ORIGINAL ARTICLE

Inflammatory and fibrinolytic states in cats with and without cardiogenic atrial/arterial thromboembolism stratified by the presence and type of congestive heart failure

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Objective: To assess whether cats with cardiogenic pleural effusion have less systemic inflammation or an enhanced systemic fibrinolysis, preventing cardiogenic atrial/arterial thromboembolism compared to cats with cardiac disease without pleural effusion.

Materials and Methods: Cross-sectional study evaluating cats presented with cardiac disease: without congestive heart failure ($n = 246$), with cardiogenic pulmonary oedema (49) and with cardiogenic pleural effusion (94). At presentation, plasma fibrinogen and serum amyloid A were measured, and the fibrinogen:serum amyloid A (a marker of systemic fibrinolysis) was calculated. The frequency of cardiogenic atrial/arterial thromboembolism among groups was compared using the chi-squared test, whereas the other biomarkers were analysed using non-parametric tests.

Results: The prevalence of cardiogenic atrial/arterial thromboembolism was significantly higher in cats with pulmonary oedema (18/49, 36.7%) compared with cats without congestive heart failure (23/246, 9.3%) and with cardiogenic pleural effusion (9/94, 9.6%). The median serum amyloid A concentration in cats with cardiogenic pleural effusion (3.35 mg/L) was significantly higher than that in cats without congestive heart failure (0.65 mg/L), whereas no significant differences were found between cats with pulmonary oedema (1.4 mg/L) and those with pleural effusion or without congestive heart failure. After excluding 50 cats with cardiogenic atrial/arterial thromboembolism, there were 223 cats without congestive heart failure, 31 with pulmonary oedema, and 85 with pleural effusion. In the 85 cats without cardiogenic atrial/arterial thromboembolism and with cardiogenic pleural effusion, the median fibrinogen:serum amyloid A ratio (58) was significantly lower than the fibrinogen:serum amyloid A ratio (316) observed in the remaining 254 cats without cardiogenic atrial/arterial thromboembolism from the other two groups combined.

Clinical Significance: Enhanced systemic fibrinolysis may play a role in the lower cardiogenic atrial/arterial thromboembolism risk of cats with cardiogenic pleural effusion.

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INTRODUCTION

Cats with cardiac disease are prone to atrial thrombi and arterial thromboembolism (ATE), which is thought to originate from the fragmentation or dislodgment of left atrial (LA) or LA appendage thrombi (Schoeman, 1999). The exact aetiology of cardiogenic atrial/arterial thromboembolism (cATE, *i.e.* atrial thrombi and/or arterial thromboembolism) is unknown. However, it is believed that thrombi form due to endothelial injury (Liu, 1970), systemic activation of platelets and the coagulation cascade with hypercoagulability (Bédard et al., 2007; Helenski & Ross, 1987; Stokol et al., 2008), and local stasis of blood flow due to LA mechanical dysfunction and enlargement (Virchow's triad) (Schober & Maerz, 2006). Cats with cATE have a significantly larger LA compared to those without cATE, and LA enlargement is a known risk factor (Laste & Harpster, 1995; Payne et al., 2015; Rush et al., 2002; Schoeman, 1999). However, we recently showed that the presence of cardiogenic pleural effusion is associated with a lower risk of presenting with ATE alone, regardless of LA size (Busato et al., 2022).

Inflammation and haemostasis are closely connected processes, and inflammation can induce coagulation via several mechanisms, such as pro-inflammatory cytokine-mediated platelet production and hyperreactivity and increased tissue factor expression on endothelial cells and monocytes (Levi et al., 2003). Acute-phase proteins, including serum amyloid A (SAA) and fibrinogen, are synthesised in the liver in response to pro-inflammatory cytokines and are used as an inflammatory marker in cats (Rossi, 2023). Moreover, anticoagulant activity is diminished in an inflammatory state (Levi & Van Der Poll, 2005). Inflammation-dependent activation of the coagulation system is part of the host response to pathogens, and it is termed immunothrombosis (Stark & Massberg, 2021). Thromboinflammation, which is the aberrant and excessive activation of immunothrombosis, not only contributes to the thrombotic complications of acute infectious diseases but is also present in sterile inflammatory conditions, such as non-infectious cardiovascular diseases, where it plays a crucial role as a trigger for thrombosis (Stark & Massberg, 2021).

In humans, dogs and horses, intracavitary effusions have been shown to have an intrinsic fibrinolytic activity due to the presence of all the proteins that participate in coagulation and fibrinolysis (Agarwal et al., 2000; Delgado et al., 2009; Diab et al., 2005; Glauser et al., 1976; Henderson et al., 1980; Zoia, Drigo, Piek, et al., 2017) and due to the activities of mesothelial cells that further increase anticoagulation (Iakhiaev & Idell, 2006; Idell et al., 1992; Ivarsson et al., 1998; Mutsaers & Wilkosz, 2007). Dogs with abdominal and pleural effusions have also enhanced systemic fibrinolysis (Zoia et al., 2012, 2018, 2019, 2022; Zoia, Drigo, Simioni, et al., 2017), possibly due to reabsorption of these pathological effusions (Zoia et al., 2012). This hyperfibrinolytic state may enhance bleeding risk and decrease thrombotic events (Chapin & Hajjar, 2015). In a similar way to the use of the fibrinogen:CRP ratio in humans and dogs (Kim et al., 2006; Zoia et al., 2022), the fibrinogen:SAA ratio can be employed as a marker of fibrinolysis, as both acute phase proteins increase in response to inflammatory diseases, but only fibrinogen concentration decreases due to fibrinolysis.

