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Development of a model based scoring system for diagnosis of canine disseminated intravascular coagulation with independent assessment of sensitivity and specificity

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ABSTRACT

A template for a scoring system for disseminated intravascular coagulation (DIC) in humans has been proposed by the International Society on Thrombosis and Haemostasis (ISTH). The objective of this study was to develop and validate a similar objective scoring system based on generally available coagulation tests for the diagnosis of DIC in dogs. To develop the scoring system, 100 dogs consecutively admitted to an intensive care unit (ICU) with diseases predisposing for DIC were enrolled prospectively (group A). The validation involved 50 dogs consecutively diagnosed with diseases predisposing for DIC, admitted to a different ICU (group B). Citrated blood samples were collected daily during hospitalisation and diagnosis of DIC was based on the expert evaluation of an extended coagulation panel. A multiple logistic regression model was developed in group A for DIC diagnosis. The integrity and diagnostic accuracy of the model was subsequently evaluated in a separate prospective study at a different ICU (group B) and was carried out according to The Standards for Reporting of Diagnostic Accuracy (STARD) criteria. Thirty-seven dogs were excluded from group A and four from group B due to missing data.

Based on expert opinion, 23/63 dogs (37%) had DIC. The final multiple logistic regression model was based on activated partial thromboplastin time, prothrombin time, D-Dimer and fibrinogen. The model had a diagnostic sensitivity and specificity of 90.9% and 90.0%, respectively. The diagnostic accuracy of the model was sustained by prospective evaluation in group B (sensitivity 83.3%, specificity 77.3%). Based on commonly used, plasma-based coagulation assays, it was possible to design an objective diagnostic scoring system for canine DIC with a high sensitivity and specificity.

Introduction

The International Society on Thrombosis and Haemostasis (ISTH) has developed simple diagnostic models for diagnosis of overt and non-overt disseminated intravascular coagulation (DIC) in humans. These models have been based on the generally available coagulation tests, namely, activated partial thromboplastin time (aPTT), prothrombin time (PT), D-Dimer, platelet count and fibrinogen, and have been proven to have high diagnostic accuracy (Bakhtiari et al., 2004; Toh and Downey, 2005; Toh and Hoots, 2007). With the lack of a 'gold standard' for DIC, these diagnostic algorithms have been based on consensus statements from specialist subcommittees (Taylor et al., 2001). Though similar consensus is currently lacking in veterinary medicine, the methods for development of similar scoring systems are available and could provide the basis for a similar approach to the diagnosis of DIC in dogs.

Laboratory testing in the management of any clinical condition is only relevant if it can be used to diagnose, indicate or guide the appropriate institution of therapeutic measures. As such, meaningful progress in DIC testing in dogs over the years has largely faltered, as there have been no universally accepted diagnostic criteria or therapy for DIC and no noticeable progress in how recognition of haemostatic dysfunction in dogs with DIC could affect outcome. A plausible explanation for the lack of progress is that haemostatic function tests are not used in a consistent manner in veterinary medicine for the diagnosis of DIC. Thus, diagnosis is traditionally based on three or more abnormal haemostatic parameters, including aPTT, PT, fibrinogen, D-Dimer, platelet count and erythrocyte morphology, along with a predisposing disease, which

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is a sensitive but unspecific approach (Bateman et al., 1999; Feldman et al., 1981).

With no 'gold standard' for DIC diagnosis in dogs, we decided to adopt the approach used by Bakhtiari et al. (2004) in the current study. Advancing our knowledge base would have several advantages. Firstly, early diagnosis of DIC would facilitate prompt and precise treatment and increase chance of survival. Secondly, consensus on DIC diagnosis would provide an important basis for treatment optimisation in dogs and make it possible to conduct multi-centre therapy studies with a minimum risk of systematic misclassification of patients. Thirdly, characterisation of DIC in dogs using an ISTH-like method could help to establish spontaneous DIC in dogs as a validated and clinically relevant spontaneous animal model of human DIC.

The objectives of the present study were (1) to develop a simple, sensitive and specific diagnostic model for canine DIC based on the ISTH diagnostic criteria and (2) to validate the model prospectively in an independent population of dogs in order to assess its diagnostic accuracy.

Materials and methods

The study was approved by the Small Animal Ethics and Administrative Committee at the Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark and by the IACUC committee at Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA, USA.

