Haemostasis screening

Introduction

Haemostasis comprises the balanced orchestration of vascular integrity, circulating procoagulant and anticoagulant proteins, and cellular blood components (Moore and Rangarajan, 2009). Disturbances of one or more of these integrated compartments can lead to abnormal bleeding. The main reasons for requiring haemostasis screening tests are preoperative checks, investigation of bleeding or monitoring of anticoagulant therapy.

Haemostasis screening almost always begins with a coagulation screen comprising prothrombin time, activated partial thromboplastin time and estimation of fibrinogen activity. In some circumstances a thrombin time may also be performed. A platelet count is crucial to initial assessment of haemostasis, which in some circumstances may be followed up with morphological and/or functional assessment.

Although the cascade theory of coagulation has been superseded by the cell surface-based model for in-vivo coagulation (Hoffman, 2003), the prothrombin time and activated partial thromboplastin time are designed with the cascade theory as their backdrop in order to isolate specific compartments in vitro, as shown in *Figure 1*.

Prothrombin time

The prothrombin time uses thromboplastin reagent containing tissue factor, phospholipid and calcium ions to initiate in-vitro coagulation via the extrinsic pathway by activation of factor VII. The activated factor VII activates factor X to take the 'common pathway' to completion. The end point is a fibrin clot, the time taken to clot being the prothrombin time itself. Abnormalities that impair the factors involved in the test cause an elevation of the prothrombin time above the upper value of the reference range. Other than therapeutic anticoagulation, the main causes of an elevated prothrombin time are:

- Hereditary or acquired deficiencies of factors II, V, VII, X or fibrinogen (reduced production or impaired function)
- Autoantibodies against the above clotting factors (increased destruction)
- Vitamin K deficiency (reduced production of vitamin K-dependent factors)
- Liver disease (decreased production)
- Disseminated intravascular coagulation (consumption > production).

Activated partial thromboplastin time

Coagulation is initiated via an activator of the contact system (typically kaolin or silica) to begin the intrinsic pathway, and a partial thromboplastin containing phospholipid but not tissue factor to exclude involvement of the extrinsic pathway. Calcium ions are also present. The intrinsic pathway culminates in factor X activation which then enters the common pathway and the result is reported as the time taken to clot. Other than therapeutic anticoagulation, the main causes of an elevated activated partial thromboplastin time are:

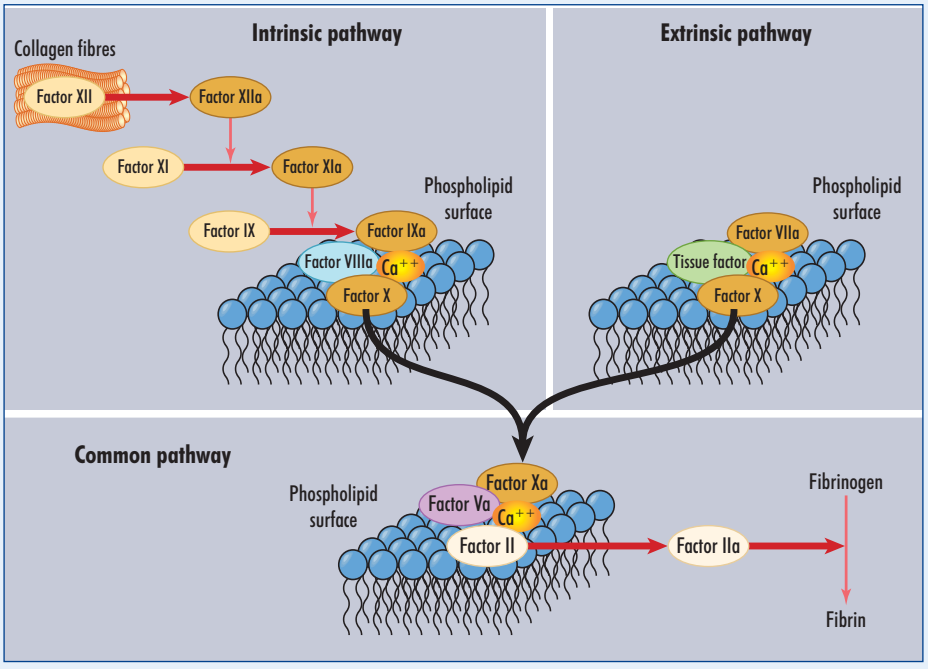
- Hereditary or acquired deficiencies of factors II, V, VIII, IX, X, XI, XII or fibrinogen
- Some sub-types of von Willebrand's disease (as a result of associated factor VIII deficiency)
- Autoantibodies against the above clotting factors
- Vitamin K deficiency
- Liver disease
- Disseminated intravascular coagulation
- Lupus anticoagulants.

Lupus anticoagulants classically elevate the activated partial thromboplastin time but the variability in reagent sensitivity means that not all lupus anticoagulants prolong routine activated partial thromboplastin times and thus specialized assays are needed to detect them. Thromboplastin reagents contain concentrated phospholipid so it is rare for lupus anticoagulants to be detected in the prothrombin time (Moore et al, 2005).

Result interpretation

The compartment where a deficiency resides is narrowed down based on pro-

Figure 1. Simplified coagulation cascade. From Moore et al (2009).