Therefore, this study aimed to assess if cats with cardiogenic pleural effusion have less systemic inflammation, as assessed by SAA concentrations, or have enhanced systemic primary fibrinolysis, as assessed by fibrinogen:SAA ratio, preventing cATE in comparison with cats with cardiogenic pulmonary oedema or with non-congestive cardiac diseases.

MATERIALS AND METHODS

Study design and population inclusion criteria

Cross-sectional study retrospectively evaluating client-owned cats with cardiac disease presented to the "San Marco Veterinary Clinic" between June 1, 2005 and December 31, 2018. Records were retrieved from the electronic medical database, P.O.A System-Plus9.0®, searching for echocardiographic reports in cats. Inclusion criteria were the presence of a complete medical record (patient signalment, history and full physical examination findings), thoracic radiographs (if their clinical condition allowed this procedure safely) and echocardiography that diagnosed their cardiac disease (with the report available for review). In addition, all included cats had to have undergone blood sample analysis and had the SAA concentration and plasma fibrinogen concentration available. Cats with no reported LA to aortic root ratio (LA:Ao) or LA diameter were excluded. Only the data from the first presentation for cardiac disease and diagnosis of cATE, if present, were included.

Ethical statement

All diagnostic and therapeutic procedures reported in this cross-sectional study were performed by the attending clinician solely for the cat's benefit, with prior informed written owner consent. Euthanasia or any kind of animal sacrifice were not required for any part of the study. All the procedures performed complied with the European legislation "on the protection of animals used for scientific purposes" (Directive 2010/63/EU) and with the ethical requirements of Italian law (Decreto Legislativo 04/03/2014, n. 26). Accordingly, this type of study did not require an authorization or an ID protocol number from an institutional animal care and use committee.

Group definitions and diagnosis of cATE

Three groups of cats were created based on the presence and type of congestive heart failure (CHF). Group 1, cats with cardiac diseases but no evidence of CHF; Group 2, cats with evidence of cardiogenic pulmonary oedema but no pleural effusion and Group 3, cats with evidence of cardiogenic pleural effusion with or without cardiogenic pulmonary oedema. Cats with concurrent cardiogenic

pulmonary and pleural effusion, even when the pleural effusion was minimal, were included in Group 3 in order to evaluate the effect of pleural effusion on the risk of developing cATE.

Pleural effusion and pulmonary oedema were attributed to CHF when clinical history, findings on physical examination, ultrasonographic evidence of cardiac disease and thoracic radiographs were consistent with this complex clinical syndrome (Ferasin & DeFrancesco, 2015). Thoracic radiographs in cats with CHF were taken in most cases if their clinical condition allowed this procedure safely or if thoracic ultrasonography did not identify the cause of respiratory distress. When it was considered necessary by the attending clinician, thoracentesis was performed in cats with pleural effusion for both therapeutic and diagnostic purposes.

Atrial thrombi were diagnosed through direct visualisation on echocardiography. Arterial thromboembolism was diagnosed based on the presence of typical clinical findings (*i.e.* absent femoral pulse, cold distal limbs, nail beds pallor or cyanosis, pelvic limb paralysis/paresis, and pain), and it was confirmed in most cases by abdominal Doppler ultrasonography (*i.e.* identification of a thromboembolus and/or absence of blood flow in the distal aorta or its branches) (Klainbart et al., 2014; Quinn et al., 1998). Finally, based on the presence of atrial thrombi or ATE, cats were divided into those with and those without cATE.

SAA and fibrinogen measurement

All tests were performed at the "San Marco Laboratory", and results were retrospectively extracted from the medical record. According to the clinic's standardised sampling protocol, a blood sample was routinely obtained at the time of initial examination from each cat via jugular venipuncture using a 10 mL syringe connected to a 21G × 5/8″ needle. Three millilitres of blood were typically placed in a plain glass tube (Vacutainer® 5.0 mL, BD, Plymouth, UK) for measurement of a routine serum biochemistry panel including SAA, measured via quantitative assays (LZ-SAA, Eiken Chemical, Tokyo, Japan) using an automated wet chemistry analyser (OLYMPUS AU600, Olympus Europe GmbH, Hamburg, Germany). Two millilitres of blood were placed in a plastic tube containing 3.2% sodium citrate, with a final anticoagulant-to-blood volume ratio of 1:9 (Vacuette® 9NC Coagulation Sodium Citrate 3.2%, 2.0 mL, Greiner Bio-One S.r.l. Kremsmünster, Austria) for measurement of fibrinogen. Sodium citrate tubes were centrifuged at 1950*g* for 5 minutes, plasma was harvested, and coagulation profile analysis, including fibrinogen concentration, was performed within 1 h after blood sample collection. Plasma fibrinogen concentration was determined via quantitative assays (STA Fibrinogen, Diagnostica Stago, Asnières sur Seine, France), with an automated analyser (STA-R Evolution, Diagnostica Stago, Roche, Bäsel, Switzerland). According to the manufacturer's kit instructions, quantification of these two analytes is not affected by the presence of concurrent free haemoglobin, bilirubin and triglycerides.

