

SEED Coagulation

Sysmex Educational Enhancement and Development
July 2012

Haemophilia – a laboratory diagnostic approach

What is haemophilia?

Haemostasis is the biological balance between clotting and bleeding. A deficiency of any clotting factor will shift this balance towards a bleeding tendency. Haemophilia is a sex linked hereditary bleeding disorder caused by the deficiency of either coagulation factor VIII (FVIII) or factor IX (FIX), called haemophilia A and B respectively. The genes for FVIII and FIX are located on the X chromosome which means that with very rare exceptions only males are affected. As females have two X chromosomes, even if one chromosome carries the haemophilia mutation, the second normal chromosome is able to produce sufficient quantities of factor for carrier females to remain clinically asymptomatic. The inheritance pattern of haemophilia is illustrated in Fig. 1. However, up to one third of haemophiliacs have no family history and result from spontaneous mutation.

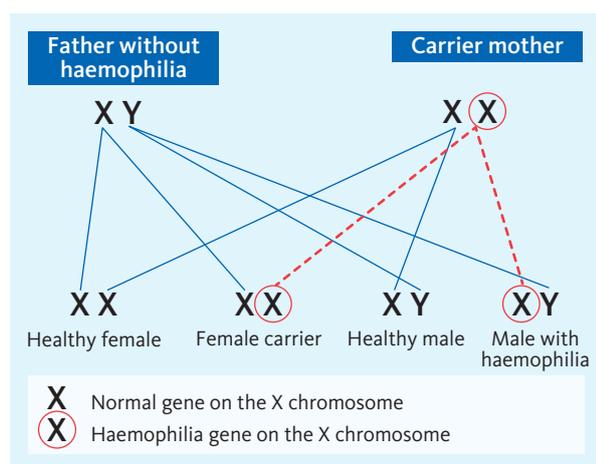


Fig. 1 The inheritance pattern of haemophilia

How common is haemophilia?

Haemophilia A is the most common of the hereditary clotting factor deficiencies occurring in about 1 in 5000 live male births. Haemophilia B is less common with a prevalence of about 1 in 30,000 in live male births. The clinical presentation, laboratory diagnosis and management of haemophilia A and B are identical. For ease of reading we will focus on haemophilia A and FVIII because it is more common, however all references to FVIII can be substituted with FIX to be applicable to haemophilia B.

When would one suspect that a patient may have haemophilia?

Haemophilia should be suspected in male patients who present with the following:

- a history of bleeding episodes that first became prominent during infancy at the time when the child first becomes mobile
- a lifelong history of easy bruising
- spontaneous bleeding into joints and subcutaneous soft tissue
- a family history of bleeding, particularly in males although this may be absent

What are the clinical manifestations of haemophilia?

The major clinical symptoms of haemophilia are recurrent bleeding episodes. The severity of bleeding is directly related to the level of plasma FVIII. Patients with the lowest level of FVIII will therefore have a more severe bleeding tendency and worse clinical picture. Patients with severe haemophilia will have more frequent bleeds with a greater degree and severity of complications. Conversely patients with mild haemophilia will experience far fewer bleeds with minimal or no complications. Patients with moderate haemophilia will present with an intermediate clinical picture. Infants may present with profuse post circumcision haemorrhage, soft tissue bleeds and excessive bruising, especially when

they first start crawling. Recurrent spontaneous joint bleeds and muscle haematomas are a common complication and if left untreated the joints can develop chronic arthritis progressively leading to disability. Complications from uncontrollable bleeds may arise during major trauma or surgery.

Factor VIII activity	Severity of haemophilia	Clinical manifestations
<1%	Severe haemophilia	<ul style="list-style-type: none"> ■ Frequent spontaneous bleeds
>1% – <5%	Moderate haemophilia	<ul style="list-style-type: none"> ■ Post traumatic bleeding ■ Occasional spontaneous bleeds
5% – 30%	Mild haemophilia	<ul style="list-style-type: none"> ■ Post traumatic bleeding ■ Rare spontaneous bleeds

Tab. 1 Clinical classification of haemophilia according to factor activity

How does one diagnose haemophilia?