The study was a prospective multi-centre observational study, performed over a 2 year period from 2004 to 2006 at the Department of Small Animal Clinical Sciences, University of Copenhagen (group A) and Cummings School of Veterinary Medicine at Tufts University (group B). In order to optimise reproducibility and quality of the validation study, the work was designed to comply with the criteria of The Standards for Reporting of Diagnostic Accuracy (STARD) initiative (Bossuyt et al., 2003a) (Table 1).

Study population and inclusion/exclusion criteria

Inclusion criteria in both the developmental and validation study were (1) an underlying disease known to predispose to DIC (severe infection, trauma, organ destruction, severe immunological and toxic reactions or malignant disease) and (2) a clinical suspicion of DIC causing the primary clinician to request a coagulation profile. Dogs with a bodyweight <8.5 kg were excluded to eliminate the potential effects of multiple blood sampling. Animals treated with heparin, aspirin or any other anticoagulant therapy within 48 h prior to sampling, as well as those diagnosed with haemophilia, von Willebrand factor (vWF) deficiency, chronic cardiac disease or chronic hepatic disease were excluded from the study. Dogs that had been started on anticoagulant treatment during the study period were also excluded.

Sampling

Client consent was obtained before collection of blood samples. Blood samples in both populations were collected upon admission to the intensive care unit (ICU) and then daily in connection with routine monitoring until death or discharge. Whole blood was collected by careful jugular venepuncture, using minimum stasis and a 21-gauge butterfly needle. Blood samples were collected into one serum, two citrated and one EDTA Vacutainer plastic tubes (Vacuette, Greiner Bio-One International) in that order. The total amount of blood drawn daily was 10.4 mL. The EDTA blood sample was used for platelet count. The 3 mL citrate tubes were inverted carefully five times after sampling to ensure mixing of 3.2% trisodium citrate and blood in a 1:9 ratio centrifuged immediately at 4000 *g* for 120 s. The plasma was collected from the centrifuged tubes within 30 min of sampling and a coagulation profile was performed immediately and additional plasma was stored at −80 °C for an extended coagulation profile analysis.

Coagulation assays

A coagulation screen (aPTT, PT, D-Dimer, fibrinogen and platelet count) was performed daily in Copenhagen for group A and in North Grafton for group B for all dogs during hospitalisation. An extended coagulation panel was performed in one batch for the two groups of dogs at the Central Laboratory, Department of Small Animal Clinical Sciences, Faculty of Life Sciences, University of Copenhagen. Samples from Tufts University were shipped to Copenhagen on dry ice with a certified courier and transit time for the shipment was <24 h. The extended coagulation profile included aPTT, PT, fibrinogen, D-Dimer Protein C (PC), Protein S (PS), α2-antiplasmin, plasminogen and a platelet count.

We measured aPTT, PT, PC, PS, α2-antiplasmin, plasminogen and fibrinogen using an automated haemostasis analyser (ACL 9000, Instrumentation Laboratory). Platelet concentrations were determined using an automated haematology instrument (Advia 120, Bayer A/S). Concentrations of D-Dimer were measured using an immunometric flow-through principle (D-Dimer Single Tests, NycoCard READER II, Medinor A/S). A list of reagents and normal values used is provided in Table 2. A pooled sample of plasma from five clinically healthy dogs was analysed together with the samples and used as internal control and reference material. Results of the chromogenic assays were defined as abnormal if they had ⩽80% of the activity of the reference pool values. Plasma samples were thawed at 37 °C in a water bath immediately before analysis and centrifuged at 4000 *g* for 3 min (to avoid remnants of cryoprecipitate in plasma after thawing); the supernatants were then used for analysis.

At Tufts, a semi-automated coagulation analyser (STart4, Diagnostica Stago) was used for PT (Stago Neoplastin, Diagnostica Stago, ref.: 6.2–9.3 s) and aPTT (Dade Actin, Dade Behring, ref.: 8.9–16.3 s). Fibrinogen was measured with standard heat precipitation (ref.: 2.0–4.0 g/L) and platelet counts were determined with an automated haematology instrument (Cell-Dyn, Abbott Diagnostics).

Diagnosis of DIC

The approach previously used by Bakhtiari et al. (2004) was used to identify dogs with DIC using an expert panel to provide the 'gold standard' for DIC diagnosis. The experts were given the results of a wide range of haemostasis assays and asked to identify patients with DIC based on published criteria, namely that a patient suffering from DIC should have evidence of procoagulant activation, inhibitor consumption and increased fibrinolytic activity (Bick et al., 1999). The expert panel consisted of a physician (PIJ) and two veterinarians (ATK, ALJ), all with more than 10 years experience working with patients with inflammatory and haemostatic abnormalities. If there was a discordant opinion between the experts, a simple majority was used to determine whether a dog had DIC.

