

ORIGINAL ARTICLE

Retrospective study on diagnostic yield and medical impact of diagnostic imaging in dogs with immune-mediated haemolytic anaemia in Belgium

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OBJECTIVES: To assess the incidence of associative immune-mediated haemolytic anaemia in dogs diagnosed with immune-mediated haemolytic anaemia and to evaluate whether performing diagnostic imaging, specifically thoracic radiographs and abdominal ultrasound, influences outcomes in non-associative immune-mediated haemolytic anaemia.

MATERIALS AND METHODS: Medical records (2017 to 2023) were reviewed for dogs diagnosed with immune-mediated haemolytic anaemia. Dogs were classified as associative immune-mediated haemolytic anaemia or non-associative immune-mediated haemolytic anaemia. Survival odds at 3, 6 and 12 months were assessed for non-associative immune-mediated haemolytic anaemia. Multivariable logistic regression was used to identify factors associated with a risk of decreased survival.

RESULTS: Among 117 immune-mediated haemolytic anaemia cases, 7 (6%) had associative immune-mediated haemolytic anaemia, and 110 (94%) had non-associative immune-mediated haemolytic anaemia. In non-associative immune-mediated haemolytic anaemia dogs, thoracic radiographs were performed in 63 dogs (57.3%) and abdominal ultrasound in 90 dogs (81.8%), with unremarkable thoracic radiographs in 55.5% of cases and unremarkable abdominal ultrasound in 17.8%. Diagnostic imaging did not significantly affect the risk of decreased survival at 3, 6 and 12 months ($P > .05$). Increasing age (OR [95% CI]: 1.31 [1.06 to 1.61] at 12 months), elevated serum alanine aminotransferase (OR [95% CI]: 3.93 [1.11 to 14.0] at 3 months) and a higher number of packed red blood cell transfusions (OR [95% CI]: 2.10 [1.09 to 4.08] at 3 months and 1.91 [1.02 to 3.56] at 6 months) were associated with an increased risk of decreased survival.

CLINICAL SIGNIFICANCE: Performing diagnostic imaging was not associated with a change in survival odds in dogs with non-associative immune-mediated haemolytic anaemia in this cohort. These findings suggest individual case-based diagnostic imaging may be appropriate in clinical practice.

Journal of Small Animal Practice (2026);01–8
DOI: 10.1111/jsap.70159

Accepted: 3 June 2026

INTRODUCTION

Immune-mediated haemolytic anaemia (IMHA) is one of the most commonly diagnosed immune-mediated diseases in dogs (Piek, 2011). The pathogenesis of IMHA involves autoantibodies targeting erythrocytes, resulting in their destruction through macrophage-mediated extravascular haemolysis or complement-induced intravascular haemolysis (Berentsen & Sundic, 2015). IMHA is classified as either associative (aIMHA) or non-associative (naIMHA), depending on whether a likely underlying cause is identified or not (Garden et al., 2019). Potential triggers of aIMHA include infectious agents, neoplastic or inflammatory diseases, drugs, toxins and vaccination (Garden et al., 2019). Reported incidence rates of aIMHA generally range from 20% to 25% of all IMHA cases (Duclos et al., 2024; Jackson & Kruth, 1985; Piek et al., 2008).

Diagnostic imaging (DI), such as thoracic radiography (TXR) and abdominal ultrasound (AUS), is frequently performed in dogs with IMHA to diagnose or rule out underlying causes, aiding clinicians in selecting optimal treatments (Piek, 2011). However, imaging techniques can be costly and are not always readily accessible. Recent studies suggest TXR and AUS do not consistently provide clinically significant information in IMHA cases (Andres et al., 2019; De Laet et al., 2022; Woodward & White, 2020). While TXR rarely reveals abnormalities in dogs with IMHA, abnormal AUS findings are frequent, though in only a small subset of cases do they provide additional diagnostic information (Andres et al., 2019; De Laet et al., 2022; Woodward & White, 2020). In one study, AUS identified possible triggers in only 7.3% of dogs, while TXR detected none (Woodward & White, 2020). Similarly, the utility of imaging techniques has also been explored in immune-mediated polyarthritis in dogs, where TXR and AUS were rarely beneficial in the initial management (Tang et al., 2024).

The present study aimed to assess the incidence of aIMHA in dogs diagnosed with IMHA at a university teaching hospital located in Liège, Belgium and to evaluate the effect of performing AUS/TXR on the clinical outcome in dogs with naIMHA. We hypothesised that the incidence of aIMHA would be low and that performing a complete imaging workup would not significantly impact clinical outcome.

MATERIALS AND METHODS

Study design and inclusion criteria

This retrospective cohort study was conducted using the electronic medical records from the university teaching hospital of Liège in Belgium and included cases from 2017 to 2023. Records were searched using the following keywords: “anaemia”, “regenerative anaemia”, “hemolytic anaemia”, “immune-mediated hemolytic anaemia”, “associative immune-mediated hemolytic anaemia”, “non-associative immune-mediated hemolytic anaemia”, “IMHA”, “hematologic disease” and “immune-mediated disease”.

