Thromboelastographic changes after gonadectomy in retired racing greyhounds

P. Vilar Saavedra, N. Stingle, C. Iazbik, L. Marín, M. A. McLoughlin, Y. Xie, G. Couto

Twenty-one healthy greyhounds with no history or clinical signs of bleeding disorders, and no abnormalities on physical examination, complete blood count, serum biochemistry profiles (in dogs more than five years of age), and SNAP-4DX test for vector borne diseases underwent routine gonadectomies at the Ohio State University Veterinary Teaching Hospital. Blood samples were collected 24 hours before and after surgery by jugular venepuncture for thromboelastography and haemostasis assays (prothrombin time [PT], activated partial thromboplastin time [aPTT], fibrinogen concentration). The magnitude of the bleeding in each patient was estimated using a bleeding scoring system recently validated in greyhounds. Eight dogs were classified as bleeders and 13 as non-bleeders. Thromboelastograph (TEG) tracings in bleeders were different to that of non-bleeders. Neither sex (odds ratio [OR]: 0.148, P=0.05), haematocrit (OR: 0.907, P=0.39), platelet count (OR: 0.996, P=0.65) or age (OR: 0.949, P=0.83) were predictors of the outcome. None of the variables that evaluated clot kinetics, and fibrinolysis (that is, aPTT OR: 0.781, P=0.51; PT OR: 1.337, P=0.63; TEG<sub>MA</sub> OR: 1.269, P=0.06; TEG<sub>LY60</sub> OR: 1.696, P=0.05; TEG<sub>LY60</sub> OR: 1.028, P=0.81) were able to predict the bleeding episodes. Only the TEG variables that represent the fibrin cross-linking of the clot (TEG<sub>MA</sub> OR: 0.903, P=0.03) and the strength of the clot (TEG<sub>LY60</sub> OR: 0.833, P=0.03) were considered predictors of the outcome.

PERIOPERATIVE haemorrhagic complications can be classified as surgical and/or non-surgical. Surgical bleeding occurs at the surgical site and results in protracted haemorrhage due to a faulty technique (for example, suturing, vessel tearing). Non-surgical bleeding reflects a failure in systemic haemostasis and is associated with oozing at the surgical site, ecchymoses, petechiae or bruising distant from the site (Adams and others 2007). The physiological cascade of events in response to the surgical injury and local inflammation results from an extensive cross-talk between the inflammatory and coagulation systems (Esmon 2004). An important cellular role in this interaction can determine the balance between bleeding and thrombosis as proposed in the cell-based model of haemostasis (Esmon 2004, Levi and van der Poll 2005, Adams and others 2007, Hoffman and Monroe 2007, Levi and others 2008). This cascade of events starts as a physiological response to a hyperadrenergic state associated with surgical stress; inflammation then triggers activation of coagulation causing the release and exposure of tissue factor (TF) from endothelial disruption and circulating cells such as monocytes. This cascade of events is basically mediated by inflammatory cytokines (that is, interleukin 6) that trigger fibrin formation, downregulate anticoagulant pathways (that is, antithrombin, protein C and TF pathway inhibitor), and increase plasminogen activation with a delayed but sustained increase in plasminogen activator inhibitor (PAI-1) concentration (Levi and van der Poll 2005, Adams and others 2007, Bateman 2009). While perioperative non-surgical bleeding complications have been well described in human beings in association with specific surgical procedures (that is, cardiac bypass, liver transplant) as well as in trauma cases, few reports have been published in veterinary medicine (Sato and others 1991, Lynn and others 2002, Paparella and others 2004, Lara-Garcia and others 2008). It has been recently demonstrated that 26 per cent of retired racing greyhounds (RRG) had excessive haemorrhage 24 hours to 48 hours after routine gonadectomy. This differs from other breeds of dogs, where the prevalence of bleeding after ovariohysterectomy (OHE) or orchietomy ranges from 0 per cent to 2 per cent (Berzon 1979, Pollari and others 1996, Burrow and others 2005, Lara-Garcia and others 2008). This high prevalence of delayed bleeding after surgical procedures in RRG has been also observed in a recent pilot study at the authors’ institution where 10 of 28 (36 per cent) greyhounds that underwent limb amputation for bone cancer had postoperative haemorrhage severely enough to require transfusion of blood components (Marin and others 2007).

