The Role of Laboratory in the Management of Hemophilia

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Hemophilia

- The most frequent hereditary coagulation disorders
- Frequency 1 in 10,000 births
- Not influenced by ethnicity, geographic, socioeconomic
- Hemophilia A is caused by deficiency of F VIII and hemophilia B by deficiency of F IX
- X linked recessive → male are more affected, female as carrier
Alexis
Hemophilia in Indonesia

- Based on the population of Indonesia around 200 millions it is estimated that the number of PWH in Indonesia 20 000
- In facts only around 1800 PWH have been registered
- Reasons: under diagnosed due to many PWH died before being diagnosed or moderate and mild hemophilia in the society have not been detected
Clinical manifestation

- Easy bruising in early childhood
- Spontaneous bleeding into joint and soft tissue
- Excessive bleeding following trauma or surgery
- Patients with mild hemophilia may not have excessive bleeding unless they have trauma or surgery
- Delayed bleeding
  - Factor VIII and F IX are not required for platelet plug formation
Site of bleeding

**Serious**
- Joints (Hemarthrosis)
- Muscle/soft tissue
- Mouth, nose, gum
- Hematuria

**Life threatening**
- CNS/ Intracranial
- Gastro intestinal
- Neck/throat
- Severe trauma
Soft tissue bleeds and bruising
- no functional impairment
- tenderness, but no severe pain
- no factor needed

Neck swelling: EMERGENCY
- potential airway compromise
- treat with a major dose of factor

Iliopsoas bleeds
- flexed hip
- pain, inability to extend the leg on the affected side
- treat with a major dose of factor

Deltoid/forearm bleed and bruising
- routine factor dose
- major factor dose if a compartment syndrome is suspected

Thigh/calf bleeds
- pain
- with/without swelling
- impaired mobility
- routine factor dose
- major factor dose if compartment syndrome is suspected

Buttock bleeds
- pain
- with/without swelling
- routine factor dose
- major factor dose if the leg on the affected side exhibits tingling or swelling
**Severity of bleeding in hemophilia**

<table>
<thead>
<tr>
<th>Severity</th>
<th>Clotting factor level % activity (IU/mL)</th>
<th>Bleeding episodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>1% (&lt;0.001)</td>
<td>Spontaneous bleeding predominantly in joints and muscles</td>
</tr>
<tr>
<td>Moderate</td>
<td>1% – 5% (0.01 -0.05)</td>
<td>Occasional spontaneous bleeding. Severe bleeding after trauma or surgery</td>
</tr>
<tr>
<td>Mild</td>
<td>5% – 40%(0.05- 0.40)</td>
<td>Severe bleeding after major trauma or surgery</td>
</tr>
</tbody>
</table>
Factor VIII (antihemophilic factor)

- Glycoprotein, not stable
- Synthesized: liver, kidney, and spleen
- Gene of F VIII located at chromosome X
- In circulation: complex with von Willebrand factor, protects from proteolytic degradation
- Function: intrinsic pathway as cofactor of F IXa in the activation of F X
von Willebrand’s factor

- multimer BM 1 - 20 x $10^6$ dalton
- synthesized: endothelial cell and megakaryocyte
- function:
  - carrier of F VIII, protects from proteolytic degradation
  - Adhesion and aggregation of platelet, as a bridge between platelet and subendothelial tissue
Factor VIII

X-chromosome

Liver sinusoidal cells
Endothelial cells

Hepatocytes

Megakaryocytes

Endothelial cells

von Willebrand factor

Chromosome 12

vWF subunit
(Mr approx 220 kDa)
combines into vWF protomer.

Factor VIII, two-chain form
Mr approx 280 kDa

vWF multimer
Mr 1 – 20 MDa

Factor VIII–vWF complex

Single chain form of factor VIII transforms into two-chain form.
Factor IX

- Synthesized: liver
- Vitamin K dependent
- Function: in the intrinsic pathway, activate
  \[ F \ X \rightarrow F \ Xa \]
The role of Laboratory in the Management of Hemophilia

- Diagnosis
- Carrier detection
- Complication detection
Diagnosis of hemophilia

Anamnesis: bleeding history, family history

Physical examination: hematoma, hemarthrosis

Laboratory diagnosis: ??
Laboratory diagnosis of hemophilia

- **Platelet count**: normal
  - no alteration in the production and consumption of platelet

- **Bleeding time**: normal
  - F VIII and IX are not required in the hemostatic plug formation
Laboratory diagnosis of hemophilia

