Hemostasis

- Hemostasis is the process of blood clot formation and represents a coordinated response to vessel injury.
- The major components of the hemostatic system are:
  - The vascular endothelium
  - Platelets
  - The coagulation and fibrinolytic systems
This dynamic process is often viewed in phases:
- Vascular spasm
- Formation of a platelet plug
- Propagation of the coagulation cascade
- Formation of a clot
- Fibrinolysis of the clot.

(Vascular — Platelet — Coagulation phase)

Vascular endothelium

The healthy endothelium is a dynamic organ:
- Maintaining a barrier to macromolecules.
- When injured, in contributing to the metabolic response and local vasoconstriction.
- Inhibiting platelets, suppressing coagulation and promoting fibrinolysis.
Vascular endothelium

- **Platelet Inhibition**
  - Prostacyclin and nitric oxide
    - Potent vasodilators
    - Platelet activation and aggregation inhibition.
- **Anticoagulation Activity**
  - Produce *heparan sulfate proteoglycans, which bind antithrombin* and accelerate the rate at which it inhibits thrombin and coagulation enzymes.

Vascular endothelium

- **Fibrinolytic activity**
  - Synthesizing and releasing tissue-type and urokinase-type plasminogen activator (t-PA and u-PA)
  - Also produce type 1 plasminogen activator inhibitor 1 (PAI-1), the regulator of t-PA and u-PA
Vascular endothelium

- Vascular tone and Permeability
  - synthesize **prostacyclin and nitric oxide**: vasodilators
  - **Endothelins** induce vasoconstriction

Platelets

- They are complex cytoplasm fragment from megakaryocyte
- Platelets have a **life span of 7 to 10 days**
- The platelets’s role is termed ‘**primary hemostasis**’
1. Platelet adhesion: adhesion to subendothelial connective tissue
2. Platelet aggregation: links platelets to each other to form clumps.

Platelets

- Granules are an important component of hemostasis and contain
  - Platelet factor 4
  - Adhesive and aggregation glycoproteins
  - Coagulation factors
  - Fibrinolytic inhibitors
Platelet adhesion

- Adhesion to subendothelial connective tissue: collagen, basement membrane, and noncollagenous microfibrils
  - adhesion creates the initial bleeding arrest plug
- Adhesion to collagen and vWF result in platelets activation.
- Release of adenosine diphosphate (ADP)
  - the primary mediator and amplifier of aggregation

Platelets adhesion:
Platelets adhere to exposed collagen and von Willebrand factor (vWF) and form a monolayer that supports and promote thrombin generation and subsequent fibrin formation
Platelet adhesion

- Induce cyclooxygenase-1 (COX-1)-dependent synthesis and release of thromboxane A, another aggregator and potent vasoconstrictor
- Release of calcium, serotonin, epinephrine, and trace thrombin
Platelet aggregation

- Platelet aggregation links platelets to each other to form clumps. (GPIIb/IIIa and its ligands; Fibrinogen and vWF)
Coagulation results in the generation of thrombin, which converts soluble fibrinogen to fibrin.

- To form clot
- ‘Secondary hemostasis’
Coagulation occurs through the action of discrete enzyme complexes, which are composed of:
- A vitamin K-dependent enzyme (II, VII, IX, X)
- A non-enzyme cofactor
- And assemble on anionic phospholipid membranes in a calcium-dependent fashion.
Fibrin, which anchors the hemostatic platelet plug, is formed from soluble plasma fibrinogen by the action of the potent protease enzyme thrombin (factor IIa).

Thrombin is formed from its inactive (zymogen) plasma precursor ‘prothrombin’ (factor II) by the action of activated factor X (Xa) and its cofactor (factor Va).

This sequence of reactions has classically been referred to as the common pathway of coagulation.
Factor X can be activated by either the tissue factor (extrinsic) pathway or the contact activation (intrinsic) pathway of coagulation.

The tissue factor pathway is now considered to be the major physiologic initiator of coagulation activation.

The fibrin mesh is stabilized by covalent cross-linking mediated by factor XIII.

---

Coagulation system based on laboratory assays (Based on **waterfall hypothesis**)

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Diagram showing the coagulation cascades and assays.
The cell-based model of coagulation

Schematic diagram of hemostasis
Fibrinolysis

- The fibrinolytic protein system consists of the zymogen plasminogen and its naturally occurring activators.
- Plasminogen is activated to the main clot-lysing enzyme, plasmin, by endogenous tissue plasminogen activator (tPA), single-chain urokinase plasminogen activator (ScuPA), and two-chain urokinase plasminogen activator (TcuPA).
- These activators are found in the endothelium as well as in granulocytes and monocytes.

