Endogenous fibrinolytic potential in tissue-plasminogen activator-modified thromboelastography analysis is significantly decreased in dogs suffering from diseases predisposing to thrombosis

Spodsberg, Eva-Maria Hohneck; Wiinberg, Bo; Jessen, Lisbeth Rem; Marschner, Clara Büchner; Kristensen, Annemarie Thuri

Published in:
Veterinary Clinical Pathology

DOI:
10.1111/vcp.12068

Publication date:
2013

Document Version
Early version, also known as pre-print

Citation for published version (APA):
Spodsberg, E-M. H., Wiinberg, B., Jessen, L. R., Marschner, C. B., & Kristensen, A. T. (2013). Endogenous fibrinolytic potential in tissue-plasminogen activator-modified thromboelastography analysis is significantly decreased in dogs suffering from diseases predisposing to thrombosis. Veterinary Clinical Pathology, 42(3), 281-290. DOI: 10.1111/vcp.12068
Endogenous fibrinolytic potential in tissue-plasminogen activator-modified thromboelastography analysis is significantly decreased in dogs suffering from diseases predisposing to thrombosis

Eva H. Spodsberg1, Bo Wiinberg2, Lisbeth R. Jessen1, Clara B. Marschner1, Annemarie T. Kristensen1

1Department of Clinical Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University Hospital for Companion Animals, University of Copenhagen, Frederiksberg C, Denmark; and 2Biopharmaceuticals Research Unit, Novo Nordisk A/S, Måløv, Denmark

Key Words
Canine, fibrinolysis, kaolin-activated TEG, native TEG, tissue-factor (TF)-activated TEG

Correspondence
Dr. Eva H. Spodsberg, University Hospital for Companion Animals, Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrøgavej 16, Frederiksberg C 1870, Denmark
E-mail: Evahs@sund.ku.dk

DOI:10.1111/vcp.12068

Introduction
Fibrinolysis is an essential part of the hemostatic process protecting against permanent thrombotic occlusion of damaged vessels in which a clot has formed. The balance between coagulation and fibrinolysis determines the size, quality, rate of dissolution, and lifespan of the clot.1,2 A balanced and healthy fibrinolytic mechanism dissolves the occlusive thrombus before permanent tissue damage occurs.1 Fibrinolysis is initiated when plasminogen is converted to plasmin by one of several plasminogen activators, with tissue plasminogen activator (t-PA) as the main intravascular activator.2 Plasmin mediates degradation of fibrinogen or soluble fibrin resulting in the formation of fibrin or fibrinogen degradation products (FDPs). Plasmin also mediates degradation of cross-linked, insoluble fibrin to produce D-dimers. As D-dimers result only from the degradation of cross-linked fibrin in a stabilized clot, they are, in contrast to other degradation products, specific for active coagulation and fibrinolysis.3 Both the FDP and D-dimer assays are plasma-based and the only tests routinely used to evaluate fibrinolysis in dogs.

In human medicine, D-dimer tests are useful for the clinical diagnosis of patients suspected of having...
deep-vein thrombosis (DVT) and patients with pulmonary thromboembolism (PTE). In patients with a low or moderate pretest probability of disease, a negative D-dimer test result helps in ruling out venous thromboembolism. In veterinary medicine, D-dimer tests have been used in the diagnosis of canine thromboembolic diseases, mainly PTE, with a negative test being considered a strong indicator of the absence of thromboembolism.

Several disease conditions have been associated with the development of thrombosis in dogs. These include immune-mediated hemolytic anemia (IMHA), protein-losing conditions, hyperadrenocorticism, neoplasias, parvovirus infection, early disseminated intravascular coagulation (DIC), and congestive heart failure. The current understanding and ability to diagnose and monitor thrombotic disease including fibrinolysis are limited. Specifically, defects of the fibrinolytic system or resistance to fibrinolysis have not been investigated, as no standardized tests are available to measure the global endogenous fibrinolytic potential of whole blood and determined risk factors for thromboembolic disease in dogs. In people, plasminogen-activator-induced fibrinolysis has been evaluated by thromboelastography (TEG) in several studies. Fibrinolysis has recently been standardized by using t-PA-induced, tissue-factor (TF)-activated TEG in human samples, and has been reported to provide rapid assessment of the endogenous fibrinolytic potential of whole blood. In another study with t-PA-modified TEG performed on whole blood from children with noncatheter-related DVT, the authors concluded that decreased fibrinolytic activity likely is a contributing factor to thrombosis. TEG is increasingly used in the diagnosis and monitoring of hemostatic disorders in dogs, including both hypercoagulable and fibrinolytic disorders. Recently, TEG was used to measure TF-initiated fibrin formation and t-PA-induced fibrinolysis in human and canine hemophilic blood after addition of the anti-fibrinolytic Solulin, a soluble analog of thrombomodulin with anti-fibrinolytic properties used to increase clot lysis time. Blood obtained from hemorrhagic dogs given Solulin intravenously was found to generate clots that are resistant to fibrinolysis.

