Hemostasis and thrombosis in the ICU

Robert Goggs
Lecturer, Emergency & Critical Care
Cornell University, College of Veterinary Medicine

IMTP

• Immune-mediated process results in premature destruction of thrombocytes

• IgG autoantibodies
  – Platelets or megakaryocytes
  – GpIIb/IIIa

• Extravascular removal
  – Mononuclear phagocytic system
  – FcR or complement mediated phagocytosis

• Antibody-mediated thrombocytopathy

A model of canine IMTP

• LeVine DN et al. JVIM
• Repeated injection of 2F9
  – Murine IgG2a monoclonal antibody to GpIIb

• All dogs developed thrombocytopenia within 2h
  – Median dose 63µg/kg (range 50.0-166.6µg/kg)
  – Pt counts 11-28 x10^3/µL
  – Platelet count remained <40 x10^3/µL for 24h
  – All dogs developed clinical bleeding

• No systemic inflammation generated
• 58 dogs with thrombocytopenia
• Dogs with <20x10^3/µL
• 36/55 diagnostic BM aspirates had <20x10^3/µL
• No definitive diagnosis made via BM evaluation
• BM evaluation unlikely to provide specific diagnostic or prognostic information in severe thrombocytopenia

• <24h post-admission, d1 after plt >40, then d4, d7, d14
• All dogs had hypocoagulable TEG tracings on admission
• All dogs developed hypercoagulable TEG tracings

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Initial</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Thrombocythemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEG Tracings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

• 24 dogs with primary IMTP
• All Tx with prednisone 1.5-2mg/kg
• 12 dogs treated with vincristine 0.02mg/kg
• Vinc Tx dogs had sig. faster plt recovery times – 4.9d ± 1.1 Vs 6.8d ± 4.5
• Vinc Tx sig. reduced duration of hospitalisation – 5.4d ± 0.3 Vs 7.3d ± 0.5
• No adverse effects noted
Platelet products

- Platelet rich plasma
- Platelet concentrate
- DMSO frozen platelets
- (Lyophilized platelets)
Any other options?

- Ralph A et al. JVECC 2013; 23(S1):S16
- 10 non-coagulopathic anemic, bleeding IMTP dogs
- 10-30mg/kg/hr ACA / NaCl placebo until Plt >30x10^3/µL
- No sig. difference in median pRBCs or Tx time

TAC / ACOT-S

Severe trauma

SAC

Death

Recovery

No complication

Death

Recovery

Severe trauma

SAC
Acute Coagulopathy of Trauma

- Tissue Trauma
- Hypoperfusion
- Hypothermia
- Sepsis
- Hemorrhage
- Injury

Coagulopathy and hemorrhage occur commonly in human patients with severe acute trauma. A prospective multicenter evaluation of coagulopathy in severely traumatized canine patients was conducted at the Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA.

The authors declare no conflict of interest.

This work was conducted at the Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA.

Keywords: Animals, Hypoperfusion, Acute traumatic coagulopathy, Hemorrhage, Coagulopathy, Dogs.
Platelet dysfunction in trauma

- Lynch A et al. JVECC 2013; 23(S1):S8-9
- 5 dogs bled to 80% and 60% normal blood volume
- Sig. changes in PCV/TS, PT, aPTT, Fg, TEG R & MA
- Plt count did not sig. alter
- Sig. decrease in AA- and ADP-induced WB agregometry AUC

Hyperfibrinolysis in trauma

- Because strategies exhibit low clot firmness and poor fibrin polymerization than
- that on admission, severely injured patients more frequently
- 5 dogs bled to 80% and 60% normal blood volume
- Prothrombin fragments (F1) ≥ 15 or Glasgow Coma Score

Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant hemorrhage (CRASH-2): a randomised, placebo-controlled trial

- 20,211 trauma patients
- TXA or placebo within 8h
- All-cause mortality:
  - 14.5% vs. 16.05
  - RR 0.91 (0.85–0.97; p=0.004)
- Death due to bleeding:
  - 4.95% vs. 5.21
  - RR 0.85 (0.78–0.96; p=0.008)
Tranexamic acid in dogs

- Blackstock K et al. JVECC 2011; 21(S1):S10
- Plasma samples spiked with:
  - EACA to 80-500µg/mL
  - TXA to 0.25-15µg/mL
- Hyperfibrinolysis simulated with tPA in Kaolin-TEG
- Therapeutic serum concentrations estimated
  - Minimum required for 0% lysis at 30min
- Minimum serum concentrations:
  - EACA 450 µg/mL
  - TEA 12.5 µg/mL
- Dogs may require higher doses of EACA and TXA cf people