SCHEMATIC REPRESENTATION OF THE FIBRINOLYTIC SYSTEM.

α2-AP, α2-Antiplasmin; PAI, plasminogen activator inhibitor; tPA, tissue plasminogen activator; uPA, urokinase plasminogen
Together, coagulation, anti-coagulation, and fibrinolysis maintain a delicate physiological balance.
Natural inhibitors of coagulation

- Protein C
- Protein S

Bleeding can occur if there is:
- abnormal platelet plug formation
- reduced thrombin generation and subsequent fibrin clot formation at the site of vascular injury

Bleeding also can occur if the platelet or fibrin clot is prematurely degraded because of excessive fibrinolysis.
Comparison of the Features of Disorders of Primary, secondary or tertiary Hemostasis

<table>
<thead>
<tr>
<th>Features</th>
<th>Primary</th>
<th>Secondary</th>
<th>Tertiary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components involved</td>
<td>Platelets, vWF and vessel wall</td>
<td>Coagulation</td>
<td>Fibrinolytic factors</td>
</tr>
<tr>
<td>Site of bleeding</td>
<td>Skin and mucocutaneous and soft tissue</td>
<td>Muscles, joints and deep tissues</td>
<td>Wounds and genitourinary tract</td>
</tr>
<tr>
<td>Physical findings</td>
<td>Petechiae and ecchymoses</td>
<td>Hematomas and hemathroses</td>
<td>Hematuria and menorrhagia</td>
</tr>
<tr>
<td>Timing of bleeding</td>
<td>Immediate</td>
<td>Delayed</td>
<td>Delayed</td>
</tr>
<tr>
<td>Inheritance</td>
<td>Autosomal dominant</td>
<td>Autosomal or X-linked recessive</td>
<td>Autosomal recessive</td>
</tr>
</tbody>
</table>

vWF: von Willebrand factor

Disorder of primary hemostasis

<table>
<thead>
<tr>
<th>Components Affected</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets</td>
<td>Quantitative or qualitative platelet disorders</td>
</tr>
<tr>
<td>vWF</td>
<td>Inherited or acquired deficiency or dysfunction of vWF</td>
</tr>
<tr>
<td>Vessel wall</td>
<td>Vasculitis or abnormalities of connective tissue supporting the vasculature</td>
</tr>
</tbody>
</table>
### Disorder of secondary hemostasis

<table>
<thead>
<tr>
<th>Component Affected</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulation factors</td>
<td>Congenital deficiency, autoantibodies, increased consumption or drugs that attenuated thrombin generation or thrombin activity</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Decreased production; increased consumption or synthesis of an abnormal protein</td>
</tr>
<tr>
<td></td>
<td>Impaired fibrin polymerization because of fibrin(ogen) degradation products or paraproteins</td>
</tr>
<tr>
<td>Fibrin cross-linking</td>
<td>Congenital or acquired factor XIII deficiency</td>
</tr>
</tbody>
</table>

### Disorder of tertiary hemostasis

<table>
<thead>
<tr>
<th>Component Affected</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasminogen activators</td>
<td>Increased t-PA or u-PA release in the genitourinary tract or other tissues</td>
</tr>
<tr>
<td>Plasmin</td>
<td>Deficiency of PAI-1 or α₂-antiplasmin, resulting in an increased plasmin concentration</td>
</tr>
<tr>
<td>Plasminogen activation</td>
<td>Enhanced plasminogen activation secondary to activation of coagulation by procoagulants, such as cancer cells, artificial surfaces or snake venoms</td>
</tr>
</tbody>
</table>
Clinical approach to the patient

- History taking
- Physical examination
  - The history alone may be useful in differentiating between platelet and coagulation factor abnormalities.
  - Platelet disorders are usually manifested as acquired petechiae, purpura, or mucosal bleeding.
  - Coagulation problems are commonly congenital, are characterized by delayed deep muscle or joint bleeding, and are seen more often in men.

