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SEED Coagulation

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The activated partial thromboplastin time test (APTT), heparin and its mechanism of action

Heparin is a drug that is used in the initial stages of treatment of patients who have developed a blood clot such as a deep vein thrombosis. As there is always the risk of clot extension, it is essential to restore the haemostatic balance as quickly as possible. Heparin is ideal for this purpose as it has a very rapid onset of action. The anticoagulant effect begins almost immediately when heparin is given intravenously. In contrast warfarin, which is an oral anticoagulant drug, not only takes a couple of days before any anticoagulant effect becomes evident, but initially induces a procoagulant state. This procoagulant effect is due to the fact that warfarin not only interferes with the production of functional clotting factors II, VII, IX and X but also the naturally occurring anticoagulant proteins C and S. Protein C by virtue of its very short half life is reduced before the clotting factors thereby paradoxically increasing the risk of clotting on the day that warfarin anticoagulant treatment is first commenced. Heparin treatment is usually continued until such time that the INR (International Normalised Ratio) is within the therapeutic range. In addition heparin is also used to prevent blockages in central lines and dialysis circuits.

Heparin is made up of complex carbohydrate molecules which are comprised of repeated sugar residues. Unfractionated heparin is comprised of a 'soup' of sugar molecules of variable lengths whereas low molecular weight heparin has been treated to contain only the shorter lengths. Each heparin molecule contains a unique pentasaccharide (5 sugar sequence) with a high affinity binding sequence for antithrombin (AT), previously referred to as antithrombin III or ATIII in old textbooks. When heparin binds to AT it induces a conformational change in AT. This in turn accelerates the inactivation of thrombin, FXa, FIXa and FXIa.

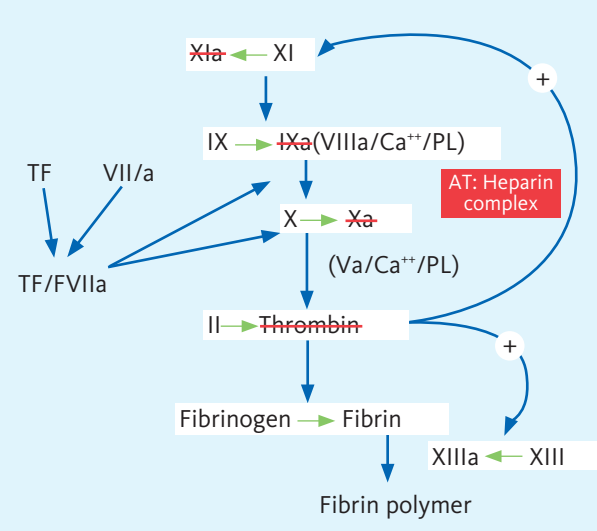

Fig. 1 Diagram illustrating the mechanism of action of heparin. Heparin binds to the anticoagulant protein antithrombin. When bound to heparin, the inhibition of the activated clotting factors XIa, IXa, Xa and thrombin by AT is potently accelerated.

Heparin has to be administered by injection, either subcutaneously or directly into a vein. When systemic anticoagulation is required, it is given either as a bolus dose every 6 hours or as a continuous infusion.

Although the action of heparin is rapid in onset the degree of anticoagulation is however not predictable. This is partly due to the fact that heparin binds non-specifically to various plasma proteins. Protein bound heparin is unable to participate in the anticoagulant action. This is particularly noticeable in the acute stages of illness due to the increase in so called acute phase proteins. Because of this patients on heparin need to undergo regular monitoring to ensure that the level of anticoagulation is within the therapeutic range. Excess heparin places the patient at risk of bleeding and too little would exacerbate the already existing prothrombotic condition.

Activated partial thromboplastin time

The activated partial thromboplastin time (APTT) is the second most frequently requested coagulation test. The APTT is a laboratory test that is most commonly used to monitor the anticoagulant effects of unfractionated heparin. It is also used to screen for various bleeding disorders caused by deficiencies in the 'intrinsic' coagulation pathway i.e. factor XI, factor IX, factor VIII, factor V, factor II (prothrombin) and fibrinogen.