Dogs were included if they met the American College of Veterinary Internal Medicine (ACVIM) consensus criteria for diagnostic or supportive IMHA, defined as anaemia (packed cell volume (PCV) <25%) with evidence of immune-mediated erythrocyte destruction (e.g. spherocytosis, positive saline agglutination test (SAT) and/or positive direct antiglobulin test (DAT)) together with at least one criterion of haemolysis (e.g. hyperbilirubinaemia and/or bilirubinuria, jaundice, haemoglobinaemia, haemoglobinuria or ghost cells). Dogs without documented evidence of haemolysis were excluded. DATs were performed by a commercial veterinary diagnostic laboratory (Antech®, Belgium), using species-specific anti-IgG, anti-IgM and anti-C3 antibodies. A positive result was defined as >1:2, following laboratory recommendations. SATs were performed using a minimal 1:4 dilution. Cases were excluded if incomplete medical records prevented accurate assessment of clinical findings or outcomes.

Two board-certified veterinary internal medicine specialists, blinded to the original diagnosis, independently reviewed each record, classifying the case as either aIMHA or naIMHA based on available clinical data. In cases where classification was equivocal, a third internist reviewed the case to provide a final classification. This population was used to assess the incidence of aIMHA. Dogs classified as naIMHA were further divided into two groups based on the extent of diagnostic imaging performed: those who underwent both AUS and TXR were categorised in the DI group, while those with only one or no imaging studies were categorised in the not complete DI group (ncDI).

Diagnostics performed

Data collected for all IMHA dogs included signalment, medical and travel history, physical examination findings, diagnostic test results (including complete blood count (CBC), biochemistry, blood smear, SAT, DAT and urinalysis), imaging findings, fine needle aspiration (FNA) and quantitative polymerase chain reaction (qPCR) testing for infectious agents such as *Babesia canis*, *Ehrlichia canis* or *Anaplasma phagocytophilum*, treatments (including immunosuppressants, antithrombotic agents and antimicrobial therapies) and packed red blood cell (pRBC) transfusions administered during initial hospitalisation.

CBC and biochemistry were performed on the IDEXX ProCyte and Catalyst Dx analyzer. Thrombocytopenia and severe thrombocytopenia were defined as platelet counts below 150,000/ μ L and 30,000/ μ L, respectively, and were confirmed by blood smear examination. Evidence of haemolysis was defined by the presence of extravascular haemolysis (characterised by the presence of spherocytosis, or hyperbilirubinaemia/bilirubinuria, or jaundice without evidence of functional hepatic disease, post-hepatic cholestasis or sepsis) or intravascular haemolysis (characterised by the presence of haemoglobinaemia/haemoglobinuria, or ghost cells on the blood smear).

All TXR and AUS procedures were performed at the university hospital and evaluated by a board-certified diagnostic imager. The decision to perform DI was based on the clinical judgement

of the supervising clinician and the owner's preferences. Some dogs were sedated for the procedure. TXR included at least two orthogonal views (latero-lateral and ventro-dorsal projections) acquired using a computed or direct radiography system (Musica DR 14s or CR35x; Agfa-Gevaert Group). AUS examinations were conducted of the entire abdomen with the patient in dorsal recumbency using a fixed ultrasound machine (Canon Aplio Alpha 450 or Hitachi Arietta 850 SE or Aloka Alpha10 or Esaote My Lab ClassC) equipped with curvilinear (4 to 11 MHz) and/or linear (3.8 to 18 MHz) probes.

Follow-up and outcomes

To determine whether a lack of DI might have resulted in missed aIMHA cases, survival status was assessed at 3-month follow-up. To rule out underlying causes of IMHA that may have temporarily responded to immunosuppressive treatment, additional follow-up visits were conducted at 6 and 12 months. Dogs discharged from hospitalisation but lost to follow-up were excluded. Regarding survival status, dogs were classified at each time point as either dead (if they died during or after hospitalisation) or alive (regardless of treatment status). For dogs with a minimum follow-up of 12 months, information regarding disease relapse and the addition of other immunosuppressants was collected. Follow-up data were obtained from owners and/or referring veterinarians if follow-up was performed in a veterinary clinic other than the university teaching hospital of Liège.

Statistical analysis

Descriptive statistics were used to summarise patient characteristics and outcomes. Continuous variables were presented as mean $\pm$ standard deviation, median with interquartile range (Q1 to Q3) and minimum and maximum values, with the choice of mean or median determined by the normality of the data distribution. Qualitative variables were summarised using frequency and percentages. Normality was assessed numerically, graphically and with the Shapiro–Wilk test. Whenever exact quantitative values were unavailable in the record, but the qualitative values (under, within or above reference range) were available, these data were excluded from descriptive statistics but were included in the qualitative logistic regression analyses.

Univariable binary logistic regressions provided unadjusted estimates for factors associated with survival at 3, 6 and 12 months. Multivariable logistic regression models identified independent risk factors. Variables included in the multivariable models were selected through a stepwise approach, and those causing quasi-complete separation of data were excluded. Statistical analyses were performed using SAS version 9.4 for Windows or equivalent standard software. A significance level of $P < .05$ was applied to all tests, with results reported at a 95% confidence level.

