Review Article: Physiology of Haemostasis

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Abstract

Haemostasis is a complex process that ensures the maintenance of blood flow under normal physiological conditions and prevents major blood loss following vascular injury. The process is tightly regulated to prevent pathological thrombosis. Normal haemostasis relies on the delicate balance of prothrombotic and anticoagulant processes, where five components play a significant role in maintaining the haemostasis, these include: 1) endothelial cells; 2) Platelets which are key to platelet plug formation; 3) coagulation factors that are essential to formation of insoluble fibrin clot; 4) coagulation inhibitors; and 5) fibrinolysis

This article will provide an overview of the current concepts of haemostasis, and through this we will explain how antiplatelets and antithrombotic drugs work, as well as provide a basic understanding of how to interpret clotting tests used to measure coagulation disorders.

Keywords: Coagulation, Haemostasis, Platelets, Bleeding, Haemorrhage, Anticoagulants, antiplatelets, clotting tests

Royal College of Anaesthetists CPD Matrix : 1A01: Physiology and biochemistry, **1A02:** Pharmacology and therapeutics

Learning Objectives

After reading this article, you should be able to:

1) Identify the key components of haemostasis
2) Describe the principles of how some major inherited bleeding disorders impact haemostasis
3) Interpret abnormal coagulation assays
4) Describe how antiplatelets and anticoagulants affect coagulation.

Introduction

The haemostatic pathway is a tightly regulated process that ensures the maintenance of blood flow under normal physiological conditions and also facilitates the prevention of significant blood loss following vascular injury. The normal haemostatic response depends on the closely linked interaction between a) blood vessel wall (endothelial cells), b) platelets, and c) blood coagulation factors. Whilst an efficient and rapid mechanism for stopping bleeding is essential for survival, it is equally important that this mechanism is tightly controlled, so that pathological thrombosis is prevented. Therefore, normal haemostasis relies on the delicate balance of prothrombotic and anticoagulant processes.

There are five Components of Haemostasis

1) Blood vessels and endothelial cells
2) Platelets
3) Coagulation factors
4) Coagulation inhibitors
5) Clot dissolution or Fibrinolysis

This article will provide an overview of the current concepts of haemostasis, and through this we will explain how antiplatelets and antithrombotic drugs work, and also provide a basic understanding of how to interpret clotting tests used to measure coagulation disorders.

Blood Vessels and Endothelial Cells

Following vascular injury, vessel walls vasoconstrict immediately to slow blood flow to the site of injury as well as prevent exsanguination from widespread damage.[4] In addition, collagen and tissue factor (TF) are brought into contact with flowing blood. Exposed collagen triggers the accumulation and activation of platelets at the site of the vessel wall damage resulting in the formation of a platelet plug, while the exposed TF initiates the activation of coagulation factors and generation of thrombin which in turn will lead to formation and stabilisation of insoluble fibrin clot.

Platelet Structure and Function

Platelets are anucleate, extremely small, discoid cells which circulate in abundant numbers in the peripheral blood. They are formed by fragmentation from megakaryocytes within the bone marrow and have a lifespan of 7-10 days. Platelets are essential in the initial formation of a mechanical plug in response to vessel injury and they achieve this through four main functions: 1) activation; 2) adhesion to the vessel wall; 3) aggregation; and 4) secretion. Activated platelets provide a surface for activation and recruitment of more platelets, as well as activation of coagulation factors, which will ultimately will lead to fibrin formation.