Echocardiography

Cardiac disease diagnoses were based on diagnostic-quality 2D colour flow Doppler and M-mode echocardiographic examinations, as recorded in the medical records, following the clinic's standard protocol. The LA:Ao was calculated at the heart base level, from a right parasternal short axis view, at the first diastolic frame in which closure of the aortic valve leaflets was evident (Abbott & MacLean, 2006). The maximal LA diameter was assessed from a right parasternal long-axis view, measured at the end of ventricular systole just prior to the mitral valve leaflets opening (Abbott & MacLean, 2006; Smith & Dukes-McEwan, 2012).

For the purposes of this study, cardiac diseases were classified based on the available echocardiographic findings into the following categories: dilated cardiomyopathy phenotype, hypertrophic cardiomyopathy phenotype, restrictive cardiomyopathy phenotype, non-specific cardiomyopathy phenotype (including all other cardiomyopathies not adequately falling into the previous categories) and other cardiac diseases (mainly acquired valvular diseases or congenital cardiac defects). Cardiomyopathies were classified according to the ACVIM 2020 consensus statement (Luis Fuentes et al., 2020).

Statistical analysis

Continuous data were assessed for normality of distribution with the Shapiro–Wilk test. Normally distributed data are reported as a mean ± standard deviation, and non-normally distributed data are reported as a median along with the 25th and 75th percentiles (interquartile range [IQR]).

The frequency of cATE among groups was compared by chi-squared test, followed by Marascuilo's procedure for comparing multiple proportions. Left atrium dimension (*i.e.* LA:Ao and LA diameter), serum SAA and plasma fibrinogen (*i.e.* markers of inflammation) and fibrinogen:SAA ratio (i.e. a marker of systemic fibrinolysis) were compared among groups by Kruskal–Wallis followed by Mann–Whitney test with Bonferroni correction.

A multivariable logistic regression model was employed to predict the relationship between the outcome variable (*i.e.* cATE) and the independent predictors previously univariately analysed, if statistically significant, after evaluation of multicollinearity and interaction between predictors. Finally, because fibrinogen:SAA ratio cannot distinguish primary from secondary fibrinolysis, we analysed inflammatory markers and the degree of primary fibrinolysis of cats with and without pleural effusion, after the exclusion of all cats with evidence of secondary fibrinolysis (*i.e*, cats with cATE) by Wilcoxon rank-sum test.

For all statistical analyses, the significance level was set to $\alpha = 0.05$.

RESULTS

Study population characteristics

Six hundred and forty-one cats with cardiac diseases were provisionally eligible for the study. None of the presented cats were on antiplatelet treatment, and few (*i.e.* <15%) of the provisionally eligible cats were receiving drugs for their cardiac disease. Twelve cats were excluded from the study, 11 due to missing LA:Ao measurements from the echocardiographic

reports, and one because it had Cor triatriatum sinister, which prevented LA measurement. An additional 240 cats without blood work were also excluded, leaving 389 cats for the final analysis. Eighteen of the excluded cats had been diagnosed with cATE (two with atrial thrombi and 16 with arterial thromboembolism; Fig 1).

At presentation, cATE was diagnosed in 50 of the 389 cats (five with atrial thrombi, 44 with ATE, and one with atrial thrombi and concurrent ATE; overall prevalence of 12.9%) and in all cases was deemed to be cardiac-related. Two hundred and forty-six cats had cardiac disease without CHF (Group 1), 49 had cardiogenic pulmonary oedema but no pleural effusion (Group 2), and 94 had evidence of cardiogenic pleural effusion (with or without concurrent cardiogenic pulmonary oedema; Group 3); ascites was also present in one of these cats (Fig 1). In the few cases of cats with concurrent cardiogenic pulmonary oedema and pleural effusion, even when the pleural effusion was minimal, they were included in Group 3 to assess the potential impact of pleural effusion on cATE risk. Forty-eight of the 94 (51%) cats with evidence of cardiogenic pleural effusion underwent diagnostic or therapeutic thoracentesis. In all cases, biochemical and cytological analysis, including serum and pleural effusion lactate dehydrogenase activities (Zoia et al., 2009; Zoia & Drigo, 2016), were consistent with transudates due to an increased hydrostatic pressure gradient, compatible with a cardiogenic origin.

There were 254 male cats (29 [7.5%] entire and 225 [57.8%] neutered) and 135 female cats (20 [5.1%] entire and 115 [29.6%] neutered). Median age at diagnosis was 120 months (IQR, 71 to 164 months). One hundred and twenty-one cats were purebreds, including Persian cats ($n = 49$), Maine Coons (13), Birmans (10), Siamese (10), British Shorthairs (10), Sphinx (7), Exotic Shorthairs (7), Chartreux (4), Scottish Folds (4) and Norwegian Forest cats (3). Bengal, Burmese, Bombay and Devon Rex were represented by a single cat. The remaining 268 cats were domestic shorthairs or longhairs. Cats were diagnosed with hypertrophic cardiomyopathy ($n = 258$), "other cardiac diseases" (62), restrictive cardiomyopathy (34), non-specific cardiomyopathy phenotype (20) and dilated cardiomyopathy (15).