RESULTS

Incidence of associative IMHA

One hundred and seventeen dogs were included in this study. Seven dogs (6%) were unanimously classified as aIMHA and 110

dogs (94%) as naIMHA. No cases showed disagreement among the internists. The median age of dogs with aIMHA was 9 years (range 4 to 13), including 5 spayed females and 2 neutered males. The median body weight was 23 kg (range 5 to 42). Breeds represented included Bernese Mountain dogs ($n=2$), Belgian Tervuren ($n=1$), beagle ($n=1$), shih-tzu ($n=1$), Jack Russell terrier ($n=1$) and 1 mixed-breed dog. IMHA was associated with a diagnosis of babesiosis (*B. canis*) in two dogs and with miscellaneous neoplastic conditions in five dogs. Of the two dogs with babesiosis, one had travelled to an endemic region (south of France). Neoplasia associated with IMHA included histiocytic sarcoma (one dog), hepatosplenic lymphoma (one dog) and three cases of metastatic cancer of unknown origin (no cytological or histopathological examination). These included one dog with a splenic mass and pulmonary metastases, one dog with a liver tumour and splenic, regional lymph node and pulmonary metastases and one patient with a lung tumour with lymph node metastases.

Non-associative IMHA

History and clinical signs

In dogs with suspected naIMHA ($n=110$), the median age was 7 years (range, 0.7 to 14.0), including 41 spayed females, 19 intact females, 24 castrated males and 26 intact males. The median weight was 15 kg (range, 2.0 to 71.0). The most common breeds included shih-tzus ($n=15$; 13.6%), English cocker spaniels ($n=9$; 8.2%) and Maltese ($n=8$; 7.3%). Notably, the prevalence of shih-tzus in the naIMHA group (13%, 95% CI: 7.2% to 20.1%) was significantly higher compared to their prevalence in the general population seen at the university hospital during the same period (2.4%, 95% CI: 2.2% to 2.5%).

Clinical findings are summarised in Table 1. Five dogs presented with abdominal pain and underwent AUS. In four cases, no underlying cause was identified, while the fifth dog showed signs consistent with duodenal ulceration. Three dogs experienced syncope, likely due to anaemia. Echocardiography and an electrocardiogram were performed in all these cases, revealing no abnormalities. None of these dogs had recurrent episodes, and all were followed for at least 6 months. One dog with severe thrombocytopenia presented with petechiae.

Table 1. Clinical findings in dogs with non-associative immune-mediated haemolytic anaemia

Abnormalities	Number of dogs ($n=110$)
Lethargy	106 (96.4%)
Pale mucous membranes	104 (94.5%)
Tachycardia	89 (80.9%)
Hyporexia/anorexia	87 (79.1%)
Bounding pulses	80 (72.7%)
Icteric mucous membranes	60 (54.5%)
Heart murmur	46 (41.8%)
Tachypnoea	41 (37.3%)
Pigmenturia	35 (31.8%)
Vomiting	21 (19.1%)
Hyperthermia	21 (19.1%)
Painful abdominal palpation	5 (4.5%)
Syncope	3 (2.7%)
Petechiae	1 (0.9%)

Table 2. Complete blood count results in dogs with non-associative immune-mediated haemolytic anaemia

Parameters	Data available	Median	Range	Reference interval	Increased	Decreased
Haematocrit (L/L)	110	0.13	0.03 to 0.25	0.37 to 0.61	0 (0%)	110 (100%)
Leucocytes (10 ⁹ /L)	104	29.68	11.3 to 129	5.05 to 16.7	90 (86.5%)	0 (0%)
Neutrophils (10 ⁹ /L)	102	19.51	5.44 to 106	2.95 to 11.6	84 (82.3%)	0 (0%)
Monocytes (10 ⁹ /L)	98	3.09	0.16 to 12.58	0.16 to 1.12	90 (91.8%)	0 (0%)
Lymphocytes (10 ⁹ /L)	95	6.54	0.2 to 46.2	1.05 to 5.1	57 (60%) [‡]	2 (2.1%)
Thrombocytes (10 ⁹ /L)	76	180	3 to 491	148 to 484	1 (1.3%)	22 (28.9%)
						<30 = 8 (10.5%)*

Bold values indicate median values that were above the upper limit of the reference interval

[‡]Lymphocytosis was a false increase in some dogs, due to elevated nucleated red blood cells detected by the IDEXX®ProCyte Dx analyzer and counted as lymphocytes

*Eight dogs had severe thrombocytopenia (platelet count <30 × 10⁹/L), representing a subset of dogs with thrombocytopenia

Table 3. Biochemistry results in dogs with non-associative immune-mediated haemolytic anaemia