Clinical approach to the patient

- A bleeding tendency may be suspected if a patient previously experienced excessive hemorrhage after surgery or trauma, including common events such as circumcision, tonsillectomy, labor and delivery, menses, dental procedures, vaccinations, and injections.
- In a patient with a history of excessive or unexplained bleeding, the initial goal is to determine whether the cause is a systemic coagulopathy or an anatomic or mechanical problem with a blood vessel.
Renal failure and the myeloproliferative disorders are associated with impaired platelet–vessel wall interactions and qualitative platelet abnormalities, connective tissue diseases and lymphomas are associated with thrombocytopenia, and liver disease causes a complex coagulopathy.

Ingestion of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) that cause nonselective inhibition of cyclooxygenase leads to platelet dysfunction. These drugs are often contained in over-the-counter preparations that patients may neglect to report without specific questioning.
Platelet or vascular disorders

In general, patients with thrombocytopenia or qualitative platelet or vascular disorders present with bleeding from superficial sites in the skin and mucous membranes and tends to occur spontaneously or immediately after trauma. These may involve

- **Petechiae**, which are pinpoint cutaneous hemorrhages that appear particularly over dependent extremities (characteristic of severe thrombocytopenia)
- ecchymoses (common bruises)
- Purpura
- gastrointestinal and genitourinary tract bleeding
- Epistaxis and hemoptysis.

Coagulation factor defect

Patient with inherited or acquired coagulation factor deficiencies, such as hemophilia, or those on anticoagulant therapy tend to bleed from deeper tissue sites and in a delayed manner after trauma. These may involve

- Hemarthroses
- deep hematomas
- retroperitoneal hemorrhage
Questions

- Bleeding disorders VS local bleeding?
- Hemostasis defects?
- Acquired VS Hereditary?
- Multiple bleeding sites?
- Onset?
- Pattern of bleeding?
- Familial history?
- Prolonged bleeding, Frequency?
- Previous medical illness and medication?
- Etc.

Physical Examination

- Vital signs
- Skin: nature of bleeding, signs of liver disease
- Mucosa: oral or nasal
- Lymphadenopathy
- Abdomen: liver size and shape, splenomegaly
- Joints: signs of previous bleeding
- Other sites of blood loss: pelvic, rectal, urinary tract
Extensive ecchymosis
Collins et al. BMC Research Notes 2010, 3:161

Hemorrhagic purpura
http://dermatlas.med.jhmi.edu

Petechiae in newborn
http://newborns.stanford.edu/PhotoGallery

Extensive ecchymosis

Laboratory evaluation of hemostatic disorder

- Complete blood count and blood smear
  - Assess the degree of disease associated with the bleeding episode
- Platelets count
  - the normal range being 150,000 to 400,000/mm³
  - Platelet counts less than 100,000/mm³ define thrombocytopenia.
Bleeding time

- Bleeding time is for determining both vascular integrity and platelet function
- The test (Ivy method)
  - Making two standard incisions 1 mm deep and 1 cm long on the volar aspect of the forearm under 40 mm Hg pressure via a blood pressure cuff.
  - The time is measured from the incision to the moment when the blood oozing from the wound is no longer absorbed by filter paper.
- Normal time is 8 minutes (Duke method; 1-3 min)
  - 8 - 10 min : borderline
  - > 10 min : abnormal
- A paper conclude that the bleeding time was not effective as a screening test, and that a normal bleeding time does not exclude a bleeding disorder.
The activated partial thromboplastin time (aPTT) tests the components of the intrinsic and common pathways, that is, essentially all factors but VII and XIII in the entire clotting cascade.

- A phospholipid source and a contact-activating agent (kaolin) are added to anticoagulated citrate plasma.
- After an incubation period that allows factor XII to become activated, calcium is added and the clotting time is recorded.
- Factor levels are often less than 40% before the PTT is prolonged.

**Reference value** 22-44 seconds (มีนาคม 2557)

- A classic approach used by many clinicians for monitoring unfractionated heparin therapy is to aim for a 1.5- to 2.5-fold increase in APTT.
The prothrombin time (PT) tests the factors of the extrinsic and common pathways.

- The patient’s anticoagulated plasma is combined with calcium and tissue factor.
- The PT detects deficiencies in fibrinogen (factor I), prothrombin (factor II), factor V, factor VII, and factor X.
- It is used to test the extrinsic pathway.
  - Or monitoring warfarin therapy
Coagulation system based on laboratory assays (Based on waterfall hypothesis)
- PT > 2 seconds or more over the control time can be considered significant.
- Results are usually reported as the international normalized ratio (INR), which compensates for differences in sensitivity of various thromboplastin reagents to the effects of warfarin.
- INR = (patient PT/mean normal PT)^[ISI]
  - Normal value: 0.75-1.3
  - Therapeutic level: 2-4.5

*ISI = International Sensitivity Index*

- Thrombin clotting time or thrombin time
  - Purified thrombin is added to plasma, and the time to clot formation is measured.
  - It is a direct measure of the conversion of fibrinogen to fibrin
  - It is a useful screening test for both qualitative and quantitative abnormalities of fibrinogen and inhibitors such as heparin and fibrin split products.

