Coagulation tests in children

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Coagulation Laboratory
Ghent University Hospital
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Introduction

The young are not just miniature adults

- Physiology of pediatric hemostasis differs from adults
- Course of hemostatic disorders may differ
- Correct interpretation of coagulation test results
Hemostasis

Introduction

BALANCE OF HEMOSTASIS

Clotting

Vessels Platelets Coagulation Fibrinolysis

Bleeding

Activators

Inhibitors

Coagulation Anticoagulation
Introduction

**Primary hemostasis**
- Platelets interact with damaged endothelium
- Primary hemostatic plug to arrest bleeding
- **Primary platelet plug**: fragile and easily dislodges

**Secondary hemostasis**
- Formation of insoluble fibrin strands
- **Secondary hemostatic plug**: a stable plug
- Involves the coagulation cascade proteins that interact with each other and the platelet plug

**Fibrinolysis**
- Once healing occurred, the clot has to be lysed to prevent occlusion of vessel
Introduction

Primary hemostasis
- Platelet count
- Platelet morphology
- Platelet function

Secondary hemostasis
- Clotting assays: aPTT, PT, thrombin time
- Fibrinogen
- Coagulation factors (FXII, FXI, FIX, FVIII; FII, FV, FVII, FX; FXIII; VWF)
- Coagulation inhibitors (AT, PC, PS)

Fibrinolysis
- Plasminogen, t-PA, α2-antiplasmin, PAI
Laboratory tests for hemostasis

Testing hemostasis in children

- **Bleeding**
  - Screening tests:
    - complete blood count
    - prothrombin time (PT)
    - activated partial thromboplastin time (aPTT)
    - (Platelet function analysis (PFA))
  - Further laboratory evaluation: additional tests

- **Thrombosis**
  - Inherited thrombophilia markers (antithrombin, protein C, protein S, FVLeiden, FIIG20210A)
  - Issues related to coagulation testing in children
Laboratory tests for hemostasis

Sample acquisition for coagulation tests

**Blood collection**
- Anticoagulated blood with citrate
- Pre-analytical variables
- Sufficient blood
- Not from a *heparinised* line
- Not in heparinised syringe
- No air bubbles
- No fluid contamination

Heparin strongly disturbs coagulation tests!
Laboratory tests for hemostasis

Citrated tubes

- buffered sodium citrate 0.109 mol/L: 3.2%
- proportion 1 part citrate/9 parts blood
- allows recalcification for testing

Hct ~ 45%
⇒ Plasma ~ 55%
⇒ **Citrate:plasma** ~ 1:5
Hemostasis in children

“Developmental hemostasis”

- **Procoagulants**
  - Liver production of FVII, FVIII, FIX, FX
  - All procoagulants detected in plasma
  - FV, FVIII within adult Fib range

- **Anticoagulants**
  - Liver production of protein C and AT
  - All anticoagulants detected in plasma
  - AT, protein C, protein S, HCII below adult levels

(Jaffray et al, Pediatr Clin N Am 2013, 60, 1407-1417)
# Neonatal versus Adult Hemostasis

<table>
<thead>
<tr>
<th>Component</th>
<th>Neonatal Functions</th>
<th>Global Effect on Hemostasis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coagulation factors</strong></td>
<td>↓ FII, FVII, FIX, FX, XI, XII</td>
<td>Decreased thrombin generation</td>
</tr>
<tr>
<td></td>
<td>← Fibrinogen, FV, FXIII</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ FVIII</td>
<td></td>
</tr>
<tr>
<td><strong>Primary hemostasis</strong></td>
<td>↑ VWF</td>
<td>Enhanced primary hemostasis</td>
</tr>
<tr>
<td></td>
<td>Normal Platelet count</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓ Platelet Function</td>
<td></td>
</tr>
<tr>
<td><strong>Fibrinolysis</strong></td>
<td>↓ Plasminogen, t-PA, α₂-antiplasmin</td>
<td>Hypofibrinolytic state</td>
</tr>
<tr>
<td></td>
<td>↑ PAI</td>
<td></td>
</tr>
<tr>
<td><strong>Natural coagulation inhibitors</strong></td>
<td>↓ AT, PC, PS</td>
<td>Reduced capacity to inhibit activated coagulation proteins</td>
</tr>
</tbody>
</table>

