an excellent prognosis.

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INTRODUCTION

Immune thrombocytopenia (ITP) is a relatively uncommon immune-mediated haematological disorder, with a reported prevalence between 3% and 18.8% in dogs presenting with thrombocytopenia (Botsch et al., 2009; Cockburn & Troy, 1986; Francés et al., 2023; Grindem et al., 1991; Jans et al., 1990; O'Marra et al., 2011; Putsche & Kohn, 2008). The exact

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pathophysiology of the disease remains largely unknown; but it is believed that the premature destruction of platelets by the reticuloendothelial system occurs secondary to the adhesion of autoantibodies to the platelet surface while in circulation, or at the level of the bone marrow (Consolini et al., 2016; Lewis et al., 1995; Lewis & Meyers, 1996; O'Marra et al., 2011). The resulting thrombocytopenia can be severe, with affected dogs being at risk of spontaneous bleeding (Putsche & Kohn, 2008; Scuderi et al., 2016).

Secondary ITP has been associated with underlying inflammatory, infectious, neoplastic or drug-induced aetiologies that result in dysregulation and inappropriate immune response (Grindem et al., 1994; Jo'Neill et al., 2010; O'Marra et al., 2011). Primary ITP is considered idiopathic (Lewis & Meyers, 1996). Detection of antiplatelet antibodies (APA) on the surface of platelets is considered supportive but not confirmatory of a diagnosis of ITP; however, these tests are not commercially available and have very variable sensitivity and specificity (Scott et al., 2002). Most commonly, a diagnosis of ITP is reached after extensive investigations and the exclusion of other potential underlying causes of thrombocytopenia (Lewis & Meyers, 1996; Provan et al., 2019). Treatment relies mainly on immunosuppression and supportive care (e.g. management of bleeding, blood products administration if required), as well as management of underlying conditions, when present. Ultimately, the goal of therapy is to stop platelet destruction and maintain normal platelet levels long-term once immunosuppressive treatment has been discontinued. The recent ACVIM consensus statements on ITP in dogs and cats includes a diagnostic algorithm, which will offer invaluable help to manage these challenging cases (LeVine, Kidd, et al., 2024).

Although the short- and long-term prognosis of ITP has been reported to be fair, with reports of over 80% of dogs surviving to discharge and achieving remission (O'Marra et al., 2011; Putsche & Kohn, 2008; Simpson et al., 2018), previous studies have also identified negative prognostic factors, such as the presence of melaena, increased blood urea nitrogen (BUN) concentration or the need for a blood transfusion at presentation.

The purpose of the current study was to describe the outcome and prognostic factors of a population of dogs diagnosed with primary or secondary ITP in a veterinary referral hospital in Ireland between 2009 and 2020. Specific emphasis was given to report the long-term prognosis of these patients. Owing to the fact that Ireland is considered an isolated ecosystem with very limited prevalence of infectious diseases (Garcia-Campos et al., 2019; Ramos, 2023), it was hypothesised that secondary ITP would mainly be associated with neoplastic conditions. Finally, it was hypothesised that a high BUN, presence of melaena and transfusion requirement would be identified as negative prognostic factors, and that the administration of vincristine would have a significant effect on hospitalisation time and survival.

MATERIALS AND METHODS

The study received approval from the research ethics committee of the author's institution. The medical records of dogs diagnosed with either primary or secondary ITP between 2009 and 2020 were retrospectively searched using the computerised medical record system and the key words "ITP," "IMT," "IMTP," "immune thrombocytopenia," "immune-mediated thrombocytopenia," "auto-immune thrombocytopenia" and "idiopathic thrombocytopenic purpura." Inclusion criteria included having complete medical records, the presence of moderate-to-severe thrombocytopaenia on presentation (platelet count ≤60 × 10^9L, confirmed on blood smear examination) and a diagnosis of primary or secondary ITP after the exclusion of other potential causes of thrombocytopenia (e.g., decreased production, increased consumption and sequestration). Based on definitions from human medicine, the ACVIM consensus on the diagnosis of ITP in dogs suggests a minimum platelet count of 100 × 10^9/L to start considering ITP as a potential diagnosis. Due to the retrospective nature of the present study, and to allow a more stringent inclusion criteria, a lower cut-off of 60 × 10^9/L was used. Dogs that did not fulfil the above criteria were excluded.

