Conventional and near-patient tests of coagulation

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Preoperative anticoagulation or perioperative coagulopathy secondary to trauma, massive transfusion, sepsis, cardiopulmonary bypass (CPB) or hypothermia may impair haemostasis. Under such circumstances, reducing the turn around time (TAT) between sampling and the result of coagulation tests could reduce inappropriate blood product administration by correct targeting of specific coagulation deficiencies.\(^1,2\) In this article, we shall describe some of the devices currently available for near-patient testing (NPT) of coagulation that have the potential to reduce coagulation result TAT.

Before considering NPT of coagulation, it is necessary to consider standard laboratory tests of coagulation and their relationship to our current understanding of the coagulation process. By convention, we will describe coagulation factors (F) by their Roman numerals and activated factors as ‘a’.

**Haemostasis and the cell-based model of coagulation**

Under normal circumstances, coagulation is initiated within seconds of a breach in the vasculature, with platelets forming a plug at the site of injury; this is termed primary haemostasis. Secondary haemostasis involves a complex interaction between plasma coagulation factors, resulting in the formation of fibrin strands to strengthen the platelet plug. This provides a mechanism of amplification to generate the necessary quantities of fibrin. In order to clot, 100 ml of blood requires 0.2 mg of FVIII, 2 mg FX, 15 mg of prothrombin (FII) and 250 mg of fibrinogen (FII). Propagation of haemostasis throughout the vasculature is prevented by inhibitors, of which tissue-factor pathway inhibitor (TFPI), protein C and antithrombin are the most important.\(^3\)

Secondary haemostasis was classically described in terms of intrinsic and extrinsic pathways uniting at a final common pathway. This model is useful to understand the actions of therapeutic anticoagulants and the derivation of the prothrombin time (PT) and activated partial thromboplastin time (APTT). In reality, this *in vitro* model disregards the interaction between primary and secondary haemostasis and does not correlate well with the *in vivo* process. The minor role that the contact factor pathway (extrinsic pathway) plays in initiation of clot formation *in vivo* is illustrated by patients with severe deficiencies of high molecular weight kininogen (HMWK), prekallikrein and FXII; all have a prolonged APTT but no bleeding disorder.

The currently accepted model of coagulation has been updated, more closely reflecting *in vivo* activity. This cell-based model places tissue factor-VIIa complex as pivotal in initiation and highlights the importance of platelet activity and recognises the key role of thrombin in both promoting and inhibiting coagulation. Rather than covering in detail the currently accepted view of haemostasis, we direct the reader to consult the article by Bombel and Spahn\(^4\) or the chapter by Laffan.\(^5\)

The process involves five steps (Fig. 1): initiation, amplification, propagation, stabilisation, and inhibition of further coagulation.

**Initiation:** Tissue factor (TF) binds to circulating FVIIa and, in the presence of FV, converts FIX to FIXa, and FX to FXa. FXa binds to prothrombin to generate a small amount of thrombin. In health, only 1% of total circulating FVII is present as FVIIa and, in the presence of FV, converts FIX to FIXa, and FX to FXa. FXa binds to prothrombin to generate a small amount of thrombin. In health, only 1% of total circulating FVII is present as FVIIa and available for the first step of initiation.

**Amplification:** The small amount of thrombin generated by initiation is insufficient to convert fibrinogen to fibrin. Thrombin-triggered feedback mechanisms occur principally on the activated platelet surface, as follows:

1. TF, thrombin, FIXa, FXa complex activates more FVII.

**Key points**

- Laboratory methods use platelet poor plasma; near-patient testing (NPT) uses whole blood.
- NPT should be thought of as expressing the effects of the prothrombin time or international normalized ratio and activated partial thromboplastin time or activated thromboplastin ratio rather than measuring them.
- The complexity of the coagulation system is unlikely to be adequately expressed by the relatively crude tests typically used.
- The thromboelastogram is one of the few tests to represent fibrinolysis.
- NPT has the potential to reduce inappropriate blood product administration.

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2. Thrombin activates FVIII to increase the action of FXIa on FX.
3. Thrombin activates FV to increase the action of FXa on prothrombin.
4. Thrombin also activates FXI to FXIa increasing FIXa.

**Propagation:** The TF/FVIIa complex ensures the supply of FIXa. FIXa with FVIIIa activates FX (intrinsic tenase complex) to ensure an adequate supply of FXa and maintains continuous thrombin generation, that is, ‘thrombin burst’.

**Stabilisation:** High levels of thrombin stimulate FXIII to cross-link the soluble monomers and the protection of the clot by thrombin-activatable-fibrinolysis-inhibitor.

**Inhibition of further coagulation:** Overwhelming pathological thrombosis is controlled by:
1. Thrombin activated protein C (aPC) cleaves FVa and FVIIIa.
2. TFPI inhibits TF-VIIa and FXa by binding them in a quaternary complex.
3. Antithrombin inhibits thrombin, FIXa, and FXa.

**Standard laboratory tests**

The standard laboratory screening tests of coagulation are the PT and the APTT. Fibrinogen level and thrombin clotting times (TCT or thrombin time, TT) may also be reported. Coagulation results can be affected adversely by poor sampling technique. To prevent clotting of the sample, whole blood is mixed with a calcium-chelating agent such as EDTA or citrate in a ratio of 9:1. Underfilling the tube or haematocrit >0.55 both reduce plasma volume in the sample and prolong clotting times artefactually due to over-anticoagulation.

These tests are performed after centrifugation on platelet-poor plasma. Most of the tests have endpoints measured in seconds but results may be expressed as referenced ratios, that is, the international normalized ratio (INR) for the PT, or a locally derived ratio termed the activated partial thromboplastin ratio (APTR) for the APTT. PT, APTT and TCT measure the time to form a fibrin clot *in vitro* after recalcification of the sample in the presence of the appropriate test reagent. Time to fibrin strand formation is detected using either a photo-optical or electromechanical device.

