Generate Knowledge

Vitamin K Dependent Coagulopathy

Paul Riley, PhD, MBA, Diagnostica Stago, Inc.
Learning Objectives

- Identify current insights in pathogenesis of Vitamin K deficiency
- Recognize clinical settings related to Vitamin K deficiency
- Describe the role of Vitamin K in development of hemorrhagic disease in newborns
- Interpret the laboratory diagnosis of impaired coagulation related to Vit K deficiency
Presentation Outline

- **Paul Riley, PhD, MBA**
  - Hemostasis refresher
  - Sources of vitamin K and biochemistry

- **Tatsiana Mardovina, MD, PhD**
  - Clinical overview of vitamin K coagulopathy
  - Causes of vitamin K coagulopathy, especially in pediatric and elderly patients
  - Studies of vitamin K deficiency and supplementation in CF patients
  - Clinical case studies
  - Clinical guidelines for nutrition to avoid vitamin K deficiency
  - Treatment of vitamin K coagulopathy
Hemostasis Refresher
Blood Circulation

- Occurs through blood vessels
- The heart pumps the blood
- Arteries carry oxygenated blood away from the heart under high pressure
- Veins carry de-oxygenated blood back to the heart under low pressure
Hemostasis

- The mechanism that maintains blood fluidity
- Keeps a balance between bleeding and clotting

2 major roles
- Stop bleeding by repairing holes in blood vessels
- Clean up the inside of blood vessels
- Removes temporary clot that stopped bleeding
- Sweeps off needless deposits that may cause blood flow blockages
Two Major Diseases Linked to Hemostatic Abnormalities

- **Bleeding** = Hemorrhage
- **Blood clot** = Thrombosis
Risk Factors for DVT and PE

- **Hypercoagulable State**
  - Malignancy
  - Pregnancy and peri-partum period
  - Oestrogen therapy
  - Trauma or surgery of lower extremity, hip, abdomen or pelvis
  - Inflammatory bowel disease
  - Nephrotic syndrome
  - Sepsis
  - Thrombophilia

- **Vascular Wall Injury**
  - Trauma or surgery
  - Venepuncture
  - Chemical irritation
  - Heart valve disease or replacement
  - Atherosclerosis
  - Indwelling catheters

- **Circulatory Stasis**
  - Atrial fibrillation
  - Left ventricular dysfunction
  - Immobility or paralysis
  - Venous insufficiency or varicose veins
  - Venous obstruction from tumour, obesity or pregnancy

- **Virchow’s Triad**
  - Stasis of blood flow
  - Endothelial injury
  - Hypercoagulability

Anatomy of a Clot


Post Thrombotic Syndrome

Physiology of Hemostasis
Wound

- PRIMARY HEMOSTASIS
  - break in vessel
  - strong clot

Sealing

- PLASMATIC COAGULATION
  - blood flow ± stopped
  - clot destruction

FIBRINOLYSIS

wound sealing → blood flow ± stopped
The Three Steps of Hemostasis

✧ Primary Hemostasis
  - Interaction between vessel wall, platelets and adhesive proteins ➔ platelet clot

✧ Coagulation
  - Consolidation of the platelet thrombus ➔ insoluble fibrin net
    • Coagulation factors and inhibitors

✧ Fibrinolysis
  - Clot lysis ➔ clot is digested
    • Fibrinolytic activators and inhibitors
**Intact endothelium** ➔ *non thrombogenic*

- Synthesis of vasodilators (prostacyclin)
- No reaction either with platelets or factors
When a vessel wall is damaged

- Exposure of the subendothelium
- Platelet adhesion
- Initiation of the mechanisms of coagulation and fibrinolysis
Primary Hemostasis

Aim is to clog the damaged vessel
(≈ bricks without cement)
Platelet Structure: Unactivated/Activated

α granules
(raw materials)
PF4, β-TG, Fibrinogen, VWF, Factor V, and PAI-1

dense granules
(energy and glue)
ATP, ADP, Serotonin, Ca$^{2+}$, Mg$^{2+}$, P

Primary Hemostasis

- Vasoconstriction occurs first

- Platelets then aggregate on the break in the vessel wall

1) adhesion
2) activation and release
3) aggregation (not reversible)
Coagulation