Tranexamic acid in dogs

- Kelmer E et al. JVECC 2011; 21(S1):S7
- Retrospective of dogs receiving IV TXA for bleeding
  - 68 study dogs and 62 controls (equivalent baselines)
  - Median Tx 2d (1-21), mean dose 8.0 ± 2.2 mg/kg
- No difference in total product usage or volume
  - 1.9 Vs 2.2 units/dog p=0.952
  - 27.2 Vs 29.6 mL/kg p=0.34

- Kelmer E et al. JVECC 2013; 23(S1):S8
  - 2 dogs administered TX 15-20 mg/kg slow IV bolus vomiting
  - 9 dogs administered TX 10 mg/kg slow IV bolus then 10 mg/kg/hr CR
- TXA sig. decreased PT, Hct, R-time, alpha angle
- TXA sig. increased TEG LY30 (unexpected)

Hemorrhagic shock resuscitation

- Current human medical practice suggests:
  - Damage control resuscitation
  - Early delivery of blood products
  - 1:1:1:1 ratios?
  - +/- TXA, rFVIIa, Fg concentrates
  - Point of care coagulation testing (TEG / ROTEM)
  - Massive transfusion protocols
TEG guided therapy in trauma

- 51-year-old male, helmeted motorcycle crash victim
- FAST - massive abdo bleeding
- Initial r-TEG shows prolonged ACT with estimated 60% lysis
- ?Consumptive coagulopathy
- Tx with large volume transfusion

- Estimated 72.7% lysis
- Products given inc:
  - 23 units PRBCs
  - 14 units FFP
  - 2 units cryoprecipitate
  - 2 units platelets

- Repeated r-TEGs provided confidence that the patient needed antifibrinolics
- 5 g of Amicar (EACA) given
- Complete reversal of fibrinolysis

Options for veterinary patients?
Liver failure

Hemostatic dysfunction liver disease

Factor deficiencies

• Chronic hepatitis
  – 1/3 dogs with CH have PT / aPTT prolongations
  – Decreased concentrations of FII, FV, FVII, FIX, FX, and FXII
  – Spontaneous bleeding rare except in advanced disease

• Acute liver disease
  – Prolonged clotting times
  – Decreased FVII, FVIII, FX and increased vWF
  – Synthetic failure complicated by DIC and thrombocytopenia

• Congenital portosystemic shunts
  – Mild prolongation of aPTT reported in some studies
  – Decreased thrombin, FV, FVII, and FX also documented
  – Bleeding rare (<5%)
Vitamin K deficiency

- Decreased bile flow
- Anorexia
- Altered GI flora

- Canine biliary disease
  - Up to 50% have PT / aPTT prolongations
  - Perioperative bleeding tendencies uncommon

- Feline biliary disease
  - Alterations in coagulation profile in 50-75%
  - Most have Vitamin K dependent coagulopathy
  - Bleeding in ~20-50% cases depending on underlying disease
  - Cause largely unknown - GI bleeding

Coagulation in hepatobiliary disease

Figure 1: Guidelines for the management of patients with hepatobiliary disease and coagulation disturbances.

PT, prothrombin time; aPTT, activated partial thromboplastin time; VK1, vitamin K1; FFP, fresh frozen plasma; PCV, packed cell volume; PLN, protein losing nephropathy; PLN, protein losing enteropathy; IMHA, immune-mediated hemolytic anemia; DIC, disseminated intravascular coagulation; Tx, treatment.

Heparin and in some reports these treatments have led to resolution of the PVT. Even in people, treatment recommendations for PVT are controversial. Recent consensus statement from the American Association for the Study of Liver Diseases recommended that patients with acute PVT (i.e., PVT diagnosed in the setting of abdominal pain) receive treatment with anticoagulants (e.g., warfarin or heparin) for at least 6 months. These recommendations are based on the observation that 50% of patients will recanalize the portal vein if anticoagulant therapy is initiated promptly, while untreated patients rarely spontaneously recanalize. The indication for treatment of chronic PVT with anticoagulants is less clear, as studies show little benefit in thrombus resolution with treatment. In addition, many human patients with hepatic disease that develop PVT are cirrhotic with preexisting portal hypertension, and the use of anticoagulants may increase the risk for hemorrhage from gastroesophageal varices. However, a recent retrospective evaluation of patients with chronic PVT showed improved survival with the use of warfarin, with no increase in clinically significant gastrointestinal hemorrhage. Platelet inhibitors have not yet been evaluated in veterinary patients with liver disease and studies are needed in this area. Further studies are also needed to determine in veterinary patients with hepatobiliary disease are at the highest risk for thrombosis and which prophylactic interventions are appropriate.

