Coagulation Testing

Erik Zager
Cornell University
Outline

• Brief Coagulation Review
• Platelet Testing
  • Platelet Counts
  • Platelet Abnormalities
  • BMBT
• In Vitro Plasma Based Testing
  • PT
  • aPTT
• Indirect Testing
  • Fibrin Split Products
  • D-Dimers
  • Fibrinogen Concentration
  • Antithrombin
• In Vivo Whole Blood Based Testing
  • TEG and PlateletMapping
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  • TEG and Platelet Mapping
Coagulation Review

In Vivo

In Vitro
AMPLIFICATION

TF Bearing Cell

Ⅷa  Xa  Ⅱa

Ⅱa

Platelet

Activated Platelet
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Platelet Testing

- Manual platelet count
- Each platelet per high power field = ~20k platelet/uL
Platelet Testing

- Cats with platelet clumps likely have adequate platelets
  - 260-587k based on 9 cats in study

http://vetbook.org/wiki/cat/images/1/16/Thrombo03.jpg
Platelet Testing

• Automated counts flawed
  • Pseudothrombocytopenia in cats due to overlap of erythrocyte and platelet volumes
Platelet Testing

- Thrombocytopenia likely greatly overestimated in cats using automated counts
  - 71% of samples in 1 year <200k in auto vs 3.1% of manual counts (similar split for <50k)
Platelet Testing

- Macrothrombocytopenia
Platelet Testing

- Macrothrombocytopenia
  - Cavalier King Charles Spaniels, Cairn and Norfolk Terrier

*Idiopathic thrombocytopenia in Cavalier King Charles Spaniels*

MK SINGH and WA LAMB

Australian Veterinary Journal Volume 88, No 11, November 2005
Platelet Testing

- Macrothrombocytopenia
  - Has been described in:
    - Chihuahua, Labrador retriever, Poodle, English Toy Spaniel, Labradoodle, Shih Tzu, Maltese, Jack Russell, Havanese, Boxer, Cocker Spaniel, and Bichon Frise

- DNA Test available through Auburn University
Platelet Testing

- Macrothrombocytopenia
- CKCS have autosomal dominant version
- 30-50% of CKCS in USA

Figure 1. Blood smear from a CKCS with macrothrombocytopenia. An enlarged platelet (arrowhead), as large as a red blood cell; a platelet of normal size (arrow); and a neutrophil granulocyte are seen (1,000×; Hemacolor stain, Merck).

Comparison of manual and automated methods for determining platelet counts in dogs with macrothrombocytopenia

Lisbeth H. Olsen, Annemarie T. Kristensen, Karen Qvortrup, Henrik D. Pedersen
Platelet Testing

- Quantitative buffy coat analysis may be superior for Cavaliers
- Cavaliers can have counts as low as 25k/uL and be ‘normal’

IC = impedance cell counter, LC = laser cell counter, QBC = quantitative buffy coat analyser, ME = microscopic estimation
Platelet Testing

- Buccal Mucosal Bleeding Time
- Measurement of formation of clot after established cut size/depth made
- Normal is <4min for dog
- Cats less established range: ~ <2.5min

https://www.atdove.org/videos/Procedure/Buccal-Mucosal-Bleeding-Time-BMBT
Platelet Testing

- Significant interobserver and intraobserver differences
  - On average 2 minute!!
  - Normal is under 4 minutes
- “The current study indicated that on 95 per cent of occasions, any two readings within a dog may differ by up to ± 2 minutes within an observer and between two observers. A single reading was accurate to within ± 80 seconds.”
Platelet Testing

- Review article of human data
  - Significant overlap between normals bleeding times and abnormal patients and vice versa
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Prothrombin Time

- Citrated blood
- Ca++ and thromboplastin (often made from rabbit brains) added
- The thromboplastin activates factor VII to VIIa
- Measures extrinsic and common pathways
Prothrombin Time

- After mixing, blood is moved through tube within spectral analyzer
- Once blood’s mobility reduced to certain point, wavelength of light changed and reading given

Activated Partial Thromboplastin Time

- aPTT uses kaolin to activate
- Spectral analyzer is the same
- Measures intrinsic and common pathway
Does PT or aPTT predict anything?