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thrombin time or activated partial thromboplastin time results:

1. Prothrombin time and activated partial thromboplastin time elevated: common pathway
2. Prothrombin time only elevated: factor VII deficiency
3. Activated partial thromboplastin time only elevated: intrinsic pathway.

Vitamin K

Factors II, VII, IX and X are termed vitamin K-dependent because their effective biosynthesis involves a step requiring vitamin K as a co-factor for the introduction of carboxyl groups to glutamic acid side chains. This section of the molecule is the Gla domain and is necessary for calcium binding which promotes interaction with the phospholipid surface of activated platelets to localize the coagulation response.

Haemophilias and von Willebrand's disease

Hereditary deficiencies of factor VIII and factor IX are haemophilia A and B respectively and present with an isolated elevated activated partial thromboplastin time. Clinical symptoms are virtually identical and specialist factor assays will isolate which of the factors is deficient and classify severity. Deficiency of factor XI is haemophilia C. More prevalent in the Ashkenazi Jewish population, it is less severe than haemophilia A or B and severity does not correlate with factor XI levels (Gomez and Bolton-Maggs, 2008).

Interestingly, factor XII deficiency is not associated with bleeding and the cell-based model accounts for effective coagulation without significant contribution from factor XII. However, factor XII deficiency will often present in the preoperative screening of an asymptomatic patient as an isolated elevated activated partial thromboplastin time and requires confirmation before invasive procedures are undertaken.

von Willebrand's disease, quantitative or functional deficiency of von Willebrand factor, is predominantly a disorder of primary haemostasis because von Willebrand factor binds activated platelets to damaged vessel endothelium (Sadler et al, 2006). von Willebrand's disease is a heterogeneous disorder that can present with an isolated elevated activated partial thromboplastin time if sufficiently severe because von Willebrand factor is the carrier molecule for factor VIII in plasma.

Mixing studies

The immediate follow-up investigation of an unexpected elevated prothrombin time or activated partial thromboplastin time is a mixing study. Equal volumes of test and normal plasma are mixed and the test(s) repeated on this mixture. If the abnormality is a factor deficiency, the normal plasma restores the deficient factor and generates a normal clotting time. If an inhibitor, in most cases it will interfere with the clotting of the normal plasma too and the clotting time of the mixture will not return to normality. Inhibitors of factor VIII are progressive because the factor VIII has to dissociate from von Willebrand factor before the inhibitor can interfere with function and so may not manifest unless incubated.

Thrombin time and fibrinogen

Addition of exogenous thrombin (activated factor II) to test plasma to directly convert fibrinogen to a fibrin clot constitutes the thrombin time. A clotting time within the reference range indicates that fibrinogen activity is not reduced. Fibrinogen can be directly quantified using a calibrated thrombin reagent in the Clauss activity assay (Mackie et al, 2003). Methods deriving fibrinogen levels from prothrombin time raw data can be unreliable. Unfractionated heparin and elevated D-dimers can also prolong the thrombin time.

Platelets

Thrombocytopenia alone is sufficient to cause bleeding symptoms, spontaneous bleeding occurring frequently when the platelet count is $<20x10^9$/litre. Thus, a platelet count via standard automated full blood count analysis is a crucial first-line investigation in haemostasis screening. Some congenital platelet disorders such as Bernard–Soulier syndrome are associated with abnormal size or morphology and mild thrombocytopenia.

Therapeutic anticoagulation

Oral anticoagulants are vitamin K antagonists and impair biosynthesis of the vitamin K-dependent factors. They are monitored using the prothrombin time which is converted to an international normalized ratio to take into account variations in reagent sensitivity. Unfractionated heparin potentiates the naturally occurring inhibitor antithrombin and is usually monitored by activated partial thromboplastin time. In clinical situations where low molecular weight heparins require monitoring, this is done by the more complex anti-Xa techniques. Results for monitoring purposes are compared with therapeutic ranges and dose is adjusted accordingly.

A false sense of security?

A patient with a positive clinical history of bleeding but a normal coagulation screen and platelet count may have the following causes:

- Mechanical or surgical
- Platelet functional disorders
- Vascular disorders
- Factor XIII deficiency – cross-linking is not necessary for clots to persist in vitro
- Reagents vary in their sensitivities to mild factor deficiencies, usually in the region of 30–40% of normal and below. In some instances of factor II, factor V

KEY POINTS

- The pairing of coagulation screen and platelet count are essential for first-line screening of haemostasis.
- Specific result patterns narrow down causes of haemostatic disturbance.
- Mixing studies will usually indicate whether an elevated prothrombin time or activated partial thromboplastin time result is the result of a factor deficiency or inhibitor.
- Prothrombin time and activated partial thromboplastin time are used to monitor warfarin and unfractionated heparin therapy respectively.
- Not all causes of bleeding will manifest in first-line screening.

and factor X deficiencies this can result in either prothrombin time or activated partial thromboplastin time being elevated but not both
- Some mutations, such as factor VII Padua, are detectable only in certain reagent sub-types
- Milder forms of von Willebrand's disease may not reduce factor VIII levels sufficient to prolong an activated partial thromboplastin time
- Fibrinolytic disorders.

Conclusions

Coagulation screening coupled with platelet assessment will detect the majority of clinically significant bleeding disorders. Informed interpretation of the results in light of clinical presentation informs subsequent diagnostic pathways. **BJHM**

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