**Prothrombin time**

The prothrombin time, introduced by Quick in 1935, is the time taken to fibrin strand formation when platelet-poor plasma is recalcified in the presence of thromboplastin (tissue factor and phospholipid). Originally considered specific for a deficiency of the extrinsic pathway, the INR is most sensitive to a decrease in FVII; however, it is also prolonged with deficiencies of FII, FV, and FX, and in liver disease, vitamin K deficiency, DIC, and high doses heparin. Different thromboplastins give different normal ranges for PT. The INR standardizes the thromboplastin reagent; it is the ratio of the patient’s PT to a control plasma PT, raised to the power of a correction factor known as the international sensitivity factor.
index (ISI) specific for each thromboplastin reagent. The amount of TF present in the initiating thromboplastins is so large that it negates the effect of TFPI and renders the test independent of FVIII, FIX, and FXI; thereby not detecting the bleeding tendency associated with haemophilia.5

**Activated partial thromboplastin time**

The term ‘partial thromboplastin’ indicates that the reagent contains phospholipids (as a substitute for the platelet membrane) but no tissue factor, distinguishing it from the PT. All procoagulant factors except FVII and FXIII are measured by this assay. Platelet-poor plasma is ‘activated’ by a 3 min pre-test incubation with the APTT reagent. In addition to the phospholipids, this reagent contains a contact activator that is a fine suspension of negatively charged particles (kaolin, celite or ellagic acid). The sample is then recalcified and the time taken to fibrin strand formation is the APTT.

APTT is used to monitor unfractionated heparin therapy and as a screening tool for haemophilia A, haemophilia B, and coagulation inhibitors. APTT is prolonged by a reduction in procoagulant activity to <30–40% of normal (factor and reagent dependent), by the presence of a specific clotting factor inhibitor, or by the presence of heparin or the lupus anticoagulant, which acts as a non-specific intrinsic pathway inhibitor. Contamination of the sample with heparin at the time of collection, or an inaccurate whole blood:citrate ratio will artificially prolong the APTT. A prolonged APTT is not necessarily predictive of clinical bleeding tendency for example lupus anticoagulant in vivo leads to prothrombotic tendency, and factor XII deficiency gives very long APTT but no bleeding tendency.

**Thrombin time**

The TCT or TT is a simple test of the fibrin polymerisation process. A standard concentration of human thrombin is added to citrated, platelet-poor plasma and time to clot formation is measured. Clot formation requires only the presence of fibrinogen and the absence of thrombin inhibitors. TCT is independent of all other factor deficiencies. The commonest thrombin inhibitor is heparin, and TCT is more sensitive than APTT for the detection of heparin. TCT is prolonged by hypofibrinogenenaemia or dysfibrinogenaemia and by the presence of fibrin degradation products and myeloma proteins. TCT is also used to monitor fibrinolytic therapy and detect heparin resistance.

**Fibrinogen level**

There are a number of tests available for fibrinogen level estimation; they vary in their complexity, TAT, and result reproducibility. The two most frequently used tests in routine clinical practice are the Clauss assay and the PT-derived fibrinogen level (denoted PT-Fg). More accurate, but time consuming, clottable protein or immunological assays are available which are used for

the investigation of congenital fibrinogen defects. The Clauss assay involves the addition of high concentration thrombin to diluted test plasma. Clotting time is compared with a calibration curve prepared by serial dilution and testing of reference plasma of known fibrinogen concentration. This gives a result expressed in g litre⁻¹. Results are adversely affected by the presence of heparin, which is a particular problem during any procedure involving an extracorporeal circuit. The heparin effect can be negated by use of an ion exchange resin or heparinase enzymes.

PT-Fg assays compare the PT of the test sample with a reference curve derived as above, indirectly measuring fibrinogen. This method is attractive as the fibrinogen level can be estimated rapidly and at no extra cost whenever a PT is requested. This technique is used in 50% of UK haematology laboratories, although significant differences in locally derived batch calibration and referencing to international standards question its suitability for routine use in coagulopathy screening.6

**Near-patient tests of coagulation**

Near-patient tests have a shorter TAT, involve less transportation, use a minimal volume of whole blood, and have no need for special preparation or personnel. Therapeutic interventions can be made more rapidly, improving convenience for staff and patients.7 The laboratory INR and APTR use platelet poor plasma. By comparison, NPT uses whole blood, and hence platelets, and often depends on changes in the viscoelastic properties of the clotting blood. NPT should be thought of expressing the effects of INR or APTR rather than measuring them.

**Hemochron activated clotting time**

The Hemochron activated clotting time (HACT) was first described in 1966 by Hattersley; it does not have a laboratory equivalent. As 2 ml of whole blood is added to a black-topped test tube containing celite and a ferro-magnetic bar, a timer is started. The tube is warmed to 37°C and gently rotated within the test well. As coagulation occurs, the bar rotates with the tube and is detected by a magnetic sensor.

The test is widely used in situations where assessment of systemic heparinization is required almost instantaneously for example cardiac surgery, cardiac catheterisation, haemofiltration. The normal (baseline) value is 120–140 s and the accepted variability of the test is 10%. There is no linear correlation between the APTR and the prolongation of the ACT. After systemic heparinization, and in the absence of aprotinin, an ACT >480 s is considered safe for cardiopulmonary bypass, whereas in the presence of systemic aprotinin, a value >700 s is required. An ACT >200 acceptable for haemofiltration. Gold-topped tubes contain kaolin as the accelerant. By effectively binding aprotinin, an ACT of 480s remains safe for CPB. Kaolin ACT results without aprotinin, tend to be 5% higher than celite ACT.
Underfilling the test tube shortens the ACT, whereas overfilling, inadequate mixing, thrombocytopenia, warfarin, and pro-coagulant (dilutional) deficiencies will prolong the ACT. Each test costs £1.30. The ACT can also be obtained from the Hemochron Jr. signature (see below). The Hemochron Response works on similar principles but, by using different test tubes, is capable of providing the ACT, PT, APTT and TT; it also calculates the INR.