Dogs were scored daily with the ISTH algorithm for overt DIC by one of us (BW). A score ⩾5 is indicative of DIC in humans (Taylor et al., 2001). Diagnosis of DIC was then derived from blinded expert evaluation of the results of the extended coagulation profile (aPTT, PT, D-Dimer, fibrinogen, PC, PS, AT, plasminogen, α2-antiplasmin, and a platelet count) from the day of the highest ISTH score of each individual. In order to limit bias, the experts were blinded to the results of the other experts (test review bias), results of the model (diagnostic review bias), and clinical information about the patients other than underlying disease (clinical review bias) (Begg, 1987; Philbrick et al., 1980).

To establish the diagnosis of DIC, the experts were asked to identify abnormalities in the coagulation profile fulfilling the criteria for the human approach to DIC defined by ISTH, i.e. indications of both activation of coagulation (PT, aPTT, platelet count), inhibitor consumption (AT, PC, PS) and increased fibrinolytic activity (plasminogen, α2-antiplasmin, D-Dimer) (Bick et al., 1999; Taylor et al., 2001).

Statistical analysis

To develop a diagnostic model for canine DIC, a multiple logistic regression analysis with backwards exclusion followed by forward inclusion was performed on the results of the extended coagulation panel in group A (Hosmer et al., 1997). The explaining variables evaluated for inclusion in the model were aPTT, PT, D-Dimer, platelet count, fibrinogen, AT and PC as continuous variables, and dichotomised D-Dimer (cut-off 0.5 mg/L), dichotomised fibrinogen (cut-off 1 g/L) and dichotomised platelet count (cut-off 100×10^9/L), including cross-products of all significant parameters. Parameter significance for inclusion in the model was set at $P < 0.15$ as recommended for this type of model development (Hosmer et al., 1997). The final model was then applied to group A and a receiver operated characteristics (ROC) curve was generated to assess the diagnostic sensitivity (Se) and specificity (Sp) for all obtained logistic *P* values. The optimal diagnostic cut-off was assessed with Youdens index optimising Se-(1-Sp). Following model development and optimisation in group A, an independent evaluation of the integrity and performance of the model was performed in a separate population (group B) to detect possible overfit.

Model integrity was tested in two steps: (1) parameters and cross-products included in the model developed in group A should all also contribute significantly to the model when applied to group B; (2) performing forward inclusion of the parameters excluded during group A model development should not result in a different model when applied to group B. Subsequently the diagnostic performance of the model was assessed by applying the model and the defined cut-off to group B. Performance was expressed as diagnostic sensitivity, specificity, positive and negative predictive values in group B, as well as the relative risk (RR) of a diagnosis of DIC when identified by the model.

To confirm that the dogs identified by the expert panel as having DIC had been diagnosed correctly, statistical analyses were performed to identify differences in the coagulation parameters between dogs diagnosed with and without DIC, i.e. a priori activation of coagulation (prolonged PT, prolonged aPTT, decreased platelet count), inhibitor consumption (decreased AT, PC and PS activity) and increased fibrinolytic activity (decreased plasminogen, increased D-Dimer) (Bick et al., 1999). For these data, distribution was assessed using the D'Agostino and Pearson

Table 1
Checklist of items that criteria should be included in the report or publication of a study of diagnostic accuracy in accordance with STARD.

Section and topic	Item	Description
Title/abstract/ keywords	I	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity')
Introduction	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups
Methods		Describe
Participants	3	The study population: the inclusion and exclusion criteria, setting and locations where the data were collected
	4	Participant recruitment: was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?
	5	Participant sampling: was the study population a consecutive series of participants defined by the selection criteria in items 3 and 4? If not, specify how participants were further selected
	6	Data collection: was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?
Test methods	7	The reference standard and its rationale
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard
	11	Whether or not the readers of the index tests and reference standard were blinded (masked) to the results of the other test and describe any other clinical information available to the readers
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals)
	13	Methods for calculating test reproducibility, if done
Results		Report
Participants	14	When study was done, including beginning and ending dates of recruitment
	15	Clinical and demographic characteristics of the study population (e.g. age, sex, spectrum of presenting symptoms, co-morbidity, current treatments, recruitment centres)
	16	The number of participants satisfying the criteria for inclusion that did or did not undergo the index tests and/or the reference standard; describe why participants failed to receive either test (a flow diagram is strongly recommended)
Test results	17	Time interval from the index tests to the reference standard, and any treatment administered between
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) against the results of the reference standard; for continuous results, the distribution at the test results against the results of the reference standard
	20	Any adverse events from performing the index tests or the reference standard
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals)
	22	How indeterminate results, missing responses and outliers of the index tests were handled
	23	Estimates of variability in diagnostic accuracy between subgroups of participants, readers or centres, if done
	24	Estimates of test reproducibility, if done
Discussion	25	Discuss the clinical applicability of the study findings