Studies assessing the ability of t-PA-modified TEG to evaluate fibrinolytic potential in healthy dogs and dogs with diseases known to predispose to thrombosis have not been reported. Early identification of dogs with altered fibrinolytic activity could ultimately help direct therapeutic measures or predict outcome. Therefore, our main hypothesis was that the addition of t-PA to TEG would activate fibrinolysis in canine whole blood from healthy dogs and dogs with diseases associated with development of thrombosis. In addition, we hypothesized that the fibrinolytic activity would be decreased in dogs with diseases associated with thrombosis when compared with healthy dogs. For that purpose, 3 different t-PA-modified TEG assays were compared.

**Materials and Methods**

The study was performed as a prospective, ex vivo study between January 2010 and March 2011 at the University Hospital for Companion Animals, Department of Veterinary Clinical and Animal Sciences (UHCA-DVCAS), Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. The study was approved by the Ethics and Administrative Committee. All laboratory analyses were performed at The Central Laboratory, DVCAS. Forty dogs were included in this study, with a control group consisting of 20 healthy dogs and a group of 20 dogs with diseases potentially associated with thrombosis.

**Animals**

Healthy dogs: Fasting blood samples from 20 clinical healthy staff- or student-owned dogs were collected. Included were clinically healthy dogs of any breed and sex, of a minimum age of one year and a minimum weight of 10 kg to ensure that collected blood volume was < 2% of the total blood volume. The dogs were considered healthy based on absence of clinical signs of illness, an unremarkable physical examination and variables of a CBC, biochemistry profile, a routine coagulation profile including APTT, PT, fibrinogen, D-dimers, TEG, and a urinalysis within the respective reference intervals (RI). Dogs with a history of receiving any medication during the preceding 2 weeks as well as bitches in estrus were excluded.

Diseased dogs: Blood samples from 20 client-owned dogs with disease conditions predisposing to thrombosis were collected between February 2010 and March 2011. Inclusion criteria for dogs with diseases associated with thromboembolic disease as defined by the literature were dogs of any breed and sex with a minimum age of one year and a minimum weight of 10 kg suffering from either (1) systemic inflammatory conditions (eg, acute pancreatitis, hepatitis, pneumonia, pyometra) resulting in systemic inflammatory response syndrome (SIRS) and confirmed by the combined results of the clinical examination (eg, hyperthermia or hypothermia, tachycardia, tachypnea), a moderate to marked increase in the acute-phase
protein C-reactive protein (CRP, RI 0–35 mg/L), and
neutrophilia or neutropenia; (2) protein-losing condi-
tions (eg, protein-losing enteropathy [PLE], protein-
losing nephropathy [PLN], vasculitis), based on results
of the clinical examination and hypoalbuminemia (RI
26–44 g/L), either alone or in combination with pan-
hypoproteinemia (total protein RI 57–82 g/L), in addi-
tion to a urine protein:creatinine (UPC) ratio of > 1 in
cases of PLN; (3) Diabetes mellitus, confirmed by
hyperglycemia, hyperfructosaminemia and glucosuria,
with or without ketonuria; (4) Hyperadrenocorticism
confirmed by the combined results of the clinical and
labaratory examinations (CBC, biochemistry profile,
urinalysis, ACTH response test, and/or low-dose dexam-
ethasone suppression test); and (5) neoplasia, con-
firmed cytotologically and/or histologically. Candidate
dogs were identified based on history, clinical exami-
nation, and the above-mentioned criteria at admit-
tance to the UHCA. All owners consented to their dogs
being included in the study. Blood samples for CBC,
biochemistry profile, a routine coagulation profile, and
TEG were collected before treatment was initiated.
Dogs with a history of medication with nonsteroidal
anti-inflammatory drugs, corticosteroids, and antico-
agulants during the preceding 2 weeks as well as bitches in estrus were excluded.

