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Platelets for anaesthetists—part 1: physiology and pathology

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Key Points

- Platelets in circulation are anucleate structures.
- Adhesion of platelets is the primary step in the platelet haemostasis followed by platelet activation and platelet aggregation.
- Von Willebrand disease is the most common inherited bleeding disorder.
- There are several tests available to diagnose qualitative platelet disorders and a haematologist should be consulted for advice on these patients and their management.
- An understanding of platelet physiology is essential to have an insight into perioperative haemostasis and the mechanism of action of antiplatelet agents.

Platelets are essential in the maintenance of haemostasis. Understanding the basic physiology of platelet function will enable anaesthetists to have an insight into the management of haemostasis in the perioperative period. This review will focus on basic physiology, testing, and disorders of platelet function.

Platelet production

Pluripotential haematopoietic stem cells present in bone marrow undergo differentiation into megakaryocytes. The platelets are released into the circulation by the fragmentation of megakaryocytes. Each megakaryocyte can produce 1000–5000 platelets. Various factors influence the production of platelets but the whole process is regulated such that the platelet count stays between 150 and 450×10^9 litre^{-1}. The most significant factor in the proliferation, differentiation, and maturation of megakaryocytes is thrombopoietin (TPO).[1]

TPO is produced in liver and kidneys constitutively at a constant rate.[2] Inducible TPO[1] (a very minor fraction) is produced by spleen and bone marrow during thrombocytopenia. Once produced, the ability of the TPO to stimulate the production of platelets depends upon the number of circulating platelets. The TPO receptor present on the surface of platelets binds to the released TPO and removes it from the plasma. The higher the number of circulating platelets, the lower the TPO. But if the circulating platelets are few, more TPO will be available to stimulate the production of platelets.[1] Approximately 100 billion platelets are produced every day.[3] About one-third of the total circulating platelets are stored in splenic sinusoids and are released into the circulation if necessary. The normal life span of platelets is ~10 days and influenced by the balance of pro- and anti-apoptotic factors in the platelet.[1]

Platelet structure

Platelets are small structures of ~2–4 μm diameter. As they are anucleate they cannot synthesize any new proteins. All the proteins stored in platelets are synthesized by the megakaryocytes and are packaged into granules to be released by the platelets into the circulation. If an antiplatelet agent irreversibly blocks

Table 1 Constituents of dense granules and alpha granules

Dense granules	α-Granules
ADP	Von Willebrand factor
Serotonin	PDGF, VEGF, TGF-b
ATP	Fibrinogen
Calcium	Platelet factor 4
Histamine	Fibronectin
Catecholamines	Thrombospondin
Various other platelet agonists	Various coagulation factors
	Vitronectin

Table 2 Glycoproteins and their ligands

Glycoproteins	Ligands
GP IIb–IIIa complex	Fibrinogen
GP Ib-IX–V complex	Von Willebrand factor
GP VI	Collagen
GP Ia–IIa	Collagen
GP Ic–IIa	Laminin, fibronectin

any of the protein receptors, the receptor will lose its function for the rest of the lifespan of that platelet. Apart from various cellular contents like mitochondrion, lysosomes etc., the two most important components in platelets are the α-granules and the dense granules[1] (Table 1).

Dense granules are ~3–8 in number per platelet.[3] These mainly contain mediators that recruit new platelets and activate more platelets. Upon stimulation these mediators can be released into the system very quickly.

α-Granules are ~50–100 in number per platelet.[3] These mainly contain various membrane receptors and proteins required for adhesion, aggregation, and coagulation.

Platelet membrane invaginates into the cytoplasm at various points to form an extensive canalicular system, which provides a large surface area on which membrane receptors and proteins are stored. This system helps the platelets to transform into various shapes and sizes during activation. They also form a membrane network called the dense tubular system which is a storage compartment for calcium and also a site for prostaglandin synthesis.[1]

Platelet receptor glycoproteins

The receptor glycoproteins are attached to the plasma membrane. Some glycoproteins are normally expressed and are attached to the surface, while other glycoproteins are attached to the canalicular system inside the platelet surface; they are then moved to the surface on platelet activation. Most of the surface receptors are in a form of complex with 2–3 glycoproteins. The major glycoproteins and their ligands are shown in Table 2.

