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Coagulation

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ABSTRACT The haemostatic system comprises four compartments: the vasculature, platelets, coagulation factors, and the fibrinolytic system. There is presently no laboratory or near-patient test capable of reproducing the complex regulated interaction between these four compartments. The prothrombin time (PT) and activated partial thromboplastin time (APTT) only test the coagulation protein compartment of the system and results have to be carefully interpreted in the context of the clinical presentation and assay limitations. This article will give a general overview of the limitations of PT and APTT and discuss specific issues that need to be considered when the tests are requested, in the context of anticoagulant monitoring, bleeding symptoms, and routine preoperative screening. Of these indications, routine preoperative screening is the most controversial and is generally not warranted in the absence of an abnormal bleeding history.

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EDUCATION

INTRODUCTION

The normal haemostatic system comprises four compartments, the vasculature, platelets, coagulation proteins and the fibrinolytic system. When a blood vessel is injured, all four compartments interact in a coordinated manner to prevent blood loss by forming a clot and localising this to the area of injury. Currently, there is no global haemostatic test that can capture the complex interaction that exists *in vivo* between these four compartments.

All existing laboratory and near-patient screening tests of haemostasis, including the coagulation screen comprising prothrombin time (PT) and activated partial thromboplastin time (APTT), have important limitations. Their clinical utility and hence, the correct diagnosis and appropriate management of the patient, is highly dependent on the clinical context and a clear understanding of the laboratory limitations.

LIMITATIONS OF PT AND APTT

Two pathways lead to the formation of a fibrin clot, the intrinsic and extrinsic pathway (Figure 1). Each pathway is initiated by a different mechanism and both converge on a final common pathway (factors II, V, and X) leading to thrombin generation and fibrin formation. The PT and APTT test the integrity of the extrinsic and intrinsic pathways, respectively, while both PT and APTT are affected by defects in the final common pathway.

The PT is the *in vitro* clotting time measured after addition of the PT reagent, which contains thromboplastin (phospholipids with tissue factor) and calcium to citrated plasma. The PT detects important deficiencies (and rarely inhibitors) of factors II, V, VII, and X. The APTT is the *in vitro* clotting time measured after addition of calcium, an intrinsic pathway activator and the APTT reagent, which contains phospholipid (a platelet substitute, also called 'partial thromboplastin' as it lacks tissue factor) to plasma. The APTT detects bleeding disorders due to deficiencies of factors II, V, VIII, IX, X, XI, XII and inhibitors including lupus anticoagulant and therapeutic anticoagulants.

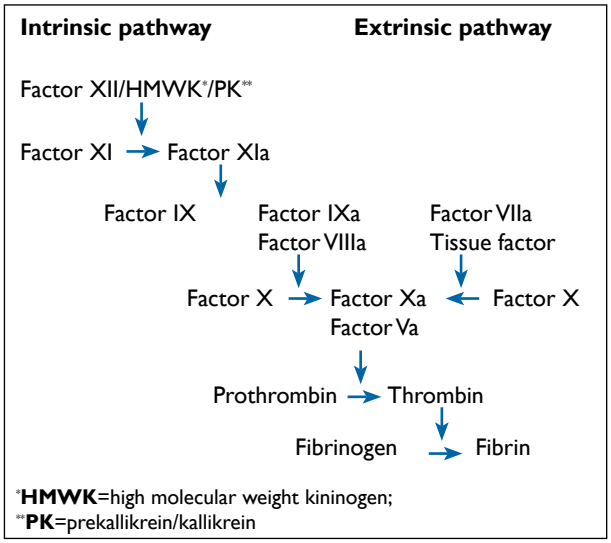

FIGURE 1 Classical coagulation cascade.

Prolongation of the PT and/or APTT only indicates a problem with the quantity and/or quality of single or multiple factors within the relevant pathways. Further specific coagulation tests are required to characterise the actual cause. Both the PT and APTT are also subject to a number of other important limitations. These include:

1. Artefact due to sample collection or contamination, e.g. inadequate volume, difficult or traumatic phlebotomy causing coagulation activation, prolonged storage, and failure to adjust for high haematocrit causing an increase in citrate to plasma volume and artefactual prolongation of PT and APTT.
2. Derivation of normal range whereby 2.5% of otherwise normal individuals are considered to be outwith the upper limit of normal.
3. Insensitivity to clinically important bleeding disorders with normal PT and APTT (e.g. mild von Willebrand disease or haemophilia A, FXIII deficiency and alpha2-antiplasmin deficiency).
4. Detection of conditions not associated with bleeding e.g. the lupus anticoagulant which can prolong both the PT and APTT, FXII deficiency which prolongs APTT.
5. The same blood sample tested in different laboratories can give variable results; mainly due to differences in commercial reagents having different responsiveness to coagulation factor deficiencies and inhibitors and to a lesser extent, the automated instrument.

