The Veterinary Journal 229 (2017) 6–12

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Original Article

# Diagnosis of canine pulmonary thromboembolism by computed tomography and mathematical modelling using haemostatic and inflammatory variables

C.B. Marschner[a,*], A.T. Kristensen[a], E.A. Rozanski[b], F.J. McEvoy[a], L. Kühnel[c,1], O. Taeymans[b,2], A. de Laforcade[b], A.F. Sato[b], B. Wiinberg[a,3]

[a] Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlaegevej 16, DK-1870 Frederiksberg C, Denmark
[b] Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, 200 Westboro Rd., North Grafton, MA 01536, USA
[c] Laboratory for Applied Statistics, Department of Mathematical Sciences, Faculty of Science, University of Copenhagen,Universitetsparken 5, DK-2100 København Ø, Denmark

ARTICLE INFO

*Article history:*
Accepted 17 October 2017

*Keywords:*
Canine
Pulmonary thromboembolism
Computed tomography pulmonary angiography
D-dimer
Prediction model

ABSTRACT

There is no evidence-based diagnostic approach for diagnosis of pulmonary thromboembolism (PTE) in dogs. Many dogs with diseases that predispose to thrombosis are hypercoagulable when assessed with thromboelastography (TEG), but no direct link has been established. The aims of this study were: (1) to investigate if diseased dogs with PTE, diagnosed by computed tomography pulmonary angiography (CTPA), had evidence of hypercoagulability by TEG; (2) to characterise haemostatic and inflammatory changes in dogs with PTE; (3) to construct models for prediction of PTE based on combinations of haemostatic and inflammatory variables; and (4) to evaluate the performance of D-dimer measurementfor prediction of PTE. Twenty-five dogs were included in this prospective observational study (PTE: $n = 6$; non-PTE: $n = 19$). Clot strength G values did not differ between the PTE and non-PTE groups in tissue factor (TF) or kaolin-activated TEG analyses. Haemostatic and inflammatory variables did not differ between the two groups. Linear discriminant analysis generated a model for prediction of PTE with a sensitivity and specificity of 100% when TF results were used as TEG data, and a model with sensitivity of 83% and specificity of 100% when kaolin results were used as TEG data. Receiver operating characteristic analysis of D-dimer levels showed that a value of >0.3 mg/L yielded a sensitivity of 100% and a specificity of 71.4%. In conclusion, the study supports CTPA as method for diagnosing canine PTE, but shows that TEG alone cannot identify dogs with PTE. Models for prediction of PTE were generated, but require further validation.



## Introduction

Several diseases in dogs are known to increase the risk of pulmonary thromboembolism (PTE) (Klein et al., 1989; Larue and Murtaugh, 1990; Johnson et al., 1999; Goggs et al., 2014). Thromboelastography (TEG) in these dogs often suggests hypercoagulability, prompting interpretations of increased thrombotic risk, but a definitive link between hypercoagulability and PTE has not been established (Otto et al., 2000; Kristensen et al., 2008; Sinnott and Otto, 2009; Donahue et al., 2011; Fenty et al., 2011; Goodwin et al., 2011; Vilar Saavedra et al., 2011; Andreasen et al., 2012; Goggs et al., 2012).

Diagnosing PTE in dogs remains a great challenge for clinicians due to non-specific clinical signs and a lack of simple and non-invasive valid diagnostic tests. Extensive evidence-based guidelines exist for human beings (Raja et al., 2015), beginning with clinical assessment of the patient based on standardised scoring systems, such as the Wells and Geneva score (Wells et al., 2000; Wicki et al., 2001; Le Gal et al., 2006). In patients with moderate clinical probability, D-dimer levels are measured and only a positive result warrants diagnostic imaging. In case of high clinical suspicion, diagnostic imaging is recommended (Raja et al., 2015). Computed tomography (CT) pulmonary angiography (CTPA), the

* Corresponding author.
*E-mail address:* clara@sund.ku.dk (C.B. Marschner).
[1] Present address: Department of Computer Science, Faculty of Science, University of Copenhagen, Universitetsparken 5, DK-2100 København Ø, Denmark.
[2] Present address: Dick White Referrals, Station Farm, London Road, Six Mile Bottom, Cambridgeshire CB8 0UH, UK.
[3] Present address: Global Research Unit, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Maaloev, Denmark.

https://doi.org/10.1016/j.tvjl.2017.10.010
1090-0233/

diagnostic standard recommended by the American College ofRadiology (Bettmann et al., 2012), has several advantages over other diagnostic imaging modalities, such as ventilation–perfusion lung scans, including greater availability, better evaluation of thoracic structures, direct visualisation of thrombi and higher inter-observer agreement (Blachere et al., 2000; Schoepf and Costello, 2004). Limitations of CTPA include risk of motion artefact and a poor sensitivity for detection of PTE in sub-segmental vessels.