(Lippi et al, Sem Thromb Hemost 2007, 33, 816-820
Guzzetta et al, Ped Anesthesia 2010, 21, 3-9)
Hemostasis in children

“Developmental hemostasis”

Maureen Andrew (1952-2001)

Mc Master University Hamilton, Canada

Reference values for coagulation tests:

- Healthy full-term infants (day1-6 months)
- Healthy children (1y-16y)
- Healthy prematures (30-36 weeks)
- Healthy fetuses (19-27 weeks)


Tribute To Dr. Maureen Andrew

Blood 1992, 80, 1998-2005
Blood 1987, 70, 165-172
Ped J Hematol/oncol 1990,12, 95-104

Monagle et al, Blood Reviews 2010, 24, 63-68
Routine coagulation assays

**Intrinsic**

neg charged surface

<table>
<thead>
<tr>
<th>XII</th>
<th>XII a</th>
</tr>
</thead>
</table>

contact activator

<table>
<thead>
<tr>
<th>XI</th>
<th>XI a</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>IX</th>
<th>a</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>IX a</th>
<th>VIII a</th>
</tr>
</thead>
</table>

| PL | Ca++ |

**Extrinsic**

<table>
<thead>
<tr>
<th>TF</th>
<th>VII a</th>
</tr>
</thead>
</table>

| PL | Ca++ |

<table>
<thead>
<tr>
<th>X</th>
<th>a</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>X a</th>
<th>V a</th>
</tr>
</thead>
</table>

| PL | Ca++ |

| II | thrombin |

| fibrin |

**Common**

<table>
<thead>
<tr>
<th>PL, CaCl₂</th>
</tr>
</thead>
</table>

| activator |

| Citrated anticoagulated plasma |
Routine coagulation assays
aPTT = is the time it takes to form a clot in activated plasma measured in seconds (M. Andrew et al, Blood 1992, 1998-2005)
Routine coagulation assays

aPTT is the time it takes to form a clot in activated plasma measured in seconds.

Contact activator and PL → CaCl₂ → Incubation 37°C → Clotting time → Fibrinogen → Fibrin

Fibrinogen → Fibrin

PK, HMWK → Tissue damage

PK, HMWK → Kall → X, Xa

Ca++, X → Xa

Xla → IX → IXa → Ca++, PL → Xla → Xa

Routine coagulation assays

**intrinsic**

neg charged surface

XII ➔ XII a ➔ XI ➔ XI a ➔ IX ➔ IX a ➔ VIII a ➔ PL ➔ Ca++

**extrinsic**

PT

TF ➔ VII a ➔ PL ➔ Ca++ ➔ X ➔ X a ➔ V a ➔ PL ➔ Ca++ ➔ X ➔ II ➔ thrombin ➔ fibrin

## Reference Values for Coagulation Tests in the Healthy Full-term Infant During the First 6 Months of Life

<table>
<thead>
<tr>
<th>PT (s)</th>
<th>day 1</th>
<th>day 5</th>
<th>day 30</th>
<th>day 90</th>
<th>day 180</th>
<th>adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.0± 1.43</td>
<td>12.4± 1.46</td>
<td>11.8± 1.25</td>
<td>11.9± 1.15</td>
<td>12.3±0.79</td>
<td>12.4±0.78</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>aPTT(s)</th>
<th>day 5</th>
<th>day 30</th>
<th>day 90</th>
<th>day 180</th>
<th>adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.9 ± 5.80</td>
<td>42.6 ± 8.62</td>
<td>40.4 ± 7.42</td>
<td>37.1 ± 6.52</td>
<td>35.5 ± 3.71</td>
<td>33.5 ± 3.44</td>
</tr>
</tbody>
</table>