THE CLINICAL CONTEXT FOR TESTING

The PT and APTT are amongst the most commonly requested laboratory assays. Typical indications include anticoagulant monitoring, routine preoperative screening, and bleeding. In addition to the limitations already described, the clinician needs to be aware of specific issues related to each of these clinical indications for testing.

(a) Anticoagulant monitoring

The problems associated with PT or APTT for monitoring vitamin K antagonists (VKA) and unfractionated heparin (UFH) relate mainly to the varying responsiveness of test reagent to single and/or multiple factor deficiencies and inhibitors. There is considerable variability in the PT thromboplastin reagent to the coagulation defect caused by VKA. Therefore, to allow PT results from different laboratories to be comparable, a calibration system that compares PT results to a World Health Organization (WHO) standard expressed as the international normalised ratio (INR) was developed. However, the INR is only valid for patients stabilised on VKA. This means that they are neither reliable nor reproducible for patients with prolonged PT for other reasons, e.g. liver disease, disseminated intravascular coagulation and congenital factor deficiency. The INR is accurate only for values within the 1.5–4.5 range as only patient samples with INRs within this range were used for calibration. This means that INR values >4.5 may no longer observe the linear relationship demonstrated for those with INRs of 1.5–4.5.

In contrast to the INR for PT, no such standardisation exists for APTT reagent, resulting in marked variation in the sensitivity of different reagents to coagulation factor deficiencies and inhibitors. To standardise the APTT used to monitor UFH use, individual laboratories should develop their own therapeutic range corresponding with accepted therapeutic UFH levels. The APTT is generally not sensitive to low molecular weight heparin (LMWH).

Although the new oral anticoagulants have been developed without the need for routine monitoring, these may be required in special circumstances, e.g. recurrent thrombosis on treatment, bleeding and preoperatively. All the new oral agents affect the APTT and PT but similar to VKA and UFH, different PT and APTT reagents show varying responsiveness. Results cannot be used for drug level monitoring or standardised across different laboratories.

(b) Investigation of bleeding

When the PT and APTT are used in the context of a patient with bleeding, the clinician should have a clear and systematic approach to the clinical and laboratory diagnosis. A detailed bleeding history including drug and family history, followed by careful examination can help to compartmentalise the haemostatic defect and direct laboratory investigations in a logical manner. However, bleeding histories in patients with mild bleeding disorders are challenging as many patients report mild bleeding symptoms, which are common in and overlap with the normal population. More recently, structured bleeding assessment tools (BATs) to objectively quantify bleeding symptoms in a standardised manner have been developed. These bleeding assessments tools have shown with good negative predictive value and their greatest clinical utility presently may lie in identifying patients who do not require further testing but validation for bleeding disorders outside von Willebrand disease is still needed. If the predictive value of the structured BATs is confirmed in prospective studies, this approach is likely to be extremely valuable in standardising and improving the specificity of determining abnormal bleeding that requires further investigations.

(c) Routine preoperative screening

Perhaps the most controversial indication for coagulation testing is in routine preoperative screening. Both the PT and APTT were designed as diagnostic tests to confirm the clinical suspicion of bleeding. This is different from their use as screening tests in otherwise healthy preoperative patients, where the prevalence of bleeding disorders is extremely low. Their use in populations with low pretest probability will invariably detect a high degree of normal results. Even when the results are abnormal, these are more likely to result from false positives or the detection of disorders not associated with bleeding e.g. FXII deficiency and lupus anticoagulant,

which have relatively high prevalence in an otherwise normal population. These false positive results cause potentially unnecessary further investigations that generate delay, anxiety, cost and harm. Additionally, 30–95% of abnormal results from screening tests are either not documented or followed-up, potentially increasing the risk of litigation. Similarly, given that the PT and APTT may not detect some clinically significant disorders, a normal result can give false reassurance. Accordingly, preoperative assessments should start with a structured bleeding history and coagulation screening performed only if there is a concern about a bleeding tendency or risk arising from the history. The majority of patients with congenital bleeding disorders are aware of their diagnosis either through a positive family history and/or a personal history of bleeding. Similarly patients with acquired bleeding risk or disorders will give a personal history of bleeding, relevant co-morbidity or anti-haemostatic medication. In the absence of an abnormal bleeding history, the utility of the coagulation screen in detecting previously unidentified individuals with a bleeding disorder is likely to be extremely low and should not be performed.