Parameters	Data available	Median	Range	Reference interval	Increased	Decreased
Creatinine (μmol/L)	61	56	23 to 286	44 to 140	1 (1.6%)	16 (26.2%)
Urea (mmol/L)	70	10	3 to 46	2.5 to 9.6	39 (55.7%)	0 (0%)
Total protein (g/L)	62	71	52 to 120	52 to 82	14 (22.6%)	0 (0%)
Globulin (g/L)	62	40	28 to 62	25 to 45	20 (32.2%)	0 (0%)
Albumin (g/L)	62	31	20 to 84	22 to 39	12 (19.3%)	1 (0.9%)
ALT (U/L)	70	142	10 to 3029	<125	38 (54.3%)	0 (0%)
ALP (U/L)	68	260	27 to 1963	<212	40 (58.8%)	0 (0%)
Total bilirubin (μmol/L)	100	34	6 to 922	<15	87 (87%)	0 (0%)

Bold values indicate median values that were above the upper limit of the reference interval

Laboratory tests

Complete blood counts and biochemistry findings are summarised in Tables 2 and 3, respectively. Among the cohort, eight dogs (7.3%) initially presented with non-regenerative anaemia. One dog exhibited azotaemia attributed to chronic kidney disease. Evidence of haemolysis is detailed in Table 4. An SAT was performed in 91.8% of dogs, yielding positive results in 64.3% of cases. The DAT was conducted in 52.7% of dogs, with a positivity rate of 77.6%. qPCR testing for infectious agents, including *B. canis*, *E. canis* and *A. phagocytophilum*, was performed in 50 dogs (45.4%).

Diagnostic imaging

Thoracic radiographs were performed in 63 dogs (57.3%) and AUS in 90 dogs (81.8%). Imaging findings are summarised in Table 5. No abnormalities were observed in 35/63 dogs (55.5%) on TXR and in 16/90 dogs (17.8%) on AUS. Fine needle aspirates of abnormal abdominal organs were obtained in 11/90 dogs (12.2%), with findings limited to extramedullary haematopoiesis in the liver and spleen, and reactive lymph node changes. Among the six dogs with a moth-eaten pattern of the spleen, three underwent splenic FNA.

One dog developed aspiration pneumonia during hospitalisation, showing a ventral alveolar pattern on TXR. AUS findings in 11 dogs suggested pancreatitis, though none showed compatible clinical or biochemical signs. Two dogs presented with signs of duodenal ulceration, suspected to be secondary to tissular hypoxia. In both dogs, the abdominal changes fully resolved, with at least 6 months of follow-up available.

Treatments

All dogs received immunosuppressive therapy with prednisolone, and 59 dogs (51.3%) were additionally treated with at least one

Table 4. Evidence of haemolysis in dogs with non-associative immune-mediated haemolytic anaemia

Parameters	Data available	Number of dogs
Spherocytosis	110	74 (67.3%)
Extravascular haemolysis	110	97 (88.2%)
Jaundice	110	60 (54.5%)
Hyperbilirubinaemia and/or bilirubinuria	104	91 (87.5%)
Intravascular haemolysis	110	46 (41.8%)
Ghost cells	110	2 (1.8%)
Haemoglobinaemia and/or haemoglobinuria	110	46 (41.8%)

other immunomodulatory agent at the clinician's discretion. These included cyclosporine (38 dogs; 64.4%), mycophenolate mofetil (eight dogs; 13.6%), azathioprine (six dogs; 10.2%), a combination of cyclosporine and azathioprine (five dogs; 8.5%) and a combination of cyclosporine and mycophenolate mofetil (two dogs; 3.3%). In total, 34 dogs (29.6%) received doxycycline or imidocarb while awaiting qPCR results. Antithrombotic therapy was administered to 85 dogs (77.3%), comprising clopidogrel (67 dogs; 78.8%), rivaroxaban (ten dogs; 11.8%), enoxaparin (five dogs; 5.9%), a combination of clopidogrel and rivaroxaban (one dog; 1.2%) and a combination of clopidogrel and enoxaparin (two dogs; 2.3%). Transfusions were given to 94 dogs (85.5%), all of which received pRBC. Dogs received a median of 1 pRBC transfusion (range 0 to 4). In the transfused dogs, 51 dogs (54.3%) received 1 pRBC transfusion, and 43 dogs (45.7%) received more than 1 pRBC transfusion.

Follow-up, outcomes and group assignment

Twenty-nine dogs (26.4%) died during hospitalisation, including 15 dogs due to cardiopulmonary arrest and 14 dogs following euthanasia. Follow-up information was available for 79, 78 and 71

Table 5. Imaging findings in dogs with non-associative immune-mediated haemolytic anaemia