**Hemochron Jr. signature**

This is a hand-held device that has the capability of deriving ACT, APTT and PT. Test-specific cuvettes are pre-warmed to 37°C, onto which 50 μl of fresh or citrated whole blood is placed. Fifteen microlitres is aspirated into the test channel and the rest drawn into a waste channel. The blood is mixed with the test-specific reagent and moved back and forth within the test channel. As coagulation occurs, optical sensors detect the impeded movement, the timer stops, and an audible tone sounds.

Heparinization for cardiac surgery (typically 4–6 IU heparin ml⁻¹ blood) is monitored using the ACT test. The reagent is a mixture of silica, kaolin, and phospholipids. ACT is derived twice as fast as the test tube celite ACT, although the result is expressed in celite equivalent values (s). Even so, the values are 10% lower than the equivalent celite ACT. The test is not affected by high dose aprotinin therapy and costs £7. The ACT-LR test uses celite as the reagent and is linear up to 2.5 IU heparin ml⁻¹, making it suitable for use with haemofiltration. It is not intended for use with aprotinin. The manufacturer does not describe the APTT reagent other than to state it is a contact activating substance formulated to provide optimal heparin sensitivity up to 1.5 IU ml⁻¹ blood. The results are displayed as plasma equivalent values. The PT test is described as using a highly sensitive thromboplastin for improved specificity and sensitivity. The results are displayed as the INR. It is used to monitor oral anticoagulant therapy.

**CoaguChek**

The Roche CoaguChek S uses 10 μl of capillary or venous whole blood to derive an INR with reference to that obtained from a control solution using reflectance photometry. The results correlate well with laboratory values for both routine samples and patients on oral anticoagulants. It is approved by the Medical Devices Agency (but not the FDA for home use in the US). Its use is well suited to patients established on warfarin for 6 months or more for general practice or home-based monitoring of warfarin. The measured INR is lower than the laboratory INR if >20 s elapse between obtaining the capillary sample and application onto the test strip or if the finger has been squeezed excessively (due to the presence of TF). CoaguChek results may be higher than laboratory values in the presence of the lupus anticoagulant, if the capillary test is contaminated with alcohol or soap residue or the patient has received heparin in the previous 2 days. A haematocrit outside the range 32–52% may increase or decrease the value for the INR.

The device is not suitable for anticoagulation control during the initiation of warfarin therapy. The device costs £399 and the tests £2.60. The test strips for newer Roche CoaguChek XS have the advantage of not needing refrigeration and incorporate controls to ensure strip integrity.

Self-monitoring of oral anticoagulation reduces thromboembolic events, major haemorrhage and mortality. Where combined with self-adjustment of therapy, thromboembolic events and death are reduced, but haemorrhagic complications are not.

**Platelet function analysing monitor**

The platelet function analysing motior (PFA 100) could be considered NPT; however, the analysis has to be performed 30–120 min after venepuncture. It distinguishes the effect of aspirin from other abnormalities of platelet function. Eight hundred microlitres of citrated whole blood introduced into a disposable cuvette is drawn through a 150 μm hole in a collagen-coated membrane to which either epinephrine or ADP is bonded. Under the biochemical and shear stress influences, platelets become activated to form a primary plug. Sealing the hole is sensed by a pressure transducer and occurs in 81–166 s with epinephrine and 54–109 s with ADP.

If the PFA 100 result is normal with epinephrine, normal platelet function can be assumed. A prolonged epinephrine result that normalizes when repeated with ADP indicates an aspirin effect. If both epinephrine and ADP are prolonged, further (more complex) investigation is needed to identify the thrombocytopathy. Laboratories use the test to screen for von Willebrand’s disease (cost £12).

**Thromboelastography (Thromboelastograph® Haemostasis Analyser)**

The Thromboelastograph® (TEG®) is based on a technique developed by Hartert in 1948. Its analyser produces a trace which quantifies the kinetics of clot formation and dissolution from the point of initial fibrin formation through to clot retraction or lysis. This differs from the majority of coagulation tests that use time to first fibrin formation as an endpoint. The TEG assesses the interaction between fibrinogen, platelets, and clotting factors with a single test.

The TEG® is produced by placing a 360 μl sample of whole blood into a pre-warmed (37°C) cup which is oscillated through an angle of 4°45’, rotating once every 10 s. This simulates a low shear environment resembling sluggish venous flow. A plastic pin is suspended in the blood sample by a torsion wire and monitored for motion. As a clot forms, the cup and pin become coupled by fibrin-platelet bonds, and the torque of the rotating cup is transmitted by the torsion wire to a mechano-electrical transducer. The resulting electrical signal is converted (nowadays by computer) into a characteristic cigar-shaped graphical output representing a function of shear elasticity against time; this is termed the TEG®.
The magnitude of the output is directly related to the strength of the fibrin-platelet bonds. A strong clot causes the pin to move directly in phase with the cup creating a broad TEG®, whereas a weak clot stretches and delays the arc movement of the pin creating a narrow TEG®. The shape of the trace allows rapid qualitative assessment of hypocoagulable, normal, or hypercoagulable states. Quantitative analysis of the TEG® is performed by assessment of five main parameters; four relating to clot formation and one to clot lysis (the LY30—see below). No single TEG® parameter correlates directly with laboratory coagulation tests but, when combined, enable identification of specific component deficiencies. Activation of the sample with celite, kaolin, or tissue factor allows earlier TEG® interpretation, as clot formation is faster. Activated TEG® samples have different normal ranges for the parameters. A normal TEG® is shown in Figure 2.