FUTURE DEVELOPMENTS

In addition to the development of structured BATs, the serious limitations of the coagulation tests have also prompted the development of alternative laboratory testing approaches. Of these, the thromboelastogram (TEG) and measurement of endogenous thrombin potential (ETP) are the most promising and most widely tested. The TEG is a near-patient test that measures the viscoelastic properties of whole blood as it clots. This test provides a more complete assessment of haemostasis as it shows the interaction of platelets with the coagulation proteins, plasma coagulation inhibitors and fibrinolytic proteins. Its application is currently limited to liver transplantation and cardio-pulmonary bypass surgery where TEG-based algorithms have been shown to reduce blood product use. Although TEG studies have expanded to many other clinical scenarios, the technique still requires proper standardisation to give reproducible and reliable results before widespread use. The ETP reflects the action of the total clotting system by measuring the ability of plasma or whole blood to generate thrombin. Studies have found correlation between thrombin generation and the severity of congenital bleeding disorders that may allow tailoring of factor replacement therapy or prediction of bleeding risk in patients on anticoagulants. Many other small studies have been performed in both congenital and acquired bleeding disorders but presently the test is still not standardised and only available in an experimental setting.

An ideal haemostatic assay would not only be capable of testing the overall function of the entire haemostatic system but also be able to define the qualitative and/or quantitative contribution of each compartment accurately and precisely. Until such a test is developed and validated for use, appropriate use of coagulation screens must involve consideration of the clinical context, disease prevalence and laboratory limitations of the tests including performance characteristics, cost and potential impact of false-positive and false-negative results.

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Highlights

- There is no global haemostatic test capable of recapitulating the complex interaction of the four compartments of the haemostatic system *in vivo*.
- The prothrombin time (PT) and activated partial thromboplastin time (APTT) test only the coagulation protein compartment. To avoid the wrong diagnosis, inappropriate investigations and treatment, results have to be carefully interpreted in the context of the clinical presentation and assay limitations.
- Both the PT and APTT have general limitations including frequent artefact due to pre-analytical factors, reagent variability, detection of disorders not associated with bleeding (e.g. the lupus anticoagulant and FXII deficiency) and insensitivity to clinically important bleeding disorders (e.g. mild von Willebrand disease and FXIII deficiency).
- Clinicians also need to be aware of specific limitations when requesting PT and APTT for anticoagulant monitoring, bleeding symptoms and routine preoperative screening.
- Indiscriminate preoperative coagulation testing is not warranted due to the poor performance characteristics of the PT and APTT in populations with low prevalence of bleeding disorders. Coagulation testing should be restricted to those with haemostatic concern following a structured bleeding history assessment.

Further reading

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SELF-ASSESSMENT QUESTIONS

1. Regarding the classical coagulation cascade, which ONE of the following statements is FALSE?

A. The extrinsic system comprises factor VII, FX, FV and FII.
B. Factors V and VIII function as cofactors in the coagulation cascade.
C. The final common pathway comprises FX, FV and FII.
D. FXIII does not participate in the coagulation cascade.
E. FXII is a component of the intrinsic system.

2. Which ONE of the following does not cause an isolated prolongation of the prothrombin time?

A. Inadequate sample volume.
B. Congenital haemophilia A.
C. Factor VII deficiency.
D. Early oral anticoagulation.
E. Mild Factor II, V or X deficiency.

3. A 24-year-old man is seen preoperatively for an elective hernia repair. He is previously well with no other significant past medical or surgical history. On systems enquiry, he describes easy bruising with recurrent epistaxis requiring nose packing and cauterisation and gum bleeding. He also describes prolonged bleeding after cuts and oozing for several days after a dental extraction two years ago. There is no family history of bleeding. He is on no medication and clinical examination is unremarkable. His full blood count (FBC) is normal. His coagulation tests are as follows:

PT 12 s (12–14.5)
APTT 42 s (27–35.6)

Which ONE of the following tests would you perform next?

A. Peripheral blood film.
B. Platelet function test.
C. FVIII and von Willebrand factor levels.
D. APTT mixing test.
E. Bleeding time.

4. Which ONE of the following is the most important component in the assessment of a patient presenting with easy bruising/bleeding?

A. Full blood count.
B. Clinical examination.
C. Bleeding history.
D. Coagulation screen.
E. Bleeding time.

5. Which ONE of the following statements regarding the international normalised ratio (INR) is INCORRECT?

A. INR is only valid for patients who are stably anticoagulated on warfarin.
B. INR allows prothrombin time results from different laboratories to be compared.
C. INR is valid for use in patients with liver failure.
D. The initial prolongation of INR in warfarin anticoagulated patients reflects the drop in FVII which has the shortest half-life.
E. Differences in thromboplastin sensitivity is the main component for variability in the prothrombin time test.

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