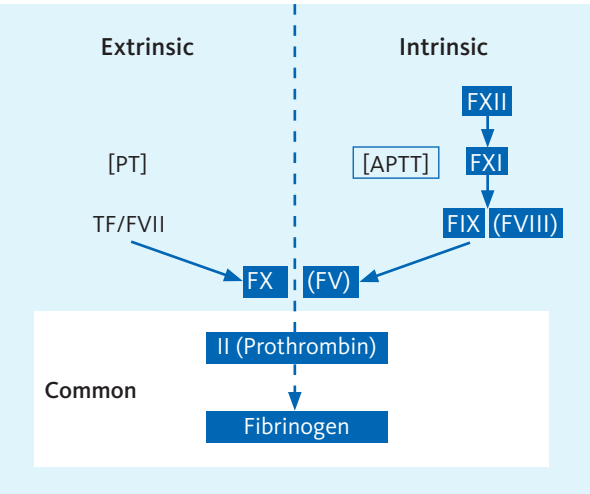

Fig. 2 Diagrammatic representation of the components of the coagulation cascade which are measured in the activated partial thromboplastin time (identified by blue boxes).

The word 'partial' refers to the fact that the reagent used in the APTT lacks the tissue factor that is a core component in the thromboplastin reagent used in the prothrombin time. It contains only the phospholipid component. The original assay, designed in 1953 to identify classical haemophilia (FVIII deficiency), was called the partial thromboplastin time (PTT) and relied on the glass of the test tube to initiate activation of the intrinsic pathway – so called contact activation. In 1961, this assay was modified to speed up the activation process by adding kaolin which replaced the test tube glass as the activation surface. This is why the clotting test in use today to assess the intrinsic pathway is referred to as the 'activated' partial thromboplastin time, namely APTT. The terms PTT and APTT are commonly used interchangeably although strictly speaking only APTT should be used.

APTT reagents

APTT reagents have two principal components; an activator and a source of phospholipid. The activator can be either particulate in nature (kaolin, celite or silica) or soluble (ellagic acid). The role of the activator is to provide a negatively charged surface which is required for the activation of the so called contact factors. The contact factors include high molecular weight kininogen (HMWK), prekallikrein and factor XII (FXII). Kallikrein is formed from prekallikrein through the action of HMWK. During this contact activation phase FXII is activated to FXIIa with the help of HMWK and kallikrein, and in turn converts FXI to FXIa. Kallikrein is formed from prekallikrein through the action of HMWK. The phospholipids are required to provide a surface for the assembly of the tenase complex (FIXa-FVIIIa), which converts FX to FXa, and the prothrombinase complex (FXa-FVa) which converts prothrombin to thrombin. The phospholipid composition is highly variable in both concentration and source. The phospholipid may be of animal or plant origin or synthetically manufactured to produce a pure form.

Consequently this diversity in concentration and composition of APTT reagents results in great variation in their responsiveness to heparin, clotting factor deficiencies and lupus anticoagulants (see later). The variability is further impacted by the type of detection systems used by automated systems i.e. mechanical versus optical. In view of the lack of uniformity of the composition and reactivity of APTT reagents, it is absolutely essential that each laboratory has a solid quality control mechanism in place and establishes its own reference range for the interpretation of APTT results generated locally. The choice of reagent must take into consideration the primary indication for APTT testing as some reagents are designed to specifically be sensitive or insensitive to lupus anticoagulants.

Lupus anticoagulant

A lupus anticoagulant is an autoantibody directed against a phospholipid-protein complex. The name 'anticoagulant' derives from the fact that these antibodies tend to cause prolongation of phospholipid dependent clotting tests such as the APTT. The name is however a misnomer as it is associated with thrombotic episodes and pregnancy loss in patients with underlying autoimmune disease.

How to perform a manual APTT

This is performed in a water bath with the same basic requirements as for the PT, except that the reagents required are an APTT reagent such as Actin FS© or Actin FSL© and calcium chloride.

a) APTT reagent choice

Actin FS reagent contains ellagic acid as the activator and purified soy phosphatides as the phospholipid. Actin FSL uses the same activator but different phospholipids, namely a mixture of soy and rabbit brain phosphatides. Actin FS is a general purpose APTT reagent whereas Actin FSL is specifically formulated to be sensitive to the detection of lupus anticoagulants. For routine APTT testing we recommend that laboratories use Actin FS reagent. Actin FS comes in a liquid formulation with an expiry of approximately 2 years and vial sizes of 2 mL or 10 mL. The stability of the reagent after opening is as follows:

- 1 week: 2 to 8° C if kept stoppered
- 2 days: 15° C (on board analyser)
- 24 hours: at 37° C if kept stoppered (in water bath)

b) Calcium chloride

Calcium chloride is an essential reagent for the APTT reaction. Calcium ions are removed from the plasma during sample collection into sodium citrated tubes. The recalcification step in the APTT is what triggers the clotting cascade.