As a clearer understanding of the role of parenchymal extinction in hepatic disease emerges in the coming years, therapy aimed at hypercoagulability may become a target for slowing the progression of liver disease.

Conclusion

Understanding and evaluating the coagulation status of patients with hepatobiliary disease is a challenge due to the liver’s dual role in the synthesis and removal of pro- and anticoagulant factors. With hepatobiliary disease antagonistic alterations may occur in platelet number and function, coagulation factors, fibrinolysis, and vascular endothelial function. Currently available coagulation tests have limitations in assessing coagulation since they fail to account for all the abnormalities occurring in vivo. New diagnostic modalities, such as TEG, may help to better elucidate the true risks of bleeding with invasive procedures.
Updates on plasma storage / stability

Comparative stability of canine and feline hemostatic proteins in freeze-thaw-cycled fresh frozen plasma

Pagé S, McElroy, DVM, Mathew W, Red, DVM, DACVIM; L. Art Infreda, VMD, DACVIM; Lee G, Keegstra, DVM, DACVIM; Holzey S, Brooks, DVM, DACVIM; Arne S, Hult, DVM and Allen Tan, VMD

Conglutination Factor and Hemostatic Protein Content of Canine Plasma after Storage of Whole Blood at Ambient Temperature


Arterial thromboembolism
**Evaluation of coagulation markers in the plasma of healthy cats and cats with asymptomatic hypertrophic cardiomyopathy**

- TAT complexes, D-dimers, FDPs, PT, aPTT, AT
- No sig. difference between groups for:
  - TAT complexes, D-dimers, FDPs
- AT activity sig. lower in HCM cats
- Hypercoagulability in 45% HCM cats

**Brief Communication**

*Platelet function in clinically healthy cats and cats with hypertrophic cardiomyopathy: analysis using the Platelet Function Analyzer-100*

- 42 clinically healthy cats and 30 cats with HCM
- Median CT of clinically healthy cats was 64 s (43–176)
- Median CT of cats with HCM was 74 s (48–197)
- No sig. difference between normal and HCM cats
- No sig. differences between mild - mod - severe HCM
“Despite the willingness of cardiologists to adopt treatment strategies for their feline HCM patients, we could find virtually no clinically relevant literature to support these decisions.”

GPIIb/IIIa inhibition
Equipoise!

- Bright JM et al. Vet Ther 2003; 4:35-46
- Magee et al. AJVR 2014; 75:309-312
- Abciximab significantly reduced in vivo feline arterial thrombus formation
- Abciximab did not significantly inhibit ADP or TRAP induced feline platelet aggregation

FATCat

- Multi-centre, double-blinded, prospective study
- Cats that experienced a CATE event and survived 1-3 months were eligible for enrollment
- Aspirin (81 mg PO E3d) / Clopidogrel (18.75 mg PO SID)
- 1^y end-point
  - Clinically evident embolic event
- 2^y end-points
  - All-cause mortality, cardiac death, ADR
• 72 cats enrolled (36 cats in each study group)
• Randomization adequate WRT: age, weight, gender, underlying cardiac disease, and ATE recovery time

<table>
<thead>
<tr>
<th>Study period</th>
<th>Endpoint</th>
<th>Aspirin (d)</th>
<th>Clopidogrel (d)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>First 12 months</td>
<td>ATE recurrence</td>
<td>192</td>
<td>&gt;365</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>ATE or cardiac death</td>
<td>128</td>
<td>346</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>All cause mortality</td>
<td>116</td>
<td>248</td>
<td>0.051</td>
</tr>
<tr>
<td>Whole study</td>
<td>ATE recurrence</td>
<td>192</td>
<td>443</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>ATE or cardiac death</td>
<td>128</td>
<td>346</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>All cause mortality</td>
<td>116</td>
<td>248</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Arterial Thromboembolism in 250 Cats in General Practice: 2004–2012
K. Bregant, J. Wright, O. Garvel, J.R. Papan, and V.L. Fauten

• 61.2% cats (n=153) euthanized at presentation
• 27.2% cats (n=68) survived >24h
• 12.0% cats (n=30) survived >7d
• MST 94d (95% CI, 42–164) (if cat alive at d7)
• 2.4% cats (n=6) alive at 1y
Rivaroxaban (Oral DTI)

**Cats**
- Dixon-Jimenez A et al. JVECC 2013; 23(S1):S12-3
- 0.7-1.3 mg/kg
- Samples at T=0, 3, 8, 24h
- Dose dependent effect
- Peak effects vs PT, aPTT at 3h
- Anti-Xa persisted >24h
- No bleeding noted