**Hemostasis in Massively Transfused Trauma Patients**

Ann. Surg. • July 1979

R. B. COUNTS, C. HAISCH, T. L. SIMON, N. G. MAXWELL, D. M. HEIMBACH, C. J. CARRICO

### Table 4. Number of Patients Having Prolonged PT or PTT

<table>
<thead>
<tr>
<th>Patient Category</th>
<th>Prothrombin Time</th>
<th>PTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1.3 × Control</td>
<td>&gt;1.3 × Control</td>
</tr>
<tr>
<td>Generalized bleeding</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>No generalized bleeding</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>15</td>
</tr>
</tbody>
</table>

For patients with bleeding, the results of tests at time of bleeding are tabulated; for non-bleeding patients, the longest PT and PTT.
Does PT or aPTT predict anything?

How Well Does the Activated Partial Thromboplastin Time Predict Postoperative Hemorrhage?

Anthony L. Suchman, MD, Alvin I. Mushin, MD, ScM
JAMA, Aug 8, 1988—Vol 256, No. 6

Table 4.—Operating Characteristics of the APTT* Test in the Clinically Defined Subgroups†

<table>
<thead>
<tr>
<th>Test Characteristic</th>
<th>Clinically Defined Subgroup</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Risk</td>
<td>Combined High Risk</td>
<td>Known Coagulopathy</td>
<td>Potential Deficiency</td>
<td>Trauma/Hemorrhage</td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>7.7</td>
<td>57</td>
<td>100‡</td>
<td>67§</td>
<td>44</td>
</tr>
<tr>
<td>Narrow</td>
<td>86.7</td>
<td>67</td>
<td>18</td>
<td>56</td>
<td>78</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>11.9</td>
<td>60</td>
<td>84</td>
<td>76</td>
<td>41</td>
</tr>
<tr>
<td>Broad</td>
<td>88.3</td>
<td>78</td>
<td>20</td>
<td>70</td>
<td>83</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>0.56</td>
<td>1.74</td>
<td>1.22</td>
<td>1.52</td>
<td>1.99</td>
</tr>
<tr>
<td>Narrow</td>
<td>0.87</td>
<td>2.75</td>
<td>1.05</td>
<td>2.50</td>
<td>2.41</td>
</tr>
<tr>
<td>Broad</td>
<td>1.07</td>
<td>0.64</td>
<td>0</td>
<td>0.59</td>
<td>0.72</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>1.02</td>
<td>0.52</td>
<td>0.80</td>
<td>0.34</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*PT indicates activated partial thromboplastin time.
†See text for definitions of subgroups and descriptions of “narrow” and “broad” definitions of hemorrhage.
‡Estimate based on two cases.
§Estimate based on three cases.

Narrow

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT Elevated</td>
<td>9</td>
<td>338</td>
</tr>
<tr>
<td>APTT Not Elevated</td>
<td>18</td>
<td>1769</td>
</tr>
</tbody>
</table>

Broad

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT Elevated</td>
<td>102</td>
<td>245</td>
</tr>
<tr>
<td>APTT Not Elevated</td>
<td>359</td>
<td>1428</td>
</tr>
</tbody>
</table>

Sensitivity

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrow</td>
<td>33.3%</td>
<td>22.1%</td>
</tr>
<tr>
<td>Broad</td>
<td>83.9%</td>
<td>85.4%</td>
</tr>
</tbody>
</table>