Organ	Finding	Number of dogs
Thoracic radiographs		n = 63
Unremarkable		35 (55.5%)
Pleural space	Pleural effusion/pleural thickening	16 (25.3%)
Lung	Bronchointerstitial pattern	14 (22.2%)
	Bronchial pattern (4), osteomas (2), bronchiectasis (1), bronchomalacia (1), ventral alveolar pattern (1)	<10%
Other	Cardiomegaly	11 (17.4%)
	Mild sternal lymphadenomegaly (4), redundant tracheal membrane (5), tracheal hypoplasia (3), pulmonary hypertension (1)	<10%
Abdominal ultrasound		n = 90
Unremarkable		16 (17.8%)
Liver and biliary tract	Hepatomegaly	42 (46.7%)
	Gallbladder sludge	33 (36.7%)
	Hyperechoic parenchyma	29 (32.2%)
	Gallbladder wall thickening	16 (17.8%)
	Heterogeneous and/or homogeneous hepatic nodules (<3 cm)	16 (17.8%)
	Mucinous hyperplasia (3), hypoechoic parenchyma (2)	<10%
Spleen	Heterogeneous and/or homogeneous nodules (<3 cm)	35 (38.9%)
	Splenomegaly	25 (27.8%)
	Moth-eaten spleen (6), cystic lesion (1)	<10%
Pancreas	Pancreatitis (11)	11 (12.2%)
Gastro-intestinal tract	Wall thickening	12 (13.3%)
	Hyperechoic mucosa (4), functional ileus (4), signs of ulceration (2)	<10%
Adrenal glands	Unilateral nodule (7), bilaterally thinner (5), bilateral nodules (4), bilateral adenomegaly (4)	<10%
Kidneys	Loss of corticomedullary layering	22 (24.4%)
	Hyperechoic cortex	17 (18.9%)
	Cortical and/or medullary mineralisation	11 (12.2%)
	Pyelic mineralisation (9), infarcts (3), cortical cyst (5)	<10%
Bladder	Hyperechoic urinary bladder sediment (cells, crystalluria)	32 (35.6%)
	Thickening of the cranio-ventral wall (3), vesical lithiasis (1)	<10%
Other	Peritoneal effusion	41 (45.5%)
	Mild abdominal lymphadenomegaly	14 (15.6%)
	Diffuse steatitis	12 (13.3%)
	Benign prostatic hyperplasia	11 (12.2%)
	Testicular nodule (2), endometrial hyperplasia (1), cystic ovaries (1)	<10%

dogs at 3, 6 and 12 months, respectively. Percentages of cases alive are illustrated in Fig 1. Among the dogs with a minimum follow-up of 12 months, 29 (40.8%) were still alive. A relapse while on treatment occurred in five dogs (16.7%), leading to the addition of a second immunosuppressive agent in two cases. Notably, 25 dogs (83.3%) were able to discontinue treatment entirely. Of these, 24 dogs did not relapse during treatment tapering. However, three of these dogs relapsed after complete treatment cessation.

A total of 60 (54.5%) dogs were assigned to the DI group and 50 (45.5%) dogs to the nDI group. The DI group included 46 (58.2%), 46 (59%) and 42 (59.2%) dogs at 3, 6 and 12 months, respectively. Further classification can be found in Table 6.

Impact of DI on the risk of decreased survival

At the assessment time points, 37/79 dogs (46.8%) were known to have died by 3 months, 39/78 (50%) by 6 months and 42/71 (59.1%) by 12 months after diagnosis. The results of the univariate binary logistic regressions assessing factors associated with the risk of decreased survival are presented in Table 7.

At 3 months: Increasing age was significantly associated with a higher risk of decreased survival (odds ratio (OR) [95% confidence interval (CI)]: 1.25 [1.07 to 1.47]). Dogs with elevated urea (OR [95% CI]: 2.58 [1.00 to 6.65]) or increased serum alanine aminotransferase (ALT) activity (OR [95% CI]: 2.58 [1.00 to 6.65]) exhibited a significantly higher risk of decreased survival. Additionally, the risk of decreased survival increased with the number of pRBC transfusions (OR [95% CI]: 1.88 [1.11 to 3.17]). Conversely, dogs receiving antithrombotic treatment had a significantly reduced risk of decreased survival (OR [95% CI]: 0.31 [0.10 to 0.92]). In the multivariate analysis, elevated serum ALT activity remained associated with an increased risk of decreased survival (OR [95% CI]: 3.93 [1.11 to 14.0]), as did the number of pRBC transfusions (OR [95% CI]: 2.10 [1.09 to 4.08]). DI was not associated with the risk of decreased survival ($P > .05$).

At 6 months: For this analysis, deaths were considered cumulatively, including all dogs that had died up to the 6-month assessment time point. Increasing age was again significantly associated with a higher risk of decreased survival (OR [95% CI]: 1.23 [1.06 to 1.43]). Consistent with the 3-month findings, the risk of decreased survival increased with the number of pRBC transfusions (OR [95% CI]: 1.70 [1.01 to 2.86]) and was significantly reduced in dogs receiving antithrombotic treatment (OR [95% CI]: 0.29 [0.09 to 0.93]). In the multivariate analysis, the number of pRBC transfusions (OR [95% CI]: 1.91 [1.02 to 3.56]) remained significantly associated with an increased risk of decreased survival. As with the 3-month results, the DI investigation was not associated with the risk of decreased survival ($P > .05$).