Sampling

Healthy dogs were fasted 12 hours prior to blood collec-
tion. From diseased dogs, blood was collected at admittance to the hospital. In both groups, blood was collected
by jugular venipuncture, by applying minimum stasis
and using a 21-gauge butterfly needle. Blood was col-
clected in the following order: one serum tube (5 mL,
Vacuette, Greiner Bio-One International AG, Kremsmu-
ster, Austria), followed by 5 citrated plastic tubes
(2.7 mL, 0.109 M, 3.2%; BD Vacutainer TM Plus Plastic
Citrate tube), followed by one EDTA tube (2 mL, Vacu-
ette, Greiner Bio-One International AG).

Routine hematology, biochemistry, and
coagulation tests

After sampling, citrate tubes were carefully inverted 5
times to ensure mixing of citrate and blood, in a 1:9
ratio. One randomly selected citrate tube was used for
the standard coagulation profile. Serum and citrate
tubes for biochemistry and coagulation profiles were
centrifuged at 4000g for 120 seconds, and serum and
plasma were separated and collected within 30 min-
utes of sampling. The CBC and biochemistry profile
were run within 2 hours of sampling. The CBC was
determined using an ADVIA 2120 automated hema-
ology analyzer (Siemens Health Care Diagnostics Inc., Deerfield, IL, USA) and the blood smear was
evaluated by a clinical pathologist. The biochemistry
profile was analyzed using an ADVIA 1800 (Siemens
Health Care Diagnostics Inc.). A coagulation profile
was performed immediately or the plasma was stored
at −80°C until analysis. APTT (apTT SynthAFax;
Instrumentation Laboratory, Bedford, MA 01730,
USA), PT (PT-Fibrinogen, Instrumentation Labora-
tory), and fibrinogen concentration (PT-Fibrinogen,
Instrumentation Laboratories) were measured using
the ACL TOP 500 analyzer (Instrumentation Labora-
tory). D-dimer concentration was measured with an
immunofiltration assay (D-dimer Single Test) on the
NycoCard READER II (Medinor A/S, Brøndby, Den-
mark) found to give accurate results for canine sam-
ples.32 A plasma pool from 10 healthy dogs was used
as a reference for plasma-based coagulations assays.
The plasma pool was stored at −80°C for no longer
than 6 months. The remaining citrate tubes were
stored in an upright position in a tube rack at room
temperature (20–25°C) for 30 minutes for subsequent
TEG analyses.

TEG analysis

Analyses using native, TF-activated, and kaolin-
activated assays, respectively, with or without t-PA
added, were performed using a computerized throm-
boelastograph (TEG 5000 Haemostasis Analyzer,
Haemonetics Corporation, Braintree, MA, USA).
Blood samples were analyzed in duplicate for healthy
dogs resulting in a total of 12 TEG analyses per animal.
For the diseased dogs, single measurements were
performed.

The analyses were performed using preheated re-
calified TEG cups (37°C, 20 μL 0.2 M CaCl₂) prior to
addition of citrated whole blood (WB). For native anal-
ysis, 340 μL citrated WB was added directly to the
cups. For the TF-activated samples, citrated whole
blood was activated with a solution of human recombi-
nant TF (Innovin, Siemens Healthcare Diagnostics,
Deerfield, IL, USA) at a final dilution of 1:50,000, as
previously described.32 Kaolin activation was initiated
by adding 1 mL citrated WB to kaolin-primed tubes
(Haemonetics Corporation), and 340 μL of this mix-
ture was added to the TEG cups. TEG analyses were
run for 120 minutes at 37°C. Six TEG parameters were
collected in the present study: the reaction time (R),
the clot formation time (K), the angle (α) representing
the kinetics of fibrin production and cross-linking, the
maximum amplitude (MA) reflecting the maximal clot
strength, and the percent clot lysis detected at 30 minutes (Ly30) and 60 minutes (Ly60), respectively, after MA is reached and representing the fibrinolytic activity.

A pilot study was performed on WB samples from healthy dogs with the addition of t-PA (Single-chain recombinant tissue plasminogen activator 10 ng/mL, Bionordika A/S, Glostrup, Denmark). A sample of 5 µL t-PA was prediluted 1:10 in a 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) buffer with 2% bovine serum albumin (BSA). Samples of 2.5 µL, 5.0 µL, 7.5 µL, or 10 µL of the prediluted t-PA were mixed with 430 µL HEPES-BSA. An aliquot of 25 µL of this mixture was added to 400 µL citrated WB to give increasing final concentrations of t-PA (3.4 pg/µL, 6.7 pg/µL, 10.0 pg/µL and 13.4 pg/µL) in a total assay volume of 340 µL. Higher t-PA concentrations showed higher inhibitory effect on clotting activity and more rapid completion of fibrinolysis. A final concentration of 10.0 pg/µL t-PA resulted in consistent and maximal fibrinolysis without affecting TEG-parameters R, K, a, and MA when compared with TEG without addition of t-PA, and thus used for the study (data not shown).