Aim is to strengthen the platelet plug
Coagulation is a balance between pro- & anti-coagulant mechanisms → bleed & clot, → hemorrhage & thrombosis

**PROCOAGULANT FACTORS**

- Triggering agents
- pro-enzyme → enzyme
  
  (serine-protease: FIIa, FVIIa, FIXa, FXa)
- Cofactors (FVa & FVIIIa)

**ANTICOAGULANT FACTORS**

- Serine-protease inhibitor: Antithrombin (AT)
- Cofactors/inhibitors: Protein C / S
- Tissue factor pathway inhibitor (TFPI)

Fibrinogen → Thrombin → Fibrin
# Coagulation factors

<table>
<thead>
<tr>
<th>Historic name</th>
<th>Factor</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>I</td>
<td>Substrate</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>II</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Proaccelerin</td>
<td>V</td>
<td>Pro-cofactor</td>
</tr>
<tr>
<td>Proconvertin</td>
<td>VII</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Anti-hemophilic factor A</td>
<td>VIII</td>
<td>Pro-cofactor</td>
</tr>
<tr>
<td>Anti-hemophilic factor B</td>
<td>IX</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Stuart factor</td>
<td>X</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Rosenthal factor</td>
<td>XI</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Hageman factor</td>
<td>XII</td>
<td>Pro-enzyme</td>
</tr>
<tr>
<td>Fibrin Stabilizing Factor</td>
<td>XIII</td>
<td>Pro-enzyme</td>
</tr>
</tbody>
</table>

Pro-enzyme = Zymogen

- Activation
- Active Enzyme
Why Coagulation Tests are Performed

- Essential role in the diagnosis, follow-up and therapeutic monitoring of hemostasis disturbances

- First line analyses, “screening” tests
  - activated partial thromboplastin time (APTT)
  - fibrinogen
  - prothrombin time (PT) / International Normalized Ratio (INR)
  - D-dimer

Lippi and Favaloro: Hemostasis testing: milestones in *Clinical Chemistry and Laboratory Medicine*
Second line assays provide further insights into abnormalities of screening tests, or more accurately measure some antithrombotic therapies
- Clotting factors assays
- VWF antigen tests
- Phospholipid-dependent coagulation assays
- Assays for heparin-induced thrombocytopenia
- Thrombophilia screening

Third line tests are intended to troubleshoot the most challenging conditions
- Coagulation factors inhibitors testing
- Analyses of rare thrombophilic mutations
- VWF collagen binding, ristocetin cofactor, VWF-FVIII binding, multimer and molecular analysis
What Coagulation Tests Cannot Tell Us

- Likelihood of bleeding will occur peri-operative or post-operative
- Prediction of blood clotting or thrombosis in the absence of vessel injury
- Confirmation/Likelihood and magnitude of most thrombophilic states
Coagulation Cascade and Screening Tests

- **PT test**
  - Thromboplastin reagent containing tissue factor, calcium, and phospholipids
  - Initiates the extrinsic coagulation pathway

- **aPTT test**
  - Negatively charged surface, phospholipid extract, no tissue factor
  - Initiates the intrinsic coagulation pathway
Prothrombin Time (PT)

- Measures how long it takes for blood to clot when TF/VIIaTF and Ca$^{+2}$ are added to the test tube to activate the extrinsic pathway
- Used to evaluate and manage bleeding and blood clotting disorders
- Other names: Protime, PT assay, PT - Prothrombin time, Quick one stage prothrombin time

Why this test is ordered
- Abnormal liver function
- HUS - Hemolytic uremic syndrome
- Anticoagulant drug monitoring
- Obstruction of biliary tree
- Blood clotting disorder
- Systemic infection
- Heat stroke
- Vitamin K deficiency
- Hemophilia
- Warfarin therapy
- Screen for inhibitors to factors VII, V, X and II
- Monitor coagulation factor replacement therapy

Follow up care
- Changes to medication or treatment plans
- Referral to a specialist
- More or less frequent monitoring
- Additional tests or procedures
Partial Thromboplastin Time, Activated (aPTT)