At 12 months: This analysis included all dogs that had died up to the 12-month assessment time point. Increasing age was significantly associated with a higher risk of decreased survival, both in the univariate (OR [95% CI]: 1.37 [1.14 to 1.64]) and multivariate (OR [95% CI]: 1.31 [1.06 to 1.61]) analyses. DI continued to show no significant impact on the risk of decreased survival ($P > .05$).

DISCUSSION

This study found a low incidence of aIMHA in this cohort of dogs. Additionally, performing AUS and TXR in dogs with nIMHA did not affect the chance of being alive at 3-, 6-, or 12-month follow-up. The incidence of aIMHA in this study was 6%, which is lower than the 20% to 25% reported in previous studies (Duclos et al., 2024; Jackson & Kruth, 1985; Piek et al., 2008). Several factors may contribute to this finding,

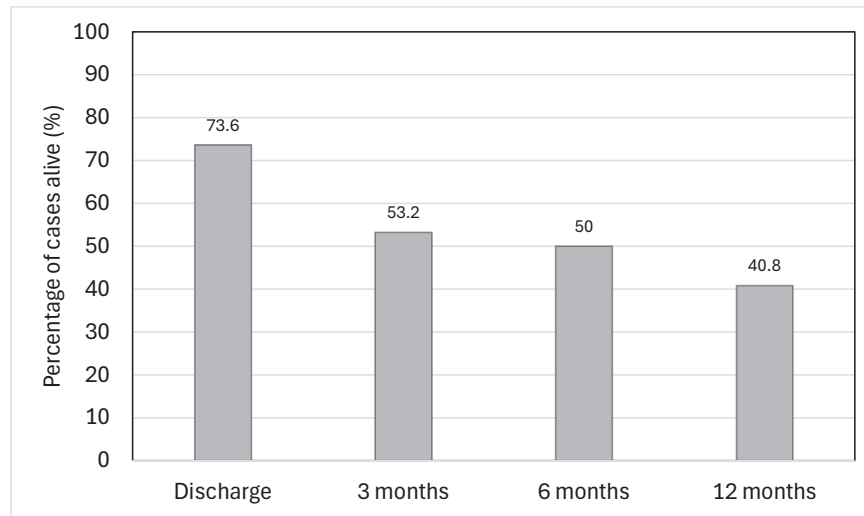


FIG 1. The percentage of cases alive at different time points of dogs diagnosed with non-associative immune-mediated haemolytic anaemia.

Table 6. Grouping of dogs with non-associative immune-mediated haemolytic anaemia into diagnostic imaging (DI) and no complete diagnostic imaging (ncDI)

	Dogs alive at discharge (n = 81)	Dogs with known outcome [‡] at 3 months (n = 79)	Dogs with known outcome [‡] at 6 months (n = 78)	Dogs with known outcome [‡] at 12 months (n = 71)
DI	43 (53%)	46 (58.2%)	46 (59%)	42 (59.2%)
ncDI				
Total	38 (46.9%)	33 (41.8%)	32 (41%)	29 (40.8%)
AUS only	22 (27.2%)	21 (26.6%)	21 (26.9%)	19 (26.8%)
TXR only	1 (1.2%)	3 (3.8%)	3 (3.8%)	3 (4.2%)
No imaging	15 (18.5%)	9 (11.4%)	8 (10.3%)	7 (9.8%)

AUS Abdominal ultrasound; DI Diagnostic imaging; ncDI No complete diagnostic imaging; TXR Thoracic radiographs

[‡]Known outcome refers to dogs for which follow-up status (alive or dead) was available at the respective time point

Table 7. Univariate binary logistic regressions assessing the risk of death of dogs with non-associative immune-mediated haemolytic anaemia based on selected parameters