Prevalence of cATE and differences in LA size among and between groups

There was a significant difference in the prevalence of cATE among cats in Group 1 (23/246, 9.3%), Group 2 (18/49, 36.7%) and Group 3 (9/94, 9.6%) (Table 1, $\chi^2 = 28.55$, $P < 0.001$).

There was a significant difference in LA:Ao among the three groups ($H = 121.24$, $P < 0.001$; Table 1). The LA:Ao in cats in Group 2 (2.1 [IQR, 1.83 to 1.63]) and Group 3 (2.0 [IQR, 1.7 to 2.37]) was significantly bigger compared to cats in Group 1 (1.4 [IQR, 1.2 to 1.63]; $P < 0.001$ for both comparisons), while no significant difference ($P = 0.36$) was found in LA:Ao for cats in Group 2 and Group 3.

There was a significant difference in LA among the three groups ($H = 109.13$, $P < 0.001$; Table 1). The LA in cats in Group 2 (19.30 mm [IQR, 16.80 to 22.40]) and Group 3 (17.55 mm [IQR, 15.43 to 19.59]) was significantly bigger compared to cats in Group 1 (13.30 mm [IQR, 11.83 to 15.38]; $P < 0.001$ for both comparisons). Moreover, cats of Group 2 also showed a bigger LA compared with cats of Group 3 ($P = 0.014$).

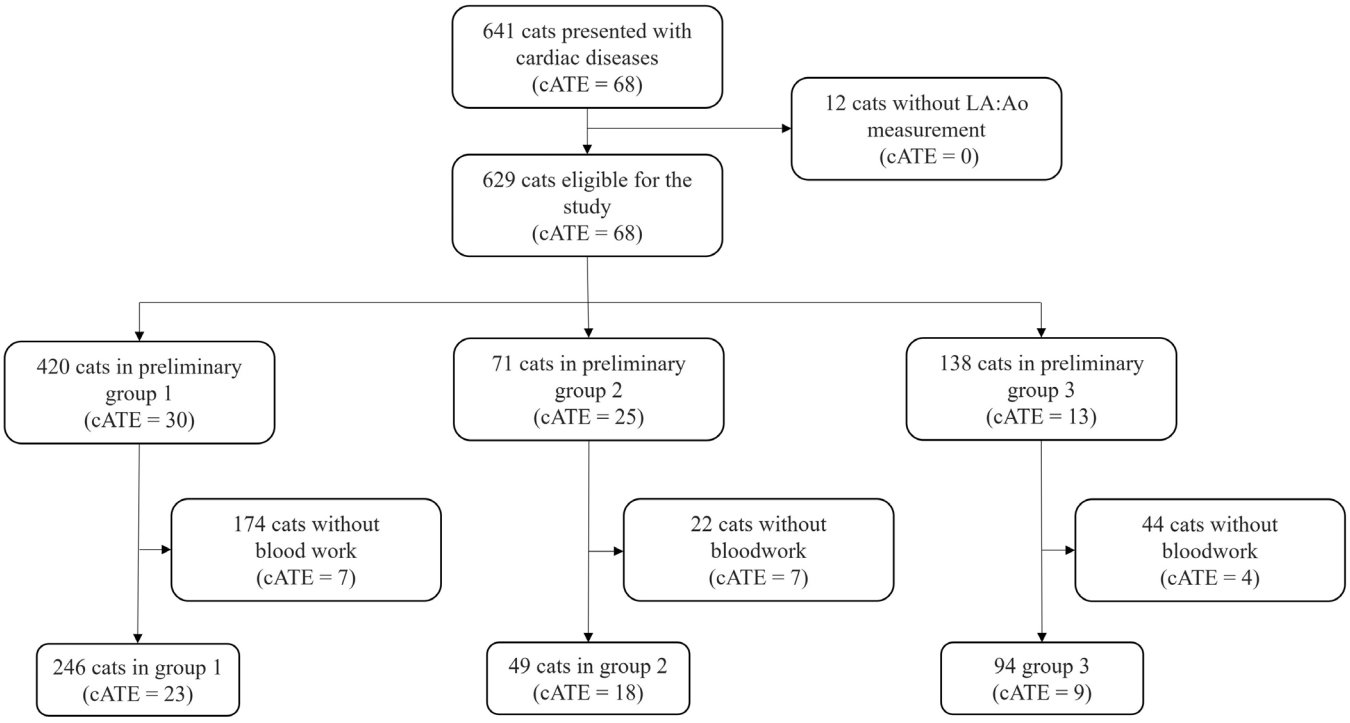

FIG 1. Flow chart of study population selection. Group 1: cats with cardiac disease but no evidence of congestive heart failure; Group 2: cats with evidence of cardiogenic pulmonary oedema; Group 3: cats with evidence of cardiogenic pleural effusion. cATE, cardiogenic atrial/arterial thromboembolism; LA:Ao, left atrial to aortic root ratio.