- **PT normal** since PT assess the **extrinsic** and common pathways.
- **APTT prolonged** because APTT assess the **intrinsic** and common pathways.
- F VIII and IX function in the **intrinsic pathway**
- **TT normal** because TT only assess the changes of fibrinogen to fibrin
## Screening for diagnosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>PT</th>
<th>APTT</th>
<th>BT</th>
<th>Platelet count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia A or B</td>
<td>normal</td>
<td><strong>prolonged</strong></td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Von Willebrand disease</td>
<td>normal</td>
<td>Normal or prolonged</td>
<td>prolonged</td>
<td>Normal or reduced</td>
</tr>
<tr>
<td>Platelet defect</td>
<td>normal</td>
<td>normal</td>
<td>prolonged</td>
<td>Normal or reduced</td>
</tr>
</tbody>
</table>
Screening and diagnosis of hemophilia

- **APTT**
  - Normal
    - Not hemophilia
  - Prolonged
    - PT
      - Normal
        - Hemophilia is possible
        - Substitution Test / TGT / Assay F VIII / F IX
Diagnosis of Hemophilia and DD/ hemophilia A or B

- Thromboplastin Generation Time
- APTTT substitution test

Based on the difference between F VIII and F IX properties.

F VIII is consumed during coagulation process → F VIII is absent in serum.

F IX is vitamin K dependent factors, is adsorbed by Ba(SO4) or Al (OH)₃ → F IX is absent in adsorbed plasma

- Factor VIII/IX Assay
Methods for Factor VIII assay

- Clotting Assay for Factor VIII:
  - One stage
  - Two stage

- Chromogenic substrate
One-stage Clotting Assay for F VIII

- Diluted sample
- F VIII deficient plasma
- APTT reagent
- CaCl2

37°C

5 min

Clotting time (second)

% activity of F VIII
Two-stage Clotting Assay for Factor VIII

1st stage  Conversion F X to activated F X

- Adsorbed patient plasma or standard
- F X, activated F IX, Phospholipid, Ca, F V in excess

2nd stage  Clot formation

- Source of prothrombin and fibrinogen
- Clot
Factor VIII assay by Chromogenic substrate

\[ \text{F VIII} \xrightarrow{\text{Thrombin}} \text{F VIIIa} \]

\[ \text{F X, FIXa, phospholipid, Ca} \xrightarrow{} \text{F Xa} \]

\[ \text{Chromogenic substrate} \xrightarrow{} \text{P-nitroaniline} \]

\[ \lambda 405 \text{ nm} \]
Indication of doing factor VIII assay

- To determine diagnosis of hemophilia
- To monitor the activity of F VIII before and after surgery
- To test the quality of cryoprecipitate. AABB guidelines recommend F VIII content in cryoprecipitate of 80 Unit per bag
- To detect carrier by phenotypic method. Ratio F VIII/vWF < 0.7 gives 80% chance being carrier
ACTIVITY OF F VIII

- Normal range: 50 - 150%
- Severe Hemophilia A: < 1%
- Moderate Hemophilia A: 1 - 5%
- Mild Hemophilia A: >5 - 40%
- von Willebrand disease: normal or low
- F VIII acute phase reactant

risk factor of thrombosis
ACTIVITY OF F IX

- Normal range : 50 - 150 %
- Severe Hemophilia B : < 1 %
- Moderate Hemophilia B : 1 - 5 %
- Mild Hemophilia B : >5 - 40 %

Low:
- deficiency of vit. K
- Vitamin K antagonist
- liver disease
- bile obstruction
### DD/ Hemophilia A and Hemophilia B

<table>
<thead>
<tr>
<th>Hemophilia A</th>
<th>Hemophilia B</th>
</tr>
</thead>
<tbody>
<tr>
<td>- APTT prolonged</td>
<td>- APTT prolonged</td>
</tr>
<tr>
<td>- F VIII ↓</td>
<td>- F VIII normal</td>
</tr>
<tr>
<td>- F IX normal</td>
<td>- F IX ↓</td>
</tr>
</tbody>
</table>
DD/ Hemofilia A dan von Willebrand’s d.