The five main TEG® parameters, determined sequentially, and their normal ranges are as follows. The scale on the x-axis is 2 mm min⁻¹, and the values are often expressed as distance in millimetres:

1. R-time (or reaction time): The time from the initiation of the TEG® until the amplitude of the trace reaches 2 mm, corresponding to initial fibrin formation. The R-time is functionally related to plasma clotting and inhibitor factor activity.
   - Normal range: 15–30 mm (whole blood); 10–14 mm (celite activated); 8–12 mm (kaolin activated).
   - Prolonged by anticoagulants, factor deficiencies, and severe hypofibrinogenaemia.
   - Reduced by hypercoagulable conditions.
2. K-time (or clot formation time): The time from the R-time until the TEG® amplitude reaches 20 mm (representing reference level clot firmness). As such, the K-time is a measure of clot formation kinetics. If amplitude does not reach 20 mm, K is undefined.
3. α-angle: This is the angle formed between the middle of the TEG® and a line drawn between the r and K-times. The angle relates directly to the K-time as they are both a function of rate of fibrin polymerisation. In the absence of a K-time being defined, the α-angle can still be expressed as the angle of a tangent to the developing TEG starting at the r-time. It represents the speed at which solid clot forms.
   - Normal range: 40–50° (whole blood); 54–67° (celite activated); 66–77° (kaolin activated).
   - Increased by increased fibrinogen level, increased platelet function.
   - Decreased by anticoagulants, hypofibrinogenaemia, thrombocytopenia.
4. MA (maximum amplitude): This is the greatest amplitude reached on the trace and is representative of maximum clot strength. It is directly related to the quality of fibrin and platelet interaction. Platelets have the most influence on final clot strength, and the MA is significantly altered by changes in platelet number or function.
   - Normal range: 6–12 mm (whole blood); 3–6 mm (celite activated); 2–4 mm (kaolin activated).
   - Prolonged by anticoagulants, hypofibrinogenaemia, thrombocytopenia.
   - Shortened by increased fibrinogen level, increased platelet function.
5. LY30: This reflects fibrinolysis and measures percent lysis 30 min after MA is reached. The measurement is based upon the reduction in area under the TEG® trace, rather than just the A30. The A30 (amplitude 30 min after MA) is a point measurement and therefore not totally representative of the rate of lysis in the preceding 30 min.
   - Normal range: <7.5%

Thromboelastography is utilized principally during orthotopic liver transplantation and cardiac surgery involving cardio-pulmonary bypass circuits. Incubation of the blood sample with heparinase I enables the TEG® to demonstrate developing coagulation abnormalities while fully heparinized or the need for more protamine when reversing heparin after cardiopulmonary bypass. TEG®-guided, protocol-based management of postoperative bleeding has been demonstrated to significantly reduce inappropriate blood component administration and re-operation rates, especially in cardiac surgery.²

Use of TEG® in other surgical settings is more limited but has been used with success in obstetrics, radical prostatectomy, neurosurgery, and trauma cases. Access to thromboelastography is of potential benefit to both anaesthetists and patients in any case involving major haemorrhage; it is the only NPT to indicate

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**Fig. 2 Normal TEG®.**
thrombolysis. However, multiple coagulation deficiencies cannot be easily distinguished. Examples of abnormal TEG tracings are shown in Figure 3.

The ROTEM is another device that produces a thromboelastogram using similar principles.

Problems of NPT

There are rigorous national standards for laboratory quality control, which have led to improved comparability of results from different laboratories. NPT, outside the main laboratory in unregulated mini-labs, may be unreliable in less experienced hands, may lack adequate calibration, have limited comparability with laboratory values, and the results may be inadequately documented or linked to the pathology data processes. Ideally, comparable standards for whole blood methods should be developed before NPT is introduced. There is no evidence that NPT shortens hospital stay.\(^7\)

Despite different methodologies, the agreement with laboratory tests is good for the PT. However, the lack of standardization of reagents for the APTT worsens the limits of agreement between laboratory and NPT. Further, it has been suggested that in situations where there is haemodilution or platelet dysfunction (e.g. after cardiac surgery), whole blood techniques are likely to differ still further from laboratory values.\(^10\)

However, the correct choice of NPT for a given clinical situation can reduce TAT, inappropriate blood product administration, 1, 2 thromboembolism, major haemorrhage, and mortality.\(^8\)

References


Please see multiple choice questions 13–17
How anticoagulants work

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KEYWORDS:
Anti-platelets;
Anti-thrombotics;
Anti-coagulants;
Pharmacokinetics;
Duration;
Reversal

Recent advances in our understanding of the mechanisms of physiological hemostasis and the limitations of existing antithrombotic drugs (ASA, Unfractionated Heparin and Oral Vitamin K antagonists) have led to the development and expanding clinical use of a number of new antithrombotic agents designed to target specific steps in the platelet and coagulation hemostatic pathways. Although these new agents hold the promise of increased efficacy, safety, and clinical utility for many patients on antithrombotic therapy, they also pose potential drawbacks and new challenges when those patients undergo neuroaxial blockade. A clear understanding of the mechanisms of action, pharmacokinetics, and duration of action and strategies to reverse the anticoagulant effect of both old and new antithrombotic agents are essential for the safe delivery of regional anesthesia/analgesia.

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Over the last 15 years, a number of new antithrombotic agents have been developed and introduced in clinical medicine to address limitations in existing drugs: ASA, Unfractionated Heparin (UFH), and Vitamin K antagonists (Warfarin). The increasingly common usage of these newer agents has raised concerns and questions among physicians involved in regional anesthesia as to the safety of neuroaxial blockade (epidural or spinal anesthesia/analgesia) in the presence of these medications. These concerns are amplified by two potential drawbacks of these new agents:

1. Many of them have minimal or no effect on routine coagulation tests (INR and aPTT), making the detection and assessment of their anticoagulant effect problematic.
2. Most of these newer agents have no specific antidote for rapid reversal of effect.