Table 1. Prevalence of cATE and comparison of LA:Ao and LA (mm) dimension in cats without evidence of CHF, cats with cardiogenic pulmonary oedema and cats with cardiogenic pleural effusion

	Group 1 (without CHF) (*n* = 246)	Group 2 (pulmonary oedema) (*n* = 49)	Group 3 (pleural effusion) (*n* = 94)	Test value P value	Comparisons between groups
cATE	23 (9.3%)	18 (36.7%)	9 (9.6%)	$\chi^2 = 28.55$ P < 0.001	G_1 versus G_2 P = 0.001 G_1 versus G_3 P = 0.998 G_2 versus G_3 P = 0.002
LA:Ao	1.4 (1.2 to 1.63)	2.1 (1.83 to 2.5)	2.0 (1.7 to 2.37)	$H = 121.24$ P < 0.001	G_1 versus G_2 P < 0.001 G_1 versus G_3 P < 0.001 G_2 versus G_3 P = 0.36
LA (mm)	13.30 (11.83 to 15.38)	19.30 (16.80 to 22.40)	17.55 (15.43 to 19.59)	$H = 109.13$ P < 0.001	G_1 versus G_2 P < 0.001 G_1 versus G_3 P < 0.001 G_2 versus G_3 P = 0.014

Data for cATE are presented as *n* (%); data for LA and LA:Ao are presented as median (IQR)
CHF Congestive heart failure, cATE Cardiogenic atrial/arterial thromboembolism, G1 Group 1, G2 Group 2, G3 Group 3, IQR Interquartile range, LA Left atrium, LA:Ao Left atrium to aortic root ratio

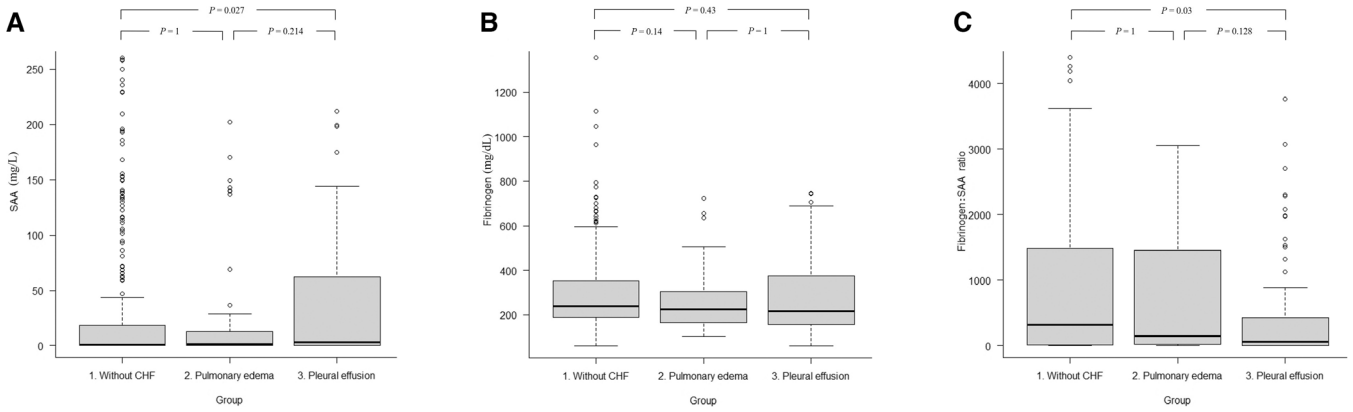

FIG 2. Differences in SAA (A), fibrinogen (B) and fibrinogen:SAA ratio (C) between cats without evidence of CHF, cats with cardiogenic pulmonary oedema and cats with cardiogenic pleural effusion. The bottom and top of the box represent the first and third quartiles, respectively; the band within the box represents the median. The whiskers correspond to the lowest datum still within 1.5 interquartile ranges of the first quartile and the highest datum still within 1.5 interquartile ranges of the fourth quartile. Circles represent outlier values (>1.5 interquartile ranges away from the closest end of the box). CHF, congestive heart failure; SAA, serum amyloid A.

SAA, fibrinogen and fibrinogen:SAA ratio among and between groups

SAA concentrations were significantly different among the three groups ($H = 7.045$, P = 0.029; Fig 2A). The SAA concentration in cats in Group 3 (3.35 mg/L, [IQR, 0.45 to 59.9]) was significantly higher compared with the SAA concentration of cats in Group 1 (0.65 mg/L, [IQR, 0.1 to 18.45]; P = 0.027). No significant differences in SAA concentrations were found between cats in Group 2 (1.4 g/L [IQR, 0.1 to 12.6]) and cats in Group 3 or Group 1 (P = 0.214 and P = 1, respectively).

No differences were found in plasma fibrinogen concentrations among cats in Group 1 (239 mg/dL, [IQR, 187.5 to 350]), cats in Group 2 (224 mg/dL, [IQR, 165 to 305]) and cats in Group 3 (216 mg/dL, [IQR, 158.5 to 371.8], $H = 3.604$, P = 0.165; Fig 2B).