An algorithm for defining the clinical probability of PTE using D-dimer measurement in dogs has been proposed but not validated (Nelson, 2005). The advantage of measuring D-dimer levels is that the test has a high sensitivity, so a negative result effectively rules out thrombosis. However, elevated D-dimer levels are not a unique feature of thrombosis and therefore are of limited value (Nelson and Andreasen, 2003). Several studies have shown promising results for diagnosing PTE in dogs with CTPA (Ben et al., 2007; Tidwell et al., 2007; Goggs et al., 2014; Ngwenyama et al., 2014). In three of these studies, general anaesthesia was performed to avoid risk of motion artefact, although this can be problematic in dogs with respiratory compromise. Although Ngwenyama et al. (2014) successfully performed CTPA in a conscious dog, alternatives to CTPA for diagnosing PTE would be beneficial in dogs with respiratory compromise or where CTPA is unavailable.

In this study, we investigated if dogs with PTE showed hypercoagulability when measured by TEG when compared to diseased dogs without PTE. Additionally, we characterised changes in inflammatory and haemostatic biomarkers in dogs with PTE to determine if a combination of haemostatic and inflammatory variables could be used to identify PTE. Finally, we evaluated the diagnostic performance of measurement of D-dimer levels as a single variable in diagnosing PTE.

## Materials and methods

### *Study design*

This study was designed as a prospective observational study and was performed as a collaboration between Foster Hospital for Small Animals, Cummings School of Veterinary Medicine, Tufts University, MA, USA (Tufts) and the Department of Veterinary Clinical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. The study was approved by the Clinical Studies Review Committee at Tufts University (approval number 109.09; date of approval 27 April 2010).

### *Animals and sampling*

Dogs were eligible for inclusion in the study if they were presented with signs of critical illness and diagnosis of a condition associated with hypercoagulability and/or risk of thrombi, including immune mediated haemolytic anaemia, sepsis, severe trauma, pancreatitis, protein-losing enteropathy, protein-losing nephropathy and neoplasia. Dogs had to be at least 6 months old and to weigh at least 5 kg. Only dogs undergoing general anaesthesia for management of a clinical condition were eligible for inclusion. Exclusion criteria consisted of treatment with prednisolone, anticoagulants or antiplatelet medication at any time in the month prior to presentation. Dogs with significant cardiac disease, as assessed by a cardiologist, were also excluded. Informed owner consent was obtained prior to inclusion.

Blood samples from each dog were collected by venepuncture of the jugular vein using minimal stasis into one 3 mL serum tube (BD Vacutainer, BD Diagnostics), two 2.8 mL sodium citrate tubes (3.2%, 0.109 M; BD Vacutainer, BD Diagnostics), one 2 mL ethylene diamine tetra-acetic acid (EDTA) tube (BD Vacutainer, BD Diagnostics) and one 4 mL sodium heparin tube (Covidien/Medtronic) in that order. Serum samples were centrifuged at 1620 *g* for 10 min and used for biochemistry analysis (Cobas 6000/501, Roche Diagnostics); the remaining serum was frozen at −80 °C for later analyses of cytokines and C-reactive protein (CRP). Citrated samples were used for TEG analysis and the remaining citrated blood was centrifuged at 1620 *g* for 10 min at room temperature; the citrated plasma was stored at −80 °C for later analyses. EDTA-treated samples were used for complete blood counts (CBCs; Advia 2120i, Siemens Healthcare GmbH) and heparinised samples were used for Multiplate platelet aggregometer (Roche Diagnostics) analysis. Computed tomography (CT) scan, blood sample collection, CBC, biochemistry, TEG and Multiplate analyses were performed at Tufts, whereas remaining plasma and serum samples were analysed at the University of Copenhagen.

### *Thromboelastography analysis*

Whole blood samples were analysed as described previously (Marschner et al., 2010) using a computerised thromboelastograph (TEG 5000, Haemoscope) with human recombinant tissue factor (TF; Dade Innovin, Siemens Healthcare GmbH) or kaolin (Kaolin tubes, Haemonetics) as activators. Both of these analyses were performed with and without the addition of tissue plasminogen activator (t-PA; Single-chain Recombinant Tissue Plasminogen Activator, Bionordika A/S) to evaluate the fibrinolytic potential, resulting in a total of four TEG analyses per dog. t-PA was pre-diluted in 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) buffer and the diluted t-PA was added to the whole blood (WB) immediately prior to analysis, yielding a final t-PA concentration in the TEG cups of 10 pg/μL. The following TEG parameters were recorded for both TF TEG and kaolin TEG analyses: reaction time (R), split point (SP), clot formation time (K), angle (α), maximum amplitude (MA), total clot strength (G) and fibrinolysis with and without t-PA at 30 min (LY30; t-PA LY30) and 60 min (LY60; t-PA LY60) after reaching MA. In addition, the TEG velocity curve (VCurve) parameters maximum rate of thrombus generation (MRTG) and total thrombus generated (TG) of the first derivative of the TEG tracing were measured.