This review will provide information on mechanisms of action of antithrombotic drugs both old and new, the pharmacokinetics and duration of action of their antithrombotic effect, and approaches to reversal of this effect to facilitate the safe performance of these procedures.

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Physiological hemostasis

Hemostasis or blood clot formation involves a series of coordinated complex interactions of injured vessels, platelets, coagulation factors, and fibrinolysis (Figure 1).

For this review, we will confine our discussion to the platelet and coagulation factor responses where the majority of the drugs pertinent to this review act.

Platelet plug formation

Along with vasospasm, activation of platelets at the site of vascular injury to form a platelet plug constitutes the primary response to hemostasis. Platelet activation triggers four processes:

- adhesion (deposition of platelets on the damaged subendothelial matrix),
- aggregation (platelet-platelet binding),
- secretion (release of platelet granules), and
- procoagulant activity (enhancement of thrombin generation) (Figure 2).

Platelet activation is mediated by a number of physiological agonists, including thrombin, collagen, ADP (adenosine diphosphate), and epinephrine. Specific receptors for these agonists exist on the platelet surface. The interaction of these receptor–agonist complexes with coupling proteins triggers transmembrane signaling leading to further intracellular enzyme reactions and exposure of surface receptors...
that are involved in platelet adhesion, aggregation, secretion, and procoagulant activity.\textsuperscript{2}

**Platelet adhesion**

Damage to the vascular endothelium exposes subendothelial collagen and von Willebrand’s factor (vWF). Plasma vWF binding to collagen becomes a strong adhesive protein which binds or anchors circulating platelets via a platelet glycoprotein surface receptor GP Ib-IX-V to the area of vascular damage, localizing the platelet plug formation.

**Platelet aggregation**

Following activation, platelet surface protein GP IIb-IIIa undergoes a critical conformational change and becomes a high affinity receptor for fibrinogen as well as vWF and other adhesive proteins. The binding of bivalent fibrinogen molecules to “activated” GP IIb-IIIa on adjacent platelets bridges or links them together forming platelet aggregates.\textsuperscript{2} Functional activation of the IIb-IIIa receptor is the final common pathway in platelet aggregation, and its blockade is the target of the class of IIb-IIIa inhibitor antiplatelet agents.

**Platelet secretion**

After activation, platelets release a number of intracellular granules which modulate platelet interactions. These include: ADP and serotonin which will activate and recruit additional platelets; fibronectin and thrombospondin—adhesive proteins that stabilize platelet aggregates; fibrinogen and vWF to promote further platelet adhesion, aggregation and fibrin clot formation; Factor V important in the coagulation process; and growth factors like platelet derived growth factor that mediates tissue repair and probably atherosclerosis.\textsuperscript{3} In addition, Thromboxane A\textsubscript{2} is synthesized and released, which promotes vasoconstriction and platelet aggregation.

**Procoagulant activity**

There is a very close interaction between the coagulation cascade and activated platelets. Platelet activation results in the exposure of “procoagulant” anionic phospholipids (phosphatidylserine) on platelet membrane surfaces. This serves as a template to facilitate the “surface assembly” of coagulation factor enzyme complexes: Factor X activating complex (X-VIIla/IXa/Ca\textsuperscript{2+}/H\textsubscript{11001}) and Prothrombinase complex (II–Xa/Va/Ca\textsuperscript{2+}) in a localized catalytically efficient environment for thrombin generation (Figure 3). Thrombin generation is 300,000 times more efficient by surface complex assembly than random circulating coagulation factor interactions.\textsuperscript{2,3} The end result of these interactions is the efficient amplification and localization of the coagulation process to the area of platelet plug and vascular injury.

**Coagulation**

The coagulation process, also known as the coagulation cascade, is a series of enzymatic reactions involving the sequential activation of a number of circulating plasma proenzyme proteins—the coagulation factors. Each activated coagulation factor in turn activates a subsequent proenzyme in the sequence. The ultimate result is the generation of fibrin which stabilizes and reinforces the hemostatic platelet plug. The key feature of this cascade is amplification. Activation of a small number of initial molecules in the sequence will ultimately generate many thousand-fold thrombin molecules, the final active coagulation enzyme for the conversion of fibrinogen to fibrin. This process is facilitated by the surface assembly of sequential enzyme complexes at the activated platelet membranes (Figure 3).

Although it has been traditional (and useful for the interpretation of in vitro clotting tests INR, aPTT) to divide the coagulation cascade into the intrinsic and extrinsic pathways with independent trigger mechanisms (Figure 4), we now know that physiological coagulation is triggered by the exposure of tissue factor at the injury site. Tissue factor (TF) then immediately binds to small amounts of circulating Factor VIIa. This leads to rapid auto-catalytic conversion of Factor VII to additional VIIa. This TF-VIIa complex then activates Factor X directly, thereby initiating the classic extrinsic pathway and as well indirectly by activating trace amounts of Factor IX, inducing activation of the intrinsic pathway via formation of intrinsic Factor X activating complex (X-VIIla/IXa/Ca\textsuperscript{2+}).\textsuperscript{4} This dual pathway of activation leads to more efficient generation of Factor Xa. Factor Xa can further activate additional Factor VII, amplifying the response. In essence, coagulation is triggered by tissue factor release and extrinsic pathway activation. This process is then amplified by the intrinsic pathway coagulation factors which have been activated by TF-VIIa in a positive feedback loop. Both pathways converge at the level of Factor X activation, leading to activation of the final common path-

![Figure 2](image-url)  
**Platelet Activation**

- **Adhesion**
- **Collagen**
- **vWF**
- **Secretion**
- **Conformational Activation of GPIIb/IIIa**
- **Aggregation**
Platelet-Coagulation Factor Interactions
“Surface Assembly of Complexes”

Figure 3  Platelet-coagulation factor interactions. “Surface Assembly of Activation Complexes.”