**Dogs**
- 8 dogs: Placebo or 2 mg/kg rivaroxaban SID or BID
- Rivaroxaban significantly affected PT, aPTT, CAT, AXA and TEG
- Difference between SID Vs BID noted with PT and CAT
- Rivaroxaban BID maintains 24h anticoagulant efficacy

Pulmonary thromboembolism

- Identifying risk factors
- Quantifying consequences
- Definitive thrombus imaging

Risk factors for PTE

<table>
<thead>
<tr>
<th>Disease process / Risk factor</th>
<th>Hypercoagulable state</th>
<th>Flow abnormalities</th>
<th>Endothelial dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIC</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIC</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypercortisolemia</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMHA</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indwelling catheters</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Neoplasm</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTE / PNL</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Septic</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Trauma</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Pulmonary Thromboembolism in 29 Dogs: 1985–1995
Lynelle R. Johnson, Michael R. Lappin, and Dale C. Baker

- PME confirmed PTE in 29 dogs
- No pathognomonic clin-path or radiographic findings
- PTE suspected antemortem in 11/29 (38%) dogs
- PTE was only a differential in 11/17 dogs despite compatible clinical signs
- All had diseases previously associated with PTE

- Arterial blood gases measured in 15/29 (52%) dogs:
  - 12/15 exhibited hypoxaemia
  - 15/15 had increased A-a gradient
- Response to supplemental O₂ was variable
- At PME:
  - 86% dogs had grossly visible emboli
  - 59% dogs had multiple disease processes
  - 55% dogs had additional pulm. pathology

Identifying risk factors
Biomarkers of PTE

- A-a gradient
- $\text{PaO}_2$:$\text{PaCO}_2$
- $\text{PaCO}_2$:ETCO$_2$
- cTn
- BNP
- FDPs
- D-dimers
- TAT complexes
- P-selectin
- PLAs
- Protein C
- TFPI

Identifying consequences
Comparison of computed tomography pulmonary angiography and point-of-care tests for pulmonary thromboembolism diagnosis in dogs

R. Goggs, D. L. Chan, L. Benigni, C. Hirst, L. Kellett-Gregory and V. L. Fuentes
Department of Clinical Science and Services, Royal Veterinary College, North Mymms, AL9 7TA
R. Goggs’s current address is Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, 14853, USA
C. Hirst’s current address is Fitzpatrick Referrals, Halfway Lane, Godalming, Surrey, GU7 2QQ

OBJECTIVES: To evaluate the feasibility of CT pulmonary angiography for identification of naturally occurring pulmonary thromboembolism in dogs using predefined diagnostic criteria and to assess the ability of echocardiography, cardiac troponins, D-dimers and kaolin-activated thromboelastography to predict the presence of pulmonary thromboembolism in dogs.

METHODS: Twelve dogs with immune-mediated haemolytic anaemia and evidence of respiratory distress were prospectively evaluated. Dogs were sedated immediately before CT pulmonary angiography using intravenous butorphanol. Spiral CT pulmonary angiography was performed with a 16 detector-row CT scanner using a pressure injector to infuse contrast media through peripheral intravenous catheters. Pulmonary thromboembolism was diagnosed using predefined criteria. Contemporaneous tests included echocardiography, arterial blood gas analysis, kaolin-activated thromboelastography, D-dimers and cardiac troponins.

RESULTS: Based on predefined criteria, four dogs were classified as pulmonary thromboembolism positive, three dogs were suspected to have pulmonary thromboembolism and the remaining five dogs had negative scans. The four dogs identified with pulmonary thromboembolism all had discrete filling defects in main or lobar pulmonary arteries. None of the contemporaneous tests was discriminant for pulmonary thromboembolism diagnosis, although the small sample size was limiting.

CLINICAL SIGNIFICANCE: CT pulmonary angiography can be successfully performed in dogs under sedation, even in at-risk patients with respiratory distress and can both confirm and rule out pulmonary thromboembolism in dogs.

INTRODUCTION
Pulmonary thromboembolism (PTE) is the obstruction of the pulmonary artery or its branches by thrombi and is a major cause of morbidity and mortality in dogs with immune-mediated haemolytic anaemia (IMHA) (Reimer et al. 1999, Scott-Moncrieff et al. 2001). Dogs with IMHA are predisposed to PTE potentially because of an associated hypercoagulable state (Fenty et al. 2011, Goggs et al. 2012) that may result from increased intravascular tissue factor expression secondary to the marked inflammatory response that accompanies the disease (Piek et al. 2011, Kidd & Mackman 2013). There is also evidence of platelet activation in
Characterisation of changes in the haemostasis system in dogs with thrombosis

OBJECTIVE:
To prospectively assess (2008 to 2009) all phases of coagulation including thromboelastography in dogs with thrombosis to determine if thrombosis is associated with hypercoagulable states.