- Measures how long it takes for blood to clot when an activator, PL, Ca\(^{+2}\) is added to activate the intrinsic pathway.
- Used to assess bleeding or blood clotting problems and to monitor treatment with heparin.
- Other names: APTT, APTT - Activated partial thromboplastin time, PTT activated, PTT assay, PTT - Partial thromboplastin time.
- Why this test is ordered:
  - Acute lower gastrointestinal bleed - Liver Disease
  - Bleeding disorder - Hantavirus pulmonary syndrome
  - Disseminated intravascular coagulation (DIC) - Heat stroke
  - Hemophilia - Heparin therapy
  - Hereditary factor XI deficiency disease - Reye's syndrome
  - Hemolytic uremic syndrome (HUS) - Vitamin K deficiency
  - Meningococcal blood infection - Systemic infection
  - Screen for inhibitors to factors VIII, IX, XI, XII, X, and V, and II
  - Screen for the presence of a lupus anticoagulant
## Assessment of Prolonged aPTT

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Increased Bleeding</th>
<th>Increased Bleeding</th>
<th>Increased Bleeding</th>
<th>No Hemostatic Problems</th>
<th>Thrombophilia, DVT, PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1 Mixing Study</td>
<td>Corrects</td>
<td>Corrects</td>
<td>No correction</td>
<td>Corrects</td>
<td>No correction</td>
</tr>
<tr>
<td>PT</td>
<td>Normal</td>
<td>Prolonged</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Pathology</td>
<td>Deficiency of factors VIII, IX and some cases of factor XI deficiency</td>
<td>Deficiency of factors II, V, X, fibrinogen</td>
<td>Autoantibodies to factor VIII (acquired hemophilia)</td>
<td>Deficiency of factor XII</td>
<td>Deficiency of other contact factors such as prekallikrein</td>
</tr>
</tbody>
</table>

*PT* refers to Prothrombin Time.
Fibrinogen is critical to normal hemostasis; without it no clots will form in response to tissue injury.

Fibrin clots also act as a site for the activation of fibrinolysis.

Fibrinogen is activated by thrombin, which releases fibrinopeptides in order to make fibrinogen able to spontaneously form a fibrin clot.

Mutations exist which give either a quantitative or functional deficiency called dysfibrinogenemia.
Thrombus Formation – Role of Fibrinogen and vWF

Overview of Fibrinogen Clauss Test

Thrombin and calcium are added to diluted plasma, and the clotting time is inversely proportional to fibrinogen concentration.
Overview of Derived Fibrinogen Test

- Progress curves from the PT assay on an optical coagulation analyzer are shown above.
- Fibrinogen is estimated based on the change in optical readout between the start and end point of the assay.
- The greater the difference between the start and end points, the higher the fibrinogen concentration.
Though PT-Fib measurements correlated with Clauss Fib assays, correlation is dependent on the PT reagent and the clinical state of the patient. Use of the derived Fib test is “unsafe” in labs where the Clauss technique is well established.

Applications and Guidelines For Fibrinogen Testing

- Major applications include testing for decreased levels and abnormalities of fibrinogen, along with increased levels leading to higher heart disease risk
- Derived Fibrinogen assays are not recommended for general use because of lack of standardization and accuracy and cannot be consistently interpreted if outside of normal range
- Recommended fibrinogen assays:

<table>
<thead>
<tr>
<th>Major Applications</th>
<th>Recommended Fibrinogen Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding symptoms</td>
<td>Clauss</td>
</tr>
<tr>
<td>Congenital fibrinogen defects</td>
<td>Clauss, or immunoassay</td>
</tr>
<tr>
<td>Disseminated intravascular coagulation (DIC) or acquired defects</td>
<td>Clauss</td>
</tr>
<tr>
<td>High fibrinogen levels (such as in heart disease or elevated risk of thromboembolism)</td>
<td>Clauss or immunoassay</td>
</tr>
<tr>
<td>Thrombolytic therapy</td>
<td>Clauss</td>
</tr>
</tbody>
</table>

Fibrin Under Microscope

**Fibrin Formation**

Fibrinogen $\rightarrow$ Thrombin

- FM
- + fibrinopeptides A & B

$\text{Thrombin} \xrightarrow{XIII \rightarrow XIIIa} \text{Soluble Fibrin Polymer}$

$\text{Fibrin crosslinking}$

Stabilized Fibrin clot (not soluble)
Fibrinolysis
(Digestion of Fibrin)
Fibrinolysis Overview