Positive Likelihood Ratio

<table>
<thead>
<tr>
<th></th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrow</td>
<td>2.08</td>
<td>1.51</td>
</tr>
<tr>
<td>Broad</td>
<td>0.79</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Outcome Group | Criteria
---------------|------------------|
Definite hemorrhage | Discharge diagnosis code for postoperative hemorrhage, or secondary procedure code for control of postoperative bleeding
Statistically defined hemorrhage | In top 2% of distribution (for a given procedure) of blood transfused during five-day interval beginning on day of surgery
Procoagulant administered | Fresh-frozen plasma or other procoagulant administered during five-day interval beginning on day of surgery
No hemorrhage | Does not meet any of the above criteria
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D-Dimers

- Breakdown product of *crosslinked* fibrin meshwork
  - FDPs don’t need crosslinking
- Half-life is 5 hours
  - Indicates active or ongoing fibrinolysis
D-Dimers vs FDPs

• D-Dimers are more specific and sensitive than fibrin degradation products for DIC
  • Abstract from ACVIM 1998
  • Specificity and sensitivity of D-Dimers in DIC compared to FDPs
    • 94.7% and 76.5% for D-Dimers
    • 84.2% and 73.5% for FDPs
D-Dimers

- 3 groups of dogs
  - Normal dogs
  - Sick dogs (hepatic, cardiac, renal, neoplastic, postoperative)
  - Dogs with thromboembolic disease
- Dogs with PTE had significantly elevated D-Dimers, as did liver disease. Neoplasia trended towards elevated (had influence from hemoabdomens), others were not significantly different.
D-Dimers

- Sensitivity of D-dimer concentrations >500 ng/mL for predicting TE was 100%
  - Specificity was 70%
- Sensitivity at >1,000 ng/mL was 80%
  - Specificity of D-dimer to predict TE was 94%
- Sensitivity at >2,000 ng/mL was 35%
  - Specificity of D-dimer was 98.5%
- No dog in the TE group had abnormal FDPs

J Vet Intern Med 2003;17:830-834

The Utility of Plasma D-dimer to Identify Thromboembolic Disease in Dogs
O. Lynne Nelson and Claire Andreasen
D-Dimers in Cats

D-Dimers neither sensitive nor specific for cats with DIC or cardiomyopathy.

Figure 3. Concentration of D-dimer in ill cats and cats with cardiomyopathy with and without disseminated intravascular coagulation. Black horizontal line represents the lower limit of detection and the upper limit of the reference interval of the immunoturbidimetric D-dimer assay.

Evaluation of plasma antithrombin activity and D-dimer concentration in populations of healthy cats, clinically ill cats, and cats with cardiomyopathy.
Fibrinogen

• Positive acute phase protein
• In humans, relationship between fibrinogen levels and coronary disease and thromboembolic disease have been described
Fibrinogen

• SAECC: “Acquired disorders are described with hemodilution, massive transfusion, hepatic dysfunction, DIC and sepsis”

• Difficult to find supporting evidence in animals
Antithrombin

- Protein that plays a role in hemostatic regulation
- Loss can occur with disease such as PLN
- Recent study showed that hypercoagulability in PLN is not associated with AT levels
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Thromboelastography (TEG)

- Better representation of in vivo hemostasis
  - Run on blood with platelets and clotting factor
Thromboelastography (TEG)

- Consists of heated cup (37°C)
- Citrated whole blood
- Pin connected to torsion wire
- Spinning of cup (or pin) is translated to electric signal and tracing
TEG vs ROTEM

- TEG
  - Cup spins
  - Torsion wire converted to electric signal
- ROTEM
  - Pin spins
  - Optical monitor
- Most companion animal studies performed with TEG
TEG vs ROTEM

- TEG
  - Cup spins
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  - Optical monitor
- Most companion animal studies performed with TEG
Thromboelastography (TEG)

- Coagulation activated by
  - Kaolin
    - Intrinsic pathway
  - Tissue factor
    - Extrinsic
  - Both kaolin and tissue factor
    - (r-Teg)
Thromboelastography (TEG)

- Kaolin cups are commercially available, as are r-TEG with kaolin and TF
- rhTF (recombinant human TF) used for experimental studies
  - Not available commercially
  - TF activated TEG showing narrower standard deviations of normals and lower interoperator differences
Thromboelastography (TEG)