Classical Coagulation Cascade

Figure 4  Classical coagulation cascade.
Physiological Coagulation

![Diagram of physiological coagulation](image)

Figure 5  Physiological coagulation.

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Antiplatelet Drugs

**Vessel Wall Injury**

![Diagram of antiplatelet drugs](image)

Figure 6  Antiplatelet drugs: site of action.
Anticoagulation Drugs-Targets

**Mechanism of action**

**Antiplatelet drugs**

*See Figure 6*

1. **Thromboxane A$_2$ inhibitors**

Acetylsalicylic acid (ASA) irreversibly acetylates and inactivates platelet cyclooxygenase (Cox-1), a crucial enzyme in the Prostaglandin synthesis of Thromboxane A$_2$ and Prostacyclin. This leads to permanent inhibition of Thromboxane A$_2$ production for the lifespan of the platelet. Thromboxane A$_2$ is important for platelet recruitment processes—platelet secretion and aggregation.

2. **ADP receptor antagonists**

Ticlopidine and clopidogrel are thienopyridine derivatives that irreversibly block the binding of ADP to the platelet receptor P2Y$_{12}$, thus inhibiting platelet aggregation responses by various platelet agonists: thrombin, collagen, ADP, and epinephrine.

3. **GP IIb-IIIa receptor antagonists**

Three parenteral GP IIb-IIIa inhibitors have been developed and licensed for use in North America, primarily to prevent thrombotic reoclusion following percutaneous coronary intervention (PCI): Abciximab (Reopro®), a chimeric monoclonal antibody, and two synthetic inhibitors, Eptifibatide (Integrilin®) and Tirofiban (Aggrastat®). They block GP IIb-IIIa receptors on activated platelets, preventing fibrinogen and vWF binding, the final common pathway of platelet aggregation. This blockage results in more than 80% inhibition of platelet aggregation, irrespective of the platelet agonist(s).

**Anticoagulant drugs**

*See Figure 7*

1. **Warfarin (Vitamin K antagonists)**

Warfarin is a coumarin compound that exerts its anticoagulant effect by inhibiting the synthesis of the active forms of four Vitamin K-dependent procoagulant proteins: Prothrombin (Factor II), Factors VII, IX, and X, and two anticoagulant proteins: Protein C and Protein S. The physiological activity of these proteins is dependent on $\gamma$-carboxylation by a carboxylase enzyme, which requires reduced Vitamin K. Warfarin blocks the regeneration of reduced Vitamin K. Subsequent depletion of reduced Vitamin K results in failure to synthesize any further active forms of these coagulation proteins. The anticoagulant effect results from the slow decline in the circulating active forms of the procoagulant proteins over a few days based on their respective half-lives (6 hours to 72 hours).

2. **Unfractionated heparin (UFH)**

A heterogeneous mixture of sulfated glycosaminoglycans of varying molecular fragment lengths (average mo-
lecular weight 15,000 Da) whose major anticoagulant effect is dependent on a specific pentasaccharide sequence with a high affinity for Antithrombin III (AT), the major inhibitor of Thrombin, Factor Xa, TF-VIIa, Factor IXa, and Factor XIa. Binding of UFH to AT results in a conformational change in AT, which catalyzes by a 1000-fold, the physiologically important inactivation of Thrombin and Factor Xa. Effective Thrombin (Factor IIa) inactivation requires that the heparin fragment be long enough to bind or ‘bridge’ both thrombin and AT simultaneously, an effect that occurs only if the heparin fragment chain exceeds 18 monosaccharide units, commonly found in UFH. In contrast, this type of simultaneous bridging is not required for AT inactivation of Factor Xa.

New anticoagulants: factor Xa inhibitors

1. Low molecular weight heparin (LMWH)

   Low molecular weight heparins are derived from unfractionated heparin by chemical or enzymatic depolymerization to yield fragments about one-third the size of UFH. These smaller heparin fragments (4000-5000 Da) still contain the crucial AT-binding pentasaccharide sequence and are able to effectively catalyze the inactivation of Factor Xa. However, their smaller fragment chain length preclude LMWHs from the simultaneous bridging of AT and Thrombin necessary for Thrombin (IIa) inactivation. Therefore, most LMWHs have high anti-Xa activity and minimal anti-IIa activity (anti-Xa/anti-IIa ratio ~ 2-4:1).

2. Fondaparinux®

   This is one of the new classes of selective Factor Xa indirect inhibitors recently approved for clinical use as antithrombotic prophylaxis following orthopedic surgery. It is also increasingly being used for treatment of deep vein thrombosis and pulmonary embolism and as alternative antithrombotic management in Heparin-induced Thrombocytopenia (HIT). It is a totally synthetic analog of the pentasaccharide sequence that binds to AT with high affinity, catalyzing (300-fold) the inactivation of Factor Xa, and thereby reducing thrombin generation. However, its short chain length renders it incapable of inactivating thrombin (Factor IIa) directly.

3. Danaparoid

   Derived from porcine and bovine mucosa, danaparoid is a heterogeneous mixture of heparan, dermatan, and chondroitin sulfates. Like LMWHs, it has a primarily anti-Xa effect with minimal anti-IIa activity (anti-Xa/anti-IIa ratio ~ 22:1).1

Direct thrombin inhibitors (DTIs)

Thrombin inhibitors bind directly to the active site of thrombin and block interaction with its substrate, fibrinogen, independent of AT. Unlike heparin and LMWH, DTIs can inactivate clot-bound thrombin which promotes further thrombus growth as well as circulating thrombin. To date, four parenteral thrombin inhibitors have been licensed in North America for limited indications: Lepirudin and Argatroban for treatment of HIT, Bivalirudin as a heparin alternative for percutaneous coronary interventions (PCI), and Desirudin for thromboprophylaxis post hip replacement.8