c) Test method

- Check that the temperature of water in the water bath is at 37° C. This is essential as the clotting factors are enzymes that are designed to work optimally at body temperature.
- Warm up the reagents (Actin FS and calcium chloride) to 37° C (dispense the quantity of each reagent required for the number of planned tests into separate test tubes and place them in a rack in the water bath).
- Add 100 µL of patient plasma to a glass test tube and place in the rack in the water bath.
- Add 100 µL of Actin FS APTT reagent, mix well and incubate in the water bath for 3 minutes. This allows for the contact activation phase of the clotting cascade to be optimally assembled.
- Add 100 µL of calcium chloride, immediately mix well and start looking for fibrin strands. Keep swirling the tube back into the water to ensure that the temperature of the reaction remains at 37° C. Gently tilt the tube when lifting it out the water and check for clot continuously.
- As soon as fibrin strands are observed, stop the stopwatch and record the time.
- Ideally the test should be done in duplicate and the average of the two times recorded.
- Repeat with normal control plasma.

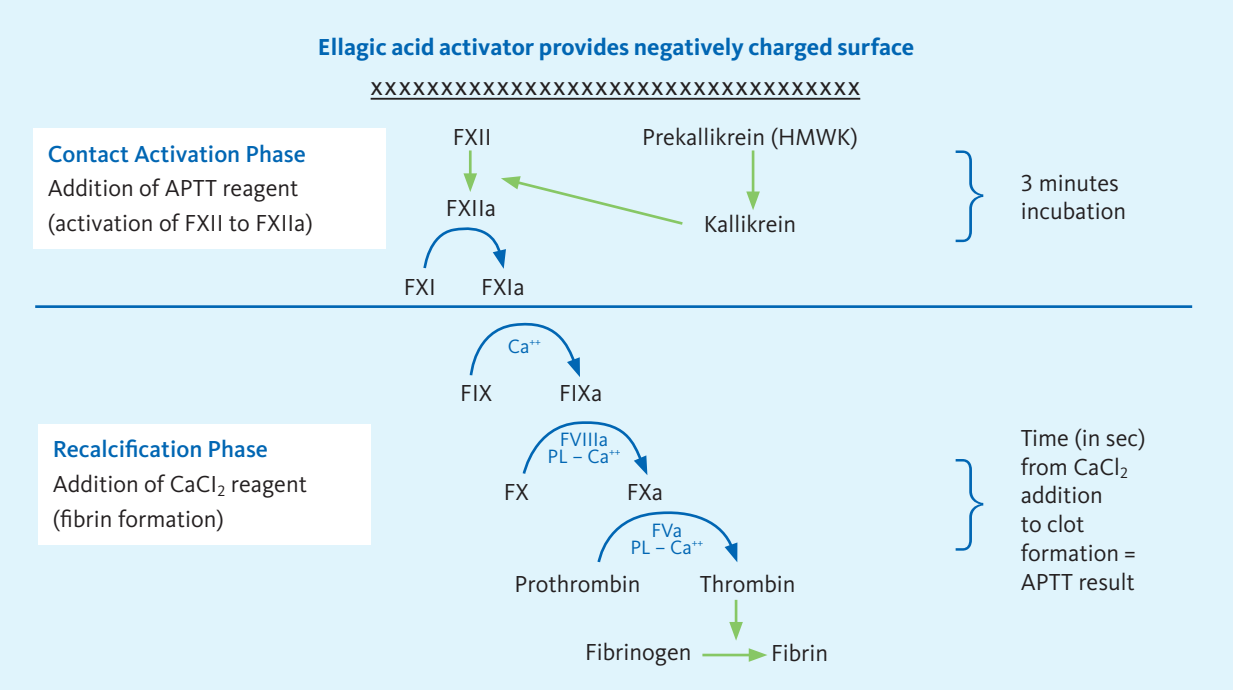

Fig. 3 *Illustration of the steps of the APTT reaction (PL = phospholipid)*

Interpretation of APTT results

The APTT results are recorded in seconds and interpreted in relationship to the normal reference range which should be established locally by each laboratory but on average would be ~24–36 seconds. A prolonged time would suggest a defect in the intrinsic or common pathways. This could be due to either a quantitative deficiency or abnormal function or an inhibitor directed against one or many clotting factors. The commonest 'inhibitor' is heparin.

When using the APTT for the purpose of monitoring heparin therapy, ideally a baseline value should be determined before heparin therapy is initiated. The target APTT value for a patient on heparin is 2 times the patients own baseline value. As patients are frequently started on heparin without a baseline value having first been obtained, a prolongation of the APTT to 1.5 – 2.5 times normal is considered to be therapeutic. In this case, normal is taken as the value obtained for the normal control sample.