Platelet Membrane: the platelet membrane invaginates into the interior of the cell and forms an extensive canalicular system, which provides a large area of numerous membrane receptors and proteins. Of particular importance in the platelet membrane are phospholipids which activate coagulation factors such as factor X (FX) and Factor II (prothrombin). Surface receptors activate intracellular pathways which lead to a conformational change in platelet structure and shape upon activation. The membrane also contains numerous glycoproteins which serve as binding sites for various other molecules such as Von Willebrand factor (vWF), Fibrinogen (adhesion) as well as binding to other platelets (aggregation). Table 1 summaries the roles and clinical significance of some of the platelet membrane proteins.[3]

Storage granules: Two important intracellular components within platelets are alpha and dense storage granules:

- α granules-contain P-selectin, fibrinogen, fibronectin, factor V, factor VIII, platelet factor IV, platelet-derived growth factor and tumour growth factor-α (TGF-α)
- dense granules-contain adenosine triphosphate (ATP), adenosine diphosphate (ADP), calcium (Ca), serotonin, histamine and epinephrine.[9]

Platelet Activation

There are numerous agonists of platelet activation such as ADP, collagen serotonin – all these lead to activation of intracellular pathways. Collagen a potent activator of platelets, is released from vessel endothelium and binds to Glycoprotein (GP) 1a and GPVI, which activates the cyclooxygenase (COX) system that will generate thromboxane A2 (TXA2). TXA2 has several haemostatic functions, such as: it causes vasoconstriction, it leads to recruitment and further activation of more platelets via thromboxane surface membrane receptors, and also causes platelet aggregation. Aspirin inhibits TXA2 production by irreversible inhibition of COX enzyme (Table 2).

Platelet Adhesion

Upon activation, platelets undergo considerable conformational change in order to maximize surface area for adhesion to other surfaces. vWF is essential in promoting platelet adhesion in high shear conditions. vWF is released from the vascular endothelium where it is continually secreted and is stored within platelet granules and in Weibel-Palade bodies within endothelial cells. vWF is a large multimeric molecule that is essential in platelet adhesion, aggregation, and also act as a carrier for coagulation FVIII. Dysfunctional, or deficiency of VWF results in a bleeding diathesis. Shear forces, stress, exercise, adrenaline and Desmopressin/DDAVP stimulates the release of vWF and thus will raise its plasma levels. DDAVP has therapeutic benefit in functional platelet disorders and in Von Willebrand disease.

Platelet Aggregation

Platelet aggregation is achieved by platelets cross linking at GPIIb/IIIa receptors on the platelet membrane. GPIIb/IIIa is the most abundant glycoprotein, and upon activation it undergoes conformational change allowing it to bind to fibrinogen, thus forming platelet-fibrinogen bridges. ADP, TXA1 and thrombin all activate GPIIb/IIIa and are potent enhancers of platelet aggregation.

Platelet Secretion

Once activated, platelets release procoagulant substances that are responsible for a 'secondary wave' of aggregation after the initial activation of platelets. The two most significant substances responsible for this positive feedback are ADP (released from dense granules), and TXA2 generated from COX pathway. Other substances released from granules include serotonin, Fibrinogen, Fibronectin, and growth factors such as PDGF. Impaired release of these mediators results in qualitative defects of platelet function which can be both congenital or acquired (e.g. drug induced). Antiplatelet therapies target various pathways of platelet activation and aggregation - their target, route of administration and their half-lives are described in Table 2.

After activation, aggregation and secretion, the membrane phospholipid becomes exposed resulting in activation of the clotting cascade. Platelet phospholipids are essential for activation of FX and FII (thrombin) and the formation of the *tenase* (Factors Xa-VIIIa- IXa) and *prothombinase* complexes (Xa-Va-IIa).

Coagulation pathway

The coagulation cascade involves the marked amplification of procoagulant proteins from relatively few initiation substances by the sequential activation of enzyme precursors (zymogens) to active enzymes. These are usually serine protease enzymes. The result is the rapid and marked generation of thrombin, which converts soluble fibrinogen into the insoluble fibrin. Fibrin enmeshes platelet aggregates and converts the unstable platelet plug into a stable fibrin clot.