**Hemofilia A**
- F VIII: N
- Bleeding time: N
- vWF level: N
- vWF: Ristosetin cofactor: N

**Von Willebrand’s d.**
- F VIII: N /
- Bleeding time prolonged
- vWF level
- vWF: Ristosetin cofactor
If laboratory facility is not available

<table>
<thead>
<tr>
<th></th>
<th>Clotting time</th>
<th>Bleeding time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia</td>
<td>Prolonged</td>
<td>normal</td>
</tr>
<tr>
<td>von Willebrand disease</td>
<td>normal</td>
<td>Prolonged</td>
</tr>
</tbody>
</table>
Carrier detection
Pattern of inheritance of X linked
Status of women in hemophilia family

- Obligat carrier
- Non carrier
- Possible carrier
Carrier of hemophilia

- Daughters of PWH are obligate carrier
- Most carrier are asymptomatic
- A few carrier may have F VIII/IX level in the hemophilia range (mostly mild), very rare in moderate or severe (Lyonisation)
- Carrier with low level of F VIII/IX → bleeding manifestation → treatment
- Menorrhagia is common manifestation
- Immediate female relative of PWH, should have F VIII/IX checked
Carrier Detection

**Phenotypic**
- Pedigree analysis
- F VIII/vWF Ratio
- Bivariate Linier Discriminant Analysis

**Genotypic**
- Direct detection of mutation
- Polymorphism gene tracking
Bivariate Linear Discriminant Analysis

Input data from possible carrier

\[ \alpha = \text{age in year} \]
\[ \beta = \text{blood group group O = 0 Non O = 1} \]
\[ \gamma = \text{vWF Ag in IU/mL} \]
\[ \delta = \text{VIII in IU/mL} \]
\[ \pi = \text{genetic probability of carriersonship} \]

Input data from non carrier reference group

\[ \mu_x = \text{mean of Ln of vWF:Ag level in IU/mL} \]
\[ \mu_y = \text{mean of Ln F VIII activity in IU/mL} \]

Calculate for possible carrier

\[ X = \ln(\gamma) - \mu_x \]
\[ Y = \ln(\delta) - \mu_y \]
Calculate the coefficient for modified discriminant:
\[ a = -0.0955 - 0.0156\alpha + 0.000196\alpha^2 + 0.0298\beta \]
\[ b = 0.649 - 0.00184\alpha + 0.000314\alpha^2 + 0.117\beta \]
Calculate predicted means of the discriminants for carrier and non carrier:
\[ c = -0.391 - 0.00571\alpha + 0.0001\alpha^2 - 0.0648\beta \]
\[ d = -0.347 - 0.00171\alpha + 0.0000473\alpha^2 + 0.0754\beta \]
Calculate the Odds ratio:
\[ e = ax + by \]
\[ = 4.28 (e-c) \]
\[ g = 7.97 (e-d) \]
\[ h = 0.623 + 0.5(f+g) (f - g) \]
\[ LR = \exp(-h), \text{ the Odds ratio favouring carriership} \]
Calculate final probability of carriership
\[ Pc = \pi LR / (\pi LR + 1 - \pi) \]
Complication Detection
Chronic Complication of Hemophilia

- **Musculoskeletal:**
  - Chronic hemophilic arthropathy
  - Contractures
  - Pseudotumor formation (soft tissue and bone)
  - Fracture
- **Inhibitor of F VIII/IX**
- **Transfusion-related infection:** HIV, HBV, HCV, parvovirus B19
Inhibitor in hemophilia

- Incidence in severe hemophilia A 20 % - 33%
- Incidence in hemophilia B 1% - 4%
- Plasma derived products: 80% high responding type, very few temporary
- Recombinant F VIII : < 50% high responding type, 1/3 temporary
- Laboratory test : Bethesda inhibitor assay
Inhibitor F VIII

Patient Plasma + Pool normal plasma

F VIII def.plasma + pool normal plasma

Incubate for 2 hours

Perform F VIII assay

Perform F VIII assay

Determine residual F VIII
Convert to Bethesda unit
Comprehensive Care Team for Hemophilia

- Team comprise: hematologist (pediatrician and adult) clinical pathologist, specialist in physical medicine and rehabilitation, orthopedic surgeon, dentist, psychiatrist, nurse

- Function of the team:
  - Coordinate care and service to patients
  - Provide education to patient and family members
  - Documentation of treatment and measurement of long-term outcome especially musculoskeletal function
  - Conduct research to improve management
Management for Hemophilia in Indonesia

- Since 1997 Comprehensive Care Team for Hemophilia was established in Dr. Cipto Mangunkusumo Hospital, Jakarta
- Meeting to solve the problem in the patient management, donation from WFH
- Surgery: TKR, implant in hip surgery
- We also have Indonesian Hemophilia Society: PWH, family, doctors, nurse, social workers.
Thank you