SEED Coagulation

Sysmex Educational Enhancement and Development
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The Prothrombin Time Test

The need for anticoagulation

As was discussed in the first SEED Coagulation edition 'Introduction to coagulation', the fundamental principle of haemostasis is to minimise blood loss at sites of vessel injury whilst maintaining blood flow at all times. This is maintained via a highly regulated fine-tuned interaction of multiple biological processes. When this balance is disturbed in favour of excessive clot formation, patients are at risk of developing pathological thrombosis which will interfere with blood circulation and may be fatal. Such patients are commonly treated with anticoagulant drugs which aim to restore the haemostatic balance and therefore minimise the risk of thrombosis. Examples of disorders for which oral anticoagulants are commonly prescribed are venous thromboembolism (deep vein thrombosis/pulmonary embolism), atrial fibrillation, coronary heart disease and ischaemic stroke.

Warfarin

The most commonly prescribed anticoagulant for long term use is warfarin. The mechanism of action of warfarin is as follows:

- Warfarin inhibits the enzyme vitamin K epoxide reductase.
- This results in an inability to convert vitamin K epoxide back into reduced vitamin K.
- Reduced vitamin K is required as a co-factor for the enzyme gamma carboxylase.
- Gamma carboxylase is an enzyme that adds carboxyl groups to the glutamic acid residues on the precursor clotting factors II, VII, IX and X as well as protein C and protein S, all of which are produced in the liver.
- If the carboxyl groups are not added, the clotting factors as well as PC and PS are non-functional.
- In the coagulation process, clotting factors need to be bound to phospholipid (the surface of activated platelets), a process that can only take place in the presence of carboxyl groups on the clotting factors and using calcium ions as a ligand.

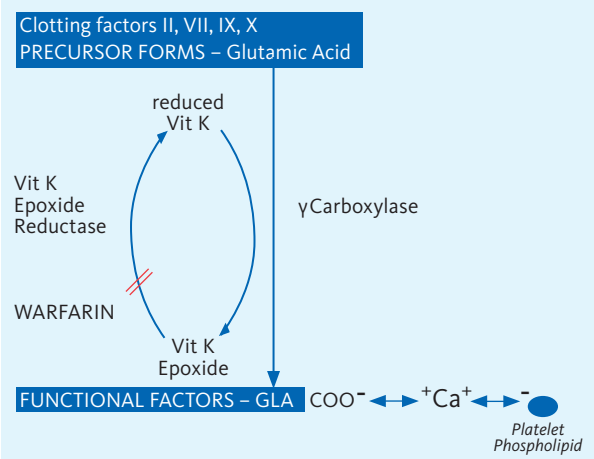

Fig. 1 *The mechanism of action of warfarin*

The great advantage of warfarin is that it is an oral drug. The disadvantage however is that there is substantial variability in its biological effect from person to person and also in a single individual over time. There are multiple factors that contribute to this including genetics, diet and concomitant drug use. As the benefits of warfarin are restricted to a narrow therapeutic window, laboratory monitoring is mandatory in order to minimise the risk of haemorrhagic complications or ongoing hypercoagulabilty.

The Prothrombin Time (PT)

The PT is a baseline screening test for patients with suspected bleeding abnormalities and is extensively used for monitoring oral anticoagulant therapy (warfarin and other coumadin drugs). It is not useful for investigating possible causes of clotting in a patient. It is used to assess for defects in the 'extrinsic' and 'common' pathways. A prolonged clotting time could be due to reduced or absent activity of one or more of factors VII, FX, FV, FII and fibrinogen. The reduced activity could be due to a quantitative deficiency or reduced function of a clotting factor due to the vitamin K absence or inhibition (warfarin) or due to an inhibitor directed against the clotting factor.