omnibus normality test. For parameters not normally distributed (PT, aPTT, platelet count, D-Dimer), the statistical analyses were carried out as non-parametric (Mann–Whitney). For normally distributed data, a *t* test with Welch correction was used. Statistical significance was set at $P < 0.05$. GraphPad Prism v4.01, GraphPad Software; MedCalc v.6.00.0012, MedCalc Software and SAS 9.2 (SAS Institute) were used for statistical analysis.

Results

Development population (group A)

From September 2004 to December 2005, 100 consecutive dogs with underlying disease known to predispose to DIC were enrolled in the development population. Thirty-seven dogs were excluded due to incomplete data. Data were assessed to be incomplete if one or more values were missing in any of the measured or recorded parameters.

Twenty-three of 63 dogs had DIC (37%) based on expert opinion and overall inter-rater agreement for diagnosis was 77%. Included were 22 males (mean age 5.8 years), two castrated males (mean age 8.8 years), 32 females (mean age 6.3 years) and seven spayed females (mean age 7.1 years). Seven of the dogs were Labrador Retrievers, five were BullTerriers, four mixed breeds, four Dachshunds, four Golden Retrievers, three Border Collies, three Bernese Mountain dogs, three Rhodesian Ridgebacks; other breeds were only represented once or twice. Table 3a shows the prevalence of underlying diseases.

Validation population (group B)

From April to July 2006, 50 dogs with predisposing diseases were included in the validation study. Four could not be assessed with the final model due to missing data. Based on expert opinion, 24/46 dogs (52%) had DIC with an overall inter-rater agreement of 76%. Only 11 had an overt ISTH score $\geqslant 5$. All dogs with an ISTH score $\geqslant 5$ had DIC, giving a sensitivity of 46% and a specificity of 100%. If the DIC positive cut-off score was lowered to $\geqslant 4$, the sensitivity increased to 73% and the specificity dropped to 79%.

Fig. 1 provides an overview of the data in the validation study. Of the 46 included dogs, there were four males (mean age 5.11 years), 17 castrated males (mean age 9.6 years), three females (mean age 8.0 years) and 22 spayed females (mean age 8.4 years). Eight dogs were Labrador retrievers, four were of mixed breeds, four were Golden Retrievers and all other breeds were only represented once or twice. Table 3b shows the prevalence of underlying disease in the 46 included dogs.

Table 2
Laboratory coagulation assays run on automated haemostasis analyser ACL 9000 and reagents used.

Factors	Methods	Units	Kits, instrumentation laboratory, USA
aPTT	Clotting time	s	APTT-SP (liquid) (0020006300)
PT	Clotting time	s	PT-fibrinogen recombinant (20005000)
Fib	Clotting time	g/L	PT-fibrinogen (9756710)
AT	Chromogenic substrate	%	Liquid antithrombin (0020002500)
PC	Chromogenic substrate	%	Protein C (0020009100)
PS	Chromogenic substrate	%	Protein S (0020002800)
Plg	Chromogenic substrate	%	Plasminogen (0020009000)
αPLI	Chromogenic substrate	%	Plasmin inhibitor (0020009200)

aPTT, activated partial thromboplastin time; PT, prothrombin time; Fib, fibrinogen; AT, antithrombin; PC, Protein C; PS, Protein S; Plg, plasminogen; αPLI, antiplasmin.

Table 3
Frequency of the underlying diseases in dogs suspected of DIC in the development (group A) and validation groups (group B).