- Destroys fibrin fibers
- Destroys the scab (*dried wound*)
- Maintains vessel integrity
Fibrinolysis Overview

Plasmin digests fibrin

Fibrin = cement fibers

Plasmin
D-dimer Formation

- **Fibrinogen**
- **Thrombin**
- **Plasmin**

**Fibrin Monomer + fibrinopeptides**
**Soluble fibrin Polymer**

**Pre-thrombotic**
**Post-thrombotic**

**Fibrin Degradation Products**
**D-Dimer**

**D-dimer Formation**
Fibrinolysis Releases D-dimers

D-dimer presence: fibrin has been formed and digested in patient's body

Normal D-dimer level: no thrombosis occurred in the patient
D-dimer, FDPs, and DIC

- **D-Dimer sensitive test for DIC, but not specific**
  - Elevated D-Dimer: Thrombin + Plasmin activity
  - Negative D-Dimer: low probability for DIC

  Cut-off value??

- **Fibrin monomers (FM; aka soluble fibrin monomers, SFM), and fibrin degradation products (FDPs; aka fibrin split products, FSPs)**
  - Manual FDP/FSP detects both fibrin and fibrinogen degradation products
  - Sensitive assay typically with cutoff adapted for DIC
D-Dimer and FDPs in DIC

- Detects both fibrin and fibrinogen degradation products
- Sensitive, cut-off adapted to DIC

Table 3. Utility of commonly used tests in the diagnosis of disseminated intravascular coagulation

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (n = 82)</td>
<td>91</td>
<td>27</td>
<td>57</td>
</tr>
<tr>
<td>PTT (n = 82)</td>
<td>91</td>
<td>42</td>
<td>57</td>
</tr>
<tr>
<td>TT (n = 43)</td>
<td>83</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>Fibrinogen (n = 71)</td>
<td>22</td>
<td>100</td>
<td>65</td>
</tr>
<tr>
<td>Platelet count (n = 82)</td>
<td>97</td>
<td>48</td>
<td>67</td>
</tr>
<tr>
<td>Schistocytes (n = 80)</td>
<td>23</td>
<td>73</td>
<td>51</td>
</tr>
<tr>
<td>FDP (n = 71)</td>
<td>100</td>
<td>67</td>
<td>87</td>
</tr>
<tr>
<td>D-dimer (n = 44)</td>
<td>91</td>
<td>68</td>
<td>80</td>
</tr>
<tr>
<td>AT (n = 21)</td>
<td>91</td>
<td>40</td>
<td>70</td>
</tr>
<tr>
<td>PT + PTT + TT (n = 43)</td>
<td>83</td>
<td>11</td>
<td>51</td>
</tr>
<tr>
<td>PT + PTT + fibrinogen</td>
<td>22</td>
<td>100</td>
<td>65</td>
</tr>
<tr>
<td>PT + PTT + FDP (n = 71)</td>
<td>91</td>
<td>71</td>
<td>86</td>
</tr>
<tr>
<td>FDP + D-dimer (n = 39)</td>
<td>91</td>
<td>94</td>
<td>95</td>
</tr>
</tbody>
</table>

PT, prothrombin time; PTT, partial thromboplastin time; TT, thrombin time; FDP, fibrinogen/fibrin degradation products; AT, antithrombin.

FM/D-Dimer in DIC: Major Differences

**FM: may be predictive**
- Appear 0-3 days after the onset of thrombosis; typically prethrombotic
- Short half-life (6 - 8 hrs)

**D-Dimer (a specific FDP): well-established DIC**
- Appear 2-10 days after the onset of thrombosis; typically postthrombotic
- Longer half-life (4 - 11 hrs)

Acquired Factor Deficiencies

- Acquired factor deficiency usually involves several of the coagulation factors
- Disseminated Intravascular Coagulation or other forms of excessive fibrinolysis
- Acquired deficiency of the vitamin K-dependent factors (II, V, VII, X)
  - Neonatal vitamin K deficiency
  - Obstructive jaundice and other liver insufficiencies
  - Intestinal malabsorption (caused by intestine resection, broad spectrum antibiotics)
  - Cystic fibrosis
  - Exposure to rat poison
  - Warfarin therapy
Vitamin K Sources and Biochemistry
Sources of Vitamin K