For the TF-activated assay, 7.5 µL prediluted t-PA was mixed with 430 µL diluted TF, and 25 µL of the TF-t-PA mixture was added to 400 µL citrated WB. 340 µL of the TF-t-PA-WB mixture was added to the TEG cups yielding a final TF:WB ratio of 1:50.000. For the kaolin-activated assay, 7.5 µL prediluted t-PA was mixed with 430 µL HEPES-BSA buffer, and 62.5 µL of the prediluted t-PA was then mixed with the kaolin-WB mixture. 340 µL of the kaolin-t-PA-WB mixture was added to the TEG cups yielding a final TF:WB ratio of 1:50.000.

Table 1. Basic data including fibrinogen, D-dimers, platelet counts, and hematocrit in 16 control dogs and 20 diseased dogs predisposed to thrombosis.

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Breed</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Clinical Status/Diagnosis</th>
<th>Fibrinogen (g/L)</th>
<th>D-Dimer (mg/L)</th>
<th>Platelets (10⁹/L)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 16</td>
<td>Different breeds</td>
<td></td>
<td></td>
<td>Clinically healthy</td>
<td>Range 1–4</td>
<td>Range 0.0–0.5</td>
<td>Range 200–500</td>
<td>Range 39–59</td>
</tr>
<tr>
<td>1</td>
<td>German Shepherd</td>
<td>5</td>
<td>M</td>
<td>Acute myocarditis</td>
<td>8.02</td>
<td>0.1</td>
<td>99 (Aggregates)</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>French Bulldog</td>
<td>1</td>
<td>M</td>
<td>Obstructive foreign body intestine</td>
<td>6.76</td>
<td>NA</td>
<td>323</td>
<td>58</td>
</tr>
<tr>
<td>3</td>
<td>Island Sheepdog</td>
<td>12</td>
<td>F</td>
<td>Severe mastitis. SIRS</td>
<td>12.7</td>
<td>0.2</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>Mixed breed</td>
<td>NA</td>
<td>F</td>
<td>Severe inflammation paw. SIRS</td>
<td>11.6</td>
<td>0.2</td>
<td>463</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>Australian Kelpie</td>
<td>9</td>
<td>F</td>
<td>Pyometra. SIRS</td>
<td>3.84</td>
<td>0.8</td>
<td>394</td>
<td>45</td>
</tr>
<tr>
<td>6</td>
<td>Labrador Retriever</td>
<td>11</td>
<td>F</td>
<td>Pyometra. SIRS</td>
<td>6.81</td>
<td>0.3</td>
<td>589</td>
<td>58</td>
</tr>
<tr>
<td>7</td>
<td>Labrador Retriever</td>
<td>4</td>
<td>F</td>
<td>Acute mild pancreatitis</td>
<td>5.37</td>
<td>0.7</td>
<td>247</td>
<td>53</td>
</tr>
<tr>
<td>8</td>
<td>Mixed breed</td>
<td>6</td>
<td>F</td>
<td>Pneumonitis. SIRS.</td>
<td>4.92–5.42</td>
<td>0.3</td>
<td>219</td>
<td>58</td>
</tr>
<tr>
<td>9</td>
<td>Basenji</td>
<td>12</td>
<td>M</td>
<td>PLN</td>
<td>4.02</td>
<td>0.5</td>
<td>720</td>
<td>48</td>
</tr>
<tr>
<td>10</td>
<td>Bichon Havanais</td>
<td>3</td>
<td>M</td>
<td>LPE. PLE</td>
<td>NA</td>
<td>NA</td>
<td>696</td>
<td>46</td>
</tr>
<tr>
<td>11</td>
<td>Boxer</td>
<td>8</td>
<td>MC</td>
<td>Chronic LPE. PLE</td>
<td>9.14</td>
<td>0.2</td>
<td>309</td>
<td>52</td>
</tr>
<tr>
<td>12</td>
<td>Mixed breed</td>
<td>7</td>
<td>FS</td>
<td>Chronic necrotizing pancreatitis. Vasculitis. SIRS.</td>
<td>4.39</td>
<td>0.7</td>
<td>285</td>
<td>40</td>
</tr>
<tr>
<td>13</td>
<td>Kleiner Münsterländer</td>
<td>6</td>
<td>MC</td>
<td>PLN. Renal amyloidosis</td>
<td>10.88</td>
<td>NA</td>
<td>240</td>
<td>32</td>
</tr>
<tr>
<td>14</td>
<td>German Shepherd</td>
<td>9</td>
<td>FS</td>
<td>Splenic+hepatic hemangiosarcoma</td>
<td>0.8</td>
<td>4.1</td>
<td>55 (Aggregates)</td>
<td>18.5</td>
</tr>
<tr>
<td>15</td>
<td>Cairn Terrier</td>
<td>12</td>
<td>FS</td>
<td>Primary thrombocytosis</td>
<td>NA</td>
<td>0.7</td>
<td>1206</td>
<td>37</td>
</tr>
<tr>
<td>16</td>
<td>English Bulldog</td>
<td>1</td>
<td>MC</td>
<td>Leukemia</td>
<td>2.31</td>
<td>1.6</td>
<td>87 (No aggregates)</td>
<td>37</td>
</tr>
<tr>
<td>17</td>
<td>Cocker Spaniel</td>
<td>11</td>
<td>F</td>
<td>Mammmary carcinoma</td>
<td>3.22</td>
<td>NA</td>
<td>905</td>
<td>31</td>
</tr>
<tr>
<td>18</td>
<td>Bernese Mountain Dog</td>
<td>8</td>
<td>M</td>
<td>Primary histiocytic sarcoma</td>
<td>4.81</td>
<td>2.6</td>
<td>92 (No aggregates)</td>
<td>42</td>
</tr>
<tr>
<td>19</td>
<td>Danish-Swedish Farmdog</td>
<td>6</td>
<td>FS</td>
<td>Diabetic ketosis</td>
<td>4.51</td>
<td>&lt; 0.1</td>
<td>461</td>
<td>48</td>
</tr>
<tr>
<td>20</td>
<td>Labrador Retriever</td>
<td>6</td>
<td>M</td>
<td>Hepatic cirrhosis. SIRS</td>
<td>1.44</td>
<td>0.2</td>
<td>218</td>
<td>41</td>
</tr>
</tbody>
</table>