The bleeding in RRGs typically begins at the surgical site, and in some dogs, it may progress to a generalised bleeding disorder associated with profound generalised bruising, mild thrombocytopenia, haemolysis and increases in liver and muscle enzyme activities (Lara-Garcia and others 2008). This syndrome resembles HELLP (haemolysis,
TABLE 1: Bleeding score system used to classify the bleeding episodes postgonaectomy

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No new bleeding</td>
</tr>
<tr>
<td>1</td>
<td>Questionable new petechiae or bruising</td>
</tr>
<tr>
<td>2</td>
<td>New cutaneous and/or mucosal haemorrhagic lesions</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe cutaneous or mucosal bleeding without measurable decline in haematocrit (HCT)</td>
</tr>
<tr>
<td>4</td>
<td>Severe external bleeding of sufficient magnitude to decrease HCT by &gt;6% points</td>
</tr>
</tbody>
</table>

Blood samples were collected by jugular venepuncture using a 21 G needle and a 3 ml syringe, then placed into a 2.7 ml Vacutainer tube (2.7 ml, 3.2 per cent buffered sodium citrate Vacutainer BD) mixed gently and stored at room temperature in a tube rack. TEG tube (2.7 ml, 3.2 per cent buffered sodium citrate Vacutainer BD) was used. The purpose of the present study was to evaluate perioperative haemostatic features in RRG using the TEG, in order to characterise the properties of the clot, and determine whether variables could be identified that would predict bleeding in greyhounds undergoing surgical procedures.

Materials and methods

Twenty-one healthy RRGs with no history or clinical signs of bleeding disorders, and no abnormalities on physical examination (PE), complete blood count (CBC), serum biochemistry profiles (in dogs more than five years of age), and SNAP (SNAP-4DX, IDEXX Laboratories) test for common vector borne diseases (that is, Anaplasma phagocytophilum, Ehrlichia canis, Dirofilaria immitis) were evaluated. The dogs included in the study were part of a third-year veterinary students’ neuter clinic; the operative practice laboratory has a current animal use protocol on file, the study was approved by the institutional review board, and informed consent for the adoption group was obtained.

Blood samples were collected by jugular venepuncture using a 21 G needle and a 3 ml syringe, then placed into a 2.7 ml Vacutainer tube (2.7 ml, 3.2 per cent buffered sodium citrate Vacutainer BD) mixed gently and stored at room temperature in a tube rack. TEG test were run approximately 30 to 45 minutes after sampling, then CBCs were performed with 0.8 ml of citrated blood in a haematology analyser (LaserCyte; IDEXX Laboratories), as previously reported (Morales and others 2007). The remnants of the samples collected into the citrated tubes were centrifuged (1380 g for 15 minutes) to obtain plasma for haemostasis assays (that is, prothrombin time [PT], activated partial thromboplastin time [aPTT], fibrinogen concentration) in a coagulation analyser (ACL-200 Automated Coagulation Laboratory; Instrumentation Laboratory). Plasma samples were stored at ~30°C and analysed no later than one month after collection.

The preanaesthetic protocol consisted of 0.05 mg/kg buprenorphine (Buprenorphine HCl, Bedford Laboratories) and 0.05 mg/kg acepromazine (Aceproject; Butler Animal Health Supply) intramuscularly in addition to a prophylactic dose (22 mg/kg) of intravenous cefazolin sodium (Cephalzin sodium; Sandoz). Induction of general anaesthesia was accomplished with 5 mg/kg ketamine (Ketaset; Fort Dodge Animal Health) and 0.25 mg/kg diazepam (Diazepam; Hospira) intravenously and maintained using isoflurane (Ilosol; Vedco) in 100 per cent oxygen. Respiration was supported with intermittent positive-pressure ventilation and intraoperative fluid therapy with lactated Ringer’s solution (10 ml/kg/h intravenously) (Ringer Lactate Solution; Baxter Healthcare Corporation). Postoperative analgesia consisted of a single intramuscular injection of carprofen (4 mg/kg) (Rimadyl; Pfizer). All dogs were kept at the authors’ institution for a minimum of four days and underwent daily PEs.