Fibrinogen:SAA ratios were significantly different among the three groups ($H = 10.782$, P = 0.005; Fig 2C), with this ratio in cats in Group 1 (316, [IQR, 13 to 1447.5]) significantly higher compared to that of cats in Group 3 (56, [IQR, 6 to 407], P = 0.03). No significant differences were found in fibrinogen:SAA ratio for cats in Group 2 (143, [IQR, 18 to 1450]) and cats in Group 3 or Group 1 (P = 0.128 and P = 1, respectively).

Multivariable logistic regression model for the risk of cATE occurrence

The final multivariable model included the following variables with significant P values in the univariate analysis: presence and type of CHF (with cats with cardiogenic pleural effusion as the reference category), LA:Ao, LA dimension, SAA concentration and fibrinogen:SAA ratio. The SAA concentration and LA dimension were then excluded due to their high autocorrelation with the fibrinogen:SAA ratio and LA:Ao, respectively, as well as significant interaction between predictors. The final

Table 2. Result of multivariable logistic regression model for the risk of developing cardiogenic thromboembolic disease

Variable		P value	Odds ratio (95% CI)
Groups	1 (without CHF) (*n* = 246)	P = 0.004	OR = 4.28 (95% CI = 1.57 to 11.64)
	2 (pulmonary oedema) (*n* = 49)	P = 0.001	OR = 5.31 (95% CI = 1.93 to 14.63)
	3 (pleural effusion) (*n* = 94)	– (reference category)	– –
LA:Ao		P < 0.001	OR = 12.23* (95% CI = 5.70 to 26.25)

CHF Congestive heart failure, CI Confidence interval, LA:Ao Left atrium to aortic root ratio, OR Odds ratio.
*Odds ratio for each 1.0 unit increase of LA:Ao.

multivariable logistic regression model (Logit cATE = −7.8 + 2.5 × LA:Ao + 1.45 × $Group_{G3vsG1}$ + 1.67 × $Group_{G3vsG2}$; Cox and Snell R^2 = 0.171; Nagelkerke R^2 = 0.32) indicated that cats with cardiac disease but without effusion (*i.e.* cats in Group 1 and Group 2) and cats with an increased LA:Ao had a higher risk of developing cATE (Table 2).

Fibrinogen, SAA and fibrinogen:SAA ratio in cats without cATE stratified by the presence of pleural effusion

After excluding the 50 cats with cATE, 339 cats remained in the analysis: 254 without pleural effusion (including 223 with cardiac disease without evidence of CHF and 31 with cardiac disease and evidence of cardiogenic pulmonary oedema) and 85 cats with cardiac disease and evidence of cardiogenic pleural effusion.

There was no difference in plasma fibrinogen concentration between the 85 cats with pleural effusion and without cATE (228 mg/dL; IQR, 158.5 to 379.5) compared to the 254 cats with a cardiac disease but without pleural effusion or cATE (238 mg/dL; IQR, 186 to 341; *W* = 11,546, P = 0.337).

The serum SAA concentration was significantly higher in the 85 cats with pleural effusion and without cATE (2.9 mg/L [IQR, 0.5 to 53.25]) compared to the 254 cats with cardiac disease but without pleural effusion or cATE (0.7 mg/L; IQR, 0.1 to 16.2; *W* = 12,770, P = 0.011).

The fibrinogen:SAA ratio was significantly lower in the 85 cats with pleural effusion and without cATE (58; IQR, 8 to 362) compared to the 254 cats with cardiac disease but without pleural effusion or cATE (316; IQR, 18 to 1478; *W* = 8420, P = 0.002; Fig 3).

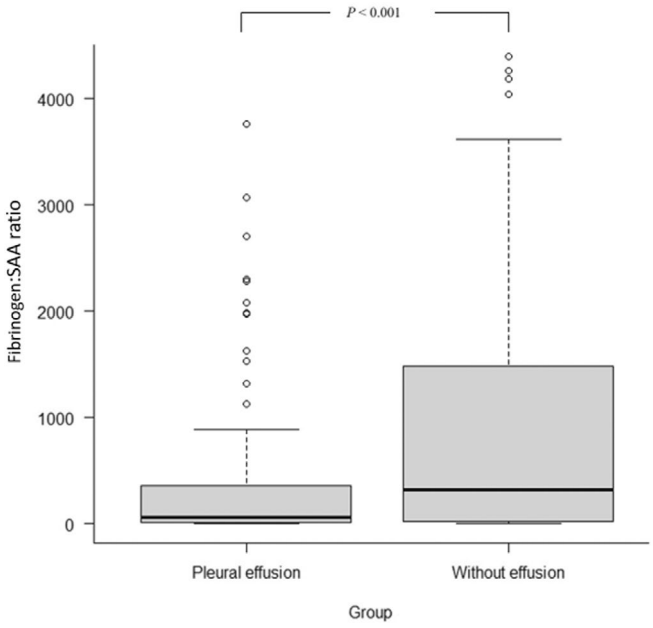

FIG 3. Difference in fibrinogen:SAA ratio in cats without cardiogenic thromboembolic disease stratified by the presence of pleural effusion. The bottom and top of the box represent the first and third quartiles, respectively; the band within the box represents the median. The whiskers correspond to the lowest datum still within 1.5 interquartile ranges of the first quartile and the highest datum still within 1.5 interquartile ranges of the fourth quartile. Circles represent outlier values (>1.5 interquartile ranges away from the closest end of the box). SAA, serum amyloid A.