The variables evaluated included signalment, previous history, physical examination findings, presenting signs, medication given prior to referral, diagnostic tests findings, presence or absence of an underlying cause, treatment administered during hospitalisation, hospitalisation time and information regarding mortality and survival. Haematological variables included platelet count, mean platelet volume (MPV), platelet distribution width (PDW), haematocrit (HTC), red blood cell (RBC) indices, reticulocyte count, total white blood cell (WBC) count and individual leukocyte count. Blood smears were manually examined to confirm the platelet count. Serum biochemical variables included BUN, creatinine and total bilirubin. Coagulation screening tests included prothrombin time (PT) and activated partial thromboplastin time (aPTT). Findings from thoracic and abdominal imaging were recorded, as well as the results of any other miscellaneous testing ordered by the attending clinician at the time of the initial investigation. Infectious disease screening was performed for common canine vector-borne diseases including *Echrlichia canis, Ehrlichia ewingii, Anaplasma phagocytophilum, Anaplasma platys, Borrelia burgdorferi and Dirofilaria immitis*, using a commercially available test (SNAP® 4DX, IDEXX). Testing for *Angiostrongylus vasorum* (*A. vasorum*) antigen was also performed using a commercially available test (Angio Detect™, IDEXX) and/or a Baermann faecal examination. These tests were performed at the clinicians' discretion based on the patient's travel history or clinical signs. Follow-up of the cases was performed by contacting the initial referring veterinary surgeons via telephone or email. The time and cause of death (ITP-related death or non-ITP-related death) or the time to the last available follow-up were recorded, as well as the suspected relapse, when available.

The cases were retrospectively classified as having either primary or secondary ITP, according to the recommendations from the recent ACVIM consensus statement (LeVine, Kidd, et al., 2024). This classification was based on physical examination findings, clinicopathological results, and thoracic and abdominal imaging findings. In the event of ambiguity or incomplete workup, cases were reviewed extensively by two of the authors (A.D and E.L.B) to determine the best classification for each individual. Cases were considered primary ITP when there were no investigative findings following screening as described above, including post-mortem investigation when available, or if workup was only partial, an appropriate response to empirical therapy with immunosuppressive medication was also considered supportive for primary ITP.

Statistical analysis

Commercially available statistical software (Stata® StataCorp LLC version 17.0) was used for data analysis. Kaplan–Meier curves were obtained by assessing all-cause mortality. Dogs lost to follow-up or alive at the end of the study were censored. Univariable Cox proportional hazards regression models with Breslow method for ties were used to screen for prognostic factors. All the variables with $P < 0.2$ on univariable analysis and all potential confounding variables were entered into the multivariable analysis. Backwards elimination was used for model selection; variables were removed by ranking the clinical relevance, with the least relevant variable being removed first. Likelihood ratio tests were performed following removal of each variable to test variable significance to the model. The process was repeated until all the variables retained in the model were significant or the P-value of the likelihood ratio test became <0.05. Hazard ratios (HRs) were calculated and presented with 95% CI. For all Cox regression models, the proportionality assumption was confirmed by the test of proportional-hazards assumption.

Additionally, univariable regression models were used to screen for factors associated with hospitalisation duration. As for the survival study, all the variables with $P < 0.2$ on univariable analysis were entered into the multivariable analysis and backwards elimination and likelihood ratio test results were used to select the final model. Gaussian distribution and homoscedasticity of the residuals were assessed by graphical assessment of frequency distribution histograms and residual plots, respectively.

RESULTS

Population

A total of 153 records were retrieved after the initial database search, of which two were mislabelled feline cases, and therefore not eligible for the study. Eighteen cases were excluded as the diagnosis was presumed Evan's syndrome, and another 84 cases were excluded due to the medical records not being complete enough to allow us confidently to rule out other causes of thrombocytopenia. Forty-nine cases fulfilled the inclusion criteria and were included in the study. The median age was 7 years [minimum-maximum (Min-Max) 0.6 to 13 years], and the median weight was 17 kg (Min-Max 3.5 to 53 kg). Twenty-five dogs were male (51%) and 24 were female (49%). Seventeen dogs (34.7%) were entire and 32 (65.3%) were neutered. Eighteen different breeds (including Crossbreed) were represented (Table 1).

History and clinical signs

The most common clinical signs at presentation included: lethargy ($n = 29$, 59.2%), petechiae and/or ecchymoses ($n = 20$, 40.8%), inappetence or anorexia ($n = 19$, 38.8%), melaena ($n = 16$, 32.6%), gingival bleeding ($n = 12$, 24.5%) and pyrexia ($n = 12$, 24.5%). Less common clinical signs included haematuria ($n = 8$, 16.3%), epistaxis ($n = 7$, 14.3%), vomiting ($n = 7$, 14.3%), weight loss ($n = 5$, 10.2%), diarrhoea ($n = 4$, 8.2%), hyphaema ($n = 3$, 6.1%) and haematemesis ($n = 2$, 4.1%). Information regarding immunosuppressive therapy prior to referral was available for 41 dogs. Of these, 18 dogs (44%) had received immunosuppressive therapy before referral.