- Venous clotting time
  - Tests the extrinsic and normal pathways.
anti-Xa assay

- The plasma Anti-Xa assay is a test that is used for monitoring patients on LMWHs or UFH.
  (Patients not on heparin: 0 U/ml)
- **Clot Solubility**
  - The result of clot solubility testing may be the only abnormality in disorders involving factor XIII deficiency and some abnormal fibrinogen. A washed clot is incubated in urea or acetic acid.
  - If the clot is not properly cross-linked, it dissolves.

- **Mixing study (often 1:1)**
  - Mixing studies based on the PT or aPTT are interpreted based on the fact that a 50% level of any coagulation factor alone gives normal PT and aPTT values.
  - Abnormal PT or aPTT
    - Normal value after normal plasma mixing (immediate or 2hr): Coagulation factor(s) deficit
    - Abnormal value after normal plasma mixing (immediate or 2hr): Coagulation factor inhibitor
Factor Level Assays

- Factor levels are determined either by bioassay, in which the ability of the sample of plasma to normalize controlled substrate-deficient plasma is evaluated, or by immunologic assay.
# Laboratory Evaluation of Abnormalities of Coagulation

### Protein aPTT and PT Screening Tests

<table>
<thead>
<tr>
<th>Long aPTT Normal PT</th>
<th>Long PT Normal aPTT</th>
<th>Long aPTT Long PT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Associated with bleeding:</strong></td>
<td><strong>Anticoagulants</strong></td>
<td></td>
</tr>
<tr>
<td>Factor VII, IX</td>
<td>DIC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin K deficiency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Massive transfusion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dysfibrinogenemia</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Not associated with bleeding:</th>
<th>Lupus anticoagulant (LA)</th>
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<th>Long aPTT Long PT</th>
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<td></td>
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<tr>
<td>Factor VII, IX</td>
<td>DIC</td>
<td></td>
</tr>
<tr>
<td>Acquired inhibitor of FVIII</td>
<td>Liver disease</td>
<td></td>
</tr>
<tr>
<td>Amyloid adsorb of factor IX</td>
<td>Vitamin K deficiency</td>
<td></td>
</tr>
<tr>
<td>FXI</td>
<td>Massive transfusion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antibodies to factor V</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Not associated with bleeding:</th>
<th>Lupus anticoagulant (LA)</th>
<th>Common pathway deficiency (rare)</th>
<th>Dysfibrinogenemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single factor deficiency</td>
<td>Single factor deficiency</td>
<td>Single factor deficiency</td>
<td>Perform specific inhibitor assay</td>
</tr>
<tr>
<td>Perform specific inhibitor assay</td>
<td>Perform specific inhibitor assay</td>
<td>Perform specific inhibitor assay</td>
<td></td>
</tr>
<tr>
<td>Multiple factor deficiency</td>
<td>Multiple factor deficiency</td>
<td>Multiple factor deficiency</td>
<td></td>
</tr>
<tr>
<td>- Pan-Inhibitor: LA, Immunoglobulin</td>
<td>- Pan-Inhibitor: LA, Immunoglobulin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Common coagulation studies

- CBC and smear (EDTA—purple top)
- Platelet count (EDTA—purple top)
- Bleeding time
- Prothrombin time (citrate—blue top)
- Partial thromboplastin time (citrate—blue top)
- Other coagulation studies: fibrinogen level, thrombin time, clot solubility, factor levels, inhibitor screens
- As necessary: electrolytes, glucose, BUN, creatinine, type and crossmatch

Bleeding disorders

- Hemorrhagic diathesis may be caused by
  - Increased blood vessel fragility
  - Platelet disorder
  - Coagulation defect
Bleeding by vessel wall

- Increased vascular fragility
  - Bleeding can occur with
    - inflammation or malformations of the blood vessels
    - abnormalities of the connective tissue supporting the blood vessels.
      - Henoch-Schonlein purpura
      - vasculitis that occurs with paraproteins or cryoglobulins or in patients with systemic lupus erythematosus or other immune disorders.
      - Hereditary hemorrhagic telangiectasia is an inherited disorder associated with malformations of the capillaries.
  - Can also occur with infection
    - Meningococcus and rickettsia
    - Vasculitis or DIC