F indicates female, FS, female spayed, LPE, lymphocytic-plasmacytary enteritis, M, male, MC, male castrated, PLE, protein-losing enteropathy, PLN, protein-losing nephropathy, SIRS, systemic inflammatory response syndrome, NA, not analyzed.

The bold numbers indicate values outside the reference range.
was added to the TEG cups, yielding the final t-PA concentration of 10 pg/uL in the cup.

Statistical analysis

Descriptive statistics were used to summarize dog characteristics at UHCA admission as well as findings of the clinical examination, CBCs, and serum biochemical analyses for dogs with disease conditions predisposing to thrombosis. GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA) was used for statistical analyses. To determine distribution of the data, D’Agostino-Paerson normality test was applied. For normally distributed data, repeated measures ANOVA was used followed by a Bonferroni multiple comparison posttest. For nonparametric data, a Friedmann test was used followed by a Dunns posttest. We used a Mann–Whitney test for comparison of the 2 unmatched groups. Statistical significance was set at \( P < .05 \). Coefficients of variation for the 3 types of assays were determined for the samples from the healthy dogs, as these were measured in duplicate.

Results

Gender, breed, age, individual final diagnosis, fibrinogen, D-dimer, platelet count, and HCT of all dogs are listed in Table 1. The control group consisted of 5 male (4 castrated males, 1 intact male) and 15 female dogs (10 intact females, 5 spayed females) with a mean age of 4.2 years (range 1.1–7.1 years). Of the 20 control dogs that were initially recruited, 3 dogs were excluded due to abnormal serum biochemistry results, and one dog was excluded due to a urinary tract infection. In one dog, the age was unknown. In another dog, hematology results were missing. In 2 dogs, results of a routine coagulation profile were missing, and in another 2 dogs, D-dimers could not be analyzed due to lipemia. Urinalysis was conducted as part of the diagnostic procedure in 15 of the dogs. No sampled animals were retrospectively excluded from the study.

Figure 1. After the addition of tissue-plasminogen activator, percent clot lysis after 30 minutes (Ly30) was significantly lower in the diseased dogs compared with healthy dogs in all 3 assays, indicating a decreased fibrinolytic activity and potential predisposition to thrombosis. CK indicates Kaolin-activated assay; CN, citrated native-activated assay, TF, tissue-factor-activated assay. *** = \( P < .0001 \)

Table 2. Results of 3 different thromboelastography assays without addition of tissue-plasminogen activator with blood from control dogs and diseased dogs predisposed to thrombosis.