Clinicopathological and diagnostic imaging results

Haematology and serum biochemistry were performed in all 49 dogs (Table 2). The median platelet count on presentation was 8 $\times 10^9$/L (Min-Max 0.25 to 60), the median MPV was increased at 17.3 fl (Min-Max 8.6 to 42.5) and the median PDW was within the reference interval at 57.5% (Min-Max 13.4 to 79.2). Thirty-four dogs (69.4%) were anaemic (HCT < 37%), with a median HCT value at presentation of 29% (Min-Max 5 to 49), and median reticulocyte count of 137.1 $\times 10^9$/L (Min-Max 0.7 to 696). Leucocytosis was present in 25/47 dogs (53.2%), with 28/48 dogs (58.3%) presenting with neutrophilia. The median neutrophil count was 13.2 $\times 10^9$/L (Min-Max 0.82 to 63.4), with band neutrophilia present in 15/49 (30.6%) dogs. Increased BUN was documented in 11/47 cases (23.4%), with median BUN value of 6 mmol/L (Min-Max 1.9 to 28.5). Median creatinine was 61 µmol/L (Min-Max 21 to 492). Hyperbilirubinaemia was present in

Table 1. Breeds represented in a population of 49 dogs diagnosed with immune thrombocytopenia

Breed	*n*
Crossbreed	10
English Springer Spaniel	6
Cocker Spaniel	6
Bichon Frisé	4
Cavalier King Charles Spaniel	3
Golden Retriever	3
Rottweiler	3
Labrador Retriever	2
German Shepherd	2
Toy Poodle	2
Miniature Schnauzer	1
Cairn Terrier	1
Jack Russell Terrier	1
Airedale Terrier	1
Alaskan Malamute	1
Welsh Springer Spaniel	1
Pembroke Welsh Corgi	1
Lurcher	1

4/46 cases (8.7%), with a median total bilirubin of 5.5 µmol/L (Min-Max 0.7 to 61.4). The PT and aPTT were assessed in 37 dogs (75.5%) and 36 dogs (73.5%), respectively, and prolongation of PT and aPTT was present in 4/37 (10.8%) and 4/36 (11.1%) dogs, respectively; however, the prolongations were mild and deemed not clinically relevant.

Screening testing for common canine vector-borne diseases (SNAP® 4DX, IDEXX) was negative in all 15 dogs (30.6%) tested. *A. vasorum* antigen testing (Angio™ detect, IDEXX) was positive in 1 out of 31 dogs tested (3.2%).

Thoracic radiographs were performed in 42 dogs (85.7%), with abnormalities identified in 27 of them (64.2%). These abnormalities included mixed alveolar and interstitial pulmonary pattern in one dog (3.7%) with confirmed *A. vasorum* infection, intrathoracic lymphadenomegaly in two dogs (7.4%) with multicentric lymphoma, aspiration pneumonia or pulmonary haemorrhage in one dog (3.7%), and pulmonary nodules suspicious for metastatic disease in one dog (3.7%) with suspected splenic neoplasia. Abdominal ultrasound was performed in 40 dogs (81.6%), with 38 cases (95%) documenting clinically relevant findings such as lymphadenopathy in six dogs (15.8%), mild volume of peritoneal effusion in seven dogs (18.4%), hepatomegaly, splenomegaly and heterogenous splenic parenchyma in five dogs (13.1%), heterogenous splenic mass in one dog (2.6%) and diffuse thickening of the urinary bladder wall and suspected intraluminal blood clot in one dog (2.6%).

Table 2. Clinicopathological results in a population of 49 dogs diagnosed with immune thrombocytopenia

Clinicopathological variables	Units	Median	Range	Reference interval
Platelets	10^9/L	8	0.25 to 60	150 to 500
MPV	fL	17.3	8.6 to 42.5	7 to 11
PDW	%	57.75	13.4 to 79.2	55.7 to 66.9
HCT	%	29	5 to 49	37 to 55
Reticulocyte	10^9/L	137.1	0.7 to 696	0 to 60
WBC count	10^9/L	17.6	2.86 to 74.1	6 to 17
Neutrophil count	10^9/L	13.2	0.82 to 63.41	3 to 11.5
Band neutrophils count	10^9/L	0.75	0.22 to 5.78	0 to 0.45
Lymphocyte count	10^9/L	1.43	0 to 14	1 to 3.6
PT	seconds	11.1	7.1 to 16.2	7 to 14
aPTT	seconds	15.3	9.2 to 37	12 to 25
BUN	mmol/L	6	1.9 to 28.5	3.6 to 8.6
Creatinine	µmol/L	61	21 to 492	20 to 120
Total bilirubin	µmol/L	5.5	0.7 to 61.4	0 to 10