Prolongation of the baseline coagulation screening tests would identify a patient with a clotting factor deficiency. Specific clotting factor assays are needed to identify the specific factor that is deficient. The expected coagulation test result findings in a patient with haemophilia are shown in Tab. 2.

Prothrombin time (PT)	Normal
Activated partial thromboplastin time (APTT)	Prolonged
Thrombin time (TT)	Normal
Fibrinogen	Normal
Platelet count	Normal

Tab. 2 Expected findings for baseline laboratory tests in haemophilia

When the APTT is prolonged, correction studies should be performed by mixing the patient’s plasma with normal pooled plasma in a ratio of 50:50. This so-called mixing or correction study is used to determine whether the prolongation of the APTT is due to a factor deficiency or due to an inhibitor such as heparin. The addition of normal pooled plasma will replace any factors that are missing in the patient plasma and consequently will ‘correct’ the APTT to within normal range if the original prolongation was due to a factor deficiency. In contrast if the prolonged APTT is due to an inhibitor then no ‘correction’ will take place.

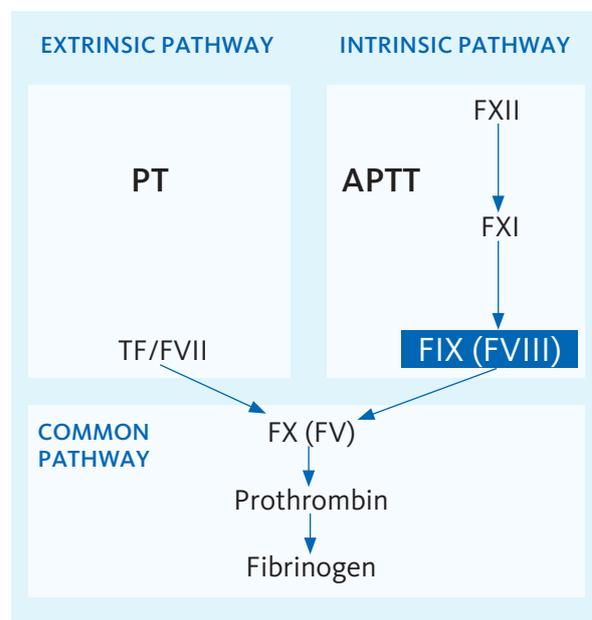


Fig. 2 Clotting pathway showing the factors measured by the APTT and the role played by FVIII. A deficiency of FVIII prolongs the clotting time of the APTT to above the normal range.

How does one confirm a suspected diagnosis of haemophilia?

Individual factor assays must be performed to confirm a diagnosis of haemophilia. As haemophilia A is five times more common than haemophilia B, it would be most cost effective to first perform a FVIII assay, followed by a FIX assay if the FVIII level is normal. The test principle and method are essentially the same for both factors with the exception that FIX deficient plasma will be used for the FIX assay.

a) Test principle of the FVIII assay

The activated partial thromboplastin time (APTT) assesses the collective function of coagulation factors that make up the intrinsic pathway and common coagulation pathway (FXII, FXI, FIX, FVIII, FX, FV, FII, fibrinogen). For the APTT to be within the normal range all the participating factors need to be present in normal or near normal quantities. As all factors are essential for the ultimate generation of a fibrin clot, a deficiency of any one will result in a prolonged APTT. The clotting time of the APTT will be prolonged in direct proportion to the extent of the deficiency i.e. the lower the level of factor, the longer the clotting time. Therefore if the only variable altering the APTT of a patient sample is the level of a specific coagulation factor, then the value of the APTT can be used to calculate the concentration of the missing factor. In the FVIII assay the patient sample with an unknown FVIII level is mixed with a reference plasma which contains normal quantities of all clotting factors except the factor that is being measured, i.e. FVIII in this case. This plasma is referred to as FVIII deficient plasma. By performing this mix, all clotting factor levels are normalised except for FVIII which is the unknown quantity under investigation. Any prolongation of the APTT can therefore be solely attributed to the concentration of FVIII in the patient sample.