Role of platelets in haemostasis

Platelets are pivotal in haemostasis. During the normal laminar blood flow in blood vessels, platelets tend to occupy the peripheral part of the blood column. This way they come in contact with any vessel injury relatively quickly and start the haemostatic process. Endothelium, which lines the vessel wall, is a crucial barrier in separating platelets and potent prothrombotic factors (collagen and subendothelial matrix) underneath the endothelium. In addition, the endothelium actively inhibits platelet activation by the production of prostacyclin and nitric oxide.

Within the haemostatic plug, platelets exist in predominantly two states of activation (fibrin coated and aggregating) caused by differences in local rheology, exposure to agonists, and anatomical factors, complicated by the presence of platelets in various stages of apoptotic activation.[4]

Platelet adhesion

An injury to the vessel wall exposes platelets to the subendothelial matrix. Platelets immediately attach to the exposed collagen on the injured vessel wall via the collagen receptor (GPVI). Platelets then undergo a change in shape and bind to the vessel wall. This may be sufficient under low shear conditions. For vessels with high shear conditions, Von Willebrand factor (VWF) is essential for the stabilization of the platelet adhesion.[1]

VWF is a large multimeric protein that is synthesized by the endothelium and megakaryocytes. The synthesized VWF is either released into circulation or stored in the Weibel–Palade bodies of endothelium and in the α-granules of platelets.[1] VWF essentially has two main functions: (i) it acts as a carrier protein for the circulating factor VIII and prevents it from proteolytic degradation; and (ii) it assists in platelet adhesion at the site of injury. When there is an exposure of subendothelial matrix, VWF binds to the collagen to create scaffolding for the platelets to adhere to them. The GP Ib-IX–V is the receptor that binds the platelets to the VWF.[5] In order for the GP Ib-IX–V receptor to bind with the VWF, the VWF should have already been bound to the collagen. Circulating VWF has a coiled structure, concealing the platelet-binding site. With subendothelial damage and consequent collagen exposure, VWF changes its shape, exposing the platelet-binding site to which platelets bind, via the GP Ib-IX–V receptor. This VWF–platelet binding is stabilized by the binding of GP VI receptor to the collagen or subendothelial matrix directly (Fig. 1). GP Ia–IIa receptor stabilizes this VWF–platelet binding in high shear conditions.

Platelet activation

The binding of platelets via GP Ib-IX–V with the VWF and GP VI receptors to the collagen stimulates a cascade of reactions releasing various mediators leading up to platelet activation (Fig. 2).

(i) Thromboxane A2: the activation of the cyclooxygenase system leads to the release of arachidonic acid from the platelet membrane and the formation of thromboxane A2 (TXA_2) in platelets. Platelets produce TXA_2 predominantly because they express more of thromboxane synthetase enzyme when compared with prostacyclin (PGI_2) synthetase. On the contrary, the endothelial cells produce PGI_2 (which inhibits platelet aggregation) as they express more of the PGI_2 synthetase. TXA_2 is a potent vasoconstrictor and activates further platelets present in the vicinity by binding to a thromboxane (TP) receptor (a G-protein-coupled receptor) present on the surface of platelets. Binding to the TP receptor leads to activation of phospholipase C which cleaves the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2), present in the platelet membrane, leading to formation of diacyl glycerol (DAG) and inositol triphosphate (IP_3).[6] IP_3 causes the release of calcium ions stored in the dense tubular system. Both DAG and cytoplasmic calcium activates protein kinase C which causes protein phosphorylation and further degranulation of α- and dense granules.