Diagnosis

Nine out of 48 dogs (18.75%) were considered to have secondary ITP. Identified pathologies are represented in Fig. 1, with neoplasia ($n = 5$, 55.5%) being the most common cause. The different neoplasms identified included lymphoma ($n = 3$, 60%), multiple myeloma ($n = 1$, 20%) and unclassified splenic neoplasia ($n = 1$, 20%). Other causes of secondary ITP included vasculitis ($n = 2$, 22.2%), based on post-mortem findings, *A. vasorum* infection ($n = 1$, 11.1%), and unspecified hepatopathy ($n = 1$, 11.1%). Therefore 39/48 (81.25%) had primary ITP. Eight cases with incomplete imaging screening (five with neither radiographs nor ultrasound, three with only thoracic radiographs) were still included as primary ITP after extensive review of their medical records. All eight cases had negative *A. vasorum* antigen testing and one also had a negative SNAP® 4DX testing. All eight cases were in the lower half of our age group (4 to 8 years old). Three out of eight cases resolved only with empirical immunosuppressive treatment and had follow-up examination and haematology available up to 6 months after diagnosis. Three dogs died or were killed due to acute neurological deterioration and suspected intra-cranial bleeding precluding the performance of all the investigations: All three dogs had normal coagulation profiles. One of the three dogs had a CT of the head compatible with brain haemorrhage, and one had a post-mortem examination confirming the suspected intracranial haemorrhage. The last one was a Cocker Spaniel, a predisposed breed. That dog had normal thoracic radiographs

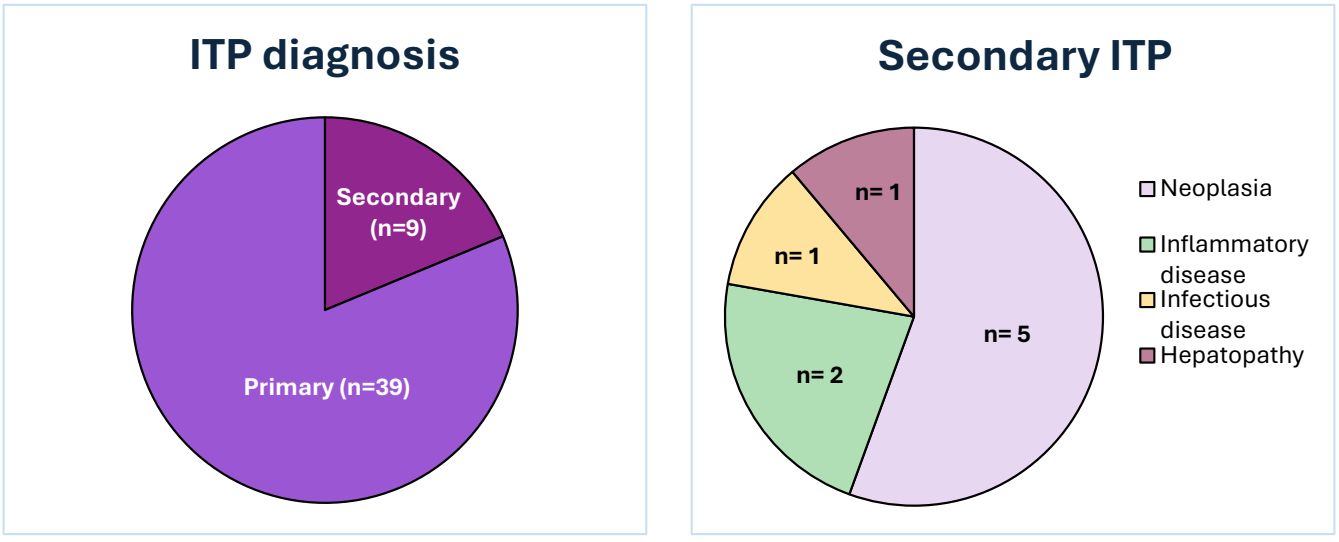

FIG 1. ITP diagnosis and associated underlying conditions in a population of 49 dogs presented with thrombocytopenia.

and died before ultrasound could be performed. Finally, two dogs died from suspected pulmonary haemorrhage after the development of acute respiratory distress, and before imaging could be fully performed – they had normal coagulation profiles, one had normal thoracic radiographs and one was from a known predisposed breed and young. One dog could not be classified under either category as screening for underlying conditions was not performed, and therefore, this dog was not included in the survival analysis.

Treatment

Treatment with corticosteroids was administered in all dogs, with either dexamethasone, prednisolone or a succession of both. A SLI was given to 13/49 dogs (26.5%). Ciclosporin (Atopica™, Elanco) was most used ($n = 6$, 46.1%), followed by mycophenolate ($n = 4$, 30.7%), and azathioprine ($n = 3$, 23.1%). Vincristine (0.02 mg/kg, intravenously) was administered in 15/49 (30.6%) dogs, of which 10 (66%) survived to discharge. Other immunomodulatory therapies, such as human intravenous immunoglobulin (hIVIg) ($n = 3$, 6.1%) or splenectomy ($n = 1$ dog, 2%) were anecdotal. Twenty dogs (40.8%) received at least one blood transfusion, and six dogs (12.2%) received two or more blood transfusions. Most dogs received a packed red blood cell (pRBC) transfusion ($n = 14$, 70%), followed by whole blood transfusion administration ($n = 6$, 30%). In two dogs that received more than one blood transfusion, information about the blood product used prior to referral was not available. Supportive medication administered during hospitalisation included proton pump inhibitors ($n = 19$, 38.7%), fenbendazole ($n = 16$, 32.6%), sucralfate ($n = 14$, 28.6%), antimicrobials ($n = 6$, 12.2%), ranitidine ($n = 3$, 6.1%) and vitamin K ($n = 1$, 2%).