Significantly different from adults

*(M. Andrew et al, Blood 1987, 70, 165-172)*

![Diagram](image_url)
Routine coagulation assays

“Developmental hemostasis”

- Age-dependent reference ranges
- Definition of healthy children
- Diagnosis of bleeding and thrombotic disorders
- Monitoring anticoagulant therapy

- Labs use published reference ranges
  - Blood samples: ethical stand points, costs
  - Reported reference ranges on small groups
  - Not reagent or instrument adapted
Age-dependent reference ranges

- Neonates
- 1 month-1year
- 1-5 y
- 6-10 y
- 11-16y
- <1 year
- Premature neonates
- Low birth weight vs healthy children

(Ignjatovic et al, J Thromb Haemost 2012, 10, 298-300)
Coagulation tests in healthy infants and children, compared with adults

<table>
<thead>
<tr>
<th>Coagulation tests</th>
<th>Day 1 of life Mean (boundary)</th>
<th>Day 3 of life Mean (boundary)</th>
<th>1 to 12 months Mean (boundary)</th>
<th>1 to 5 yr Mean (boundary)</th>
<th>6 to 10 yr Mean (boundary)</th>
<th>11 to 16 yr Mean (boundary)</th>
<th>Adult Mean (boundary)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT(s)*</td>
<td>15.6 (14.4-16.4)*</td>
<td>14.9 (13.5-16.4)*</td>
<td>13.1 (11.5-15.3)</td>
<td>13.3 (12.1-14.5)*</td>
<td>13.4 (11.7-15.1)*</td>
<td>13.8 (12.7-16.1)*</td>
<td>13 (11.5-14.5)</td>
</tr>
<tr>
<td>INR</td>
<td>1.26 (1.15-1.35)*</td>
<td>1.20 (1.05-1.35)*</td>
<td>1.00 (0.86-1.22)</td>
<td>1.03 (0.92-1.14)*</td>
<td>1.04 (0.87-1.12)*</td>
<td>1.08 (0.87-1.30)*</td>
<td>1.00 (0.80-1.20)</td>
</tr>
<tr>
<td>APTT(s)*</td>
<td>38.7 (34.3-44.6)*</td>
<td>36.3 (29.5-42.2)*</td>
<td>39.3 (35.1-46.3)*</td>
<td>37.7 (33.6-43.7)*</td>
<td>37.3 (31.8-46.1)*</td>
<td>39.5 (33.9-48.1)*</td>
<td>33.2 (28.6-38.2)</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.80 (1.92-3.74)</td>
<td>3.30 (2.83-4.01)</td>
<td>2.42 (0.82-3.83)*</td>
<td>2.82 (1.62-4.01)*</td>
<td>3.04 (1.99-4.09)</td>
<td>3.15 (2.12-4.33)</td>
<td>3.1 (1.9-4.3)</td>
</tr>
<tr>
<td>Factor II (U/mL)</td>
<td>0.54 (0.41-0.69)*</td>
<td>0.62 (0.50-0.73)*</td>
<td>0.90 (0.62-1.03)*</td>
<td>0.89 (0.70-1.09)*</td>
<td>0.89 (0.67-1.10)*</td>
<td>0.90 (0.61-1.07)*</td>