b) Reagents and controls required for the FVIII assay

- APTT reagents – the same reagents that are used for baseline APTT testing are required for the intrinsic pathway clotting factor, namely the APTT reagent (Actin FS[®]) and calcium chloride.
- Reference plasma – this reference plasma (Standard Human Plasma[®]), which contains a known amount of FVIII, is used to generate a standard curve (calibration curve) against which the clotting time of the patient sample is compared and converted into an absolute value (%).
- Factor VIII deficient plasma – this plasma contains normal amounts of all coagulation factors but is completely deficient in FVIII.
- Normal and abnormal controls – Control Plasma N[®] and Control Plasma P[®]

The names above followed by '®' are the Siemens brand names of reagents used for the FVIII assay on Sysmex analysers

c) Automated FVIII analysis on Sysmex analysers

Individual clotting factor assays can be performed on all Sysmex coagulation analysers. The analyser needs to be calibrated using Standard Human Plasma whenever the lot number of reagents changes. Please refer to the instructions for use or consult your local Sysmex representative.

d) Results interpretation

The FVIII results are reported as a percentage value. The normal reference range can be quite wide (~50 – 150%) but this is not relevant for the diagnosis of haemophilia. As indicated in Tab. 1, a diagnosis of haemophilia requires there to be an isolated FVIII level below 30% in a male patient. The level of factor will determine the clinical severity and consequently the approach to treatment.

How are bleeding episodes in haemophilic patients treated?

a) Restoring the haemostatic balance

Haemophiliacs bleed because they have a partial or complete absence of a single clotting factor; namely FVIII or FIX. It should therefore not be surprising that the mainstay of treating bleeding episodes is to replace the missing clotting factor. This is usually given in the form of a single factor concentrate (FVIII or FIX) which is either derived from human blood through a process of purification or is manufactured by means of genetic engineering – so called recombinant factor. The use of recombinant products is favoured as they are completely free of the risk of transfusion associated infections, such as HIV and Hepatitis B and C, which although extremely rare, cannot be entirely eliminated from any blood derived products even in countries with the highest blood transfusion safety standards. Recombinant products are however expensive and not widely available therefore factor concentrates or even fresh frozen plasma, both obtained from blood transfusion services, are used for treatment in most countries.

The amount of factor that needs to be given will depend on the severity of the bleed. For spontaneous bleeds a FVIII level of 30 – 50% is usually adequate but for any major trauma or surgery a level of 100% is required. Once the

bleeding has stopped, FVIII levels should be maintained at about 50% until healing has occurred.

b) General measures

As is the case in non-haemophiliacs, standard measures of external compression at the bleed site, rest and elevation are applied to minimise the extent of bleeding.

c) Preventative measures

Patients with haemophilia are advised not to take aspirin or non-steroid anti-inflammatory drugs for pain relief. These drugs can make the bleeding worse by affecting platelet function. Intramuscular injections must also be avoided as this may precipitate bleeding.

d) Prophylactic factor replacement therapy

Ideally, haemophiliacs should receive factor replacement on a regular basis to prevent bleeding rather than just when bleeding has already occurred. This has been proven to be enormously effective in preventing long term complications but it is exorbitantly expensive and is therefore an unattainable goal for the vast majority of haemophiliac patients.

What is the role of the laboratory in haemophilia care?

Not only is the FVIII assay essential to confirm the diagnosis, but is also an essential component of on-going haemophilia care. As mentioned above, the infusion of factor concentrate is the mainstay of treatment but levels have to reach a certain minimum level in order for haemostasis to be effectively restored. It is therefore vital that factor levels are checked at specific time intervals after factor has been administered. Algorithms exist which assist in determining how many units of FVIII need to be infused. The formula uses the baseline FVIII level as well as body weight. This is used to determine the starting dose but the response to treatment has to be confirmed by repeating the factor assay post-infusion. If the recovery of the factor level is sub-optimal, then a top up dose must be given, especially if there are still clinical signs of bleeding. If patients do not respond as expected or require ever increasing doses to control bleeding in comparison to previously, the possibility that inhibitors have developed must be explored.