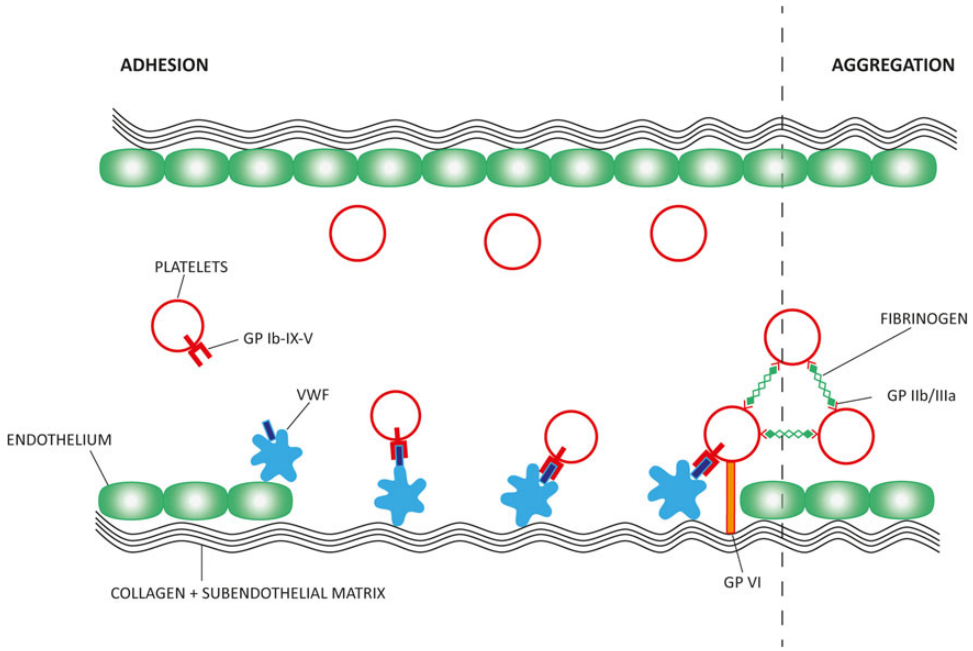

Fig 1 Schematic representation of platelet adhesion.

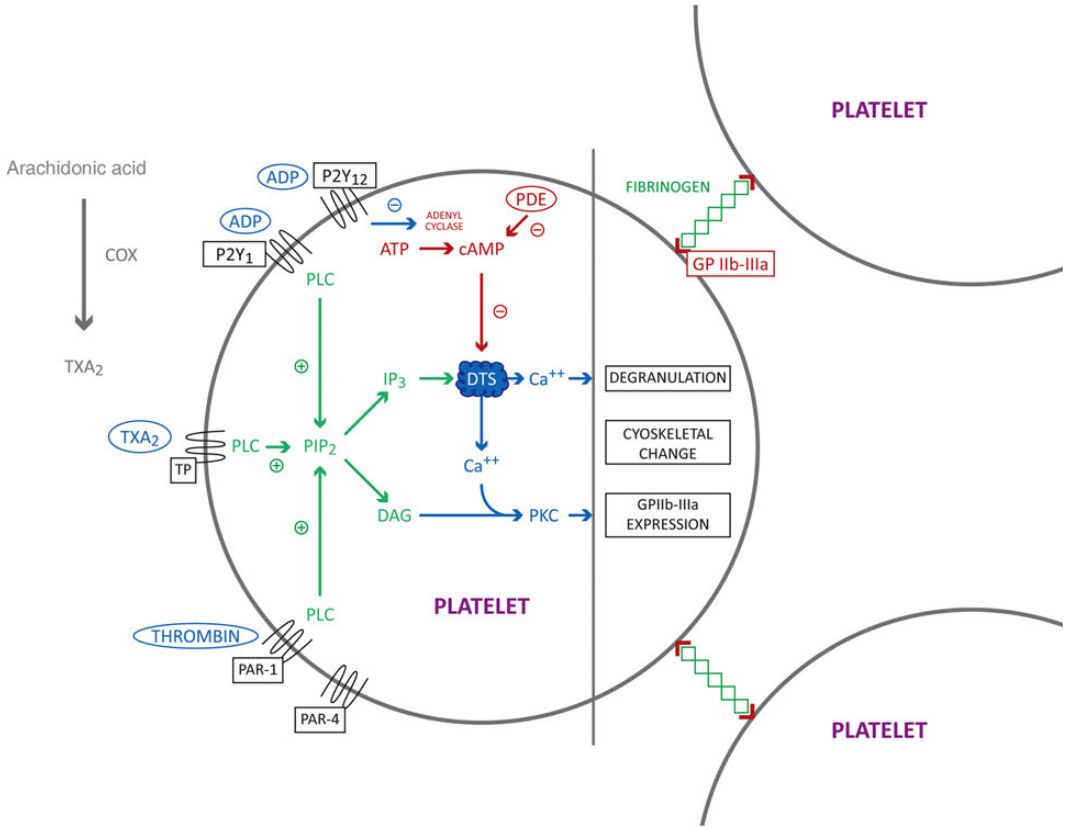

Fig 2 Platelet activation and aggregation. ADP, adenosine diphosphate; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; COX, cyclooxygenase; DAG, diacyl glycerol; DTS, dense tubular system; IP_3, inositol triphosphate; PAR, protease activated receptor; PDE, phosphodiesterase; PIP_2, phosphatidylinositol 4,5-biphosphate; PKC, protein kinase C; PLC, phospholipase C.