- Normal flora in the gastrointestinal tract
- Leafy green vegetables (kale, collards, broccoli, etc.)
- Meat, fish, liver, eggs, cereals

Vitamin K Mechanism Cycle (K = Koagulation)
Vitamin K Dependent Factors

- **IX**
  - Intrinsic pathway

- **X**
  - Common pathway

- **II**
  - Fibrinogen

- **VIIa**
  - Vitamin K dependent proteins

The diagram illustrates the interactions of Vitamin K dependent factors within the coagulation cascade.
### Other Vitamin K Dependent Proteins

<table>
<thead>
<tr>
<th>Coagulation Factor</th>
<th>MW (KDa)</th>
<th>Coagulation Factor</th>
<th>MW (KDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Proteins</td>
<td></td>
<td>Nephrocalcin</td>
<td>14</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>58</td>
<td>PRGP1</td>
<td>23</td>
</tr>
<tr>
<td>Matrix Gla protein</td>
<td>10</td>
<td>PRGP2</td>
<td>17</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td>TIMG3</td>
<td>25.8</td>
</tr>
<tr>
<td>Gas 6 Protein</td>
<td>75</td>
<td>TIMG4</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Not only are proteins involved in hemostasis dependent on vitamin K but also others, giving rise to glycosylation disorders, Noonan syndrome, liver function issues, among others.
Vitamin K Antagonist (VKA)

VKA = Vitamin K Antagonist

- Vitamin K structure

- Vitamin K Antagonist structure
  - Examples: derivatives from
    - HydroxyCoumarin
      (Warfarin, Coumadin/Acenocoumarol, Sintrom),
    - Indane 1-2 dione
      (Fluindione, Previscan)

adapted from HJ Kolde, 2010
Coagulation Factor Function

- charged groups
  (e.g. γcarboxyglutamic residues synthesized via vitamin K)

The factor binds to phospholipids with its negatively charged residues through Calcium (++)

(injured, stressed platelets or endothelium)
Vitamin K Mechanism Cycle

Precoagulation factor
CH2
CH2 — COOH

PIVKA
Protein Induced by Vitamin K Antagonist

Carboxylase
O₂, CO₂

reduced vitamin K* epoxide

Vitamin K epoxide reductase

Coagulation factor
CH2
CH2 — COOH

VKA blocks

Stago
PIVKAs are non-carboxylated or partially-carboxylated forms of factors II, VII, IX, X
- Are non-functional; have no procoagulant activity
- Present in all coumadin patients
- Present in patients with liver dysfunction and certain cancers
- Present in vitamin K deficient patients

Recombinant PT reagents are sensitive to PIVKAs
Rabbit brain PT reagents are insensitive to PIVKAs
PIVKA antigen can correlate to PT

“….rabbit brain Thromboplastins of good sensitivity have an advantage over recombinant human Thromboplastin”.
Denson K et al. Clin Lab Haemat 1998, 10:315-328
Elevated levels of PIVKA correlate with lower cumulative metastasis-free rates (left), and when combined with tumor markers like alphafetoprotein (AFP), the PIVKA diagnostic robustness increases (right).

Bae et al. BMC Cancer 2011, 11:435
http://www.biomedcentral.com/1471-2407/11/435
PIVKA Proteins and Cystic Fibrosis

PIVKA levels were elevated in CF patients but decreased when vitamin K supplementation was provided.

Vitamin K Dependent Coagulation Factors: PL & Ca++

- Calcium links factors to phospholipids

- Vitamin K → Factors II, VII, IX, X* carboxyl residues (= anchor)

* (and Protein C, Protein S, and Protein Z)
Vitamin K Dependent Coagulation Factors: PL & Ca++

Coagulation factor

If no carboxyl residues (= anchor), no link to Ca++/PL → no activity

Factors II, VII, IX, X and Protein C & Protein S

Calcium links factors to phospholipids

Phospholipids
Example of Vitamin K Dependent Factors and Deficiency

Sufficient Vitamin K

Vitamin K deficiency or Warfarin treatment

Factor anchoring region

NO Factor anchoring region
Thank you! Questions?
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  - Virtual exhibit hall

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- 30 – 45 min including 15 min discussion

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  - Tablet only

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TIME FOR A BREAK