EDITORIAL

Clinically relevant concepts of haemostasis and detection of coagulopathy after trauma and surgery

Conceptos clínicalemente relevantes de hemostasia y detección de coagulopatías postraumáticas y posquirúrgicas

N.A. Windeløv*, L.S. Rasmussen*1

Department of Anaesthesia, Centre of Head and Orthopaedics, Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark

Introduction

Major bleeding is associated with a risk of coagulopathy which increases mortality after trauma and surgery. Coagulopathy in these settings is assumed to be caused by a combination of dilution, hypothermia, and acidosis. However, it has recently become clear that coagulopathy is insufficiently explained by this triad since alterations of platelet and endothelial function also are important. In this paper, we would like to contribute to the celebration of the 60th Anniversary of the Revista Española de Anestesiología y Reanimación by describing some new aspects of coagulopathy with particular relevance for medical doctors working within Anaesthesiology, Critical Care, and Emergency Medicine.

This new appraisal of coagulation underlines the importance of evaluating haemostasis in whole blood. Traditional tests like prothrombin time (PT)/international normalized ratio (INR), and activated partial thromboplastin time (aPTT) assess coagulation only in plasma and does so with an important time delay. Platelet count may be relevant but platelet function must also be considered and that is now possible, also in emergency situations. In the present review, we aim to delineate the current model of coagulation, and describe recent advances in monitoring coagulation.

A contemporary view on coagulation

In 2001, Hoffman and Monroe introduced a cell based model of haemostasis.1 This model has since been refined and expanded and emphasizes that coagulation as presented in the traditional plasma based model of haemostasis, often referred to as the "coagulation cascade", is insufficient to provide functional haemostasis in vivo, and that activation of the endothelium and platelets is pivotal for physiological haemostasis. Many elements of the "coagulation cascade" remain valid and are implemented in a cell based context.

The endothelium—gatekeeper of coagulation initiation

Vascular injury activates the endothelium from its resting state. This is critical for the coagulation process, as the resting endothelium secretes platelet deactivating mediators (e.g. prostacyclin and nitric oxide) and anchors a web of glycoproteins called the endothelial glyocalyx (Fig. 1a, additional material online). A major component of this web is the heparin-like heparan sulphate that activates the circulating antithrombin and creates a non-adherent barrier between the vascular wall and blood cells. Moreover, the resting endothelium presents thrombomodulin and...
endothelial protein C receptor that greatly amplify the ability of thrombin to activate protein C. Activated protein C cleaves activated coagulation factor V and VIII and up-regulates fibrinolysis through inhibition of plasminogen activating inhibitor-1. Following activation, the endothelium sheds its glyocalyx, protein C activating receptors, stops secretion of platelet deactivating mediators, and releases the platelet anchoring von Willebrand Factor (vWF) from intra-cellular vesicles called Weibel-Palade bodies (Fig. 1b, additional material online). The endothelium is therefore a dynamic regulator of haemostasis. In its resting state, it mitigates platelet activation and coagulation activity, and increases fibrinolysis when exposed to thrombin. This process is reversed upon activation where the endothelium promotes coagulation and adhesion of platelets. As the resting endothelium limits the spread of coagulation, it is pivotal that activation of the endothelium does not disseminate. However, systemic inflammation following trauma, shock, sepsis or major surgery can induce widespread activation of the endothelium. Although incompletely understood, this may lead to coagulopathy with high circulating levels of anti-thrombotic mediators like heparan sulphates and activated protein C, and with later depletion of antithrombotic mediators and increased risk of thrombosis and organ failure. Endothelial function remains difficult to assess and no reliable technique is currently available to the clinician; however, circulating components of the glyocalyx (syndecan-1), and endothelial adhesion molecules are promising indicators of systemic endothelial activation.

The platelet plug

Following vascular injury, platelets rapidly form a soft plug that covers the exposed sub-endothelial connective tissue and provides a thrombin generating platform for clot formation. Formation of the platelet plug is called primary haemostasis and is a complex and highly controlled process involving the adherence, activation and aggregation of platelets. The exposed sub-endothelial collagen binds circulating von Willebrand Factor (vWF) causing the large protein to unveil a binding site for the platelet glycoprotein (GP) Ib-IX-V complex. The platelet is tethered to the vascular wall by binding of the immobilized vWF allowing for direct adhesion of the platelet to collagen by GPIIbIIIa complexes and GPVI (Fig. 2, additional material online). The binding of GPVI to collagen activates the platelet that cause reconfiguration of the GPIIbIIIa complex enabling firm adhesion to the vascular wall by vWF and to other platelets through fibrinogen or vWF. The activation process is reinforced by several important mechanism including binding of GPIIbIIIa to its ligands; from nearby coagulation activity activating the protease activated receptor (PAR) 1 and 4; and auto- or paracrine signalling of adenosine-diphosphate (ADP) secreted from dense granules binding to the P2Y1 receptor and thromboxane A2 (TXA2) synthesized from arachidonic acid by cyclooxygenase (COX) 2 binding to the TXA2 receptor.