(a) Predisposing diseases in the development group A ($n = 63$)

Angiostrongylus vasorum (9)	IMTP (2)	Mammary gland tumour	Pulmonary carcinoma
Acute hepatitis (6)	Splenic tumour (2)	Mastocytoma	Pyometra
Lymphoma (6)	Cold agglutination disease	Meningitis	Snakebite
Mammary adenocarcinoma (4)	Fever of unknown origin	Multitrauma	Nasal squamous cell carcinoma
Sepsis (4)	Heart base tumour	Oral carcinoma	Squamous papilloma vulva
Acute renal failure (3)	IMHA	Pancreatitis	Vesical tumour
Haemangiosarcoma (3)	Intra abdominal sarcoma	Perianal adenocarcinoma	
Haemorrhagic gastroenteritis (3)	Malignant histiocytosis	Pneumonia	

(b) Predisposing diseases in the validation group B ($n = 46$)

Multitrauma (5)	Haemothorax (2)	Leukaemia
Sepsis (6)	Pancreatitis (2)	Lung torsion
IMHA[#] (4)	Acute hepatic failure	Lymphoma
Gastric dilatation volvulus (3)	Acute renal failure	Pleural effusion
Haemangiosarcoma (3)	IMHA + IMTP	Rattlesnake bite
Pericardial effusion (3)	Heartbase tumour	Thoracic tumour
Splenic tumour (3)	Haemorrhagic gastroenteritis	
Malignancy with pulmonary metastasis (2)	IMTP	

IMTP, immune mediated thrombocytopenia; IMHA, immune mediated haemolytic anaemia.

Development

The final optimised model for the development population included fibrinogen, PT, aPTT, dichotomised D-Dimer (cut-off 0.5 mg/L) and the vector cross-product of aPTT and PT, giving the following equation:

$$\begin{aligned}\text{Logit}(DICprob) &= 15.99 - 0.14 \times \text{Fib} - 2.52 \times \text{PT} - 2.13 \times \text{aPTT} \\ &\quad + 0.28 \times (\text{aPTT} \times \text{PT}) + (5.41, \text{when D-Dimer} > 0.5\ \text{mg/L})\end{aligned}$$

The model was observed to have significant diagnostic abilities with ROC area under the curve of 0.958 (95% CI; 0.874; 0.992).The optimal diagnostic cut-off was assessed to be a *P* value (*DICprob*) of 0.401, with an observed diagnostic sensitivity and specificity of 90.9% (95% CI: 70.8–98.6) and 90.0% (95% CI: 76.3–97.1), respectively.

Example of how the model is used

A dog with an underlying disease known to predispose to DIC has the following test results: aPTT 21 s, PT 17 s, fibrinogen 6 g/L, D-Dimer 1.2 mg/L. This dog has a *DICprob* of:

$$\frac{e^{15.99-(0.14\times6.0-2.52\times17.0-2.13\times21.0+0.28\times(17.0\times21.0)+5.41)}}{1+e^{15.99-(0.14\times6.0-2.52\times17.0-2.13\times21.0+0.28\times(17.0\times21.0)+5.41)}} = 1.0,$$

which is > 0.401

Thus the dog has a high risk of DIC. A dog with normal values, such as aPTT 10.4 s, PT 7.5 s, fibrinogen 4.0 g/L and D-Dimer <0.5 mg/L, has a *DICprob* = 0.023 and thus a low probability of DIC.

Validation

The validation study fulfilled all 25 criteria of the STARD initiative (Bossuyt et al., 2003a,b). The model developed in group A passed the integrity test when applied to group B. When applying the developed model and cut-off to the new population (group B), a diagnostic sensitivity and specificity of 83.3% (95% CI: 62.6–95.2) and 77.3% (95% CI: 54.6–92.1), respectively, was observed. Using an ROC curve, the model was found to have good diagnostic abilities in the validation population, with ROC area under the curve of 0.817 (95% CI; 0.678; 0.917) and a positive predictive value of 80% (95% CI; 0.593; 0.932) and a negative predictive values of 81% (95% CI; 0.581; 0.946) at a prevalence of 54%. The relative risk of DIC when *DICprob* > 0.401 was RR = 3.7 (95% CI; [1.7; 8.1], $P < 0.0001$).