1. Lepirudin®

   Lepirudin is a recombinant form of hirudin—a potent anticoagulant produced by the medicinal leech. It is a bivalent inhibitor of thrombin that binds irreversibly. Lepirudin is cleared by the kidney and must be adjusted for renal insufficiency. It is approved for use as an alternate antithrombotic therapy in HIT.8

2. Bivalirudin (Hirulog®)

   This is a bivalent synthetic analog of hirudin which, in contrast, binds transiently to thrombin. Its transient thrombin binding, short half-life, and only partial dependence on renal excretion potentially lowers its risk of bleeding compared with other thrombin inhibitors. It has been approved for use as a heparin alternative in PCI.8

3. Argatroban

   This is the smallest molecule in the class of DTIs. It is a synthetic derivative of arginine that binds reversibly to the active site of thrombin. It is primarily metabolized by the liver making it useful in renal failure patients. It has been approved for use in HIT patients.7,8

Pharmacokinetics

(See Table 1)

Antiplatelet drugs

ASA is rapidly absorbed except with enteric-coated formulations where absorption is delayed. It undergoes rapid hydrolysis and is further metabolized in the liver. Although it only has a half-life of 0.4 hours, its inhibition of platelet aggregation is irreversible and lasts for the life span of the exposed platelet.10 The reversal of effect requires synthesis of an adequate number of new functioning platelets (approximately 20% of total circulating platelets) and this may take 2 to 4 days.11 Ticlopidine displays nonlinear pharmacokinetics and reduced clearance on repeated dosing.15 It requires hepatic biotransformation to become active and the onset is delayed. The effect persists for the life of the platelet resulting in long duration of action.13 Clopidogrel undergoes extensive and rapid hydrolysis to its active metabolites.14 The maximum platelet inhibition of 40% to 60% occurs after 3 to 5 days; however, the onset of action can be shortened by giving a loading dose.15 Similar to ticlopidine, it irreversibly modifies the ADP receptor, and the platelets are affected for the remainder of their lifespan (7-10 days).16

Abciximab must be administered intravenously as an infusion. It has a short half-life in plasma probably related to rapid binding to the platelets.17 When the infusion is stopped, glycoprotein IIb-IIIa receptor occupancy on platelets decreased to 60% within the first 6 hours.18 Platelet function generally recovers within 24 to 48 hours; however, low levels of inhibition may last for 15 days after discontinuation of infusion.19
It is not affected by renal or hepatic impairment. The pharmacokinetics of Eptifibatide are linear at the usual dosages.\textsuperscript{20} It requires continuous intravenous infusion due to its short half-life. Promptly after the initiation of infusion, greater than 90\% of platelets are inhibited within 15 minutes.\textsuperscript{21} The effect on platelets is rapidly reversible such that the normal platelet function is usually restored within 4 hours.\textsuperscript{20} Approximately 50\% of administered drug is eliminated in urine and 27\% is broken down in plasma into naturally occurring amino acids.\textsuperscript{22} The antiplatelet effect is prolonged in patients with renal impairment. The pharmacokinetic properties of Tirofiban are very similar to eptifibatide. It reaches maximum plasma concentrations and onset of action rapidly after intravenous loading infusion.\textsuperscript{23} It is mainly eliminated by renal excretion with limited metabolism. Thus, plasma clearance is increased by >50\% in patients with severe renal impairment, however, is not affected by hepatic impairment. Platelet function is expected to return to normal in 4 to 8 hours after discontinuation of infusion.\textsuperscript{24}

### Anticoagulant drugs

Warfarin pharmacokinetics are highly variable from person to person, especially when the plasma concentrations are not always related to the anticoagulant effects.\textsuperscript{25} The dosage requirement is unpredictable. After oral administr-
tion, it is rapidly absorbed from the gastrointestinal tract with high bioavailability. Based on Warfarin’s mechanism of action, its anticoagulant effect is dependent on the depletion of circulating functional Vitamin K-dependent factors. Similarly, when warfarin is discontinued, reversal of effect is dependent on the generation of new functional forms of these factors. In patients with severe hepatic impairment, the synthesis of coagulation factors and metabolism of warfarin may be impaired. In addition, some patients may have a defective variant of Cytochrome P450 2C9, a hepatic enzyme that metabolizes warfarin. Not only are they more sensitive to warfarin, but they also have a slower rate of elimination.

The dose requirement of UFH is also unpredictable due to variable binding to plasma lipoprotein, globulins, and fibrinogen. The onset is immediate following intravenous infusion, but slightly delayed when given subcutaneously. The elimination involves a combination of a rapidly saturable mechanism at low doses and a slower first-order mechanism at higher doses. As a result, the half-life increases as the dose increases. The effect may be prolonged with renal and hepatic impairment.

Conversely, LMWHs have predictable pharmacokinetics such that they can be given without close monitoring of anticoagulant effect. Several pharmacologically distinct agents are commercially available. However, their anti-IIa and anti-Xa activities are sufficiently different that they are not considered interchangeable. The onset is typically slower and duration of action is longer than UFH, requiring once to twice daily dosing. They are eliminated renally, thus the half-life is expected to be prolonged in patients with renal insufficiency.

Fondaparinux pharmacokinetics is linear and predictable. It specifically binds to AT and does not bind significantly to other plasma proteins or red blood cells. The onset is rapid after subcutaneous injections, and the half-life is sufficiently long for once daily administration. It is primarily eliminated via the kidneys with up to 77% of the dose recovered in urine as unchanged drug. Total clearance is 25% lower with mild renal impairment, 40% lower with moderate impairment, and 55% with severe impairment. Danaparoid has predictable pharmacokinetics when given in usual dosages. Its anti-Xa activity is linearly related to the dose. It reaches maximum anti-Xa effect in approximately 2 to 5 hours. The half-life of its anti-Xa and antithrombin (IIa) activity are 25 and 7 hours, respectively. Renal excretion is the main route of elimination accounting for 50% of total clearance, and half-life is prolonged with renal impairment.