Hospitalisation time, survival and long-term outcome

Thirty-three dogs (67.3%) survived to discharge. The cause of death was attributed to ITP in all the dogs that did not survive to discharge ($n = 16$, 32.6%). Intracranial bleeding and pulmonary haemorrhage were presumed as the cause of death in three dogs (18.75%) and two dogs (12.5%), respectively. The cause of death in one other dog was not recorded.

Kaplan–Meier survival analysis was performed to assess for short- and long-term survival in both primary and secondary ITP cases (Fig. 2). The survival rate was 69% at 2 weeks [95% CI: 0.54 to 80; primary ITP only: 72% (95% CI: 0.55 to 0.83); secondary ITP only: 56% (95% CI: 0.20 to 0.80)], 63% at 3 months [95% CI: 0.48 to 0.75; primary ITP only: 67% (95% CI: 0.50 to 0.79); secondary ITP only: 56% (95% CI: 0.20 to 0.80)], and 51% at 2 years [95% CI: 0.37 to 0.65; primary ITP only: 55% (95% CI: 0.38 to 0.69); secondary ITP only: 44% (95% CI: 0.14 to 0.72)]. The overall median survival time was 985 days. However, primary ITP had a median survival time of 1084 days, when secondary ITP had a median survival time of 225 days. Long-term follow-up until death or until the end of the study was available for 20 dogs (40.8%). Four dogs were known to be alive at the end of the study period. When excluding dogs who died within 30 days of diagnosis, the median survival time was 3686 days (primary ITP only: 3686 days; secondary ITP only: 1473 days).

Variables assessed for their impact on survival in the univariable analysis included: age, gender, neutered status, weight, clinical signs (i.e., lethargy, inappetence/anorexia, vomiting, haematemesis, diarrhoea, melaena, weight loss, pyrexia, petechiae and/or ecchymoses, epistaxis, hyphaema, haematuria, haemoptysis and gingival bleeding), prior immunosuppressive

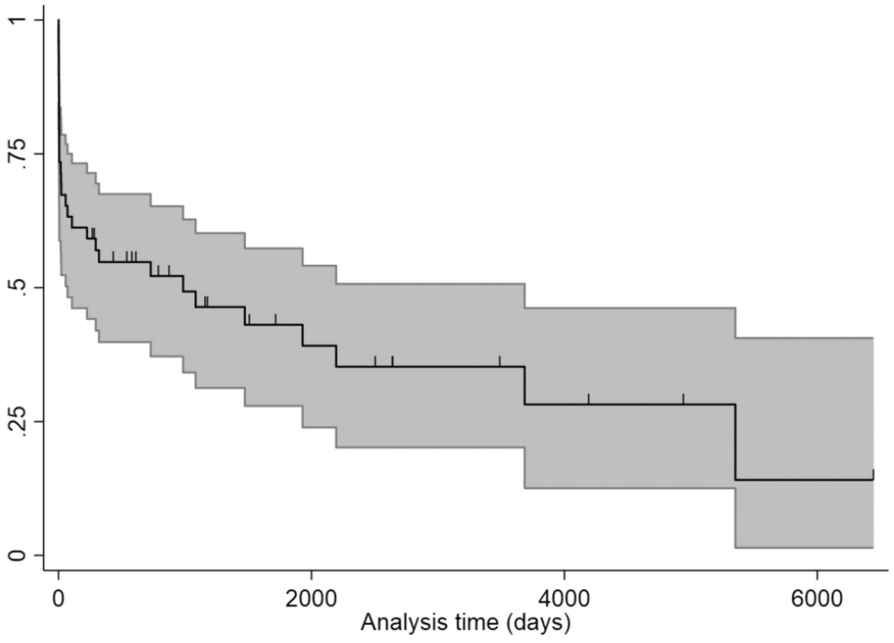

FIG 2. Overall Kaplan–Meier survival analysis of dogs with ITP presented to a single referral hospital in Ireland. Median survival time was 985 days. Survival rate was 69% at 2 weeks [95% CI 54 to 80), 63% at 3 months [95% CI 48 to 75), and 51% at 2 years [95% CI 37 to 65].

treatment, platelet count, PDW, HCT, anaemia, reticulocytosis, neutrophilia, high band cell count, hyperbilirubinaemia, BUN, creatinine, treatment with antacids, sucralfate, hIVIg, vincristine or second-line immunosuppressive agent (SLI), number of blood transfusions and secondary ITP diagnosis. Those variables with a $P < 0.20$ are included in Table 3. Variables with $P < 0.20$ but with very few events (i.e., haemoptysis, hyphaema and hIVIg use) were excluded from the multivariable analysis (Table 4). Following the multivariable analysis, the presence of a lower haematocrit had a significant negative impact on survival (HR: 0.96, 95% CI: 0.92 to 0.99, $P = 0.02$). Dogs with neutrophilia had a 66% increase in the likelihood of surviving as compared to dogs without neutrophilia (HR: 0.44, 95% CI: 0.20 to 0.96, $P = 0.04$). The use of SLI was not associated with survival (Table 3).