Circulating inactive platelets are recruited to the clot by vWF bound to the GPIIbIIIa receptors of the activated and adherent platelets. Recruited platelets have little or no access to collagen, and activation occurs by paracrine signalling from coagulation activity by thrombin or by ADP and TXA2 released from activated platelets. As the platelet plug grows, the activating signal weakens and the shear stress from the blood increases causing activated platelets to return into circulation. Detached platelets may circulate for days; however, the initial activation exhausts their haemostatic capability by shedding of the external domains of GP Ibα and GPVI, the PAR-1 thrombin receptor, and by emptying of granules. A recent in vitro study indicates that whilst platelet degranulation is enhanced at low pH—as often found hypop- erfused tissue—adherence is reduced. This emphasizes that platelet counts in some patients may provide false safety when assessing primary haemostatic capacity.

Formation of the clot

Activated platelets form the physiological platform for thrombin generation. Nevertheless, coagulation is not initiated on platelets but on sub-endothelial tissue factor (TF) bearing cells. TF is a transmembrane protein that binds and activates circulating coagulation factor VII forming the tenase complex that activates factor IX and X. Activated factor X binds and activates factor V forming the prothrombinase complex that cleaves pro-thrombin to thrombin (Fig. 3, additional material online). However, due to low concentrations coagulation factors, only small amount of thrombin are produced on the TF-bearing cell, and the coagulation process is rapidly shut down by anti-thrombotic mediators (e.g. tissue factor pathway inhibitor). The thrombin must therefore traverse to adjacent platelets that can amplify the process.

Activated platelets present an anionic outer membrane that attracts coagulation factors, including the minute amount of thrombin produced on the TF-bearing cell. The action of thrombin is dual: it activates factor V, IX and XI and also promotes a strong procoagulant state of the platelet with extensive expression of receptor that binds the coagulation factors in highly active complexes enhancing thrombin formation with up to 2 × 10^6 fold when compared to plasma (Fig. 4). The activated factor VIII and IX forms a tenase complex on the platelet membrane activating large amounts of factor X, that again binds to activated factor V resulting in massive burst of thrombin (Fig. 5). The large amount of thrombin then splices fibrinogen to fibrin that polymerizes into a strong mesh imbedding the platelets. Finally, the thrombin activates factor XII that cross-links the fibrin increasing resistance to fibrinolysis.

Clot formation as a defence against pathogens

It is increasingly acknowledged that the immune system and the coagulation system overlap, and that both systems can potentiate the effect of the other. Beyond its role in haemostasis, clot formation provides an important defence of the innate immune system in limiting pathogens from systemic dissemination. Platelets present pathogen recognizing toll like receptors that activate the platelet following binding to bacterial antigens, alarmins of dying cells or activated neutrophils. The activated endothelium
binds activated platelets and leukocytes and secretes protein disulphide isomerase that activates TF of adherent monocytes. The presence of active TF on the monocytes and activated platelets on the pro-coagulant endothelium can drive functional clot formation. This may be further enhanced by release of tiny cell membrane vesicles called microparticles released from activated monocytes that can provide a circulating source of TF and thus potentiate coagulation on systemic scale. Although these mechanisms may play important roles in the physiological defence against invading pathogens, they may—if not controlled—cause the severe complications of inflammatory induced dissemination of coagulation and thrombosis.

**Monitoring haemostasis**

Several assays are available to monitor coagulation in whole blood. Widely used is the Thrombelastograph (TEG™, Haemonetics, Braintree, MA, USA) that employs a rather simple principle. By immersing a pin into whole blood following activation by tissue factor or kaolin, the gradual clotting of the blood will attach the pin to the cup. By then oscillating the cup, the firmness of the clot can be measured by the movement of force transferred to the pin. Movement of the pin is registered continuously and allows for a myriad of different results including time from activation to clot initiation, velocity of clot growth, maximal clot strength and fibrinolysis. Another almost similar technique is called rotational thrombelastometry (ROTEM™, TEM™, Munich, Germany) in which the cup is hold still while the pin rotates and measures the resistance provided by the growing clot. TEG and ROTEM can be employed at the point of care and provide results within minutes of sampling and increasingly considered the golden standard for monitoring haemostasis in the peri-operative, trauma and ICU setting. Other devices, like the Sonoclot™ (Sienco, Boulder, CO, USA) or Reofox™ (MediRox, Nyköping, Sweden), also provide information about clot formation and fibrinolysis and they may offer better insight in the early formation initiation of coagulation; however, they remain less investigated and not as widely used.