<td>1.10 (0.78-1.38)</td>
</tr>
<tr>
<td>Factor V (U/mL)</td>
<td>0.81 (0.64-1.03)*</td>
<td>1.22 (0.92-1.54)*</td>
<td>1.13 (0.94-1.41)</td>
<td>0.97 (0.67-1.27)*</td>
<td>0.99 (0.56-1.41)*</td>
<td>0.89 (0.57-1.41)*</td>
<td>1.18 (0.78-1.52)</td>
</tr>
<tr>
<td>Factor VII (U/mL)</td>
<td>0.70 (0.52-0.86)*</td>
<td>0.85 (0.67-1.07)*</td>
<td>1.28 (0.63-1.60)</td>
<td>1.11 (0.72-1.50)*</td>
<td>1.13 (0.70-1.56)</td>
<td>1.18 (0.69-2.00)</td>
<td>1.29 (0.61-1.99)</td>
</tr>
<tr>
<td>Factor VIII (U/mL)</td>
<td>1.82 (1.05-3.29)</td>
<td>1.59 (0.83-2.74)</td>
<td>0.64 (0.54-1.45)*</td>
<td>1.10 (0.35-1.65)*</td>
<td>1.17 (0.52-1.82)*</td>
<td>1.20 (0.59-2.00)*</td>
<td>1.60 (0.52-2.90)</td>
</tr>
<tr>
<td>vWF (U/mL)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>0.62 (0.60-1.20)</td>
<td>0.95 (0.44-1.44)</td>
<td>1.00 (0.46-1.53)</td>
<td>0.92 (0.50-1.58)</td>
</tr>
<tr>
<td>Factor IX (U/mL)</td>
<td>0.48 (0.35-0.56)*</td>
<td>0.72 (0.44-0.97)*</td>
<td>0.71 (0.43-1.21)*</td>
<td>0.85 (0.44-1.27)*</td>
<td>0.96 (0.48-1.45)*</td>
<td>1.11 (0.64-2.06)</td>
<td>1.30 (0.59-2.54)</td>
</tr>
<tr>
<td>Factor X (U/mL)</td>
<td>0.55 (0.46-0.67)*</td>
<td>0.60 (0.46-0.75)*</td>
<td>0.65 (0.47-1.22)*</td>
<td>0.88 (0.72-1.25)*</td>
<td>0.97 (0.68-1.25)*</td>
<td>0.30 (0.53-1.22)*</td>
<td>1.24 (0.96-1.71)</td>
</tr>
<tr>
<td>Factor XI (U/mL)</td>
<td>0.30 (0.70-0.41)*</td>
<td>0.57 (0.24-0.79)*</td>
<td>0.89 (0.62-1.25)*</td>
<td>1.13 (0.65-1.62)</td>
<td>1.13 (0.65-1.62)</td>
<td>1.11 (0.65-1.39)</td>
<td>1.12 (0.67-1.96)</td>
</tr>
<tr>
<td>Factor XII (U/mL)</td>
<td>0.58 (0.43-0.80)*</td>
<td>0.53 (0.14-0.80)*</td>
<td>0.79 (0.20-1.35)*</td>
<td>0.85 (0.35-1.35)*</td>
<td>0.81 (0.26-1.37)*</td>
<td>0.75 (0.14-1.17)*</td>
<td>1.15 (0.35-2.07)</td>
</tr>
<tr>
<td>XIIIa (U/mL)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>1.08 (0.72-1.43)</td>
<td>1.09 (0.65-1.51)</td>
<td>0.99 (0.57-1.40)</td>
<td>1.05 (0.55-1.55)</td>
</tr>
<tr>
<td>XIIIIs (U/mL)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>1.13 (0.69-1.56)</td>
<td>1.16 (0.77-1.54)*</td>
<td>1.02 (0.60-1.43)</td>
<td>0.87 (0.57-1.37)</td>
</tr>
</tbody>
</table>

