LECTURE 8. HAEMOSTASIS

OVERVIEW

1. Normal haemostasis
2. Clinical signs of haemostatic disorders
3. Sample collection and storage
4. Laboratory evaluation of haemostasis
5. Haemostatic disorders

1. NORMAL HAEMOSTASIS
DEFINITION

Haemostasis is a complex sequence of physical and biochemical changes induced by damage to tissues and blood vessels, which transform the blood into a clot, and, later, bring about the repair of damaged vascular endothelium.

FUNDAMENTAL STEPS IN HAEMOSTASIS

<table>
<thead>
<tr>
<th>STEP</th>
<th>ELEMENTS INVOLVED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary haemostasis</td>
<td>blood vessels</td>
</tr>
<tr>
<td></td>
<td>platelets (PLT, thrombocytes)</td>
</tr>
<tr>
<td>Secondary haemostasis</td>
<td>plasma coagulation factors</td>
</tr>
<tr>
<td>Fibrinolysis</td>
<td>plasma coagulation factors</td>
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</tbody>
</table>

OVERVIEW OF HAEMOSTASIS

Vascular damage

- collagen exposure
- release of tissue thromboplastin

WVF (von Willebrand Factor)

PLT aggregation

- intrinsic pathway
- extrinsic pathway

THROMBIN

FIBRIN

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PRIMARY HAEMOSTASIS

VASCULAR PHASE
- Vessel constriction
- Pressure by external blood lost into surrounding tissues

PLATELET PHASE
- Immediate accumulation of PLT at the site of blood vessel damage
- PLT adhesion to the subendothelial collagen by means of exposure of vWF
- PLT shape changes and release of its internal substances (ADP, serotonin…) which induce aggregation of further PLTs

Formation of primary platelet plug

SECONDARY HAEMOSTASIS

Factor XII
Factor XI
Factor IX
Factor VIII
Factor X + Factor V
Prothrombin
Thrombin
Fibrinogen
Fibrin

FIBRINOLYSIS

endothelial damage and activation of some coagulation factors
Plasminogen → Plasmin → fibrin digestion (clot lysis)
Fibrin(ogen) degradation products (FDP)
bleeding tendency
HAEMOSTASIS MODULATION

Activators and inhibitors of coagulation and fibrinolysis are normally in dynamic equilibrium. Failure of the equilibrium may result in:
- abnormal bleeding or
- increased risk of intravascular coagulation (thrombosis)

EXAMPLE OF MODULATION OF HAEMOSTASIS: Effects of Antithrombin III (AT-III)

- AT-III mainly inhibits the action of Thrombin
- Heparin potentiates the activity of AT-III

2. CLINICAL SIGNS OF HAEMOSTATIC DISORDERS
CLINICAL HISTORY

- Tendency to prolonged bleeding (in the past or recently)
- Patient's age
- Exposure to drugs, poisons, and chemicals

CLINICAL EVALUATION

Bleeding type can help to identify the cause:

- Capillary bleeding: Defects in primary haemostasis (inadequate platelet numbers/function)
- Haemorrhages, massive bleeding, haematomas: Defects in secondary haemostasis (coagulation factors)

EXAMPLES OF BLEEDING ON MUCOSAL OR CUTANEOUS SURFACES
(Usually due to defect in primary haemostasis)
EXAMPLES OF BLEEDING IN TISSUES
(Usually due to defect in secondary haemostasis)

*cutaneous haematoma*  *intraocular haemorrhage*

EXAMPLES OF BLEEDING FROM NATURAL ORIFICES
Can be due to primary and/or secondary defects

*epistaxis*  *haemoptysis*

3. SAMPLE COLLECTION AND STORAGE
SAMPLE COLLECTION

– Clean venipuncture from a large vein
– Discard haemolyzed or clotted samples
– Anticoagulants:
  • EDTA for PLT count
  • Sodium citrate for coagulation assays
– Centrifuge and separate citrated plasma as soon as possible

Sample collection is critical for reliable results

SAMPLE CARE AND STORAGE IS CRITICAL FOR RELIABLE RESULTS

– If possible, perform the coagulation assays asap
– Store and deliver citrated plasma with care
– Citrated plasma from a healthy animal is sometimes required as a control

4. LABORATORY EVALUATION:
   - Platelets
   - Coagulation factors
   - Fibrinolysis
LABORATORY EVALUATION OF PLATELETS

PLATELET EVALUATION
- PLT count, morphology, and mean platelet volume MPV (see Lecture 4)
- Bone marrow examination (see lecture 9)
- PLT function tests
  - Clot Retraction Time
  - Bleeding time

PLATELET COUNT (PLT) INTERPRETATION
- PLT < 100 x 10^9/L = thrombocytopenia
- PLT 40-70 x 10^9/L = bleeding may be present
- PLT < 10-20 x 10^9/L = bleeding in all affected animals