<table>
<thead>
<tr>
<th></th>
<th>Control Dogs</th>
<th></th>
<th>Diseased Dogs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CK</td>
<td>CN</td>
<td>TF</td>
<td>CK</td>
</tr>
<tr>
<td>R [minutes]</td>
<td>5.1 (3.3–7.9)</td>
<td>6.1 (3.9–11)</td>
<td>5.3 (3.7–7.6)</td>
<td>5 (3.3–7.7)</td>
</tr>
<tr>
<td>(CV: 8.6%)</td>
<td>(CV: 29.8%)</td>
<td>(CV: 4.9%)</td>
<td>(CV: 4.9%)</td>
<td>(CV: 4.9%)</td>
</tr>
<tr>
<td>K [minutes]</td>
<td>1.9 (1.2–2.8)</td>
<td>2.4 (1.7–7.7)</td>
<td>1.7 (1.4–2.8)</td>
<td>1.3 (0.8–5.5)</td>
</tr>
<tr>
<td>(CV: 20.4%)</td>
<td>(CV: 31.2%)</td>
<td>(CV: 11.5%)</td>
<td>(CV: 11.5%)</td>
<td>(CV: 11.5%)</td>
</tr>
<tr>
<td>Angle (deg)</td>
<td>63.1 (45.6–71.9)</td>
<td>54.1 (29.3–65.7)</td>
<td>62.7 (52.9–70)</td>
<td>69.9 (36.9–78.6)</td>
</tr>
<tr>
<td>(CV: 5.5%)</td>
<td>(CV: 10.1%)</td>
<td>(CV: 8.8%)</td>
<td>(CV: 8.8%)</td>
<td>(CV: 8.8%)</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>58.6 (48.5–66.7)</td>
<td>56.9 (42.5–65.8)</td>
<td>59 (48.8–67.6)</td>
<td>71.9 (36.8–83.9)</td>
</tr>
<tr>
<td>(CV: 7%)</td>
<td>(CV: 7%)</td>
<td>(CV: 2.3%)</td>
<td>(CV: 2.3%)</td>
<td>(CV: 2.3%)</td>
</tr>
<tr>
<td>Ly30 (%)</td>
<td>0 (0–5.7)</td>
<td>0 (0–4.8)</td>
<td>0 (0–6.7)</td>
<td>0 (0–4.8)</td>
</tr>
<tr>
<td>(CV: 133.7%)</td>
<td>(CV: 755.2%)</td>
<td>(CV: 147%)</td>
<td>(CV: 147%)</td>
<td>(CV: 147%)</td>
</tr>
<tr>
<td>Ly60 (%)</td>
<td>1.5 (0–14.3)</td>
<td>0.8 (0–11)</td>
<td>1.1 (0–12.8)</td>
<td>1.5 (0–9.6)</td>
</tr>
<tr>
<td>(CV: 74.6%)</td>
<td>(CV: 134.7%)</td>
<td>(CV: 67.4%)</td>
<td>(CV: 67.4%)</td>
<td>(CV: 67.4%)</td>
</tr>
</tbody>
</table>

Data are means (range) and CV in control dogs (duplicate assays). CK indicates Kaolin-activated assay; CN, citrated native-activated assay, TF, tissue-factor-activated.
Table 3. Thromboelastography results with addition of tissue plasminogen activator with blood of healthy control dogs and diseased dogs predisposed for thrombosis.

<table>
<thead>
<tr>
<th></th>
<th>Control Dogs</th>
<th>Diseased Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CK</td>
<td>CN</td>
</tr>
<tr>
<td>R (minutes)</td>
<td>5.2 (3.9–6.7)</td>
<td>6.3 (4.8–11.9)</td>
</tr>
<tr>
<td>(CV: 3.9%)</td>
<td>(CV: 30.1%)</td>
<td>(CV: 7%)</td>
</tr>
<tr>
<td>K (minutes)</td>
<td>2.5 (1.3–5)</td>
<td>3.7 (2.2–10.4)</td>
</tr>
<tr>
<td>(CV: 23.9%)</td>
<td>(CV: 31.6%)</td>
<td>(CV: 15%)</td>
</tr>
<tr>
<td>Angle (deg)</td>
<td>58.3 (35.8–70.6)</td>
<td>45.6 (18.3–59.2)</td>
</tr>
<tr>
<td>(CV: 5%)</td>
<td>(CV: 10.1%)</td>
<td>(CV: 9.3%)</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>49.8 (32.9–60.2)</td>
<td>45.5 (9.7–54.7)</td>
</tr>
<tr>
<td>Ly30 (%)</td>
<td>45.7 (18.1–73.3)</td>
<td>62.6 (27.7–88.3)</td>
</tr>
<tr>
<td>(CV: 7.6%)</td>
<td>(CV: 7%)</td>
<td>(CV: 10.2%)</td>
</tr>
<tr>
<td>Ly60 (%)</td>
<td>73 (42.9–87.1)</td>
<td>81.8 (58.4–93.7)</td>
</tr>
<tr>
<td>(CV: 7.2%)</td>
<td>(CV: 43%)</td>
<td>(CV: 12.1%)</td>
</tr>
</tbody>
</table>

Data are means (range) with CV in control dogs (duplicate assays).