All factors except fibrinogen are expressed as units per milliliter, where pooled plasma contains 1.0 U/mL. All data are expressed as the mean, followed by the upper and lower boundary encompassing 95 percent of the population. Between 20 and 67 samples were assayed for each value for each age group. Some measurements were skewed due to a disproportionate number of high values. The lower limit, which excludes the lower 2.5 percent of the population, is given.

PT: prothrombin time; APTT: activated partial thromboplastin time; VIII: factor VIII procoagulant; vWF: von Willebrand factor; n/a: data not available.

* Normal range for PT and APTT should be based upon the standards set by individual clinical laboratories.
• Denotes values that are significantly different from adults.

Routine coagulation assays

Example of aPTT

- Adult reference range: 28.9-38.1 sec
- 5 - 30 dag reference range: 32.0 - 55.2 sec

- “abnormal aPTT”
  - Repeat testing
  - Mixing test
  - FVIII, FIX, FXI, FXII, VWF, lupus anticoagulant

- Unnecessary costs, cancellation of surgery, additional clinical consults, overtreatment

- Misdiagnosis of bleeding or clotting disorder
Prolonged aPTT

- Increased bleeding tendency
  - Von Willebrand
  - Factor XI def.
  - FX, FV, FII, Fg
  - Hemophilia

- No bleeding
  - Artefact
  - F XII def.
  - Def. prekallikrein
  - LAC

- Thrombosis
  - LAC
Table I. Summary characteristics of the rare coagulation disorders. The laboratory criteria for definition of disease severity are as proposed by the European Network of Rare Bleeding Disorders (EN-RBD), which does not specify suggested upper limits of factor activity for the rare coagulation disorders (Peyvandi et al, 2012b).

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Worldwide prevalence</th>
<th>Gene(s) involved</th>
<th>EN-RBD disease severity</th>
<th>Clinical-laboratory correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen deficiency (F1D)</td>
<td>1:1 million (AR)</td>
<td>FGA, FGB, FGB</td>
<td>Undetectable</td>
<td>0.1–1 g/l</td>
</tr>
<tr>
<td></td>
<td>Unknown (AD)</td>
<td></td>
<td></td>
<td>&gt;1 g/l</td>
</tr>
<tr>
<td>Prothrombin deficiency (F2D)</td>
<td>1:2 million</td>
<td>F2</td>
<td>Undetectable</td>
<td>≤0.1 iu/ml</td>
</tr>
<tr>
<td>Factor V deficiency (F5D)</td>
<td>1:1 million</td>
<td>F5</td>
<td>Undetectable</td>
<td>&lt;0.1 iu/ml</td>
</tr>
<tr>
<td>Factor VII deficiency (F7D)</td>
<td>1:0.5 million</td>
<td>F7</td>
<td>&lt;0.1 iu/ml</td>
<td>0.1–0.2 iu/ml</td>
</tr>
<tr>
<td>Factor X deficiency (F10D)</td>
<td>1:1 million</td>
<td>F10</td>
<td>≤0.1 iu/ml</td>
<td>0.1–0.4 iu/ml</td>
</tr>
<tr>
<td>Factor XI deficiency (F11D)</td>
<td>1:1 million (AR)</td>
<td>F11</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1:30 000 (AD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor XIII deficiency (F13D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor V + VIII deficiency (F5F8D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin K dependent coagulation factor deficiency (VKDCF)</td>
<td>1:1 million</td>
<td>GGCX, VKORC1</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

AR, autosomal recessive; AD, autosomal dominant.

Mild decreased FXI: normal aPTT

Guideline for the diagnosis and management of the rare coagulation disorders

A United Kingdom Haemophilia Centre Doctors’ Organization guideline on behalf of the British Committee for Standards in Haematology
### Sensitivity for factor deficiencies

<table>
<thead>
<tr>
<th>Procoagulant</th>
<th>Reference value</th>
<th>Level for normal aPTT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fg</td>
<td>200-400 mg/dl</td>
<td>60 mg/dl</td>
</tr>
<tr>
<td>Prothrombine (FII)</td>
<td>70-120%</td>
<td>15%</td>
</tr>
<tr>
<td>F V</td>
<td>70-120%</td>
<td>40%</td>
</tr>
<tr>
<td>FVII</td>
<td>70-130%</td>
<td>-</td>
</tr>
<tr>
<td>F VIII</td>
<td>60-150%</td>
<td>35%</td>
</tr>
<tr>
<td>F IX</td>
<td>60-150%</td>
<td>20%</td>
</tr>
<tr>
<td>F X</td>
<td>70-120%</td>
<td>25%</td>
</tr>
<tr>
<td><strong>F XI</strong></td>
<td><strong>60-140%</strong></td>
<td><strong>30%</strong></td>
</tr>
<tr>
<td><strong>F XII</strong></td>
<td><strong>60-150%</strong></td>
<td><strong>20%</strong></td>
</tr>
</tbody>
</table>