Variables	At 3 months		At 6 months		At 12 months	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Age (years)	1.25 (1.07 to 1.47)	.004*	1.23 (1.06 to 1.43)	.008*	1.37 (1.14 to 1.64)	.001*
Sex (female spayed)						
Female intact	0.26 (0.05 to 1.40)	.11	0.213 (0.04 to 1.14)	.07	0.185 (0.03 to 1.06)	.058
Male intact	1.14 (0.35 to 3.72)	.82	0.948 (0.29 to 3.10)	.93	0.647 (0.19 to 2.23)	.49
Male castrated	3.34 (0.96 to 11.6)	.06	3.73 (1.00 to 13.9)	.05	3.24 (0.76 to 13.8)	.11
Weight (kg)	0.98 (0.95 to 1.02)	.39	0.99 (0.96 to 1.03)	.67	0.993 (0.96 to 1.03)	.71
Haematocrit (L/L)	0.99 (0.91 to 1.08)	.82	1.00 (0.92 to 1.09)	>.99	1.02 (0.93 to 1.13)	.63
Non-regenerative anaemia	0.36 (0.04 to 3.63)	.39	0.316 (0.03 to 3.18)	.33	0.329 (0.03 to 3.81)	.37
Leucocytosis	0.46 (0.13 to 1.55)	.21	0.533 (0.16 to 1.81)	.31	0.222 (0.04 to 1.11)	.066
Neutrophilia	0.37 (0.12 to 1.15)	.09	0.443 (0.14 to 1.36)	.15	0.280 (0.07 to 1.11)	.070
Monocytosis	0.523 (0.13 to 2.03)	.35	0.590 (0.15 to 2.29)	.45	0.143 (0.02 to 1.21)	.075
Thrombocytopenia	2.79 (0.91 to 8.55)	.07	2.42 (0.79 to 7.42)	.12	2.04 (0.6 to 6.96)	.26
Increased urea	2.58 (1.00 to 6.65)	.049*	2.10 (0.82 to 5.40)	.12	2.17 (0.78 to 5.99)	.14
Hyperglobulinaemia	1.87 (0.63 to 5.57)	.26	1.58 (0.53 to 4.72)	.41	1.76 (0.54 to 5.76)	.35
Increased ALT	2.58 (1.00 to 6.65)	.049*	2.10 (0.82 to 5.40)	.12	1.87 (0.68 to 4.96)	.23
Increased ALP	1.64 (0.65 to 4.14)	.29	1.34 (0.53 to 3.38)	.54	1.11 (0.42 to 2.94)	.83
Hyperbilirubinaemia	0.829 (0.24 to 2.85)	.77	0.969 (0.28 to 3.33)	.96	0.544 (0.13 to 2.31)	.41
Number of pRBC transfusions	1.88 (1.11 to 3.17)	.018*	1.70 (1.01 to 2.86)	.044*	1.62 (0.93 to 2.81)	.089
Antithrombotic drugs	0.31 (0.10 to 0.92)	.035*	0.29 (0.09 to 0.93)	.037*	0.257 (0.07 to 1.01)	.051
Imaging (DI group)	1.10 (0.45 to 2.70)	.84	1.24 (0.50 to 3.05)	.65	1.04 (0.40 to 2.72)	.94

ALT Alanine aminotransferase; ALP Alkaline phosphatase; CI Confidence interval; DI Diagnostic imaging; OR Odds ratio; pRBC Packed red blood cells

*P values < .05 were considered statistically significant

including the geographic distribution of infectious agents commonly associated with IMHA, such as *B. canis*. While babesiosis appears to be uncommon in the region of our hospital, one

affected dog in this study had not travelled to an endemic area. This finding highlights the importance of considering *B. canis* infections in Belgium, even in dogs without a history of travel.

No impact on survival rate was observed with a complete imaging workup compared to partial or no imaging. This suggests that targeted imaging in suspected naIMHA cases does not result in underdiagnosis of aIMHA, which could negatively impact prognosis. Thoracic radiographs showed no abnormalities in 55.5% of cases, and AUS was unremarkable in 17.8%, indicating that many dogs exhibit abnormalities without affecting the clinical diagnosis of naIMHA. These findings align with recent literature showing that imaging provides additional diagnostic information related to their IMHA in only a small subset of cases (Andres et al., 2019; De Laet et al., 2022; Woodward & White, 2020). The results suggest limited benefit of routine TXR and AUS in the diagnostic management of IMHA in dogs, supporting the notion that selective use of these imaging modalities is more appropriate. This interpretation is consistent with the 2019 ACVIM consensus statement, which recommends investigating potential underlying causes of IMHA while selecting diagnostic tests, including thoracic and abdominal imaging, based on the individual clinical context (Garden et al., 2019). A recent study on immune-mediated polyarthritis in dogs reported similar findings, where numerous abnormal findings on TXR and AUS did not significantly impact case management (Tang et al., 2024). This further suggests a trend across immune-mediated conditions, supporting the selective use of imaging to reduce unnecessary costs and resource allocation in veterinary practice.

The clinical history and presenting signs in this cohort were consistent with prior studies (Duclos et al., 2024; Goggs et al., 2015; Piek, 2011). The median age was 7 years, and higher age was associated with an increased risk of decreased survival at all time points in the univariate analysis, and only at 12 months in the multivariate analysis, though it has not been consistently reported as a prognostic factor in IMHA. This association should therefore be interpreted with caution, as it may partly reflect euthanasia decisions rather than a purely biological prognostic effect. The overrepresentation of shih-tzus in the naIMHA cohort suggests a potential breed predisposition, consistent with previous research (Ishihara et al., 2010). Shih-tzus were significantly more likely to present with IMHA than the general hospital population. Further research into genetic or breed-specific factors may be warranted in this breed.

CBC results were generally consistent with the literature. The presence of thrombocytopenia was common (28.9%), as has been reported before. Severe thrombocytopenia (platelet count $<30,000/\mu\text{L}$) was observed in 10.5% of the dogs in this cohort, although only one of them presented with clinical signs (petechiae). Thrombocytopenia is often described as a negative prognostic factor, but it was not in this study (Duclos et al., 2024; Ishihara et al., 2010; Piek et al., 2008). The prevalence of lymphocytosis (60%) was notably higher than previously reported (e.g. 20% in Duclos et al., 2024). IDEXX strongly recommends concurrent dot plot review during analysis. At our institution, all CBC analyses are routinely complemented by dot plot evaluation and blood smear examination. However, the retrospective nature of the study precluded precise determination of lymphocytosis prevalence, and this parameter was excluded from further statistical analyses.