Platelet disorder

- Thrombocytopenia
  - Thrombocytopenia
    - is defined as a platelet count less than the normal range, typically below 150,000/µL.
Thrombocytopenia

- Platelets/mm$^3$
  - $100,000$: can surgery
  - $<100,000$: prolonged bleeding time
  - $<50,000$: bleeding after trauma/surgery
    (depends on operation/procedure)
  - $<10,000-20,000$: spontaneous bleeding
  - $<5,000$: increase risk of intracranial hemorrhage

Thrombocytopenia

- There is no absolute threshold for spontaneous bleeding due to thrombocytopenia.
- It may occur at higher counts when fever, sepsis, severe anemia, and other hemostatic defects are present or when platelet function is impaired by medication.
Thrombocytopenia

- General mechanisms of thrombocytopenia
  - Increased Platelet Destruction
  - Platelets Sequestration
  - Decreased Platelet Production
  - Other e.g. dilution: massive transfusion

Mechanisms of Platelet Destruction

<table>
<thead>
<tr>
<th>Type of thrombocytopenia</th>
<th>Specific example(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune mediated</td>
<td></td>
</tr>
<tr>
<td>Autoantibody-mediated platelet destruction by reticuloendothelial system (RES)</td>
<td>Primary immune thrombocytopenic purpura</td>
</tr>
<tr>
<td></td>
<td>Secondary immune thrombocytopenic purpura such as collagen vascular disease; Infectious mononucleosis; HIV</td>
</tr>
<tr>
<td>Alloantibody-mediated platelet destruction by RES</td>
<td>Neonatal alloimmune thrombocytopenia posttransfusion purpura</td>
</tr>
<tr>
<td></td>
<td>passive alloimmune thrombocytopenia alloimmune platelet transfusion refractoriness</td>
</tr>
<tr>
<td>Drug-dependent, antibody-mediated platelet destruction by RES</td>
<td>Drug-induced immune thrombocytopenic purpura (e.g., quinine)</td>
</tr>
<tr>
<td>Platelet activation by binding of IgG Fc of drug-dependent IgG to platelet Fc receptor</td>
<td>Heparin-induced thrombocytopenia</td>
</tr>
</tbody>
</table>
# Mechanisms of Platelet Destruction

<table>
<thead>
<tr>
<th>Type of thrombocytopenia</th>
<th>Specific example(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-immune mediated</td>
<td></td>
</tr>
<tr>
<td>Platelet activation by thrombin or proinflammatory cytokines</td>
<td>Disseminated intravascular Coagulation septicemia/systemic inflammatory response syndromes</td>
</tr>
<tr>
<td><strong>Platelet destruction via ingestion by macrophages (hemophagocytosis)</strong></td>
<td>Infections; certain malignant lymphoproliferative disorders</td>
</tr>
<tr>
<td>Platelet destruction through platelet interactions with altered von Willebrand factor</td>
<td>Thrombotic thrombocytopenic purpura hemolytic-uremic syndrome</td>
</tr>
<tr>
<td><strong>Platelet losses on artificial surfaces</strong></td>
<td>Cardiopulmonary bypass surgery use of intravascular catheters</td>
</tr>
<tr>
<td>Decreased platelet survival associated with cardiovascular disease</td>
<td>Congenital and acquired heart disease cardiomyopathy pulmonary embolism</td>
</tr>
</tbody>
</table>

## Problems that lead to platelets destruction

- **Cardiopulmonary bypass surgery**
- **Use of intravascular catheters**
  - Platelets adhere to extracorporeal synthetic surface
  - Fibrinogen and other plasma proteins adhere to the artificial surfaces that provide a substrate for platelets adhesion
  - Hemodilution
Immune thrombocytopenic purpura (ITP)

- It is an autoimmune disease characterized by immune-mediated platelet destruction.
  - **Acute ITP**
    - Platelet autoantibody
    - Destruction occurs in the spleen
    - Patients with acute ITP usually have a rapid onset of bleeding symptoms
    - Often in children with a history of a recent upper respiratory infection, febrile illness or immunization
      - Rubella, CMV, viral hepatitis, infectious mononucleosis
  - **Chronic ITP**
    - In more than 70% of adults, ITP persists beyond the 6-month period and becomes chronic.