The same variables as those assessed for their impact on survival were included in the univariable analysis to assess for their impact on hospitalisation length. Those variables with a $P < 0.20$ are included in Table 5, and the variables included in the multivariable analysis are summarised in Table 6. The presence of band neutrophilia was significantly associated with shorter hospitalisation time (regression coefficient: −3.56, 95% CI: −5.70 to −1.42, $P = 0.003$). The presence of petechiae and/or ecchymoses was significantly associated with longer hospitalisation time (regression coefficient: 2.41, 95% CI: 0.41 to 4.42, $P = 0.02$). Using SLI was also significantly associated with longer hospitalisation (regression coefficient: 2.11, 95% CI: 0.11 to 4.12, $P = 0.04$). Vincristine neither had a significant effect on hospitalisation time ($P = 0.48$) nor survival ($P = 0.89$).

DISCUSSION

This retrospective study describes the presentation and short- and long-term outcome of dogs diagnosed with primary and secondary ITP in Ireland between 2009 and 2020. The population was similar to previously described populations of dogs with ITP: adult and neutered dogs with a wide age span (6 months to 13 years) and a median age of 7 years (Jans et al., 1990; Kohn et al., 2000; O'Marra et al., 2011). Unlike previous studies (Botsch et al., 2009; Putsche & Kohn, 2008), where females appeared to be overrepresented, this population had an equal distribution between male and female genders. The two more prevalent pure breeds in our population were English Springer Spaniel and Cocker Spaniel, which agrees with previous reports (Botsch et al., 2009; Lewis & Meyers, 1996; Putsche & Kohn, 2008).

Table 3. Variables assessed for association with survival in the univariable analysis with a P <0.2

Parameters	*n*	Hazard ratio (HR)	95% confidence interval	P value
Lethargy	49	1.70	0.78 to 3.69	0.18
Pyrexia	48	1.72	0.75 to 4.00	0.2
Melaena	48	1.87	0.86 to 4.04	0.11
Hyphaema	49	2.46	0.71 to 8.42	0.15
Haemoptysis	49	3.89	0.88 to 17.10	0.07
HCT (%)	49	0.97	0.94 to 1.01	0.11
Neutrophilia (>11.5 ×10^9/L)	48	0.58	0.28 to 1.20	0.14
Hyperbilirubinaemia (>10 µmol/L)	46	2.54	0.86 to 7.50	0.09
hIVIg administration	**49**	**5.53**	**1.56 to 19.60**	**0.008**
Number of blood transfusions	49	1.16	0.98 to 1.36	0.08

These variables were included in the multivariable model, and those with a $P < 0.05$ are indicated in bold. Haemoptysis, hyphaema and hIVIg were excluded from the multivariable analysis due to the low number of events (2, 3 and 3, respectively).

Table 4. Variables assessed for association with survival using multivariable analysis

Parameters	n	Hazard ratio (HR)	95% confidence interval	P value
Lethargy	45	0.95	0.37 to 2.41	0.92
Pyrexia	45	1.70	0.68 to 4.24	0.25
Melaena	45	1.64	0.7 to 3.86	0.25
HCT (%)	**45**	**0.96**	**0.92 to 0.99**	**0.02**
Neutrophilia (>11.5 ×10^9/L)	**45**	**0.44**	**0.2 to 0.96**	**0.04**
Hyperbilirubinaemia (>10 µmol/L)	45	2.13	0.57 to 7.92	0.26
Number of blood transfusions	45	1.22	0.61 to 2.48	0.57

Variables significantly and independently associated with survival ($P < 0.05$) are indicated in bold.

Table 5. Variables assessed for their effect on hospitalisation duration in the univariable analysis with a P <0.2

Parameters	*n*	Regression coefficient	95% confidence interval	P value
Lethargy	44	−1.11	−3.04 to 0.82	0.14
Petechiae and/or ecchymoses	44	1.44	−0.43 to 3.32	0.13
Anaemia (HCT <37%)	44	1.35	−0.62 to 3.32	0.14
Band neutrophilia (>0.45 ×10^9/L)	21	−2.79	−5.48 to −0.09	0.04
BUN (mmol/L)	43	0.14	−0.05 to 0.34	0.15
Creatinine	43	0.01	−.002 to 0.02	0.11
Second-line immunosuppressive agent use	**44**	**3.24**	**1.28 to 5.20**	**0.002**

These variables were included in the multivariable model, and those with a $P < 0.05$ are indicated in bold.