DISCUSSION

This cross-sectional study aimed to determine whether cats with evidence of cardiogenic pleural effusion have less inflammation or enhanced systemic fibrinolysis compared with cats with cardiac disease but without pleural effusion that could explain the lower risk found in these cats in a previous study (Busato et al., 2022) of developing ATE alone. The first result of this study suggested that inflammation is unlikely to play a major role in the development of cATE, as SAA concentrations were highest in cats with evidence of cardiogenic pleural effusion, the group that had the lowest risk of cATE. The second result of the current study suggested that systemic primary fibrinolysis may contribute to the lower risk of cATE in cats with evidence of cardiogenic pleural effusion because, in this group, plasma fibrinogen concentrations were similar to those of cats without pleural effusion despite elevated serum SAA concentrations, after excluding cats with cATE from the analysis. This result is further strengthened by the lowest fibrinogen:SAA ratio in this group of cats, again when excluding those presented with cATE from the analysis.

As previously demonstrated in a different cohort of 366 cats extracted from the same initial population of 629 cats with cardiac disease presented to the "San Marco Veterinary Clinic" between 2005 and 2018, where LA enlargement was identified as a risk factor for arterial thromboembolism only, this cohort of 389 cats similarly found that LA enlargement was a risk factor for cATE. Consistent with the previous study, cats with CHF due to pulmonary oedema and/or pleural effusion had a significantly larger LA dimensions compared with those without CHF (Busato et al., 2022). Finally, consistent with a previous study in which the presence of pleural effusion was associated with a lower risk of arterial thromboembolism only (Busato et al., 2022), our findings also showed that pleural effusion was associated with a reduced risk of cATE.

Due to the known association between inflammation and the haemostatic systems (Levi et al., 2003; Smith, 2010), to assess if the reduced risk of cATE in cats with cardiogenic pleural effusion was due to an increased level of systemic inflammation, we studied the SAA concentration of cats with cardiac diseases, which is one of the major acute phase proteins in this species (Kajikawa et al., 1999). Acute phase proteins are considered sensitive circulatory biomarkers for inflammatory conditions (Liu et al., 2020), and recently, acute phase protein concentrations have been found to increase in cats with CHF in comparison with cats with cardiac disease but without CHF (Liu et al., 2020). Similarly, in our study, SAA concentration was significantly higher in cats with cardiogenic pleural effusion compared with cats with cardiac disease but no evidence of CHF and, despite the larger LA size of the cats with cardiogenic pleural effusion, the prevalence of cATE was not significantly different in this group of cats. Furthermore, no significant difference was found in SAA concentration and LA size between cats with evidence of cardiogenic pulmonary oedema and cats with cardiogenic pleural effusion, despite the prevalence of cATE being significantly higher in cats with cardiogenic pulmonary oedema. Altogether, these results would suggest that systemic inflammation is unlikely to play a role in the genesis of cATE, being SAA the highest in cats with the lowest risk of cATE occurrence (*i.e.* in cats with evidence of cardiogenic pleural effusion). Finally, the overlap in SAA concentrations among the three groups of cats with different types of severity or presentations of cardiac disease may suggest that additional factors not considered in this study, whether cardiac (such as the specific disease phenotype) or non-cardiac (such as concurrent extracardiac conditions), could also influence this inflammatory marker.

Fibrinogen is a minor to moderate acute-phase protein in cats (Paltrinieri, 2008); therefore, its increase in inflammatory conditions takes a longer time in comparison with the major acute-phase protein and does not reach more than a ten-fold magnitude. Fibrinogen concentration is also affected by its conversion by thrombin into fibrin in the activation of the coagulation process (Mosesson, 2005) and by a direct lysis by plasmin in primary fibrinolysis (Zoia et al., 2022). We found no statistical difference in fibrinogen concentrations between the three groups of cats studied. One possible explanation is that, being a minor to moderate acute-phase protein, the time elapsed from the insurgence of CHF to the hospital admission and the magnitude of rise that this protein can have during an inflammatory process was not enough to allow differences between groups. Another possible explanation is that the ongoing secondary fibrinolysis, due to the high frequency of cATE in cats with cardiogenic pulmonary oedema, and the ongoing primary fibrinolysis present in the groups of cats with cardiogenic pleural effusion may have mitigated the expected rise in plasma fibrinogen in these two groups of cats. The lowest fibrinogen:SAA ratio found in cats with evidence of cardiogenic pleural effusion may support the possible presence of ongoing primary fibrinolysis in this group, at least when compared to cats with cardiac disease without CHF, given that both groups demonstrated a similarly low prevalence of cATE/secondary fibrinolysis (see following discussion). Although the fibrinogen:SAA ratio was also lower in cats with cardiogenic pleural effusion compared to those with pulmonary oedema, the difference did not reach statistical significance. This could potentially be explained by a higher prevalence of cATE and secondary fibrinolysis in the pulmonary oedema group, which may have levelled out any underlying increase in primary fibrinolysis in cats with pleural effusion. The overlap in fibrinogen:SAA ratios observed between groups may reflect the combined influence of both primary and secondary fibrinolysis under the current analytical approach.