Direct thrombin inhibitors

The pharmacodynamic effect of Lepirudin is directly proportional to the plasma concentration because one lepirudin molecule binds to one thrombin molecule. The response is rapid after intravenous injection. The half-life is approximately 1 hour, and the clearance is directly proportional to creatinine clearance. Severe renal impairment can prolong the half-life to up to 2 days. Many patients (~40%), treated with Lepirudin for more than 5 days, can develop antilirudin antibodies that extend the drug’s half-life because of reduced clearance of the active molecule. Bivalirudin similarly has rapid onset following intravenous administration. It is only partially excreted by the kidneys. It also undergoes hepatic metabolism and rapid proteolysis at other sites. In patients with normal renal function, the half-life is about 25 minutes. The half-life is increased to 34 minutes with moderate renal impairment, 57 minutes with severe impairment, and 3.5 hours in dialysis-dependent patients. Argatroban, unlike lepirudin and other antithrombins, is primarily metabolized by the liver. As such it provides a safe alternative for patients with renal insufficiency. Its quick onset and short duration of action makes it preferable in unstable patients.

Reversal strategies

Antiplatelet drugs

The perioperative risk of bleeding with antiplatelet agents varies and depends on the surgical procedure. In the event of acute hemorrhage, discontinuing the oral antiplatelet (ie, ASA, clopidogrel, ticlopidine) is often inadequate due to their irreversible effect on circulating platelets. The antiplatelet agents do not have a specific antidote, thus acute reversal of their effect often relies on transfusion of platelets and other blood products. The antiplatelet effects of Eptifibatide and Tirofiban are dose-dependent. As plasma concentrations decrease, platelet function is restored in 4 to 8 hours. Thus, discontinuation of these drugs is usually sufficient. Note that they have low affinities for the GP IIb-IIIa receptors, thus they exist predominately as free drug in plasma. Given the large number of free drug molecules compared with available platelets, platelet transfusion is unlikely to reverse the effect. In contrast, Abciximab has high affinity for GP IIb-IIIa receptors with little drug unbound in plasma. Therefore, platelet transfusion is expected to reverse the antiplatelet effect.

Anticoagulant drugs

Reversing the effect of warfarin can be done with several modalities, depending on the urgency. In patients where rapid reversal is not required, correction of INR can be achieved with 1 to 5 mg of vitamin K₁ (phytonadione) given orally or intravenously. When oral vitamin K is not available, the correct amount in intravenous formulation can be administered orally. Subcutaneous administration is not recommended due to lower efficacy compared with intravenous route and unpredictable response. Normalization of the INR can be expected within 24 hours. Larger doses (ie, greater than 10 mg) of vitamin K₁ should render the patient warfarin-resistant for up to a week or more. In acute bleeding, vitamin K should be administered along with fresh frozen plasma to rapidly reverse the anticoagulant effect. Factor concentrates may also be used (Prothrombin Complex Concentrates and recombinant factor VIIa). Ideally, the INR should be <1.5 before proceeding to invasive procedures.

Protamine sulfate is a highly basic protein which combines with strongly acidic heparins and rapidly neutralizes both the anti-Xa and the anti-IIa anticoagulant effect of...
UFH. Since plasma UFH concentrations decrease rapidly, the dose of protamine required also decreases as time elapses. If UFH is given as an infusion, the dose of protamine required is based on the amount administered in the previous 4 hours at 1 mg per 100 units of UFH. If 90 minutes (ie, one half-life) have elapsed since the last bolus injection, then 0.5 mg per 100 units of UFH should be given. Note that the half-life of protamine is shorter than UFH, when UFH is given subcutaneously, thus repeated doses of protamine may be required. Protamine should only be given by slow IV infusion, with no more than 50 mg over a 10-minute period.

There is no specific antidote for Fondaparinux or Danaparoid. Protamine sulfate is unreliable and ineffective in reversing the anticoagulant effect of these drugs. Due to their long duration of action, they are less favorable for patients at high risk of bleeding. Two studies in healthy volunteers have shown a possible reversal strategy for Fondaparinux with recombinant factor VIIa. Plasma and prothrombin complex concentrates may also be considered, although data are lacking. Danaparoid should be discontinued and blood products should be transfused as needed.

Direct thrombin inhibitors

There is no specific antidote for Lepirudin, Bivalirudin, or Argatroban. However, bleeding is expected to stop soon after discontinuing the drug due to their short half-life. In patients with renal failure, the duration of action for both lepirudin and bivalirudin can be prolonged. Animal models suggest that hemofiltration may be useful in removing these drugs from plasma. Current research is focused on using recombinant factor VIIa, but supportive data are still lacking.

Summary

Rapid advances in our understanding of the mechanisms of physiological hemostasis and the limitations of existing antithrombotic agents have triggered the development and expanding clinical use of a number of new and novel antithrombotic agents which target specific sites in the hemostatic pathways and promise increased efficacy, safety, and clinical utility. However, with progress come new challenges. Routine coagulation assays (INR, aPTT) do not detect the presence of these new agents nor reflect the true extent of their effect when therapeutic levels of these agents are present. The lack of specific antidotes for rapid reversal of the antihemostatic effect of these newer agents and the potential for drug accumulation and delayed elimination in clinical conditions like renal insufficiency compound the concerns about bleeding risks with invasive procedures. The risk of a potentially catastrophe complication of spinal hematoma in neuraxial blockade in the presence of antithrombotic drugs mandates the following:

- Careful drug history to detect the presence of any antithrombotic agent and timing of the last dose given;
- Understanding of the pharmacokinetics and duration of antihemostatic activity of these agents; and
- Adequate time has elapsed to allow metabolic elimination of the antithromostatic effect.

These are all vital for safe administration of neuraxial blockade for anesthesia and analgesia. Excellent consensus guidelines are available that discuss in detail the neuraxial anesthetic management in patients receiving antithrombotic agents.

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