Always rule-out a pseudothrombocytopenia due to PLT clumping
COMPARED IMAGES OF PLATELET PRECURSORS AND OSTEOBLAST IN THE BONE MARROW

MEGAKARYOBLASTS

MEGAKARYOCYTE

PROMEGAKARYOCYTE

OSTEOBLAST

CLOT RETRACTION TIME (CRT) (CLOT LYSIS)
– To evaluate thrombocytopenia (crude test)
After few minutes from sampling

P1 C1 P2
P1 (defective clot retraction) C1 P2 (normal clot retraction)

BLEEDING TIME (BT)
Time taken for bleeding to stop following a standardised incision

Incisions can be made on:
– mucosae of the upper lip (buccal mucosal bleeding time/BMBT) or vaginal mucosae (cattle) (vaginal mucosal bleeding time),
– alternatively the toenail may be clipped a little shorter (cuticle bleeding time/CBT).

An increased time indicates vascular abnormalities, lack of platelets or defects of platelet function
**PROTHROMBIN TIME (PT)**

- It is prolonged by alterations of factors involved in extrinsic (Factor VII) and common pathways (Factors X, V, II, and I)
- Always performed with the activated partial thromboplastin time (aPPT)

**ACTIVATED PARTIAL THROMBOPLASTIN TIME (aPTT, PTT)**

- It is prolonged by alteration of factors involved in intrinsic (Factors XII, XI, IX, and VIII) and common pathways (Factors X, V, II, and I)
- Always performed with PT

**ALTERNATIVE TESTS FOR aPTT**

**COAGULATION TIME (CT, WBCT)**
- A prolongation indicates deficiency of factors in the intrinsic pathway

**ACTIVATED COAGULATION TIME (ACT)**
- ACT is prolonged by defects in intrinsic and common pathways
- Less sensitive than aPTT
- Prolongation may be caused by thrombocytopenia
LABORATORY EVALUATION OF FIBRINOLYSIS

FIBRIN (FIBRINOGEN) DEGRADATION PRODUCTS (FDPs) AND D-DIMER

- FDPs derive from the action of plasmin on fibrinogen and fibrin.
- D-dimer originates only from the action of plasmin on (insoluble) fibrin
FDPs and D-DIMER

Fibrinogen \( \Rightarrow \) Fibrin
\[ \downarrow \] Plasmin \[ \downarrow \] Plasmin
FDPs \hspace{1cm} \text{D-dimer and FDPs}

Raised values of FDPs and D-dimer indicate increased fibrinolysis (i.e. both are increased in Disseminated Intravascular Coagulation)

TESTS RECOMMENDED FOR INVESTIGATION OF HAEMOSTATIC DISORDERS

- For platelet evaluation:
  - Platelet count and morphology
  - Bleeding time (vascular-Plt function)
- For coagulation factor evaluation:
  - PT (extrinsic and common pathways)
  - aPTT (intrinsic and common pathways)
- For fibrinolysis evaluation:
  - FDPs or D-dimer (fibrinolysis)

5. HAEMOSTATIC DISORDERS
CLASSIFICATION OF HAEMOSTATIC DISORDERS

- vascular
- platelet
  - Alterations in platelet count
  - Alterations of platelet function
- coagulation factors
  - Inherited disorders
  - Acquired disorders
- fibrinolytic system
  - Disseminated Intravascular Coagulation
  - Thrombosis

PLATELET DISORDERS

- Acquired
  • thrombocytopenia
    a) ↓ production (bone marrow disorders)
    b) Consumption (Disseminated Intravascular Coagulation, DIC)
    c) ↑ destruction (immune-mediated)
  • thrombocytosis
  • thrombocytopenia
- Inherited
  (i.e. thromboasthenic thrombocytopenia)

LABORATORY FINDINGS

<table>
<thead>
<tr>
<th>THROMBOCYTOPENIA</th>
<th>THROMBOCYTOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count:</td>
<td>↓ markedly and persistently ↑</td>
</tr>
<tr>
<td>BMBT:</td>
<td>↑ or N or N</td>
</tr>
<tr>
<td>PT</td>
<td>in ref. range or in ref. range</td>
</tr>
<tr>
<td>aPTT</td>
<td>- or -</td>
</tr>
<tr>
<td>FDPs</td>
<td>- or -</td>
</tr>
</tbody>
</table>

Platelet count: ↓ markedly and persistently ↑ (> 1,000 x 10^9/L)
BMBT: ↑ or N or N
PT: in ref. range or in ref. range
aPTT: - or -
FDPs: - or -
THROMBOCYTOPATHY:
LABORATORY FINDINGS
- Platelet number: in reference range
- BMBT: ↑
- PT, aPTT and FDPs in reference ranges
- alteration of PLT function assays
  (clot retraction, and more specific assays eg. PLT aggregation to various inducers)