Descriptive data

The significant results of the haemostasis assays to measure sensitive markers for coagulation between dogs with and without DIC are shown in Fig. 2. Significant differences in the coagulation parameters between dogs diagnosed with and without DIC were observed for most coagulation parameters. Thus, dogs with DIC had significantly prolonged PT, prolonged aPTT, decreased platelet count (activation of coagulation), decreased AT and PC (inhibitor consumption) and decreased plasminogen and increased D-Dimer (increased fibrinolytic activity). Overall mortality was 46%. Only 26% of dogs without DIC died versus 59% of dogs with DIC. The

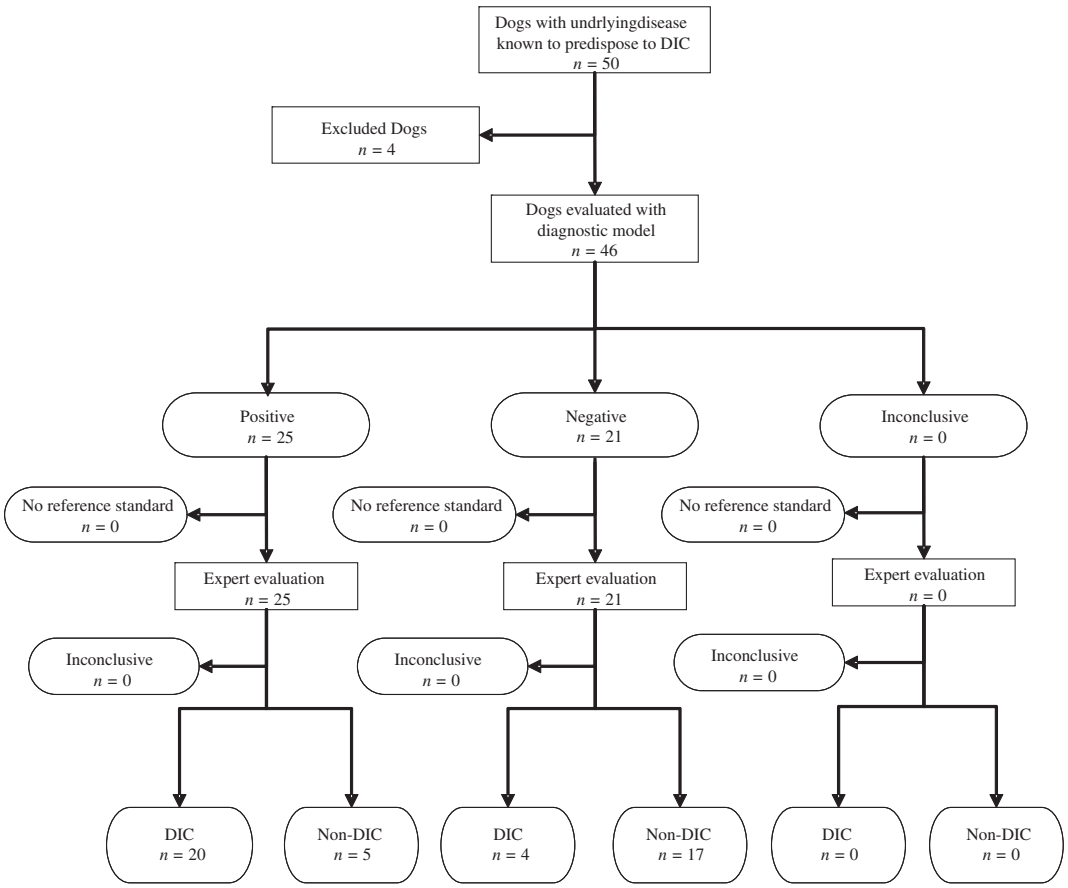

Fig. 1. Flow chart of data collection and results for validation study on canine DIC.

relative risk of death when *DICprob* > 0.401 was RR = 2.84 (95% CI; 1.227:6.590, *P* 0.0148).

Discussion

Based on commonly available coagulation assays, it was possible to design a simple (i.e. based on readily available parameters), objective and robust diagnostic model for canine DIC. The model had both a high sensitivity and specificity when evaluated in a group of dogs independent of the population with which the model was developed.

The relative risk of death in the group diagnosed with DIC was markedly increased, as would be expected. From a clinical perspective, the model enables the clinician to diagnose DIC with almost the same degree of accuracy as a group of three experts evaluating a much more extensive panel of coagulation assays. Importantly this can be done based on the results of only aPTT, PT, fibrinogen and D-Dimer, which are generally available in veterinary medicine and which have traditionally been used to diagnose DIC. Conversely, the human ISTH overt DIC score does not seem to be directly applicable to dogs when using a cut-off value of $\geqslant 5$. If the score is dropped to $\geqslant 4$, the ISTH scoring system performs better in dogs, but the model developed in our study is still superior to the ISTH score.