**Clinical features of ITP**

- Adult, female, easy bruising or nosebleeding, petechial hemorrhage, internal hemorrhage (melena, hematuria)
- The most serious complication of acute ITP is intracranial hemorrhage (ICH)
- Diagnosis
  - Clinical
  - Blood smear: thrombocytopenia
  - Bone marrow biopsy: increased megakaryocyte
  - Bleeding time: prolonged
  - PT and aPTT: normal
- ITP is a diagnosis of exclusion.
Immune thrombocytopenic purpura (ITP)
- the most common and most effective pharmacologic therapies for acute ITP include intravenous immunoglobulin (IVIG), Rh immune globulin (RHIG) and corticosteroids

Drug-induced thrombocytopenia
- Immune-mediated platelet destruction
- Drug acting as hapten
- Drug withdrawal leads to clinical improvement
  - e.g.
    - Acetaminophen, Ampicillin, Simvastatin, Ibuprofen Ranitidine, Naproxen, Phenytoin, Rifampin, Ethambutol, Trimethoprim–sulfamethoxazole, Valproic acid, Carbamazepine, Haloperidol, Oxaliplatin
- Heparin-induced thrombocytopenia
HIT is caused by platelet-activating immunoglobulin G (IgG) antibodies that bind to multimolecular complexes of platelet factor 4 (PF4) bound to heparin.

Whereas DITP is strongly associated with petechiae and purpura, HIT is strongly associated with thrombosis.
D-ITP: Drug-induced thrombocytopenia
HIT: Heparin-induced thrombocytopenia

Thrombotic thrombocytopenic purpura (TTP)

- A syndrome consisting of
  - microangiopathic hemolytic anemia (MAHA)
  - Thrombocytopenia
  - End-organ damage secondary to microvascular thrombi.
- The pathophysiology of TTP is an acute deficiency of von Willebrand factor (VWF)-cleaving metalloprotease ADAMTS13 [a disintegrin and metalloprotease with thrombospondin type 1 motif, 13]) due to
  - ADAMTS13 inhibitor
  - Genetic dysfunction
- ADAMTS13 deficiency results in the accumulation of ultra-large VWF multimers, which bind platelets, and leads to both thrombi in the microvasculature and thrombocytopenia.
Autopsy: from patient with ITP

Microthrombi: arrows

Schistocyte: arrow

Microangiopathic hemolytic anemia (MAHA)

Schistocyte: arrow
Thrombotic thrombocytopenic purpura (TTP)

- The classic pentad of anemia, thrombocytopenia, fever, neurological signs and renal failure is infrequently present.
- Therapeutic plasma exchange (TPE) is the primary therapy for TTP
Hemolytic uremic syndrome (HUS)

- Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy (TMA) characterized by thrombocytopenia, acute renal failure and microangiopathic hemolytic anemia.
- TMA is defined by endothelial cell damage, resulting in platelet-associated thrombosis and vessel obstruction.
- Red blood cell (RBC) destruction occurs in the vessels narrowed by these thrombi, leading to schistocyte formation.
- HUS is most commonly associated with bloody diarrhea caused by shiga-like toxin-producing bacteria (~90% of HUS in children).
Platelet sequestration

- Platelets may be sequestered when the spleen enlarges owing to portal hypertension or infiltrative diseases.
- This in turn can result in moderate thrombocytopenia.
- Because hypersplenism never causes a platelet count less than 40,000 to 50,000/µL, bleeding due to thrombocytopenia from hypersplenism alone is unusual.
Decreased platelet production

- Occurs in
  - Primary diseases of the bone marrow, such as acute leukemia and aplastic anemia
  - Myelophthisic processes in which marrow is affected by metastatic carcinoma, fibrosis, or other clonal hematopoietic disorders.
  - Following chemotherapy and/or radiation therapy;

- Ethanol toxicity
- Infections with viruses such as Dengue virus, HIV, cytomegalovirus (CMV), Epstein-Barr virus (EBV), and varicella.
- Thrombocytopenia also occurs when normal megakaryocyte proliferation is impaired by myelodysplasia.
Marked reduction in all three cell lines (erythroid, myeloid, and megakaryocytic), with replacement by normal fat, characterize the classic picture of aplastic anemia.