METHODS:
Coagulation reaction in dogs with thrombosis (n=7) diagnosed by diagnostic imaging or histopathology was compared with 56 control dogs. Dogs pretreated with antiplatelet and anticoagulation drugs were excluded. Thromboelastographic G-values >10·3 Kdyn/cm$^2$ were used to define a hypercoagulable state.

RESULTS:
Compared with the controls, there was a significantly higher mean platelet component indicating lower platelet activation status (17·99 ±1·36 versus 20·48 ±2·04 g/dL, P=0·0004), increased thromboelastographic G-value (6·4 ±1·6 versus 13·1 ±6·2 Kdyn/cm$^2$, P=0·0029), activated partial thromboplastin time, fibrinogen, D-dimers (all: P<0·0001) as well as decreased antithrombin (P=0·0049), factor VIII (P<0·0001), protein C (P<0·0001), protein S (P=0·0373) and activated protein C-ratio (P=0·0013). On the basis of thromboelastographic G-value, three of six thromboelastographic tracings were classified as normocoagulable and three as hypercoagulable.

CLINICAL RELEVANCE:
In dogs with thrombosis, both normo- and hypercoagulable states are present and activated protein C resistance is common.

INTRODUCTION
Characteristics of thromboembolic disease are of growing interest in both human and veterinary medicine. Three factors – the so called Virchow's triad – may be responsible for the development of thrombosis including venous stasis, injury of the vascular endothelium and a hypercoagulable state (Favaloro & Lippi 2011). However, in dogs and humans a hypercoagulable state is difficult to detect during routine coagulation analysis. The routinely measured coagulation variables (coagulation times, fibrinogen, D-dimers) have been evaluated previously in dogs with thrombosis (Nelson & Andreasen 2003, Rosser 2009). To our knowledge all phases of the coagulation process have not been assessed to date.

Increased platelet function and activation status may contribute to thrombophilia (i.e. a prothrombotic state), but the measurement of platelet function and platelet activation status is more difficult to perform on a routine base. During each haematological examination, the ADVIA 120/2120 (Siemens Healthcare Diagnostics) laser-based haematology analyser, provides a variety of unique platelet morphology indices. In dogs, ADVIA 120/2120 platelet morphology indices have been rarely evaluated (Bauer et al. 2012, Moritz et al. 2003a,b). Previous investigations in dogs (Moritz et al. 2005) and humans (Chapman et al. 2003) demonstrated that one of these indices – the mean platelet component concentration (MPC) – can be used to assess platelet activation status because of its correlation with P-selectin.

The assessment of single coagulation variables has the disadvantage that only one phase of the haemostasis process is determined and it is difficult to detect hypercoagulable states. In contrast, thromboelastography (TEG) reflects the whole coagulation process and was used previously to detect thrombophilia in dogs with disseminated intravascular coagulation (DIC) (Wiinberg et al. 2009), neoplasia (Kristensen et al. 2008),

www.bsva.com
Identifying thrombi

TEG identification of non-overt DIC

15 dogs with primary IMHA
- Standardized IMHA therapy and CD UFH 150U/kg SC, n=7, or IAD UFH, n=8
- CD UFH q6h to d7, then q8
- IAD based on anti-Xa activity
- IAD daily to d7, then weekly to d28
- 7 IAD dogs alive at 180d
- 1 CD dog alive at 180d
- TE in 5/7 CD dogs Vs 2/8 IAD dogs
Was IAD actually effective?

PK / PD of LMWH (dalteparin)

- 150IU/kg q8h
- aPTT & TT not useful
- AT activity 102 → 91
- Plt count 317 → 281
- Hct 51 → 43

Take home messages…

- Consider vincristine / IVIG for IMTP
- Frozen platelets are a poor substitute for fresh platelets
- TAC definitely occurs in dogs, ACOT-S may too
- If TAC occurs, consider DCR, early transfusion and TXA
- Myriad hemostatic disorders accompany hepatobiliary disease
- Plasma products may be more stable than we thought
- No evidence-based acute therapy for FATE
- Current recommendation = clopidogrel +/- aspirin for FATE prevention
- ID at-risk patients to improve PTE detection
- CTPA is the future of PTE diagnosis
- TEG may aid DIC identification
- UFH more likely to be efficacious if dose is individualized