(ii) Adenosine diphosphate (ADP). ADP stored in the dense granules is released upon initial activation of the platelets and they bind to the ADP receptor $P2Y_{12}$ predominantly and also to another ADP receptor $P2Y_1$ present on the surface of platelets. For a maximal response both the receptors have to be activated by ADP.[7] $P2Y_{12}$ receptors belong to the Gi group

of G-protein-coupled receptors. Binding of ADP to this receptor causes inhibition of adenylate cyclase resulting in decreased levels of cAMP enabling further release of calcium ions from the dense tubular system. $P2Y_1$ receptors belong to the Gq group of G-protein-coupled receptors. Binding of ADP to this receptor causes degranulation and activation of various other proteins through the phospholipase C pathway as described above.

(iii) Thrombin: thrombin generated through the coagulation pathway binds to the surface of platelets through PAR (protease activated receptor)—mainly PAR-1 and PAR-4 receptors.[6] The PAR is also a G-protein-coupled receptor having a similar second messenger system to the $P2Y_1$ receptor.[4]

Activated platelet

Platelet secretion occurs through receptor activation of some platelet and paracrine activation then triggers a chain reaction of further platelet activation. The platelet secretion varies within the haemostatic plug and can include the release of α-granules, dense granules, and lysosomal enzymes.[4] Phosphorylation of the proteins present in the cytoskeleton of platelets leads to a change in the shape of the platelets for aggregation. This leads to a population of (fibrin) coated platelets, supporting thrombin generation and aggregating platelets.[4]

Fibrin coated, activated platelets promote coagulation by providing a scaffold for the various stages of coagulation cascade. The thrombin generated through this mechanism creates a cyclical positive feedback to activate more platelets resulting in the generation of huge amounts of thrombin, called 'thrombin burst'. The thrombin converts fibrinogen to fibrin to form a clot. Also platelets release various factors from its granules for the stabilization of the clot.

Platelet aggregation

Aggregating platelets are predominantly important in clot retraction and express activated glycoprotein receptors.[4] A change in shape of the platelets is the primary response of platelets to the activation by ADP or thrombin or TXA_2. This is achieved through conformational changes in its cytoskeleton.[8] The effect of ADP, TXA_2, and thrombin leads to the activation and surface expression of the receptor GP IIb–IIIa. Fibrinogen acts as a ligand in bridging two platelets via glycoprotein receptor GP IIb–IIIa. Linking of several platelets leads to platelet aggregation.

Assessment of platelet count

This is a selection of the commonly available laboratory tests to help quantify platelet function. Current methods are designed to detect reduced platelet function with no consensus with regard to the interpretation of occasionally apparent increased platelet function. All functional methods become invalid when the platelet count is critically reduced with the thresholds varying between the different methods. Other than the full blood count a haematologist should be consulted to request and interpret the more differentiated platelet function tests.[9]

Full blood count

Platelets can be assessed by automated Coulter count which uses the changes to electric conductivity induced by platelets passing through a narrow channel to measure platelet size and number (impedance).[10] This method is universally available but can be flawed if there is artifactual platelet clumping (EDTA or other anticoagulant induced).[11] Where the patient is found to have an abnormally low platelet count that is unexpected this should be confirmed with a blood film. Where there is platelet clumping this should be discussed with the laboratory as an alternative anticoagulant such as citrate or ThromboExact® (Sartstedt, Sarstedt, Germany) and minimizing the time delay for the sample to get back to the laboratory may help overcome this.