As introduced above, the fibrinogen:SAA ratio can be considered a marker of fibrinolysis because while both acute phase proteins rise in response to inflammatory diseases, only fibrinogen concentration decreases due to primary fibrinolysis (*i.e.* direct plasmin degradation of fibrinogen) or secondary fibrinolysis (*i.e.* degradation of fibrinogen by plasmin after its conversion to cross-linked fibrin) (Zoia et al., 2022), allowing detection and quantification of these two events indirectly. Therefore, we used this ratio to assess if cats with cardiogenic pleural effusion have enhanced systemic fibrinolysis, more specifically primary fibrinolysis, preventing cATE in comparison with cats with cardiogenic pulmonary oedema or with non-congestive cardiac diseases. To achieve this aim, we excluded all cats with documented secondary fibrinolysis (*i.e.* cats with cATE) because the fibrinogen:SAA ratio decreases both in cases of primary and secondary fibrinolysis. This led to the analysis of the fibrinogen:SAA ratio in only 339 cats: 254 without pleural effusion and 85 with pleural effusion. Cats with cardiac disease but no evidence of CHF and cats with evidence of cardiogenic pulmonary oedema were joined in a single group for this analysis for two reasons: (a) our aim was to assess whether the presence of cardiogenic pleural effusion in cats with cardiac disease was associated with enhanced systemic fibrinolysis compared to cats with cardiac disease but without pleural effusion and (b) the exclusion of cats with cATE decreases substantially only the number of cats with cardiac diseases with evidence of cardiogenic pulmonary oedema, limiting the ability to detect the level of fibrinolysis in comparison to the other two groups, of these cats due to the small sample size (*i.e.* $n = 31$). The results of our analysis suggest that, similarly to dogs with pleural effusion of different origins (Zoia et al., 2018), cats with cardiogenic pleural effusion also exhibit enhanced fibrinolysis, at least compared to cats with cardiogenic pulmonary oedema or with non-congestive cardiac diseases.

Some limitations are present in this study. First, while cardiogenic thromboembolic disease was confirmed in all cases by direct ultrasound visualisation, ATE was initially suspected based on clinical signs and subsequently confirmed by ultrasound in most cases. Second, the presence of systemic fibrinolysis was diagnosed indirectly by measuring the fibrinogen:SAA ratio. Thus, the activities of fibrinolytic enzymes such as plasmin, serum tryptase or non-plasma polymorphonuclear elastase were not measured, either in pleural effusion or plasma. Similarly, inflammation was not demonstrated directly by the determination of pro-inflammatory cytokines, but it was indirectly determined by serum acute-phase protein concentrations. Third, the retrospective nature of the study did not allow the inclusion of all the 626 cats for which ultrasonographic determination of the LA was available because blood sampling was not performed in

all cases. Nevertheless, because the lack of blood sampling collection depended upon different reasons (*e.g.* financial constraints, clinician's perception of blood sample benefit in some of these animals, cat's non-compliant behaviour to blood sampling, others), it is unlikely that a systemic bias affecting study results may have occurred. The similarity between the previously published multivariable logistic regression model derived from the full cohort of 629 cats (Logit ATE only = $-5.46 + 1.84 \times$ LA:Ao $+ 1.26 \times \text{Group}_{G3vsG1} + 2.03 \times \text{Group}_{G3vsG2}$; Cox and Snell $R^2 = 0.134$) (Busato et al., 2022) and the model obtained from the subset of 389 cats with available SAA and fibrinogen concentrations supports that the selection criterion did not introduce significant selection bias. Fourth, the cats included in the study were stratified by presence and type of CHF without considering any other possible concomitant disease and/or treatment that may have impacted the level of systemic inflammation/fibrinolysis markers. This choice was made to evaluate the "real presentation" of cats with cardiac disease, making our results applicable to the general population without restriction. Finally, in rare cases, the type of CHF may have been miscategorised as a small volume of pleural effusion may not have been identified on thoracic radiographs, especially if concurrent concomitant pulmonary oedema was present. However, as all cats underwent echocardiographic evaluation, it is unlikely that the presence of pleural effusion was missed.

The LA dimension represents a risk factor for cATE in cats with cardiac disease, while the presence of cardiogenic pleural effusion is independently associated with a reduced risk for this outcome in multivariate analysis, after controlling for confounding factors such as left atrial size. Notably, this association was observed not only in comparison to cats with pulmonary oedema, who had a higher unadjusted cATE prevalence, but also in comparison to those without CHF, despite a similar unadjusted cATE prevalence. Systemic fibrinolysis may play a role in the low cATE occurrence in cats with evidence of cardiogenic pleural effusion in comparison with cats with cardiac disease but without effusion, while a decreased inflammatory response does not play a role in the lower risk of cATE found in cats with cardiogenic pleural effusion.

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Author contributions

F. Busato: Conceptualization, data curation, software, methodology, writing – original draft, writing – review and editing. **M. Drigo:** Conceptualization, data curation, formal analysis, supervision, writing – original draft, writing – review and editing. **A. Zoia:** Conceptualization, data curation, formal analysis, software, supervision, methodology, writing – original draft, writing – review and editing.

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.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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