Defective platelet function

- **Congenital disorder**
  - Defective platelet adhesion
    - Bernard-Soulier Syndrome: an abnormality in the platelet GPIb/V/IX complex
  - Defective platelet aggregation
    - Glanzmann Thrombasthenia: a quantitative or qualitative defect in the GPIIb/IIIa complex
  - Disorders of platelets secretion
Defective platelet function

- **Acquired disorders**
  - Many drugs may affect platelet function.
  - Aspirin irreversibly acetylates and inactivates the platelet cyclooxygenase, leading to inhibition of synthesis of Thromboxane A2 (TxA2) and endoperoxides (PGG2 and PGH2).
  - Several other nonsteroidal antiinflammatory drugs (NSAIDs) also impair platelet function by inhibiting the cyclooxygenase enzyme and may prolong bleeding time.
  - Compared with aspirin, inhibition of cyclooxygenase by these agents is generally short-lived and reversible.
Defective platelet function

- Ticlopidine, Clopidogrel, and Prasugrel are orally administered thienopyridine derivatives that inhibit platelet function by inhibiting the binding of ADP to the platelet P2Y12 receptor.

- Uremia
  - The pathogenesis remains unclear.
  - But platelet dysfunction and impaired platelet–vessel wall interaction are considered the major causes.
  - Bleeding time is prolonged in uremia.
Hemorrhagic diathesis related to abnormalities in clotting factors

- **Clinical features**
  - Large ecchymosis or hematoma after injury
  - Prolonged bleeding after a laceration or surgical procedure
  - Bleeding of GI, Urinary tract, Weight bearing joints.

- **Hereditary abnormalities**: Hemophilia, von Willebrand disease, etc.

- **Acquired abnormalities**: Liver disease, Dissiminated intravascular coagulation, vitamin K deficiency

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**von Willebrand disease**

- vWF serves as a carrier protein for factor VIII
- And an adhesive platelet ligand that tethers the platelet to exposed collagen at sites of vascular injury.
- von Willebrand disease is a group of inherited bleeding disorders related to qualitative or quantitative defects of vWF.
### Classification of von Willebrand disease

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Inheritance</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Partial quantitative deficiency of vWF. Mild abnormalities in multimer structure or distribution may occur.</td>
<td>AD</td>
<td>70-80%</td>
</tr>
<tr>
<td>2</td>
<td>Qualitative vWF defects.</td>
<td>AD, AR</td>
<td>15-30%</td>
</tr>
<tr>
<td>2A</td>
<td>Decreased vWF-dependent platelet adhesion and deficiency of HMW vWF multimers.</td>
<td></td>
<td>10%</td>
</tr>
<tr>
<td>2B</td>
<td>Increased affinity for platelet GPIbα.</td>
<td></td>
<td>&lt;8%</td>
</tr>
<tr>
<td>2M</td>
<td>Decreased vWF-dependent platelet adhesion with a normal multimer distribution.</td>
<td></td>
<td>&lt;2%</td>
</tr>
<tr>
<td>2N</td>
<td>Decreased affinity for FVIII.</td>
<td></td>
<td>&lt;1%</td>
</tr>
<tr>
<td>3</td>
<td>Almost complete deficiency of vWF.</td>
<td>AR</td>
<td>rare</td>
</tr>
</tbody>
</table>
von Willebrand disease

- Mild type I vWD may not be diagnosed until adulthood
- Individuals with vWD primarily complain of excessive mucocutaneous bleeding, such as
  - Bruising without recognized trauma
  - Prolonged, recurrent nose bleeds
  - Oral cavity bleeding, including bleeding from the gums after brushing or flossing the teeth or prolonged bleeding after dental cleaning or extractions.
  - Prolonged excessive bleeding after surgery or trauma
- Affected females frequently experience menorrhagia, usually since menarche, and have prolonged or excessive bleeding after childbirth.

von Willebrand disease

- In general, the management of vWD can be divided into three main categories:
  - Localized measures to stop or minimize bleeding;
  - Pharmacologic agents that provide indirect hemostatic benefit; e.g. Tranexamic acid
  - Treatments that directly increase plasma vWF and FVIII levels; e.g. Desmopressin (DDAVP), vWF : FVIII concentrates.
Hemophilia

- The hemophilias include hemophilia A and hemophilia B, caused by deficiencies or defects in clotting factor VIII (antihemophilic factor) and factor IX (antihemophilic factor B, or Christmas factor), respectively.
- Hemophilia A and B are X-linked recessive disorders.
  - The prevalence in Thailand is between 1 of 13,000 to 20,000 people.
- Factor XI deficiency (Hemophilia C) is an autosomal dominant disorder (severe form is an AR disorder)
  - The prevalence is 1:1,000,000 in general population.
- A deficiency of either of these intrinsic coagulation pathway proteins results in inadequate formation of thrombin at sites of vascular injury.