CK indicates Kaolin-activated assay; CN, citrated native-activated assay, TF, tissue-factor-activated.

Comparisons of Ly30 and Ly60 values before and after addition of t-PA in both groups of dogs were performed using Friedmann test and Dunn’s post hoc tests. The Friedmann test showed highly significant differences in both healthy and diseased dogs (all P < .0001) when comparing the 3 different assays for Ly30 and Ly60, respectively. Dunn’s post hoc test showed that addition of t-PA significantly decreased Ly30 and Ly60 measurements (all P < .05) in both healthy and diseased dogs, regardless of assay type (Figure 1).

There were no significant correlations between fibrinogen, D-dimer, platelet count, hematocrit, and lysis parameters Ly30 and Ly60 before or after addition of t-PA (results not shown). TEG variables before and after addition of t-PA in both groups are reported in Tables 2 and 3 before and after addition of t-PA, respectively. There were no significant differences in lysis parameters among the 3 assays or between the 2 groups of dogs before addition of t-PA.

The results of the Mann–Whitney test showed that Ly30 and Ly60 were significantly different after addition of t-PA (all P < .05) between healthy and diseased dogs, regardless of the assay, but with the highest significance (P < .0001) in the native assay. Examples of a t-PA-modified TF-activated TEG tracing from a clinically healthy dog and a diseased dog with resistance to fibrinolysis are shown in Figure 2.

Threshold values for Ly30 and Ly60 for all 3 assays were defined as lysis values (%) below the lower end of the range of the healthy control group depending on the assay.

CVₐ values were only obtained for healthy dogs based on duplicate analyses and are reported in Tables 2 and 3 before and after addition of t-PA, respectively. CVₐ before addition of t-PA was lowest in the TF-activated assays for all measured parameters except Ly30 and highest in the native assays for all measured parameters except MA. After addition of t-PA, the kaolin-activated assays had the lowest analytical variation for all measured parameters except K and MA.

Discussion

The results of this study show that t-PA-modified TEG can be used to assess fibrinolysis in canine whole blood from both healthy dogs and dogs with diseases known to predispose to thrombosis. However, the diseased dogs were significantly more resistant to t-PA-induced fibrinolysis than the healthy control group.

The majority of dogs in the diseased group had lysis values significantly below the values obtained from healthy dogs. Although there was an overlap between some of the dogs in the diseased group and the healthy dogs, a substantial number of diseased dogs were clearly resistant to activation of fibrinolysis, independent of the assay used. Interestingly, dogs with fibrinolytic activity below the lower range of healthy dogs in all 3 assays after the addition of t-PA had all been diagnosed with either a systemic inflammatory condition or a protein-losing disorder. The lowest individual lysis variables were observed in dogs with PLN, necrotizing pancreatitis, SIRS and hepatic cirrhosis.
with SIRS due to pneumonitis, and mammary carcinoma, respectively.

As the majority of dogs with decreased fibrinolytic activity suffered from conditions associated with systemic inflammation, inhibition of fibrinolysis secondary to acute inflammation could be one of the major reasons for our findings. Acute inflammation activates systemic coagulation by TF-mediated thrombin generation, down-regulation of physiological anticoagulant mechanisms, and inhibition of fibrinolysis, resulting in enhanced fibrin formation and impaired fibrin degradation.33

Sepsis has previously been associated with reduced activity of endogenous anticoagulants in dogs, suggesting an imbalance of hemostasis with a tendency toward a procoagulant state in affected dogs.18 For instance, significantly increased thrombin-activatable fibrinolysis inhibitor (TAFI) activity was determined in dogs with bacterial sepsis.34