Traditionally the coagulation pathway was classified into extrinsic, intrinsic and common pathways. This classical model still remains useful in interpreting in vitro coagulation screening tests (i.e. prothrombin time [PT], activated partial thromboplastin time [APTT]), however, the classical model does not incorporate the central role that cell surfaces play in coagulation. Further, this system does not explain why some patients with coagulation factor deficiencies have bleeding tendencies (for example why individuals with factor IX or factor VIII deficiency have severe bleeding even though their extrinsic and common pathways are normal which should be sufficient for haemostasis) and more importantly, it does not predict which patients are at risk of bleeding or thrombosis.

The Cell Based Model of Haemostasis

The 'cell based' model of haemostasis has replaced the classical pathway and it is now the most widely accepted model of in vivo coagulation. The cell-based model proposes that the coagulation process takes place on different cell surfaces and, occurs not as a cascade but in 3 overlapping stages which include: 1) initiation, 2) amplification, and 3) propagation.[5,6]

Initiation

The primary event of *in vivo* coagulation is the exposure of tissue factor (TF) which will lead to activation of FVII in flowing blood after vascular injury. The TF:FVIIa complex catalyses the activation of FIX and FX. The activated FX which escapes the cell surface environment is rapidly inhibited by tissue factor pathway inhibitor (TFPI) and antithrombin (AT), whereas that which remains on the TF bearing cell will activate a tiny amount of thrombin from prothrombin. This initial thrombin is essential for the activation of more platelets, as well as activation of FVIII and FV, thus setting the scene for the large-scale thrombin generation. The small initial thrombin generated will also activate factor XI in a positive feedback manner, leading to amplification

Amplification

Platelets provide the surface on which the amplification and propagation phases take place. During the amplification phase the procoagulant signal shifts from TF-bearing cells to the surface of platelets as these become activated, whilst in the propagation phase, a large burst of thrombin is generated on the surface of activated platelets (Figure 1).

The interaction of platelet GPVI receptor with exposed collagen from the vessel wall damage and platelet GP Ib-V-IX receptor with collagen-bound von Willebrand factor (VWF) promotes adhesion of platelets to the site of injury. These binding processes partially activate platelets and localize them near

the site of TF exposure. Moreover, binding of VWF to GP-Ib-V-IX receptor[6] localizes FVIII to the surface of platelets where its activation will support the thrombin generation process

Independently, the small amount of thrombin formed during the initiation phase enhances platelet adhesion and fully activates platelets as well as FV, FVIII, and FXI. Thrombin induces platelet activation by binding to protease-activated-receptor 4 (PAR4) on the platelet surface which in turn causes the release of adenosine diphosphate, serotonin and thromboxane A2 from platelets. These agonists activate other platelets and in so doing they release FV from α granules. FV is then fully activated by thrombin or FXa.

Propagation

During the propagation phase, FVIIIa combines with FIXa (generated by TF:VIIa complexes) to form the intrinsic 'tenase' complexes on the surface of activated platelets. This complex (FVIIIa/FIXa) is a powerful and a major activator of FX (10 fold more active than FIXa alone). FXa in combination with its co-factor FVa (and calcium ions) forms 'prothrombinase' complexes which will subsequently catalyse prothrombin to thrombin. This complex is 300,000-fold more active than FXa alone in catalysing prothrombin activation.

Coagulation inhibitors

It is fundamental that clot formation is regulated and localized to the site of injury, so that arterial or venous thrombosis are prevented. Hence, a crucial element to a balanced haemostasis is played by the naturally occurring inhibitors to coagulation, which include *Tissue factor Pathway Inhibitor* (TFPI), heparin co-factor II, antithrombin (AT) and protein C and protein S activation .

TFPI is the principle regulator of the thrombin generation initiation phase (i.e. inhibits FXa , FVIIa and TF), whereas AT attenuates thrombin activity and its generation (inhibits FIIa, FXa, FIXa, and FXIa). TFPI is synthesized in endothelial cells and is mostly stored in platelets. A small amount is in free circulation in the plasma. Plasma concentrations of TFPI are greatly increased with heparin. Heparins bind to antithrombin and potentiates its action by 1000-4000 fold.