- R time = reaction time
  - Time to formation of initial fibrin
- K time = kinetic time
  - Time for fibrin cross linkage to reach 20mm (from 2mm)
- $\alpha$ Angle =
  - Angle from baseline to the slope of tracing represents clot formation

Explicative TEG tracing (modified from TEG 5000 User’s Manual, Haemoscope, Niles, IL)
Thromboelastography (TEG)

- **MA**
  - Maximum amplitude of tracing
- **G**
  - Calculated value of clot strength
    \[
    \frac{(5000 \times \text{MA})}{(100 - \text{MA})}
    \]
- **LY 30**
  - Clot lysis at 30 min following MA

Explanatory TEG tracing (modified from TEG 5000 User's Manual, Haemoscope, Niles, IL)
TEG Interpretation

- **R time**
  - Correlation to PT
  - Not reproducible in many studies
TEG Interpretation

- K time and $\alpha$ Angle
- Platelet concentration and function
- Fibrinogen concentration and function
- Clotting factor activity
TEG Interpretation

- K and MA both affected by platelet count
- Logarithmic relationship
- May not be true at higher platelet counts

Influence of platelet count and activity on thromboelastography parameters

*Platelets* (June 2003) 14(4), 219-224

Virginia A. Bowbrick, Dimitri P. Mikhailidis, Gerard Stansby

**Figure 1.** Control subjects. Plot of MA (mm) against Log₁₀ [platelet count/ml].

**Figure 2.** Control subjects. Plot of K time (min) against Log₁₀ [platelet count/ml].
TEG Interpretation

- Hypercoagulability may cause reduced R and K values and increased angle and increased MA.
TEG Interpretation

- Multiple hypercoagulable states have had increased MA, including parvovirus, IMHA, and hyperadrenocorticism
TEG Interpretation

- In human study of postsurgical patients, the use of G of r-TEG showed 100% sensitivity and 45% specificity for thromboembolic event.
TEG Interpretation

- LY30 correlates to clot breakdown
  - Depending on study, also correlates to d-dimers, FDPs
- ROTEM ML% (maximum lysis) in one study predicted post-traumatic hyperfibrinolysis better than plasma-based tests (FDPs, d-dimers)
TEG Interpretation

- Study of Dogs in DIC showed no correlation between LY30 and d-dimer concentrations.
- Study of people in DIC showed prolonged lytic measurements despite higher d-dimers.
Thromboelastography (TEG)
Considerations

- Factor XIIa is not calcium dependant
- Delay in running sample results in thrombin formation from factor XIIa
- HCT changes create artifactual changes in TEG tracings due to changes of viscosity
- Decreased blood viscosity creates hypercoagulable TEG tracings

*Effects of hematocrit and red blood cell–independent viscosity on canine thromboelastographic tracings*

Aimee C. Brooks, Julien Guillaumin, Edward S. Cooper, and C. Guillermo Couto

Volume 54, March 2014  TRANSFUSION  727
• Special use of TEG to determine the function on platelets in the presence of different activators
Activation of Platelets

[Diagram showing the process of platelet activation with various molecules and pathways involved.]
TEG PlateletMapping

- Requires 4 cups
  - Cup with heparinized blood combined with XIIIa and reptilase (similar to thrombin) to form fibrin scaffold without thrombin
    - Measures clot formation from non-platelet activation dependent parts
  - Cup with ADP platelet activations and arachidonic acid activator
  - 4th cup with heparinase and kaolin activator
  - Equations are used to determine the effect of each activator on MA
Conclusions

• Platelet testing - 20k/HPF is better#
  • EXTREMELY important part of coag picture
• PT/aPTT - not very sensitive tests for clinical bleeding
• D-Dimers much more specific for thrombosis than FDPs
• Fibrinogen - Correlates with lots but not super helpful
• TEG - Many factors interfere with results, but good test of overall hemostatic system