Surgery consisted of a 3-clamp technique with a 10 to 15 cm midline incision through the skin and subcutaneous tissues, from 1 cm caudal to the umbilicus to the level of the most caudally located mammary glands; and a three-clamp technique with small prescrotal incision through the skin and subcutaneous tissues for closed castration. All the surgeries were done under direct supervision of an ACVS Diplomate surgeon.

The magnitude of the bleeding in each animal was estimated immediately after surgery, 24 hours and 48 hours after surgery using a bleeding scoring system adapted from that proposed by Buchanan and Adix for children with idiopathic thrombocytopenic purpura and recently evaluated in greyhounds (Buchanan and Adix 2002, Lara-Garcia and others 2008). Greyhounds with a bleeding score of greater than or equal to 2 at 24 to 48 hours after surgery were designated as bleeders, whereas those with a bleeding score of less than 2 at 24 to 48 hours after surgery were designated as non-bleeders. The bleeding score system is shown in Table 1.

FIG 1: Postoperative bleeding in a greyhound 24 hours after surgery

The TEG parameters routinely evaluated include: the TEGmax, which is the time from addition of the agonist (CaCl2) until the clot starts to form; TEGγ, represents a measure of the speed to reach a certain level of clot strength; TEGref, is related to the fibrinogen concentration and the rapidity of fibrin build-up and cross-linking; TEGmax, is the maximum amplitude, or ultimate strength of the fibrin clot and the contribution of platelet aggregation to clot formation; and TEGγ represents the viscoelastic shear of the clot. Finally, TEGγref, represents the percentage or proportion of clot lysis (clot retraction or fibrinolysis), measured as the decrease in area under the TEG tracing, from the maximum amplitude at 60 minutes (Haemoscope 1995).
TABLE 2: Odds ratio (OR), 95% confidence interval (CI), P value, mean (sd) difference in values before and after gonadectomy, mean (sd), and female/male ratio (F:M) of the parameters evaluated as possible predictors of bleeding episodes in greyhounds

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR</th>
<th>CI</th>
<th>P</th>
<th>Mean (sd) difference</th>
<th>Mean (sd) F:M</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT (%)</td>
<td>0.907</td>
<td>0.724-1.136</td>
<td>0.39</td>
<td>0.097 (0.29)</td>
<td></td>
</tr>
<tr>
<td>PLT (%)</td>
<td>1.269</td>
<td>1.027-1.583</td>
<td>0.04</td>
<td>0.47 (0.72)</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>1.001</td>
<td>0.995-1.008</td>
<td>0.67</td>
<td>0.15 (0.39)</td>
<td></td>
</tr>
<tr>
<td>aPTT (seconds)</td>
<td>1.337</td>
<td>0.402-4.421</td>
<td>0.63</td>
<td>0.03 (0.75)</td>
<td></td>
</tr>
<tr>
<td>LY60 (%)</td>
<td>0.627</td>
<td>0.43-0.928</td>
<td>0.01</td>
<td>0.35 (0.63)</td>
<td></td>
</tr>
<tr>
<td>Sex (female v male)</td>
<td>0.625</td>
<td>0.465-0.841</td>
<td>0.01</td>
<td>0.23 (0.51)</td>
<td></td>
</tr>
</tbody>
</table>