Protein C and Protein S are both serine protease enzymes, whose activation is essential for inhibition of FVa and FVIIIa. Protein C/S pathway is activated by thrombin which binds to thrombomodulin (an endothelial cell surface receptor), and activate protein C - this in turn will inactivate FVa and FVIIIa. Protein S is a cofactor to activated protein C (APC), potentiating its action.

Almost all currently available anticoagulant drugs will inhibit either FXa or FIIa (thrombin) – Figure 1 gives and overview of the Cell based model of haemostasis and the common targets of anticoagulant agents.

<u>Fibrinolysis</u>

Following haemostasis, in order for vessel patency and blood flow to resume, the fibrin clot must be removed by proteolytic enzymes. Fibrinolysis is triggered in response to vessel injury, and requires

activation of plasminogen to plasmin by the tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA).

t-PA is stored in the endothelium and is released following endothelial damage or stimulation by thrombin or vasoactive agents (adrenaline, bradykinin etc.). Fibrin acts as a cofactor for t-PA to enhance the activation of plasminogen. Both TPA and u-Pa are used in clinical practice for thrombolysis – TPA has been manufactured in recombinant form and urokinase isolated from human urine. Streptokinase is a peptide isolated from haemolytic streptococci which converts plasminogen to plasmin.

The main regulators of fibrinolytic systems are: plasminogen activator inhibitor-1 (PAI-1) which targets u-PA and t-PA; and α2-antiplasmin that targets plasmin. Deficiencies of PAI or alpha-2-antiplasmin are rare disorders which result in a severe bleeding diathesis due to uninhibited fibrinolysis.

The antifibrinolytic drug Tranexamic acid is a synthetic analog of Lysine - which reversibly binds to plasminogen and prevents its activation to plasmin. This therefore prevents plasmin from degrading fibrin.

Standard clotting Tests and their interpretation

The most commonly used clotting assays include prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and Clauss fibrinogen assay. All these tests have limitations in that they are performed in plasma, and thus they do not reflect the *in vivo* haemostasis. As such, it is important to emphasize that when interpreting their results, the assessment of patient's bleeding history (i.e. current bleeding symptoms, prior bleeding events in responses to haemostatic challenges, such as surgery or dental extraction), drug history (use of anticoagulant/antiplatelet drugs), and family history of bleeding disorder must be taken into account.

Prothrombin Time

The PT measures the time taken for clot formation when TF (thromboplastin) and calcium are added to platelet depleted plasma. There are several thromboplastin reagents commercially available, and each of them have a variable sensitivity. Hence, in order to standardize the PT assay, individual thromboplastin reagents are compared with a World Health Organization standard reference and assigned an ISI (International Standardised Index). The ISI of each PT reagent is then used to calculate the international normalized ratio (INR – see formula below), which is essential for determining the anticoagulant status of patients taking vitamin K antagonist agents (such as warfarin)

INR is calculated from the following formula:

$$\textbf{INR} = \left[\boldsymbol{Test\ PT} \div \boldsymbol{Reference\ PT}\ (\boldsymbol{Mean\ of}\ \mathbf{20}\ \boldsymbol{normal\ Donors})\right]^{\boldsymbol{ISI}}$$

PT measures the activity of extrinsic and common pathway, and therefore will be affected by plasma concentration of FVII, FV, FX, FII (prothrombin) and fibrinogen.

Activated Partial Thromboplastin Time

The APTT Measures the activity of the intrinsic pathway, thus it will be affected by abnormalities of factors XII, XI, VIII, IX as well as the common pathway factors V, X. II (prothrombin) and fibrinogen.