- Acquired hemophilia A (AHA) is a very rare disease, caused by the development of autoantibodies, directed against circulating factor VIII of coagulation.
Hemophilia

- The severity of bleeding depends upon the percentage of circulating clotting factor activity.
  - 5–40% are classified as having mild hemophilia
  - 1–5% : moderate
  - less than 1% : severe
- Approximately 60% of all cases of hemophilia A are clinically severe, whereas only 20 to 45% of cases of hemophilia B are severe.

Hemophilia

- Severe disease, characterized by frequent spontaneous bleeding events in joints (hemarthrosis) and soft tissues and by profuse hemorrhage with trauma or surgery.
- Although spontaneous bleeding is uncommon in mild deficiencies (> 5% normal activity), excess bleeding typically occurs with trauma or surgery.
Hemophilia

- Diagnosis of hemophilia
  - A family and personal bleeding history
  - Laboratory detection of prolongation of the aPTT (with normal PT)
  - Factor assay

- If mild hemophilia is clinically suspected in an individual with a normal aPTT, a specific factor should be measured.

- Factor antibody
  - History of factor replacement
  - Differential diagnosis: Mixing study, Factor antibody

Hemophilia

- Treatment and prevention of acute bleeding are based on replacement of the missing factor.
  - Recombinant factor VIII or factor IX concentrate
  - Cryoprecipitate (there is no FIX)
  - Fresh frozen plasma
  - Recombinant factor VIIa (For use in patients with inhibitors to factor VIII, IX or XI)
  - Etc.
Disseminated Intravascular coagulation

- DIC is an acquired syndrome characterized by the intravascular activation of coagulation without a specific localization and arising from different causes.
- It can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction.
- At the microcirculatory level, both thromboses and hemorrhage into the organ result in ischemia, tissue damage, and progressive organ failure.
Cause of DIC

- Infectious
  - Meningococcemia
  - Bacterial infection (staphylococcal, streptococcal, Escherichia coli, Salmonella)
  - Rickettsia, fungus, Malaria, virus
- Tissue injury: Massive head injury, massive burn, profound shock, etc.

Cause of DIC

- Venom or toxin e.g. Russell's viper (งูแมวเซา)
- Microangiopathic disorder: Severe TTP/HUS, Giant hemangioma
- Gastrointestinal: Pancreatitis, Fulminant hepatitis, etc.
- Miscellaneous: Placental abruption, Acute hemolytic transfusion reaction, ATIII or homozygous Protein C deficiency etc.
Treatment of DIC

- Identify and eliminate the underlying cause
  - No treatment if mild, asymptomatic, and self-limited
  - Hemodynamic support, as indicated, in severe cases
  - Blood component therapy
    - Indications: active bleeding or high risk for bleeding
    - Fresh-frozen plasma
    - Platelets
  - In some cases, consider cryoprecipitate, antithrombin III
- Drug therapy
  - Indications: heparin for DIC manifested by thrombosis or acrocyanosis;
  - antifibrinolytic agents generally contraindicated except with life-threatening bleeding and failure of blood component therapy

Coagulation defects in liver disease

- Abnormalities in coagulation
  - Decreased synthesis of coagulation factors
  - Impaired vitamin K–dependent γ-carboxylation
  - Dysfibrinogenemia
  - Disseminated intravascular coagulation
  - Increased fibrinolytic activity
- Abnormalities in platelets
  - Thrombocytopenia (hypersplenism)
  - Abnormal platelet function
Vitamin K deficiency

- Vitamin K is required for γ-carboxylation of glutamic acid residues of the procoagulant factors II (prothrombin), VII, IX, and X and the anticoagulant factors protein C and protein S.

Anticoagulant

- Heparin
  - Action: Inhibit thrombin (IIa), Inhibit Factor Xa, Inhibit Factor IX and XI
  - Heparin binds to the enzyme inhibitor antithrombin III (AT)
    - results in its activation
  - Low molecular weight heparin (LMWH)
    - Inhibit FXa >> FIIa
Monitoring UFH e.g. aPTT, anti-Xa assay
Monitoring LMWH e.g. anti-Xa assay

- Warfarin
  - Action
    - inhibit vitamin K epoxidase, Vitamin K dependent factor depletion (II, VII, IX and X)
  - Dose adjustment by INR (adjusted PT ratio to ISI)