Routine quality controls with normocoagulable and hypo-coagulable tracings (level I and level II) and e-test controls were run in each TEG channel eight hours before each sampling test in order to achieve the recommended quality assurance (Haemoscope 1995). A TEG test (single test) was performed by the same operator (PVS) to confirm that PT and aPTT are not predictors of bleeding in greyhounds. In a previous study involving postoperative TEG variables were expressed as a percentage of changes compared to baseline (‘positive’ [+] if they increased and ‘negative’ [−] if they decreased), there were virtually no changes for TEGG (median before v after surgery: 4.7 v 4.5 minutes), in the non-bleeders; however, there was a +43 per cent change in TEGG (5.7 v 2.1 minutes), a +20 per cent change in both TEGtime (49.5 v 60.5 degrees) and TEGmax (48.8 v 59.8 mm), and a +58 per cent change in TEGK (4771 v 7451 dyn/cm²), supporting an increase in clot strength. In contrast, in the bleeders, there was a +251 per cent prolongation in TEGG (2.9 v 10.2 minutes), and +62 per cent change in TEGK (3.2 v 5.2 minutes), a −27 per cent change in TEGtime (52.2 v 38.3 degrees), a +6 per cent change in TEGmax (42.5 v 81.6 mm) and +13 per cent change in TEGK (4719 v 5339 dyn/cm²), supporting slower clot kinetics. These results are shown in Fig 2 and Table 3.

**Discussion**

In human beings, approximately 55 per cent of postoperative complications are related to bleeding or thrombotic events; postoperative thromboembolism is more common than bleeding, and it likely relates to a transient hypercoagulable postoperative period. In addition, during the convalescent period, leg motion is limited in bedridden patients, who are at high risk of developing deep vein thrombosis (Siemens and others 1999, Kaplan and others 2002, Adams and others 2007). Postoperative haemostatic complications in dogs undergoing gonadectomy are common after surgery (Vipond and others 1990, Millis and others 1992, Lara-Garcia and others 2008). Increases in TEG time, platelet count and fibrinogen concentration and decreased fibrinolytic activity (that is, tissue plasminogen activator) are common after surgery in human beings. Fibrinogen is an acute phase protein that plays a key role in blood clotting; hyperfibrinogenaemia leading to hypercoagulability is associated with inflammation or stress induced by surgery (Vipond and others 1990, Millis and others 1992, Lara-Garcia and others 2008). Increases in TEGmax and TEGK observed in the non-bleeding greyhounds support the formation of a stronger clot, an expected physiological hypercoagulable response to surgery as shown in Fig 3 (Siemens and others 1999, Okamura and others 2007). Although the fibrinogen concentration was not a predictor of bleeding, TEGK has been shown to have an excellent correlation (TEGk r²= 0.940) with the functional fibrinogen concentration (Carroll and others 2005). Therefore, the total concentration of fibrinogen may not correlate as well as the TEGk value does with the amount of functional fibrinogen or with fibrin networking (that is, fibrin assembly). A previous study in dogs reported postoperative hyperfibrinolysis with a peak of fibrinolysis (that is, plasminogen concentration) 24 hours after surgery (Laneveschi and others 1996). However, in the present study the specific TEG variable that represents clot fibrinolysis (that is, TEGfibrinolysis) was not predictive of the outcome.

The use of NSAIDs and their effect on haemostasis could have had an impact in the present results. In a previous study involving dogs of various breeds, treatment with carprofen (4 mg/kg orally every 24 hours) resulted in prolonged TEGtime and decreased TEGfibrinolytic (Brainard and others 2007). The effect of carprofen on haemostasis and the individual response to this drug could partially