APTT measures the time taken for clot formation in platelet poor plasma after the addition of phospholipid (partial thromboplastin), a contact activator (a negatively charged substance that will activate the contact system) and calcium. It is important to note that deficiency of FXII (or contact factor) will cause marked prolongation of the APTT, but this condition does not carry any bleeding risk.

Another common pitfall to be aware of when APPTT is prolonged, are the lupus anticoagulant antibodies, which are inhibitory antibodies associated with the Antiphopholipid Syndrome (rarely can affect the PT). Lupus anticoagulant antibodies are associated with thrombotic tendency rather than bleeding risk.[7,8]

Thrombin Time

The Thrombin time assesses the conversion of fibrinogen to fibrin and is measured simply by adding exogenous thrombin to platelet depleted plasma.

Fibrinogen Level

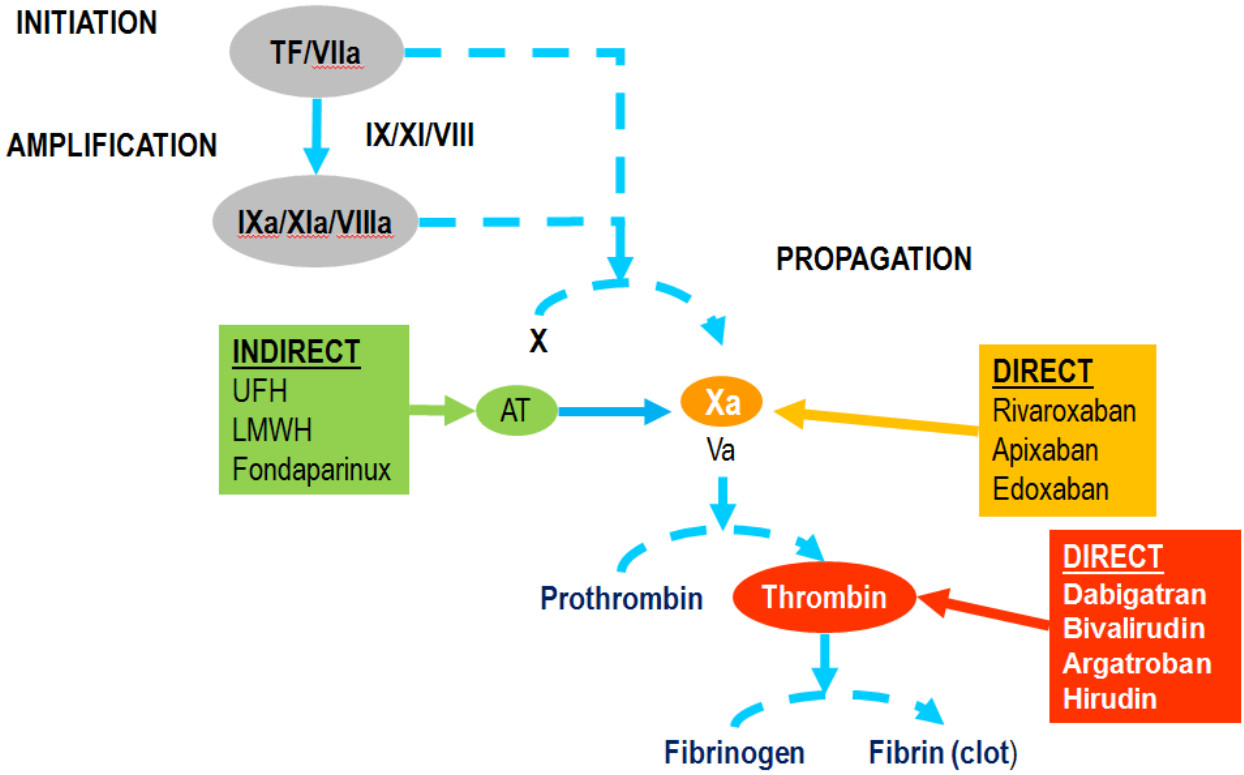
Fibrinogen levels are a useful part of the investigation of prolonged APTT or PT. There are different methods of assaying fibrinogen – the two commonest methods are either Clauss Fibrinogen – a functional assay based on the time for a fibrin clot formation and the PT derived fibrinogen – where fibrinogen level is derived based upon the prothrombin time.