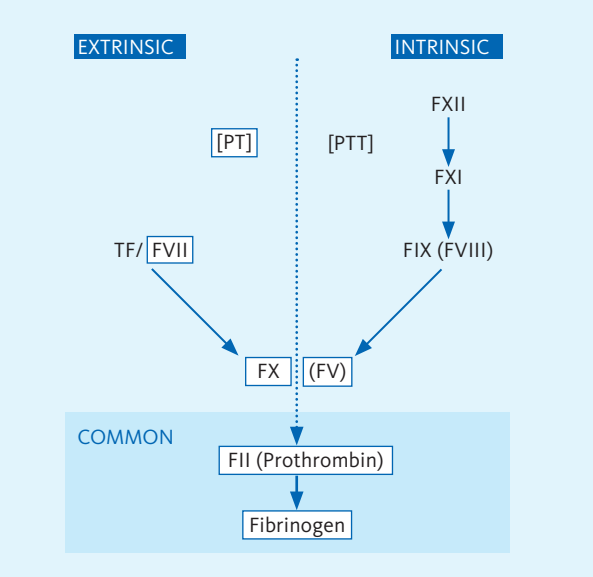

Fig. 2 *Clotting pathway tested by the prothrombin time*

The thromboplastin reagent used to start the clotting reaction in the PT test is in fact the equivalent of tissue factor (TF).

The manual PT method

a) Requirements

- Waterbath at 37°C
- Thermometer
- Pipettes – 100 µL and 200 µL
- Pipette tips
- Stop watch x 2
- Glass test tubes – 5 mL
- Thromboplastin reagent (e.g. Innovin® – Siemens)
- Spun down patient plasma
- Normal patient plasma
  - Pooled normal plasma or
  - Citrol Level 1 (non-assayed or assayed)
- Control plasma – Citrol Level 1 assayed
- Scientific calculator

b) Innovin®

- Thromboplastin reagent containing 'tissue factor', phospholipid (platelet substitute) and calcium chloride
- Lyophilised
  - Very long expiry times ~ 2–3 years
- Needs to be reconstituted with preservative free distilled water
- Must warm up to 37°C before use.
- Stability after reconstitution
  - 10 days – 2–8°C if kept stoppered
  - 5 days – 15–25°C if kept stoppered
  - 24 hours – at 37°C if kept stoppered (in waterbath)
- Refer to the package insert for comprehensive handling instructions

c) Manual Method

- Check that temperature of water in waterbath is at 37°C.
- Warm up reagent (Innovin®) to 37°C (dispense quantity required for number of planned tests into test tube, place test tube in rack submerged in waterbath).
- Add 100 µl of patient plasma to glass test tube in waterbath.
- Add 200 µL of Innovin to patient test tube and start stop watch immediately.
- Immediately mix well and start looking for fibrin strands. Keep swirling tube back into the water to ensure that the temperature of the reaction remains at 37°C. Gently tilt tube when lifting it out the water and check for clot continuously.
- As soon as fibrin strands are observed, stop the stop watch 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. It is best to use pooled normal plasma to obtain the mean normal PT time, but if this is not available then use a commercial normal control that is compatible with the thromboplastin reagent eg. Citrol Level 1.

Prothrombin time and INR

PT results are commonly reported as an international normalised ratio (INR). The World Health Organisation introduced the concept of the INR in order to standardise reporting of PT results for patients on warfarin (or equivalent coumadin derivatives) oral anticoagulant therapy. Different thromboplastin reagents have different sensitivities, i.e. will result in slightly different clotting times on the same plasma samples. As the degree of prolongation of the PT is used to assess whether the patient is adequately anticoagulated, the results in seconds and hence the interpretation will be influenced by the reagent used. In order to compensate for these differences reagents are assigned an 'international sensitivity index' or ISI value. The ideal reagent would have an ISI value of 1. It is always advisable to use a reagent with an ISI value as close to one as possible.

The PT is converted to an INR using the following formula:

INR = (Patient PT/MNPT)ISI

MNPT – mean normal PT time (obtained from a pool of 20+ normal plasmas or alternately from a commercial normal control such as Citrol Level 1). For manual testing, each technologist must determine his/her own MNPT time. A scientific calculator is required to calculate the final INR value.

The INR should only be used for patients on anticoagulant therapy.

Interpretation of INR:
INR 2–3 – therapeutic
INR <2 – underdosed, at risk of clotting
INR >3 – overdosed, at risk of bleeding

For non anticoagulated patients, the PT numerical value in seconds should be used in conjunction with normal reference ranges for interpretation. The normal reference range should be established locally by each laboratory but on average will be ~10–13 seconds. A prolonged value suggests a defect in the extrinsic and/ or common pathway. A low value usually has no clinical significance but should be reviewed case by case.

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