Table 6. Variables assessed their effect of hospitalisation duration using multivariable analysis

Parameter	*n*	Regression coefficient	95% confidence interval	P value
Lethargy	21	1.90	−0.29 to 4.09	0.08
Petechiae and/or ecchymoses	**21**	**2.41**	**0.41 to 4.42**	**0.02**
Anaemia (HCT <37%)	21	0.99	−2.58 to 4.56	0.56
Band neutrophilia (>0.45 ×10^9/L)	**21**	**−3.56**	**−5.70 to −1.42**	**0.003**
BUN (mmol/L)	21	−0.11	−0.39 to 0.18	0.43
Creatinine (µmol/L)	21	0.006	−0.03 to 0.04	0.72
Second-line immunosuppressive agent use	**21**	**2.11**	**0.11 to 4.12**	**0.04**

Variables significantly associated with hospitalisation duration ($P < 0.05$) are in bold.

Primary ITP was more common in this cohort, and as previously described (Jackson & Kruth, 1985; Kohn et al., 2000). Previous studies reported neoplasia, and in particular round-cell neoplasia, as the most common trigger associated with ITP (Grindem et al., 1994). Similarly, in the present study, four out of five dogs with ITP secondary to a neoplastic process had a confirmed diagnosis of a round-cell neoplasia. There are several mechanisms other than immune-mediated destruction that can participate in neoplasia-associated thrombocytopenia, including disseminated or local platelet consumption, splenic sequestration, myelophthisis and tumour-elaborated oestrogens (Grindem et al., 1994; Lewis & Meyers, 1996). In the present study, cases were included only if these other causes were considered unlikely or excluded.

All dogs received treatment with corticosteroids, which is still considered the mainstay of treatment for ITP, and a smaller percentage of dogs received additional treatment with a SLI. Dogs receiving a SLI were hospitalised for longer than those receiving corticosteroids alone, but survival was similar between the two groups. This is likely representative of the fact that dogs receiving a SLI had a more severe disease and/or a disease more difficult to control and thus remained hospitalised for longer. The effect of different treatment protocols on hospitalisation time and survival has only rarely been evaluated specifically (Kristiansen & Nielsen, 2021). Indeed, the only immunomodulatory treatment found to be associated with decreased hospitalisation time in dogs was hIVIg in a single study (Bianco et al., 2009). The reason for starting a second immunosuppressive agents in the present study was not recorded, and therefore, it is not possible to know whether the SLI was started based on clinician's preference, because the disease was deemed more severe, or because it was considered refractory to treatment with corticosteroids alone. Therefore, because SLI may or may not have been administered to more severely affected dogs, it is difficult to appreciate (or further speculate on) their effect on outcome.

In contrast to previous studies, the administration of vincristine in this population neither reduced the hospitalisation time significantly, nor had a positive effect on survival. Vincristine is often administered to increase the number of circulating platelets through stimulation of thrombopoiesis and accelerated megakaryocytic breakdown. Other hypothesised mechanisms include inhibition of platelets' phagocytosis, and interference with antiplatelet antibody formation and binding (Balog et al., 2013; Park et al., 2015; Rozanski et al., 2002; Weigert et al., 2010). Previous studies have reported a faster increase in platelet count and shorter hospitalisation time with vincristine use (Rozanski et al., 2002), and a prolonged effect has been reported with vincristine-loaded platelet therapy compared to single intravenous vincristine injection (Park et al., 2015). Following evaluation of the evidence available in the literature, the recent ACVIM consensus statement on the treatment of ITP in dogs and cats also recommends the use of vincristine as a first-line emergency adjunctive treatment for dogs with ITP and clinically relevant bleeding (LeVine, Goggs, et al., 2024). When compared to the administration of hIVIg, the effect in platelet recovery and hospitalisation time has been reported to be similar between vincristine and hIVIg (Bianco et al., 2009). Out of the 15 dogs treated with vincristine in the present study, eight (53.3%) survived to discharge. Vincristine was administered at the discretion of the clinician in charge of the case, and therefore, a standardised protocol was not followed. It is possible that vincristine was only used in dogs with more severe disease and therefore more likely to require longer hospitalisation times and less likely to survive.