Where platelets are unusually large the Coulter counters may be unable to quantify the number of platelets and a flow cytometry-based count may be helpful such as in gestational thrombocytopenia or some forms of idiopathic thrombocytopenic purpura.[12]

Assessment of platelet function

Light transmission aggregometry

This method is labour intensive and not well standardized and so should not be requested without the help and advice of a haematologist. In addition, it is the mainstay of the assessment of congenital platelet dysfunction and Von Willebrand disease (VWD).[9]

Point of care methods

PFA 100. The PFA 100® (Siemens, Frimley, UK) is a flow chamber-based test that assesses the time taken for whole blood to form a clot over a small opening in a membrane within a cartridge, in the presence of different agonists and collagen (e.g. epinephrine, ADP). It has been shown to be sensitive enough to detect some forms of VWD but is poorly predictive in some cases of platelet dysfunction and is therefore not routinely used. It is potentially useful in the assessment of haemostatic treatments such as DDAVP but caution is advised in its interpretation and ideally should be accompanied by haematological advice.[13]

Multielectrode aggregometry. Multielectrode aggregometry (MEA) (Multiplate®, Roche, Rotkreuz, Switzerland) is a technology that uses electrical impedance in whole blood after exposure of the patients' blood to specific platelet agonists. It is rapidly available and potentially at the point of care. As it is an open system, it can potentially be adapted to be used with a wide variety of platelet agonists. It is much more rapid than light transmission aggregometry as it can use the whole blood, however it is also more sensitive to changes in platelet count over time.

Its main application has been in assessing the patient's response to aspirin or clopidogrel. It is not validated to predict a clinical bleeding phenotype but its use is advocated by a number of experts in the perioperative setting.[14]

Platelet mapping. Thromboelastography (TEG) (Haemonetics®, Niles, NY, USA) can be modified to make the clot formation reaction dependent on the action of platelets in response to specific agonists such as ADP or arachidonic acid. There have only been a limited of number of studies and the test is not validated to predict a bleeding phenotype.[15]

Verify now. Verify now® (Douglasville, PA, USA) is a closed system that allows the assessment of platelet aggregation response to ADP and arachidonic acid and its use is therefore limited to assessment of aspirin or clopidogrel on patients. It is insufficiently validated to predict a patient's bleeding phenotype.

Flow cytometry. Flow cytometry can measure the response to ADP or the cell surface expression of markers of platelet activation.

Table 3 Causes of thrombocytopenia

Decreased platelet production	Increased platelet destruction
B12, folic acid deficiency	Immune related
Drugs	Idiopathic thrombocytopenic purpura
Bone marrow failure	Autoimmune diseases
Cancer, leukaemia, radiation	Infection—HIV, Dengue
Aplastic anaemia, Myelodysplastic syndromes	Drugs—Heparin, Post transfusion purpura
Infection—HIV	Non immune related
Myeloma	Disseminated intravascular coagulation
	Thrombotic thrombocytopenic purpura

Table 4 Causes of thrombocytosis

Primary thrombocytosis	Reactive thrombocytosis
Essential thrombocythaemia	Disseminated malignancy
Polycythaemia vera	Drugs
Myelofibrosis	Inflammation
Leukaemia	Surgery
	Iron deficiency
	Blood loss or haemolysis

Compared with the other assays samples are stable and dependent on assay for up to 48 h. A wide variety of different markers can be tested such as VASP or p-selectin expression. In addition, it remains a research tool. Flow cytometry-based platelet counting may be used to obtain a more accurate platelet count in patients with abnormally large platelets by using a platelet specific antibody (CD41, CD61) rather than platelet size and impedance (Coulter method) for platelet counting.

Platelet disorders

Quantitative platelet disorders

The normal platelet count is in the range 150–450 × 10^9 litre^{-1}. A platelet count of <150 × 10^9 litre^{-1} constitutes thrombocytopenia. Thrombocytopenia can be either due to decreased production or increased destruction of platelets as illustrated in Table 3. Other causes of low platelet count include increased platelet sequestration by the spleen due to hypersplenism and dilution of the blood volume by massive blood transfusion.

Treatment

Treatment was primarily aimed towards the management of the underlying cause. The options include platelet transfusion, immunosuppression with steroids, immunoglobulin, TPO stimulation with TPO agonists (romiplostim, eltrombopag), discontinuation of suspected drug or plasma exchange

High platelet count—thrombocytosis

High platelet count can either be due to primary (essential) bone marrow pathology or a reactive process secondary to a systemic condition. Table 4 illustrates some examples of thrombocytosis.

Treatment

Primary thrombocytosis: should be treated with aspirin. Cytoreductive treatment (e.g. hydroxycarbamide) to control platelet count should be considered in liaison with a haematologist as well as extended thromboprophylaxis with new oral anticoagulants or fractionated heparin.