In non-heparinised patients the APTT is seldom performed in isolation. It is almost always requested together with a prothrombin time with the results being interpreted together. The finding of a prolonged APTT result requires further investigation. As the APTT is a global screening assay which assesses the integrity of the intrinsic coagulation cascade, it is important to determine which aspect of the cascade is defective. The quickest way to achieve this is to perform 'correction studies'.

APTT correction studies

The aim of correction studies is to determine whether the primary cause of prolongation is due to one or more clotting factor deficiencies or due to an inhibitor. Correction studies are also called 'mixing studies' because the patient plasma sample under investigation is mixed in a 1:1 ratio with normal plasma. The normal plasma used is most commonly sourced from pooled normal plasma (PNP) which as the name implies, is prepared by pooling the plasmas from several normal individuals. This plasma is then aliquotted into 3 – 5 mL volumes and frozen for later use. This provides the laboratory with a uniform source of normal plasma for mixing studies and other laboratory investigations.

The APTT is then repeated on the 1:1 patient/PNP mix. The addition of PNP to the patient sample aims to replace any clotting factors that may have been missing or reduced in the patient sample. If this was indeed the cause of the original APTT prolongation, then the APTT would 'correct' or normalise or at the very least become significantly shorter even if not completely within the normal range again. If the APTT mix fails to correct, then the primary cause for the original APTT prolongation is almost certainly due to the presence of an inhibitor. The explanation for this is any inhibitor present in the patient plasma will equally inhibit the clotting factors of the added PNP and therefore the APTT remains prolonged.

a) Suspected clotting factor deficiencies

If the mixing studies show correction, the next step would be to identify which factor/factors may be deficient. In order to do so specific factor assays need to be undertaken. As clotting factor assays are not inexpensive to perform, the order of testing is selected based on the joint interpretation of the PT, APTT and mixing studies of each. If the APTT is prolonged, but the PT is completely normal, then this strongly suggests that the defect lies in the intrinsic pathway involving factors above the common pathway – namely FXII, FXI, FIX or FVIII. If the PT is normal, it is safe to conclude that the factor deficiency is likely to be isolated (multiple deficiencies due to generalised consumption would invariably have a prolonged PT because FVII has the shortest half life). If the patient has a history of bleeding, the commonest deficiency would be FVIII, then FIX, then FXI. FXI deficiency is uncommon and occurs primarily in Ashkenazi Jews. Primary FVIII and FIX deficiency are the cause of haemophilia A and B respectively which occurs only in males. FVIII deficiency can occur in severe Von Willebrands disease which occurs in both sexes. Acquired haemophilia can occur due to inhibitors to these factors but this is exceptionally rare. These are usually slow acting inhibitors which require long incubation (2 hours rather than the standard 3 minutes) of the APTT. In such a case the APTT would normally correct as the plasma primarily manifests as having a factor deficiency rather than an inhibitor that will interfere with the PNP in the mixing study. If no factor deficiency is identified, the defect could be in the contact activation factors, prekallikrein or HMWK although these do not result in bleeding problems. If one uses APTT reagents with celite, silica or kaolin as the activator and increase the incubation time to 10 minutes, and the APTT corrects, there is likely to be a prekallikrein deficiency. HMWK deficiency will not correct. Deficiencies of prekallikrein and HMWK are not detectable using ellagic acid as this activator is much more potent than the others.

b) Suspected inhibitor

This is most commonly due to heparin, even if there is no clinical history of heparinisation. The source of heparin is usually from central lines or the blood specimen having been taken with a heparinised syringe before being placed into the citrate collection tube. One way of confirming this is to perform a thrombin time test which shows marked prolongation in the presence of heparin but is insensitive to other inhibitors. If heparin is excluded, a lupus anticoagulant must be suspected. Lupus testing involves confirmation that the clot time prolongation is due to a phospholipid dependent antibody by showing normalisation by the addition of excess phospholipid. Discussion of lupus anticoagulant testing is beyond the scope of this newsletter.

Paradoxically prolonged APTT results

A prolonged APTT test result does not always translate into a bleeding tendency. Defects in the so called contact activation factors i.e. HMWK, prekallikrein and FXII may also lead to a prolonged APTT test result but these deficiencies are not linked to bleeding abnormalities. On the contrary some conditions that result in a prolonged APTT paradoxically are associated with a thrombotic tendency. FXII and the other contact factors have no role in activating the clotting cascade in vivo. In fact they are involved in activating the fibrinolytic system and therefore deficiencies would result in a prothrombotic tendency. This has been well documented for FXII deficiency. Mr. Hageman, in whom FXII (also known as Hageman factor) deficiency was first described, actually died of a pulmonary embolism. Lupus anticoagulants, as previously mentioned, are another cause of a paradoxically prolonged APTT.

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