Several studies have investigated the short- and long-term outcomes of dogs diagnosed with ITP regardless of the presence of an underlying condition (Cummings & Rizzo, 2017; Jans et al., 1990; O'Marra et al., 2011; Putsche & Kohn, 2008; Simpson et al., 2018); however, the information available is still limited and largely variable. Previous retrospective studies have shown an overall fair prognosis, with a large percentage of dogs (74% to 97%) surviving to discharge (Jans et al., 1990; O'Marra et al., 2011; Simpson et al., 2018). The most recent study describing the outcome of 45 dogs with primary ITP reported an 89% survival rate to discharge, with 77% of dogs still alive at the last follow-up (range of 603 to 675 days) (Simpson et al., 2018). The short-term survival in the present study was shorter compared to previous studies, with only 67.3% of dogs surviving to discharge. Long-term survival was fair, with nearly half of the dogs being alive 2 years after diagnosis. Differences in the reported short- and long-term survival times for dogs with ITP are likely due to the differences in duration of follow-up periods, treatment protocols and inaccuracies of retrospective data analysis. Being a secondary referral hospital, cases were all referred cases, potentially skewing the population towards more severe ITP cases. Another factor potentially resulting in shorter survival in this population could be the fact that secondary ITP, particularly neoplastic, was not excluded from the overall survival analysis. Overall, the effect of being diagnosed with a primary ITP or secondary ITP was not significantly associated with survival in our population (HR: 1.17, 95% CI: 47 to 293, P 0.73) on univariate survival analysis; however, the median survival was much shorter in secondary ITP cases. The small number of secondary ITP precluded performing survival analysis on both groups separately, but the absence of significance of the median survival times between groups might therefore be due to type 2 error. This is supported by the marked discrepancies between median survival times and the fact that long-term survival of dogs surviving 30 days post diagnosis is just over 10 years. This is likely more representative of the primary ITP population and offers an excellent prognostic marker for owners.

Despite a better understanding of the pathophysiology of the disease in recent times, the lack of a gold standard diagnostic test or biomarker to direct treatment can make the diagnosis and treatment-tailoring of dogs with ITP challenging in some cases. The presence of melaena, high BUN or blood transfusion requirement at presentation were previously identified as negative prognostic factors (O'Marra et al., 2011; Simpson et al., 2018). In this study population, neither the administration nor the number of blood transfusions had a significant impact on survival. The presence of high BUN or melaena, likely reflecting gastrointestinal mucosal bleeding, were not found to be significant negative prognostic factors. The only variable negatively associated with

survival in our population was the presence of a lower haematocrit, which is likely to be present in dogs that have had more significant bleeding and therefore are considered to be more severely affected. Similarly, dogs with petechiae and ecchymoses had a significantly prolonged hospitalisation time, reflecting the more severe nature of the disease and active bleeding in these patients. Neutrophilia was the only variable associated with a decreased risk of death, and the presence of band neutrophilia was associated with shorter hospitalisation. Neutrophilia, often with a left shift, is commonly seen in ITP and other immune-mediated diseases, reflecting a marked systemic inflammatory response. To the best of our knowledge, presence of neutrophilia and band neutrophilia have not been previously reported as positive prognostic factors. It is possible that the presence of neutrophilia and band neutrophilia reflects that the bone marrow in these animals is very active, making them more likely to respond to treatment and therefore survive.

Limitations

There were several limitations in the study, most inherent to its retrospective nature and extensive period. These include being a single-centre study, the small case number and potentially low power of some of the results explaining lack of statistical significance, as previously discussed for the survival analysis. Other common pitfalls of retrospective studies include incomplete data and follow-up information, variable treatment without standardised protocols and a lack of control group. Considering the potential triggers, vaccination was not specifically investigated based on difficulty on accessing reliable information retrospectively. In addition, there is low evidence for its association with ITP (LeVine, Kidd, et al., 2024). Treatment was also left to the discretion of the clinician and not standardised; therefore, there can be a bias based on caregivers' financial or emotional limitations. Another limitation of the study would be the assumption that the mechanism of thrombocytopenia in all secondary ITP cases diagnosed with neoplastic disease was immune-mediated and not due to other mechanisms such as consumptive thrombocytopenia. Finally, primary and secondary ITP were included in the survival analysis, as previously discussed.

In conclusion, this study investigated the short- and long-term survival, as well as prognostic factors, in a population of dogs diagnosed with primary and secondary ITP in Ireland. In agreement with previous literature, long-term survival was fair: 51% of dogs were reported to be alive 2 years after diagnosis; however, for dogs surviving 30 days post-diagnosis, the median survival time was excellent: 3686 days. A lower haematocrit was the only variable associated with a worse prognosis. The administration of a SLI and the presence of petechiae and ecchymoses were associated with a longer hospitalisation, likely reflecting a more severe disease, and neutrophilia was associated with a decreased risk of dying. In contrast to previous studies, an increased BUN, presence of melaena or blood product requirement were not associated with a worse prognosis. The administration of a single dose of vincristine did not significantly reduce hospitalisation time nor improved survival.

Author contributions

E. López-Bailén: Conceptualization (equal); data curation (lead); formal analysis (supporting); investigation (equal); methodology (equal); project administration (equal); resources (equal); writing – original draft (lead); writing – review and editing (equal). **A. Duclos:** Conceptualization (equal); data curation (equal); methodology (equal); resources (equal); writing – original draft (supporting); writing – review and editing (equal). **D. Mullany:** Data curation (supporting). **K. Le Boedec:** formal analysis (lead); investigation (equal); methodology (equal); writing - original draft (supporting); writing - review and editing (equal). **B. Cuq:** Conceptualization (equal); data curation (equal); formal analysis (supporting); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (lead); writing – original draft (supporting); writing – review and editing (equal).

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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 the paper.

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