Clinical interpretation of abnormal clotting tests are described in the Table 3.

Table 4 provides a summary of anticoagulant drugs, their mechanism of action, effect on coagulation tests and haemostatic measures to consider in management of major bleeding.

Figures

Figure 1 –Cell based model of Coagulation Pathway with targets of common anticoagulants highlighted



TF: Tissue Factor, UFH: Unfractionated Heparin, LMWH: Low molecular Weight Heparin, VIII, IX, X, XI : Coagulation factors VIII, IX, X and XI respectively.

Tables

Table 1 – Role and clinical significance of Key Platelet membrane Proteins

Membrane Protein	Role	Clinical Significance
GP1a	Binds Collagen Activated intracellular pathways leading to thromboxane A2 (TXA2) generation	Aspirin suppresses TXA2 synthesis by inhibiting COX
GP1b	Binds Von Willebrand Factor	Defective in Bernard Soulier disease
GPVI	Binds Collagen	GP VI Absence results in severe bleeding diathesis
GPIIb/IIIa	Binds Fibrinogen and Von Willebrand Factor Binding site for other platelets in aggregation	Defective in Glanzmanns Thrombaesthenia
Membrane Phospholipid	Activates coagulation factors	Activates Factor X→ Xa and Factor II → IIa
P2Y12	Activated by ADP, leads to generation of TXA2 and aggregation.	P2Y12 Inhibited by Clopidogrel and Ticagrelor

Key: GP = Glycoprotein, TXA2 = Thromboxane A2, COX= cyclooxygenase, ADP= Adenosine Triphospate, P2Y12 = Platlelet Membrane Protein Receptor

Table 2: Summary of antiplatelet drugs, mechanism of action and their limitations

Drug	Target	Binding	Route	Half Life
Aspirin	Acetylates Ser529 of COX-1	Irreversible	PO	15–20 minutes
Ticlopidine	ADP-$P2Y_{12}$	Irreversible	PO	20–50hours after a single dose
Clopidogrel	ADP $P2Y_{12}$	Irreversible	PO	7-8 hours
Prasugrel	ADP $P2Y_{12}$	Irreversible	PO	7 hours
Cangrelor	ADP $P2Y_{12}$	Reversible	IV	3–5 minutes
Ticagrelor	ADP $P2Y_{12}$	Reversible	PO	7 hours (ticagrelor) 9 hours (active metabolite)
Abciximab	GP IIb/IIIa	Irreversible	IV	10- 30 minutes
Tirofiban	GP IIb/IIIa	Reversible	IV	1.5–3 hours
Eptifibatide	GP IIb/IIIa	Reversible	IV	2 - 3 hours

Adapted from Laboratory Hematology Practice – Kandice Kottke-Marchat, Bruce Davis, Chapter 41 Anticoagulant, Antiplatelt and thrombolytic drugs Wiley –Blackwell 2012 – L Green, S Machin [(10)]