As an indicator of the soundness of the model, a comparison of the results of the haemostasis assays between the dogs diagnosed with and without DIC based on the model showed that there were significant differences between these groups for all parameters examined except PS and α2-antiplasmin activity. Thus, dogs with DIC had activation of coagulation, consumption of endogenous anticoagulants and increased fibrinolytic activity as expected. Consequently, as in humans there should be no or minimal additional value in including specific and not generally available parameters such as AT, PC or plasminogen in this model or in the development of a canine ISTH-like scoring system (Feldman et al., 1981; Levi et al., 2004; Toh and Downey, 2005).

Because the parameters used for model development were also included in the extensive coagulation panel used to establish expert diagnosis of DIC, there is a risk of dependency between the diagnosis and the explanatory variables used in the model. However, the bias introduced by this possible dependency is probably limited, as a range of additional highly sensitive and specific coagulation parameters were made available to the experts compared to those used for model development.

In the human ISTH score, a distinction is made between overt and non-overt DIC (Taylor et al., 2001). Due to the relatively small number of animals, such a distinction was not made in this study. Future work could aim to establish whether there is any gain in such a division, or perhaps from the inclusion of tests of general haemostasis in a scoring system, as has been proposed by the ISTH (Taylor et al., 2001). The motivation for inclusion of tests for overall haemostatic capability is that in humans it is generally acknowledged that aggressive intervention in the early non-overt and hypercoagulable stage of DIC increases chances of survival. Early intervention through supportive and/or antithrombotic therapy, while the underlying disease is treated, may minimise thromboembolic complications and delay or even prevent progression to overt symptoms, thus increasing chances of survival (Lindblad et al., 1987; Logan et al., 2001; Palmer et al., 1998).

Unfortunately, assessment of hypercoagulability and thrombosis is difficult with routinely used coagulation assays such as D-Di-