Figure 2. Examples of tissue-plasminogen-modified (t-PA) tissue-factor-activated thromboelastography tracings from (A) a clinically healthy dog where addition of 10.0 pg/μL t-PA resulted in a clearly visible activation of fibrinolysis; (B) from a diseased dog predisposed to thrombosis, where the resistance to fibrinolytic activity in spite of addition of 10.0 pg/μL t-PA is clearly visible. R indicates reaction time in minutes, and represents the time span from initiation until the first fibrin polymers are produced. The cut-off value is when the amplitude reaches 2 mm. K = clot formation time in minutes, measured from the end of R until an amplitude of 20 mm is reached, representing the speed of clot formation. A = angle in degrees, representing the kinetics of fibrin production and cross-linking. MA = maximum amplitude reached in millimeters, reflecting the maximal clot strength. Ly30 = clot lysis in percent at 30 minutes after MA is reached; Ly60:clot lysis in percent at 60 minutes after MA is reached.
Hemostatic abnormalities consistent with systemic hypercoagulability are common in dogs with PLE and PLN.\textsuperscript{11,35} PLN was associated with an increased risk of thromboembolism in 22.2\% of dogs in a case series of 137 dogs.\textsuperscript{36} The mechanisms leading to hypercoagulability and increased risk for thrombosis in dogs with protein-losing conditions are multifactorial; one of the proposed causes is enteric and renal loss of antithrombin (AT) as dogs with PLE and PLN have decreased AT levels.\textsuperscript{11,35} AT deficiency could lead to an increased amount of thrombin, resulting in hypercoagulability.\textsuperscript{11} Endothelial cell injury with secondary activation of the coagulation cascade and urinary loss of fibrinolytic and anticoagulant proteins are other possible causes.\textsuperscript{35}

We used and compared 3 different TEG assays in our study. For the native assay, recalcified citrated whole blood was used without an activator, and as such carrying a higher risk of analytical variation.\textsuperscript{32} In our study, the native assay had the highest analytical variation both with and without addition of t-PA. For the TF-activated assay, we used human recombinant TF as an activator to initiate coagulation through the extrinsic pathway in a concentration validated earlier.\textsuperscript{32} The TF-activated assay had the lowest CV values without addition of t-PA. Activation with kaolin initiates coagulation via the intrinsic pathway. The kaolin-activated assay had the lowest analytical variation after addition of t-PA. All 3 assays can be used for activation of fibrinolysis with the addition of t-PA. However, the results cannot be used interchangeably as the activated assays produced the least variable and most reproducible results, while the unactivated citrated native assay showed high analytical variation as seen in other canine studies.\textsuperscript{11,32} A study of 3 different TEG assays (native, TF, kaolin) on WB from clinically healthy cats confirmed the native assay having the highest CV and the kaolin assay having the lowest CV.\textsuperscript{37} In human samples, a CV between 4\% and 14\% with no significant differences between TF- and kaolin-activated TEG assays was described.\textsuperscript{38} The large analytical variation in the native assay compared with the activated assays can be explained by the absence of a standardized amount of activator as initiation of coagulation in the native assay occurs spontaneously.\textsuperscript{37}

As all TEG parameters were significantly affected in all 3 assays by the addition of t-PA in the healthy control group, standard TEG analyses cannot be replaced by t-PA-modified TEG to evaluate individual hemostatic abnormalities besides fibrinolysis.

A limitation of this study was the small number of dogs with diseases predisposing to thrombosis. Further evaluation of fibrinolysis should include a larger group of dogs, with different disease categories to study and compare the levels of t-PA-activated fibrinolysis and their potential correlation with development of thrombosis. In addition, measurement of downregulators of fibrinolysis may be useful in evaluating the cause for the decreased fibrinolytic response in dogs with diseases predisposing to thrombosis.

Addition of t-PA to TEG can be used as a tool for the assessment of endogenous fibrinolytic potential in healthy dogs and dogs with diseases associated with thrombosis. Furthermore, many dogs suffering from diseases associated with thrombosis are more resistant to t-PA than healthy controls. Dogs with the highest resistance to t-PA in the present study were either suffering from systemic inflammation or a protein-losing disease. Thus, t-PA-modified TEG may be a promising tool for the detection of decreased endogenous fibrinolytic potential or resistance to fibrinolysis.

Activated TEG assays appear to be less variable and more reproducible compared with the native assay in this study and therefore, activated assays are recommended for future studies.

The clinical consequences of decreased fibrinolytic activity are currently not known. Further investigation of decreased fibrinolytic activity in dogs may help elucidate the complex pathophysiological mechanisms leading to hemostatic dysfunction in disease and the potential development of thrombosis in dogs.

**Acknowledgment**

The authors thank Annette U. Martinsen for excellent technical assistance.

**Disclosure:** The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

**References**


32. Wiinberg B, Jensen AL, Rojkjaer R, Johansson P, Kjelgaard-Hansen M, Kristensen AT. Validation of