Table 3 – Screening Tests of Coagulation and their Clinical Implication

Test	Principle	Clinical Implication
APTT	Measure of Intrinsic Pathway coagulation Factors	*Isolated prolonged APTT:* Deficiency of FXII, XI, IX, VIII Lupus Anticoagulant antibodies Antibodies to FVIII or FIX (i.e. acquired haemophilia) *Prolonged APTT + PT:* Vitamin K antagonists (e.g, warfarin) Disseminated Intravascular Coagulpathy (DIC) Common Pathway Deficiency Afibrinogenaemia and dysfibrinogenemia Direct Thrombin Inhibitors Malabsorption (leading to vitamin K deficiency) High concentrations of unfractionated heparin Dilutional coagulopathy e.g. massive blood transfusion
PT	Measures extrinisic and common pathway Activity	Isolated Prolonged PT: Deficiency of Factor VII
Thrombin Time	Time to firbrin clot after addition of thrombin	Prolonged Thrombin Time: Hypofirbinogenaemia – eg acquired from DIC, Malignancy Liver disease Following thrombolysis Congenital deficiencies of fibrinogen Direct Thrombin inhibitors (eg dabigatran)
Fibrinogen	Assay can be functional or quantitative	Reduced DIC Liver disease Inherited deficiency of fibrinogen Thrombolysis Increased Pregnancy Female sex Acute phase response,

Table 4: **Summary of anticoagulant drugs, their mechanism of action, effect on coagulation tests and haemostatic measures to consider in management of major bleeding**

Anticoagulant Class	Examples	Route	Half-life	Mechanism of action	◆Effect on Clotting Tests	Specific assays	Reversal agents to consider in major bleeding
Vitamin K Antagonist (VKAs)	Warfarin Sinthrome Coumadin	PO	40-70 hrs	Vitamin K Epoxide Reductase inhibition – Factors II, VII, IX, X, protein C, S, Z	**PT ↑↑↑** APTT ↑↑ Fib ↔ TT↔/ ↑	INR	Vitamin K Prothrombin Complex Concentrate (PCC)
Unfractionated Heparin	Unfractionated Heparin	IV	30 mins-4h	Potentiation of Antithrombin - Targets IIa, Xa, IXa, XIa	PT ↔/ ↑ **APTT ↑↑↑** Fib ↔ TT ↔/ ↑	APTT ratio	Protamine
Low Molecular Weight Heparin	Enoxaparin Dalteparin Tinzaparin	SC SC SC	3 – 6 hrs	Potentiation of Antithrombin - targets Xa, IIa, Tissue factor pathway inhibitor	PT ↔/ ↑ **APTT ↑↑** Fib ↔ TT↔/ ↑	*Anti Xa Level using respective LMWH	Consider Protamine if <16 hr of administration
Indirect Xa Inhibitor	Fondaparinux	SC	17-20 hrs	Target Xa inhibition, through potentiation of Antithrombin	**PT↔/ ↑↑** APTT ↔/↑ Fib ↔ TT↔	*Anti Xa Level using fondaparinux	No antidote Consider Tranexamic acid + PCC in severe bleeding
Direct Xa inhibitors	Apixaban Rivaroxaban Edoxaban	PO BD PO OD PO OD	8-15 hrs 5- 13 hrs 10-14 rs	Direct Xa Inhibition	**PT ↔/ ↑↑** APTT ↔/↑ Fib ↔ TT↔	*Anti Xa Level using respective drugs	No reversal agent yet available (Andexanat Alpha awaiting licence) Tranexamic acid Consider PCC in severe bleeding
Direct Thrombin Inhibitors	Dabigatran Argartroban Bivalrirudin Hirudin	PO BD IV IV IV	12–17hrs 45 min 25 min 1-2 hrs	Direct Thrombin Inhibition	PT ↑/ APTT ↑↑ Fib ↑ **TT ↑↑↑↑↑**	*Haemoclot Assay or dilute thrombin time	Idaricizumab if available No antidote for parenteral Direct Thrombin inhibitors

*Monitoring not required.

◆The effect of anticoagulants may cause prolongation of a single or multiple clotting tests but can do so to a varying degree or not at all. Clotting tests highlighted in bold show the clotting test that would be expected to be most significantly prolonged due to the anticoagulant. Key: ↑ = Prolongation (quantitative), ↑↑= Moderate Prolongation ↑↑↑ = Marked Prolongation, ↔ = Unchanged. LMWH= Low Molecular weight heparin, PT = prothrombin time APTT= Activated partial thromboplastin time, Fib= fibrinogen, TT = Thrombin time

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