DISORDERS OF COAGULATION FACTORS
- disorders are induced by a deficiency of
  (biological) clotting activity of one or more clotting factors
- clinically characterized by large haemorrhages, haematomas and bleeding into body cavities
  (including joints and epidural spaces)
- classified as inherited or acquired

INHERITED DISORDERS OF COAGULATION FACTORS
GENERAL CHARACTERISTICS
- Usually affect young animals
- Usually affect a single coagulation factor
- Will present as a defect in secondary haemostasis (with external and/or internal bleeding)
LABORATORY FINDINGS IN INHERITED COAGULATION DISORDERS

- General tests (used as screening assays):

<table>
<thead>
<tr>
<th>Tests Results</th>
<th>Factors possibly affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ APTT and PT</td>
<td>XII, XI, IX or VIII</td>
</tr>
<tr>
<td>↑ PT and = APTT</td>
<td>III or VII</td>
</tr>
<tr>
<td>↑ PT and APTT</td>
<td>X or other factor in the common pathway (prothrombin, fibrinogen)</td>
</tr>
</tbody>
</table>

- Quantification of specific factors
- Tests to detect von-Willebrand’s disease:
  - = PT and APTT and ↑ BMBT
  - vW factor Ag assay; (usually <50%)

IDENTIFICATION OF SPECIFIC DISEASES

Factor XII (no clinical signs)

Factor XI (haemophilia C)

Factor IX (haemophilia B)

Factor VIII (FVIII:C, haemophilia A)
(vWf. von Willebrand’s disease)

Factor X (death in newborn)

Factor VII (minor clinical signs)

ACQUIRED DISORDERS OF COAGULATION FACTORS

- Vitamin K antagonism and deficiency
- Disseminated Intravascular Coagulation (DIC)
- Liver disease
VITAMIN K ANTAGONISM/DEFICIENCY: LABORATORY FINDINGS

- Platelet number: in reference range
- BMBT: in reference range
- ↑ PT and aPTT.

PT is firstly affected (FVII has a short half life and is first to become deficient producing ↑ PT)

DIC: LABORATORY FINDINGS

DIC usually induces alterations in several laboratory tests evaluating different coagulation steps:
- Platelet (↓ PLT count).
- Vascular-platelet (↑ bleeding time).
- Plasma coagulation factors (↑ PT, aPTT or ACT),
- Fibrinolysis (↑ in FDPs and D-dimers, ↓ in ATIII)

COMBINED ALTERATIONS IN LABORATORY RESULTS OF DIFFERENT PHASES OF COAGULATION ARE HIGHLY INDICATIVE OF DIC

In addition to thrombocytopenia, DIC may be associated with other haematological abnormalities:
1. Schistocytes
2. Regenerative haemolytic anaemia
3. Others findings:
   - neutrophilia with left-shift (rarely neutropenia),
   - haemoglobinaemia (intravascular haemolysis)

serum biochemistry and urinalysis may indicate organ failure, and possibly the cause of DIC
ALTERATIONS OF COAGULATION TESTS IN LIVER DISEASE

- Alteration in liver function test (bilirubin, bile acids)

+ 

- ↑ PT and aPTT (as a result of decreased synthesis of coagulation factors in the liver)

DISORDERS OF THE FIBRINOLYTIC SYSTEM

- Thrombosis

- Disseminated Intravascular Coagulation (DIC)

THROMBOSIS: LABORATORY FINDINGS

- ↓ level of ATIII

- Occasionally ↓ PT, aPTT

- Very marked ↑ of D-dimer, while FDPs are slightly ↑

Overall diagnosis is difficult and complementary methods such as diagnostic imaging (doppler ultrasound) are frequently used
**LABORATORY FINDINGS IN THE MOST FREQUENT COAGULATION DISORDERS (MAINLY DOG AND CAT)**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>BT</th>
<th>BM</th>
<th>PCT</th>
<th>PT</th>
<th>aPTT</th>
<th>D-dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia due to increased consumption or destruction</td>
<td>I</td>
<td>N/I</td>
<td>D</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Thrombocytopenia due to decreased production</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Inherited defect of intrinsic pathway (ie haemophilia A or B)</td>
<td>N*</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>N</td>
</tr>
<tr>
<td>Inherited defect of extrinsic pathway (ie VWD deficiency)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Inherited defect of common pathway (ie FX deficiency)</td>
<td>N*</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>von Willebrand disease (VWF deficiency)</td>
<td>I</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>VKK antagonism (ie warfarin poisoning)</td>
<td>N*</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>I</td>
<td>N</td>
</tr>
<tr>
<td>Disseminated Intravascular Coagulation (DIC)</td>
<td>I</td>
<td>N</td>
<td>D</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>

* = initially normal, then the bleeding could re-start; I= increased; D= decreased; N= normal; BM=bone marrow; D-dimer=D-dimer