**When the changes in postoperative TEG variables were expressed as a percentage of changes compared to baseline (‘positive’ [+] if they increased and ‘negative’ [−] if they decreased), there were virtually no changes for TEGG (median before v after surgery: 4.7 v 4.5 minutes), in the non-bleeders; however, there was a +43 per cent change in TEGG (5.7 v 2.1 minutes), a +20 per cent change in both TEGtime (49.5 v 60.5 degrees) and TEGmax (48.8 v 59.8 mm), and a +58 per cent change in TEGK (4771 v 7451 dyn/cm²), supporting an increase in clot strength. In contrast, in the bleeders, there was a +251 per cent prolongation in TEGG (2.9 v 10.2 minutes), and +62 per cent change in TEGK (3.2 v 5.2 minutes), a −27 per cent change in TEGtime (52.2 v 38.3 degrees), a +6 per cent change in TEGmax (42.5 v 81.6 mm) and +13 per cent change in TEGK (4719 v 5339 dyn/cm²), supporting slower clot kinetics. These results are shown in Fig 2 and Table 3.**
explain the results observed here; however, both bleeders and non-bleeders received NSAIDs.

It has been reported that sedation using acepromazine (0.13 mg/kg) in dogs resulted in decreased platelet count and platelet aggregation, with no clinical signs of bleeding (Barr and others 1992). In the present study, acepromazine was used at a lower dose (0.05 mg/kg). Although unlikely, idiosyncratic spurious effects of acepromazine on platelet function, which could have led to postoperative bleeding, cannot be completely ruled out. As with the NSAIDs, both groups of dogs (that is, bleeders and non-bleeders) received acepromazine preoperatively.

Postoperative changes to the HCT due to blood loss and intravascular fluid administration could have affected haemorheology and altered clot formation. Haemodilution after intravenous fluid administration of crystalloids may result in a hypercoagulable state on TEG; however, this effect is short lived and usually resolves 15 minutes after finishing the infusion. Therefore, it is unlikely to persist 24 hours after surgery, the time frame in which the clot kinetic changes were detected (Ng and others 2002, Ruttmann and others 2006, Vilar and others 2008b). Again, both groups of dogs received similar fluid dosages and rates.

The present study was not designed to assess the confounding factor of sex (Roeloffzen and others 2010). There are several variables associated with sex that may have had an impact on the outcome. These factors are mainly related to the surgical procedure (for example, length and location of the incision) and a more constant methodology (for example, same experienced surgeon) would have been desirable.

A larger sample population will be necessary to determine the effect of some of these factors on bleeding in greyhounds. An additional limitation of the present study is that the TEGs were not performed in duplicate for logistical reasons (that is, only one TEG analyser was available). However, ongoing studies at the authors’ institution (data not published) revealed a coefficient of variation of less than 10 per cent in duplicate samples for all the TEG variables.

Based on these results, the authors postulate that the pathogenesis of this bleeding disorder occurs during the postoperative reactive phase of coagulation; clot formation depends on local cellular properties (that is, endothelial cells, leucocytes) and availability of clotting factors, among others. The local thrombin concentration at the site of injury changes during clot formation; therefore, defective thrombin generation patterns may result in abnormally structured clots that are associated with an increased risk of bleeding (Wolberg and Campbell 2000). The authors also propose that because this syndrome is due to defective cell/protein interaction, the conventional quantitative and/or plasma-based coagulation test (that is, aPTT, PT), platelet count and