What are inhibitors?

One of the most serious complications that arise from haemophilia treatment is the development of antibodies (inhibitors)

against the infused FVIII. This tends to occur only in patients with severe haemophilia where there is less than 1% or a complete absence of FVIII. Because the patient has virtually no endogenous FVIII, any infused exogenous FVIII is seen by the body as a foreign substance against which antibodies are produced. These antibodies are referred to as inhibitors as they bind to the infused factor and prevent it from participating in the coagulation cascade. This means that larger quantities of infused factor are required to sustain coagulation. This occurs in approximately 5 to 10% of patients with severe haemophilia A and 2 to 4% in patients with severe haemophilia B.

How does one test for inhibitors in the laboratory?

FVIII inhibitors tend to be time dependent so when a mixture of normal plasma and patient plasma containing an inhibitor is incubated the inhibitor will gradually neutralise the FVIII over time. If the activity of FVIII in the normal plasma and the incubation time are known then the strength of the inhibitor can be determined. FVIII assays are performed on a set of patient plasma samples that have been serially diluted with normal plasma with a known quantity of FVIII. The dilution value of the sample that generates a residual FVIII level that is closest to 50% is used to calculate the amount of inhibitor present, reported in Bethesda units. One Bethesda unit is the amount of inhibitor that can inactivate 50% of 1 unit of FVIII in a pool of normal plasma after 120 minutes incubation at 37°C. The inhibitor activity is then read off the graph using the residual FVIII value (Fig. 3).

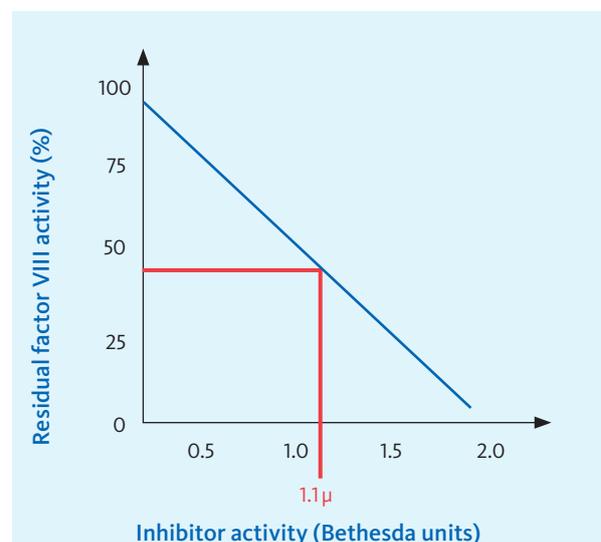


Fig. 3 The residual factor VIII that is closest to 50% is used to read the inhibitor activity off the graph. In this example it is 45%.

The final Bethesda units are then calculated by multiplying the inhibitor activity unit with the dilution factor of the results. All values that are higher than 80% residual FVIII are considered negative for inhibitors.

How does the presence of inhibitors affect treatment?

As a rule of thumb, a patient with one Bethesda unit of inhibitor will require twice the usual quantity of factor than what would be required if the patient did not have an inhibitor. As inhibitor levels rise, factor concentrates become progressively ineffective in managing bleeding episodes. When this happens alternate products such as recombinant factor VIIa, which acts directly on factor X thereby bypassing the need for FVIII (please see coagulation cascade diagram in SEED Coagulation from April 2012). This product is prohibitively expensive and is not widely available therefore the only alternative in most patients is to use massive amounts of factor concentrates whilst simultaneously targeting the production of inhibitor using immune based treatments and on occasion chemotherapy.

Take home message

Haemophilia may be a relatively uncommon in terms of the number of affected patients, however because these patients tend to have frequent bleeding events throughout their lives coupled with the need to test blood specimens within 2 hours of collection in order for FVIII levels to be accurate, there is a significant demand for FVIII and FIX assays. The widespread availability of reliable laboratory services that can perform FVIII and FIX testing is therefore an essential component for any haemophilia care programme, even in under-resourced countries.