* Depends on the reagent used
## Von Willebrand disease

<table>
<thead>
<tr>
<th>Screening tests</th>
<th>Confirmation tests</th>
<th>Tests for further classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT</td>
<td>VWF:Ag</td>
<td>RIPA (2B)</td>
</tr>
<tr>
<td>Closure time (PFA)</td>
<td>VWF:act (VWF:RCo; CBA)</td>
<td>Multimer analysis</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td>FVIII:C</td>
<td>VWF-FVIII binding assay (2N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mutation (type 2) VWF:pp</td>
</tr>
</tbody>
</table>
## Von Willebrand disease

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<tr>
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<tr>
<td>Closure time (PFA)</td>
<td>VWF:act</td>
<td>Multimer analysis</td>
</tr>
<tr>
<td>Acute phase proteins</td>
<td>VWF-FVIII binding assay (2N)</td>
<td>VWF-FVIII binding assay (2N)</td>
</tr>
<tr>
<td>Repeat testing!</td>
<td>FVIII:C</td>
<td>Mutation (type 2)</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td></td>
<td>VWF:pp</td>
</tr>
</tbody>
</table>

Repeat testing!
Prolonged aPTT

Increased bleeding tendency
- Von Willebrand
- Hemophilia
- Factor XI def.
- FX, FV, FII, Fg
- Anticoagulants
- Def. prekallikrein
- Artefact
- FV, FII, Fg

No bleeding
- Factor XII def.
- LAC
- Def. prekallikrein
- LAC

Thrombosis
- Heparin contamination
- Under filling tube
Prolonged aPTT as artefact

CRP

- Acute phase protein
- Affinity for phospholipids
  → to recognize pathogens and apoptotic cells

- Prolonged aPTT in intensive care patients
  

- Analytical interference with CRP: binds to phospholipids
  Falsely prolonged aPTT
  
Prolonged aPTT as artefact

(Schouwers, Delanghe, Devreese. Thromb Res 2010,125:102-4)
Prolonged aPTT

- Increased bleeding tendency
  - Von Willebrand
  - Factor XI def.
  - FX, FV, FII, Fg
- Hemophilia
- Artefact
  - Def. prekallikrein
- No bleeding
  - Factor XII def.
  - LAC
- Thrombosis
  - FX, FV, FII, Fg
  - Def. prekallikrein
  - LAC
Prolonged aPTT as artefact

**intrinsic**

neg charged surface

\[
\text{XII} \rightarrow \text{XII} \ a
\]

\[
\text{XI} \rightarrow \text{XI} \ a
\]

\[
\text{IX} \rightarrow \text{IX} \ a
\]

\[
\text{VIII} \ a
\]

\[
\text{PL} \ a
\]

\[
\text{Ca}^{++}
\]

**extrinsic**

\[
\text{TF} \rightarrow \text{VII} \ a
\]

\[
\text{PL} \ a
\]

\[
\text{Ca}^{++}
\]

\[
\text{X} \rightarrow \text{X} \ a
\]

\[
\text{V} \ a
\]

\[
\text{PL} \ a
\]

\[
\text{Ca}^{++}
\]

\[
\text{II} \rightarrow \text{thrombin}
\]

\[
\text{fibrin}
\]

Antiphospholipid antibodies: lupus anticoagulant
Lupus anticoagulant

- **Criterion for antiphospholipid syndrome**
  - persistently positive > 12 weeks
  - Risk for thrombosis

- **Transient LAC**
  - Incidentally found prolonged aPTT
  - Infection
  - No association with thrombosis
  - Repeat testing
A normal aPTT is not always an indication of normal hemostasis

Mild factor deficiencies with normal aPTT can be associated with significant bleeding history (eg FXI >30%, type I VWD)