### TABLE 3: TEG results (median [range]) for bleeders and non-bleeders before and after gonadectomy, and thromboelastograph (TEG) reference ranges for healthy greyhounds, and mean (sd) and range of routine haemostatic parameters (PT, aPTT, fibrinogen, platelet count and haemoglobin) for bleeders and non-bleeders before and after gonadectomy, and control plasma test group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>13 non-bleeders before surgery</th>
<th>13 non-bleeders after surgery at 24 hours</th>
<th>Eight bleeders before surgery</th>
<th>Eight bleeders after surgery at 24 hours</th>
<th>Reference range for greyhounds/plasma test</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (minutes)</td>
<td>4.7 (1.8-7.2)</td>
<td>4.5 (2.8-12.6)</td>
<td>2.9 (2.8-8.8)</td>
<td>10.2 (5.0-24.2)</td>
<td>(1.6-8.2)</td>
</tr>
<tr>
<td>K (minutes)</td>
<td>3.7 (2.2-5.8)</td>
<td>2.1 (1.5-8.3)</td>
<td>3.2 (1.8-4.7)</td>
<td>5.2 (2.1-11.8)</td>
<td>(1.1-7.1)</td>
</tr>
<tr>
<td>Angle (degrees)</td>
<td>49.5 (39.9-56.0)</td>
<td>60.5 (25.6-66.3)</td>
<td>52.2 (41.0-64.9)</td>
<td>38.3 (16.8-56.2)</td>
<td>(33.3-63.3)</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>48.8 (39.0-56.0)</td>
<td>59.8 (39.1-68.0)</td>
<td>48.5 (42.2-60.9)</td>
<td>51.6 (49.9-57.8)</td>
<td>(35.7-56.5)</td>
</tr>
<tr>
<td>G (dyn/cm²)</td>
<td>4771 (319.6-6358)</td>
<td>7451 (3217-10,614)</td>
<td>4719 (3653-7772)</td>
<td>5339 (3906-8837)</td>
<td>(2515-6227)</td>
</tr>
<tr>
<td>LY60 (%)</td>
<td>1.6 (0.0-19.2)</td>
<td>0.5 (0.0-4.7)</td>
<td>2.2 (0.0-5.8)</td>
<td>0.5 (0.0-1.7)</td>
<td>(0.0-4.4)</td>
</tr>
</tbody>
</table>

#### TEG Kinetics and Parameters

- **R (minutes)**: Time from the end of the R time to a 22 mm maximal amplitude on TEG trace deflection.
- **K (minutes)**: Kinetics time from the end of the R time to a 22 mm amplitude on TEG trace deflection.
- **Angle (degrees)**: Angle of the TEG trace at 22 mm amplitude.
- **MA (mm)**: Maximum amplitude of the TEG trace at 22 mm deflection.
- **G (dyn/cm²)**: Maximal shear stress measured at 22 mm amplitude on TEG trace deflection.
- **LY60 (%)**: Maximal lysis or percentage reduction in maximal amplitude of the TEG trace after 60 minutes.
- **PT (seconds)**: Prothrombin time.
- **aPTT (seconds)**: Activated partial thromboplastin time.
- **Fibrinogen (mg/dl)**: Fibrinogen level.
- **Platelets x 10⁹ (U/L)**: Platelet count.
- **Haemoglobin (g/dl)**: Haemoglobin level.

#### APTT and PT

- **PT**: Prothrombin time.
- **aPTT**: Activated partial thromboplastin time.

#### Fibrinogen

- **Fibrinogen (mg/dl)**: Fibrinogen level.

#### Platelet Count

- **Platelets x 10⁹ (U/L)**: Platelet count.

#### Haemoglobin

- **Haemoglobin (g/dl)**: Haemoglobin level.
fibrinogen concentration, are not affected. The TEG was the only diagnostic test (that is, TEG\textsubscript{platelet,Mg}\textsuperscript{2+}) that correlated with clinical signs of postoperative bleeding. Therefore, it may be a useful test to evaluate the perioperative risk of bleeding in greyhounds. The pathophysiological event of cell/protein interaction that results in weaker clots and a lack of response to the postoperative reactive phase of coagulation in bleeding greyhounds is currently unknown. Specific activators of coagulation (that is, TF, kaolin) can ameliorate the performances of the TEG and may also provide information about the pathway mostly involved in haemostatic defects (for example, cell-based TF/ factor VII-dependent pathway). Such coagulation activators might be used in future studies to try to identify predictors of bleeding in greyhounds undergoing surgery.

Acknowledgements
This study was supported by Fundación Pedro Barrie de la Maza-Becas de Posgrado (P. Vilar) and by Obra Social ‘La Caixa’ International Fellowship Program (P. Vilar), Spain and by the Savannah and Barry French Doodle Memorial Foundation.

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Veterinary Record 2011 169: 99 originally published online July 1, 2011
doi: 10.1136/vr.d2671

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