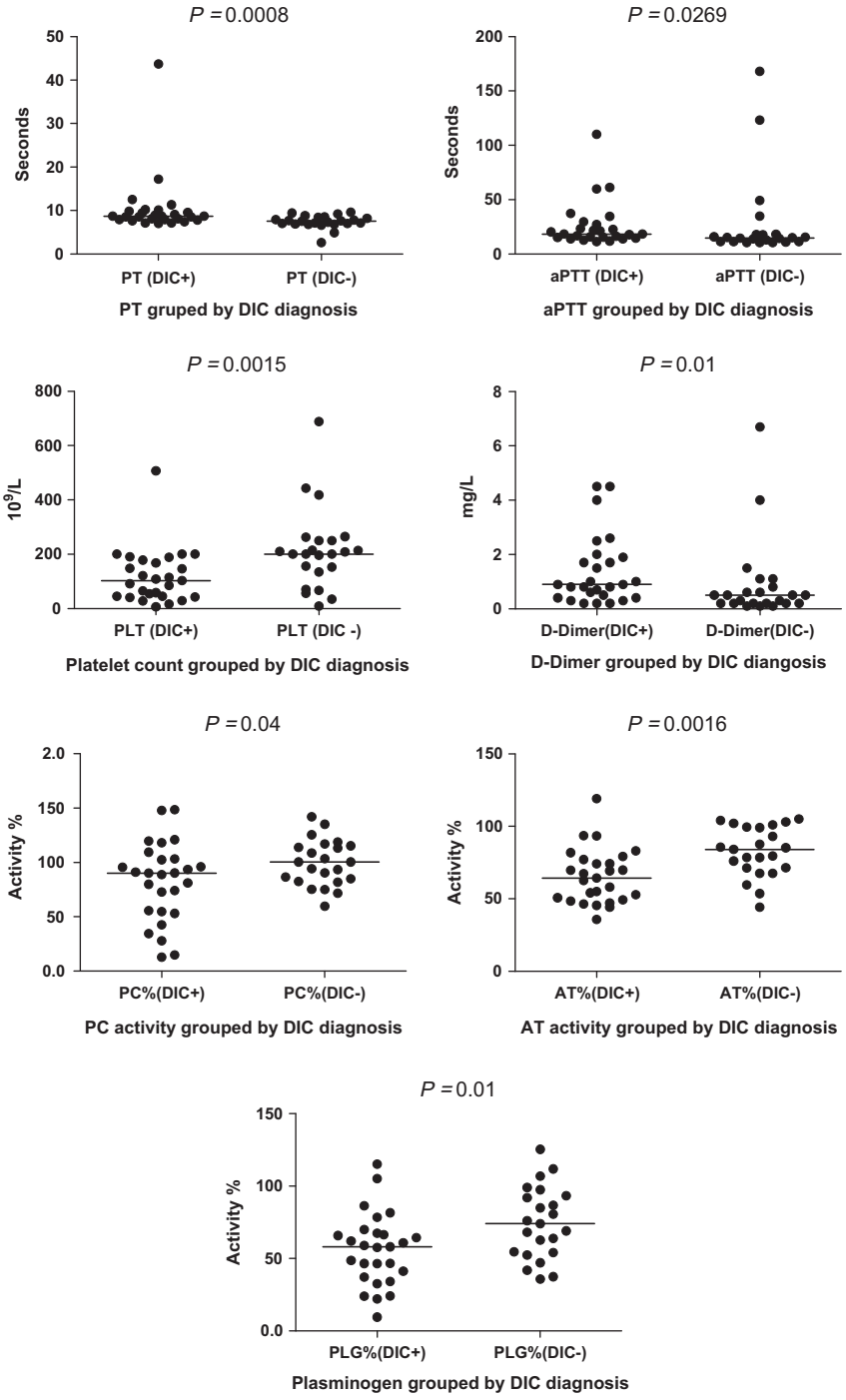

Fig. 2. Significant laboratory values of the haemostasis assays used to measure markers for coagulation activation (aPTT, PT, platelet count), endogenous coagulation inhibitors (AT, PC) and fibrinolytic activity (plasminogen, D-Dimer) in dogs with and without DIC.

mer, which has been shown to have mainly negative predictive value (Griffin et al., 2003; Nelson and Andreasen, 2003). Thus, there is an urgent need for improved assay methods that enable easy and patient near assessment of the overall haemostatic state in patients with DIC in order to identify hypercoagulability and ultimately to improve patient management. Thromboelastography (TEG) analysis is able to detect hypercoagulability in dogs and thus has the potential to aid in early diagnosis of non-overt DIC (Wiinberg et al., 2005). Interestingly, recent studies on the haemostatic capability in dogs with DIC or cancer have demonstrated that when Tissue Factor-activated TEG was used to assess the overall haemostatic state of dogs with DIC, the most common abnormality was hypercoagulability (Kristensen et al., 2008; Wiinberg et al., 2008). This observation and the finding that mortality was significantly lower in the hypercoagulable group than in dogs that were hypocoagulable supports the view that early and aggressive intervention may also be vital for outcome in canine patients with DIC and that TEG that may be particularly valuable in identifying these animals (Wiinberg et al., 2008).

The focus in treating DIC is on providing optimal therapy and care without taking unjustifiable risks. DIC therapy is often empirical and directed at correcting the imbalance in the haemostatic system, while treating the underlying disease aggressively (Bick, 2003; Franchini et al., 2006; Kienast et al., 2006; Levi et al.,

2004). Thus, treatment is not tailored to the needs of the individual patient with DIC. Unfortunately, response to treatment has been unpredictable, perhaps because no laboratory tests have been available to accurately predict or monitor the effect of treatment. With further clinical validation, the diagnostic model developed in this study may make it possible to conduct multi-centre therapy studies with minimal risk of systematic misclassification and may provide an important basis for optimising novel treatment modalities for DIC.

Several factors, such as study design, selection of patients, execution of tests and data analyses affect the validity of studies on diagnostic accuracy (Bossuyt et al., 2003b). Un-optimised studies may lead to exaggerated or misleading results which guide the veterinarian or physician to suboptimal or incorrect decisions about patient care. The objective of the STARD initiative is to improve the quality of reporting in studies of diagnostic accuracy so enabling the reader to assess general applicability and detect possible biases in the results (Bossuyt et al., 2003a). The present study was designed to minimise bias and optimise readability and reproducibility and should lead to a detailed and systematic presentation of study design and results (Philbrick et al., 1980; Ransohoff and Feinstein, 1978).

Conclusions

This study has demonstrated that it is possible to design an objective diagnostic model for canine DIC based on generally available assays. This model has both a high sensitivity and specificity when applied to another demographic population. The results suggest that it is possible to develop a scoring algorithm for the diagnosis of DIC in dogs. With further validation, this model should allow for the conduct of multi-centre therapy studies with a lower misclassification of canine patients and for the optimisation of treatment of canine DIC.

Conflict of interest statement

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

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