Most of the variables measured by ROTEM and TEG have been associated with risk of significant haemorrhage—often measured by the use of blood product transfusions. Although such associations have also been demonstrated by plasma based analysis like aPTT and PT/INR, it seems that TEG and ROTEM provide faster and superior prediction of haemorrhage in trauma patients. Moreover, as TEG and ROTEM provide measurement of each phase of the coagulation specific defects can be targeted using a so called goal directed haemostatic therapy. Here plasma or coagulation factor concentrates are transfused for correction of slow clot initiation or growth; platelet concentrates or fibrinogen concentrates for impaired maximal clot strength; or antifibrinolytics like tranexamic acid for hyper fibrinolysis. Different variants of goal-directed haemostatic therapy have been evaluated in observational studies and seem to reduce the need for transfusion of blood products and also improve outcome in patients with large blood loss.

TEG or ROTEM provides no specific measures of platelet function. However, an estimate of the potential for platelet activation to enhance clot formation can be provided by a modification where the results of the so-called FIBTEM/functional fibrinogen are subtracted from a standard assay. This estimate is not sensitive to the effect of platelet inhibiting drugs other than GPIIb/IIIa antagonists and a more specific platelet function analysis must be performed to quantify the ability of platelets to adhere and aggregate at site of injury.

**Monitoring of platelet function**

Monitoring of platelet function and aggregation capacity has traditionally been performed using quite laborious techniques such as light transmission aggregometry or flow cytometry, and has commonly been restricted to patients with suspected congenital platelet dysfunction. However, the increasing awareness of the pivotal role of platelet function in haemostasis and the expanding number of patients on different and very potent platelet inhibitory drugs have markedly expanded the area and several commercial point-of-care devices are now available (Table 1).

<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
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<tr>
<td>Verify now</td>
<td>Aggregation of platelets to fibrinogen coated beads.</td>
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<tr>
<td></td>
<td>Assessment of the ability of light to pass through the blood.</td>
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<tr>
<td>Multiplate</td>
<td>Measures the electric impedance between pairs of silver electrodes</td>
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<tr>
<td></td>
<td>imbedded in blood.</td>
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<td></td>
<td>Following activation, platelets aggregate on the electrodes.</td>
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<tr>
<td>PFA-100</td>
<td>Blood flows through a small pore coated with ADP/epinephrine or collagen/</td>
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<tr>
<td></td>
<td>epinephrine and the time to occlusion is measured as an inverse indicator of</td>
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<tr>
<td></td>
<td>platelet function.</td>
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<tr>
<td>Platelet mapping</td>
<td>Measures the dynamic build up and following disintegration of the clot</td>
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<td></td>
<td>strength in heparinised blood following platelet activation in a TEG device</td>
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Monitoring of platelet function may be valuable in surgical patients where the impact of platelet inhibiting drugs needs to be quantified and possibly compensated by platelet transfusion or desmopressin. This could be a patient taking platelet inhibiting drugs but for whom surgery is urgently needed. Moreover, it is well documented that in a third of patients, platelets do not respond sufficiently to ADP/P2Y1 blockers like clopidogrel. Identifying the optimal dose of clopidogrel or other platelet inhibitors for each patient by platelet function analysis may thus be beneficial. Indeed, excessive inhibition increases the risk of bleeding events whereas insufficient inhibition increases the risk of ischaemic events. We recently investigated performed a prospective study of platelet function in non-medicated 213 severely trauma patients. Interestingly, we found that half...
of patients had abnormal aggregation values on hospital arrival and that patients with low aggregation had high risk of requiring massive transfusion in the emergency department whereas patients with high aggregation had high risk of dying from cerebral injuries during the first 28 days following injury. 20 Although we cannot conclude regarding causation, it indicates that platelet function may be important in that setting.

Concluding remarks

Coagulation in the circulating whole blood of the patient is very different from that measured in plasma tubes; although more complex, we as clinicians should strive to monitor and treat haemostatic disorders in a physiological context. Several new modalities are providing more accurate monitoring of haemostasis and recent evidence supports their implementation in several clinical settings.

Appendix A.

Additional material available on the Web.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.redar.2013.09.001.

References