Be aware of the sensitivity of the aPTT reagent

If bleeding history is suggestive: perform factor dosage even with normal aPTT
Routine coagulation assays

intrinsic
neg charged surface

aPTT

PT

extrinsic

TF
VII a
PL
Ca++

IX a
VIII a
PL
Ca++

X a
V a
PL
Ca++

XII
XII a

XI
XI a

IX

common

II
trombin

Fibrin

Fibrin polymerisation

Routine coagulation assays

aPTT

PT

extrinsic

TF
VII a
PL
Ca++

IX a
VIII a
PL
Ca++

X a
V a
PL
Ca++

II
trombin

Fibrin

Fibrin polymerisation
**Table I. Summary characteristics of the rare coagulation disorders.** The laboratory criteria for definition of disease severity are as proposed by the European Network of Rare Bleeding Disorders (EN-RBD), which does not specify suggested upper limits of factor activity for the rare coagulation disorders (Peyvandi et al, 2012b).

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Worldwide prevalence</th>
<th>Gene(s) involved</th>
<th>EN-RBD disease severity</th>
<th>Clinical-laboratory correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen deficiency (F1D)</td>
<td>1:1 million (AR)</td>
<td>FGA, FGB, FGB</td>
<td>Undetectable</td>
<td>0.1–1 g/l</td>
</tr>
<tr>
<td>Prothrombin deficiency (F2D)</td>
<td>1:2 million</td>
<td>F2</td>
<td>Undetectable</td>
<td>≤0.1 iu/ml</td>
</tr>
<tr>
<td>Factor V deficiency (F5D)</td>
<td>1:1 million</td>
<td>F5</td>
<td>Undetectable</td>
<td>&lt;0.1 iu/ml</td>
</tr>
<tr>
<td>Factor VII deficiency (F7D)</td>
<td>1:0.5 million</td>
<td>F7</td>
<td>Undetectable</td>
<td>0.1–1.0 iu/ml</td>
</tr>
<tr>
<td>Factor X deficiency (F10D)</td>
<td>1:1 million</td>
<td>F10</td>
<td>Undetectable</td>
<td>&lt;0.1 iu/ml</td>
</tr>
<tr>
<td>Factor XI deficiency (F11D)</td>
<td>1:1 million (AR)</td>
<td>F11</td>
<td>Undetectable</td>
<td>0.1–0.3 iu/ml</td>
</tr>
<tr>
<td>Factor XIII deficiency (F13D)</td>
<td>1:2 million</td>
<td>F13A, F13B</td>
<td>Undetectable</td>
<td>0.1–0.3 iu/ml</td>
</tr>
</tbody>
</table>

**Normal aPTT, normal PT**

- FXIII activity

- Immunoassay for subunit A and subunit B
Routine coagulation assays

intrinsic
neg charged surface

XII → XII a

XI → XI a

IX a
VIII a PL Ca++

extrinsic
PT

TF VII a PL Ca++

X a V a PL Ca++

II → trombin fibrin

vitamin K dependent factors
Prolonged PT

Vitamin K deficiency

Synthesis of Non-Functional Coagulation Factors

- Vitamin K deficiency
- VII: 6h T1/2
- IX: 18-24h
- X: 40-50h
- II: 48-60h
- PS: 48h
- PC: 6-8h

Warfarine

Vitamin K
Precursors of factors II, VII, IX, X, protein C and protein S \( \Rightarrow \) PIVKA

Complete forms of factors II, VII, IX, X, protein C and protein S

\[ \text{Ca}^{2+} \]

Bind to phospholipids

Prolonged PT
Vitamin K deficiency bleeding (VKDB)

- Bleeding: factors <30%
- Mild vitamin K deficiency: PT prolonged (FVII)
- Severe vitamin K deficiency: aPTT and PT prolonged
- Diagnosis: PT, aPTT, FII, FVII, FIX, FX dosage
- < PIVKA, vitamin K dosage
- FV: differentiates liver dysfunction/vit K deficiency
# Routine coagulation assays