All dogs received immunosuppressive therapy with prednisolone, and approximately half of them received an additional immunomodulatory agent, which is in line with current therapeutic strategies for managing IMHA (Garden et al., 2019). However, treatment protocols were not standardised and were selected by the attending clinician, which may have introduced variability in treatment and follow-up assessment. The in-hospital mortality rate for the naIMHA group was 26.4%, consistent with recent literature (Duclos et al., 2024; Goggs et al., 2015).

Thromboprophylaxis was administered to 77.3% of dogs to address the thromboembolic risks associated with IMHA. The inclusion period started prior to the publication of the recent consensus guidelines on IMHA and the rational use of antithrombotics, and this may have been the reason why thromboprophylaxis was not administered to all cases (Garden et al., 2019; Goggs et al., 2019). In addition, early euthanasia in some cases may have limited the opportunity to initiate thromboprophylaxis. Dogs receiving thromboprophylaxis demonstrated a significantly lower risk of decreased survival at 3 and 6 months.

Most dogs received at least one pRBC transfusion (85.5%), with nearly half of them (45.7%) requiring more than a single transfusion. Although the number of pRBC transfusions has not previously been identified as a negative prognostic factor, this study found an increased risk of decreased survival at 3 and 6 months associated with the number of transfusions administered. It is important to note that this was not the primary focus of the study, and the analysis at each time point (3, 6, and 12 months) included patients who died during hospitalisation. This association likely reflects greater disease severity in dogs requiring repeated transfusions rather than a direct causal effect of transfusion itself, as more severely affected patients are more likely to require multiple transfusions and may also have a poorer overall prognosis. Nonetheless, these findings suggest that animals requiring higher numbers of pRBC transfusions might benefit from closer monitoring and follow-up to ensure adequate disease control.

This study has several limitations. Conducted in a tertiary care setting, the findings may not fully generalise to the broader IMHA population due to potential selection bias. The retrospective design limited standardisation of imaging and treatment protocols, and imaging decisions were influenced by clinical and financial factors, which could have led to selection bias in cases receiving complete *versus* partial workups. Additionally, in the DI group, complete exclusion of underlying causes was not always possible, as FNAs of abnormal abdominal organs and qPCR testing for infectious agents were inconsistently performed. Information regarding follow-up is also limited due to the retrospective nature of the study and the reliance on data collected from owners and/or referring veterinarians for a subset of these dogs. As a result, precise details regarding recurrence rates, timing of relapses, and the use of adjunct immunosuppressive treatments could not be provided.

In this study, we included only dogs meeting the criteria for diagnostic or supportive IMHA according to the ACVIM consensus statement. Consequently, dogs in which a rapid diagnosis was obtained *via* in-house-tests, such as a peripheral blood smear

(e.g. babesiosis) or lymph node aspirates (e.g. multicentric lymphoma), might have been missed, as owners may have declined further diagnostic tests or treatment, potentially leading to an underestimation of aIMHA cases. Moreover, this may have influenced the number of aIMHA cases referred, as some dogs could have been euthanased by the referring veterinarian without being sent for further evaluation. This underestimation may also have been exacerbated by the increasing availability of blood products *via* blood banks in Belgium, making blood transfusions more accessible to primary care veterinarians. Therefore, a selection bias in the referred patient population is possible. Survival status was evaluated at 3, 6, and 12 months after diagnosis to allow sufficient time for the detection of clinically relevant underlying diseases that may initially remain occult. However, because follow-up was limited to survival status rather than systematic re-evaluation, the presence of undiagnosed underlying diseases cannot be completely excluded.

In conclusion, although medical imaging can be considered in the management of dogs diagnosed with IMHA, the low incidence of aIMHA combined with the limited clinical utility of routine TXR and AUS suggests that selective use of these modalities (e.g. dogs exhibiting clinical signs suggestive of an intrathoracic or intra-abdominal underlying cause), rather than routine imaging for all IMHA cases, may be more appropriate and cost-effective in managing suspected naIMHA. The overrepresentation of Shih-Tzus among naIMHA cases suggests a potential breed predisposition. Further prospective studies with standardised diagnostic and follow-up protocols are needed to confirm these findings and provide clearer guidance on the role of imaging in dogs diagnosed with IMHA.

Acknowledgements

The authors have nothing to report.

Author contributions

R. Vlassenbroek: Conceptualization; investigation; writing – original draft; resources; methodology; project administration; validation; writing – review and editing; data curation; visualization; software. **C. François:** Validation; writing – review and editing; visualization. **D. Peeters:** Conceptualization; methodology; supervision; validation; writing – review and editing; investigation; visualization. **G. Bolen:** Validation; writing – review and editing; visualization. **L. Matthewman:** Writing – review and editing; supervision; validation; visualization. **K. Gommeren:**

Conceptualization; methodology; supervision; validation; writing – review and editing; investigation; writing – original draft; visualization; project administration.

Conflict of interest

No conflicts of interest have been declared.

Data availability statement

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

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