Reactive thrombocytosis: treatment should be directed at the underlying cause (e.g. infection, iron deficiency, or inflammation). Extended thromboprophylaxis with fractionated heparin or new oral anticoagulants and/or aspirin should be considered.

Qualitative platelet disorders

Inherited: plasmatic defects associated with platelet dysfunction

Von Willebrand disease. It is the most common inherited bleeding disorder. Not a platelet disorder in the true sense of the word, but a disorder of the chief platelet adhesion factor: VWF. Note that the platelet function is normal (with the exception of type 3 VWD).

- Type 1 VWD (most common subtype)—mild-to-moderate quantitative deficiency with balanced reduction of activity and factor level.
- Type 2 VWD—qualitative defects in VWF structure and function, characterized by a discrepancy in antigenic level and functional activity (subtypes A, B, N, and M).
- Type 3 VWD—severe balanced quantitative deficiency of VWF activity and level.

Patients with this type of VWD have normal platelet count but poor platelet function. Patients also have reduced factor VIII levels (VWF is essential for the prevention of proteolytic degradation of factor VIII), which may in severe cases prolong the of activated partial thromboplastin time (aPTT). A normal aPTT does however not exclude a bleeding tendency in the presence of a significant bleeding history.

Treatment

Treatment must be guided by a haematologist experienced in the management of haemophilia. Therapeutic options include desmopressin (DDAVP) which stimulates the release of VWF stored in the Weibel–Palade bodies of the endothelium (mainly useful in mild type 1 VWD) and plasma derived factor VIII and VWF concentrates in severe forms. At present there is no commercially available recombinant VWF concentrate.

Inherited platelet disorders

(i) Defects in adhesion: Bernard–Soulier syndrome, an autosomal recessive disorder caused by deficiency of the GP Ib-IX–V complex, causing a symptomatic macrothrombocytopenia.[12]

(ii) Defects in platelet secretion: storage pool disease, a heterogeneous group of disorders caused by either due to lack of dense granules or poor release of the mediators from the granules (aspirin-like defect). Rare forms such as the grey platelet syndrome affect the α-granules.

(iii) Defects in platelet aggregation: Glanzmann's thrombasthenia characterized by the deficiency of platelet receptor GP IIb–IIIa resulting in failure of platelet aggregation with normal platelet count.[14]

(iv) Platelet release and retraction: myosin heavy chain-9 (MYH9) defects (Epstein syndrome, Fechtner syndrome, May—Hegglin syndrome, and Sebastian anomaly)—characterized by platelet contractility defects associated with their premature release from the bone marrow, macro thrombocytopenia, and sometimes associated with hearing loss, renal impairment, and presenile cataracts.

Treatment should be discussed with a haematologist. Patients usually have a special transfusion requirement for HLA matched platelets to avoid alloimmunization. Adjuncts such as tranexamic acid, DDAVP, and optimization of plasmatic coagulation may be useful when treating or preventing excessive bleeding.

Acquired qualitative platelet disorders

Iatrogenic causes of acquired platelet disorders include the use of antiplatelet agents and extra corporeal circuits. The use of antiplatelet agents is one of the commonest causes of acquired platelet disorders. Use of extra corporeal circuits initially causes platelet activation and secretion leading to severe depletion of its granular contents on prolonged use. These circuits also cause extensive physical trauma to the platelets. Uraemia (end-stage renal disease) causes acquired defects in platelet function. The pathogenesis of the platelet dysfunction is multifactorial and complex secondary to defects in platelet adhesion, aggregation, and secretion. Patients with severe liver disease also develop platelet dysfunction due to abnormal platelet aggregation and reduced TXA_2 production.

Treatment

Direct treatment at the underlying cause. DDAVP and tranexamic acid can be useful. Seek haematological advice in complex patients.

Conclusion

The process of haemostasis is complex and platelets play a pivotal role. Understanding the physiology of platelets not only helps us in the management of perioperative haemostasis but also forms a basis for understanding the mechanism of antiplatelet agents.

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Declaration of interest

None declared.

MCQs

The associated MCQs (to support CME/CPD activity) can be accessed at https://access.oxfordjournals.org by subscribers to *BJA Education*.

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