## Table 1. Initial Laboratory Evaluation to Screen for Bleeding Disorders: Analysis and Interpretation

<table>
<thead>
<tr>
<th>PT</th>
<th>aPTT</th>
<th>Platelet Count</th>
<th>Possible Bleeding Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>• vWD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• <a href="#">Platelet function defect</a></td>
</tr>
<tr>
<td>Normal</td>
<td>Prolonged</td>
<td>Normal</td>
<td>• Factor XIII deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Fibrinolytic defect</td>
</tr>
<tr>
<td>Normal</td>
<td>Prolonged</td>
<td>Normal</td>
<td>• Hemophilia A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Hemophilia B</td>
</tr>
<tr>
<td>Normal</td>
<td>Prolonged</td>
<td>Normal</td>
<td>• Factor XI deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• vWD with FVIII dysfunction</td>
</tr>
<tr>
<td>Normal</td>
<td>Prolonged</td>
<td>Low</td>
<td>• Lupus anticoagulant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Heparin contamination or excess</td>
</tr>
<tr>
<td>Prolonged</td>
<td>Normal</td>
<td>Normal</td>
<td>• Early vitamin K deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Factor VII deficiency</td>
</tr>
<tr>
<td>Prolonged</td>
<td>Prolonged</td>
<td>Normal</td>
<td>• Warfarin excess</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Liver disease</td>
</tr>
<tr>
<td>Prolonged</td>
<td>Prolonged</td>
<td>Low</td>
<td>• Vitamin K deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Common pathway factor (factor X,V, prothrombin, fibrinogen) deficiency</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Dysfibrinogenemia</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>Low</td>
<td>• DIC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Liver disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Acute ITP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Chronic ITP</td>
</tr>
</tbody>
</table>

PT = prothrombin time; aPTT = activated partial thromboplastin time; vWD = von Willebrand disease; DIC = disseminated intravascular coagulation; ITP = immune thrombocytopenic purpura.

(Sarnaik et al, Clin Ped, 2010, 49, 422-431)
Platelet function

- Neonates:
  Hyporeactive to thrombin, ADP, tromboxane first 2-4 weeks
Laboratory tests for platelet function

**Platelet aggregation LTA on PRP**

-250 µL PRP/agonist = 10 mL blood

-platelet aggregometry should be repeated once to ensure reproducibility of results.

- ADP 2.5µM
- ADP 5µM
- Collagen 2.5µg/ml
- Collagen 5 µg/ml
- Ristocetin 1.5 mg/ml
- Ristocetin 0.5 mg/ml
- Arachidonic acid 1.5 mM
- Epinephrine 10µM
- Tromboxane-analogue
Screening platelet function

Closure time in seconds is a measure for function of thrombocytes

Adult:
Collagen/epinephrine 82 – 150 sec
Collagen/ADP 62 – 100 sec

Shorter in neonates

Laboratory tests for platelet function

2x 800µl citrated blood
Hct>35% and >80 000 plts/µL
The PFA-100®: a potential rapid screening tool for the assessment of platelet dysfunction


PFA-100® collagen/ADP (CADP) (a) and collagen/epinephrine (CEPI) (b) closure times in patients classified as either normal (N) or diagnosed with von Willebrand's disease (VWD), Bernard Soulier syndrome (BS) and Glanzmann's thrombasthenia (GT); Hermansky Pudlak syndrome (HPS), Storage Pool Disease (SPD), Release Defects (RD), Wiskott Aldrich syndrome (WAS) and Grey Platelet syndrome (GPS). The normal range values are shown (NR) with 2 SD normal range limits (dotted lines).
Interpretation of coagulation tests

- Interpretation with caution

- Diagnosis of bleeding disorder or thrombophilia based on
  - Clinical symptoms
  - Family history
  - Reproducible laboratory results
Coagulation tests in children: Conclusions

- Immature hemostatic system 3-6 months
- Differences between adults and children: physiological
- Age appropriate reference ranges

- Sample integrity, repeat testing

- Routine coagulation tests and additional testing

- An abnormal laboratory test result is not sufficient to define a disease
- A normal test result does not exclude a disease
THANK YOU FOR YOUR ATTENTION