### *Multiplate platelet aggregation analysis*

Multiplate analyses were performed to investigate platelet reactivity to certain agonists using a Multiplate platelet aggregometer (Multiplate, Roche Diagnostics) and were performed as previously described, with a total measurement time of 15 min (Marschner et al., 2012, 2013). Four parameters were recorded, reflecting total platelet aggregation (area under curve, AUC) using adenosine diphosphate (ADP; ADPtest, Roche Diagnostics), collagen (COL; COLtest, Roche Diagnostics) and arachidonic acid (ASPI; ASPItest, Roche Diagnostics) as agonists, as well as a buffer control (Buffer).

### *Plasma and serum analyses*

Frozen plasma and serum samples were transported on dry ice to the Veterinary Diagnostic Laboratory at the University of Copenhagen. The following parameters were analysed in citrated plasma using the ACL TOP 500 haemostasis testing system (ACL TOP 500, Instrumentation Laboratories): prothrombin time (PT; Recombiplastin, Instrumentation Laboratories), activated partial thromboplastin time (aPTT; Synthafax, Instrumentation Laboratories), fibrinogen (Fib; Recombiplastin, Instrumentation Laboratories), antithrombin (AT; Liquid antithrombin, Instrumentation Laboratories), plasminogen (PLG; Plasminogen, Instrumentation Laboratories), plasmin inhibitor (PI; Plasmin Inhibitor, Instrumentation Laboratories) and von Willebrand factor (vWF; von Willebrand Factor Antigen, Instrumentation Laboratories). Assays for AT, PLG, PI and vWF were calibrated using canine plasma pools obtained from clinically healthy dogs with the pool set to 100%. The results were extrapolated from the standard curve and reported as percentages. In addition, citrated plasma was also used to determine D-dimer levels (Nycocard D-Dimer, Alere). Serum samples were used for analyses of CRP using an Advia 1800 (Siemens Healthcare GmbH) and for measurement of interleukin (IL)-6, IL-8, IL-10, IL-18 and monocyte chemoattractant protein (MCP)-1 using a canine cytokine assay (Milliplex MAP Canine Cytokine/Chemokine Magnetic Bead Panel, CCYTOMAG-90K, EMD Millipore Corporation) and a Luminex analyzer with Bioplex Manager version 6.1 software (Luminex, Bio-Rad Laboratories).

### *Imaging protocol*

CTPA scans were performed using a multi-slice helical CT unit (Toshiba Aquilion-16, Toshiba America Medical Systems). Each dog underwent scans before, during and after intravenous injection of Iohexol-300 (Omnipaque, GE Healthcare; 600 mg I/kg, diluted to 150 mg I/mL with normal saline). The injection rate was 4 mL/s in most dogs, but in one dog, where the injection time would be shorter than the scan time, the rate was reduced to 2 mL/s to ensure arterial filling throughout the scan. Scans were triggered automatically using bolus tracking software with a region of interest (ROI) drawn on the main pulmonary artery immediately proximal to its bifurcation into the left and right pulmonary arteries. Scanning from the diaphragm to the thoracic inlet commenced when the blood in the main pulmonary artery reached 150 Hounsfield units (HU). The volume of contrast and the rate of injection allowed continuous injection of contrast during scanning, ensuring contrast filling of the pulmonary vasculature.

The pre-contrast thoracic scans were processed using a thorax algorithm and reconstructed in transverse, sagittal and dorsal planes. Scans during Iohexol-300 injections were processed using a soft tissue algorithm and also reconstructed in transverse, sagittal and dorsal planes. Delayed post-contrast transverse thoracic scans were processed using a lung algorithm and reconstructed in sagittal and dorsal planes. Slice thickness in pre-contrast and post-contrast scans varied among 2, 3 and 5 mm, depending on the size of the dog, while slice thickness from scans during the angiographic phase were 1 mm. Reconstructed images had a slice thickness of 1 mm. All scans were reviewed for presence of pulmonary emboli. The dogs were diagnosed with presence or absence of PTE on CTPA, based on unanimous radiological agreement.