Perspective

Hemostasis practice: state-of-the-art

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Abstract: Hemostasis disorders are typically classified as either hemorrhagic or thrombotic. Irrespective of the nature and underlying causes, the diagnostic approach to patients with hemostasis disturbances is multifaceted and involves accurate collection of clinical history taking and physical examination, as also combined with results of an appropriate number and type of laboratory investigations, which can be arbitrarily classified as first-line (i.e., screening), second-line (i.e., for the etiological diagnosis) and third-line (i.e., for biochemical or even molecular characterization) analyses. This possible classification actually mirrors that of laboratory services, which are now increasingly organized in "spoke" (i.e., peripheral), "hub" (i.e., core) and reference facilities. Albeit many supranational initiatives are underway to improve quality throughout the total hemostasis testing process, several important issues still plague this important branch of laboratory medicine, including the relatively modest knowledge that many laboratory professionals have of hemostasis in health and disease, the unacceptable heterogeneity of available diagnostic algorithms for both diagnosis and therapeutic management of hemostatic diseases, the inaccurate definition of reference ranges, the identification and communication of critical values, as well as the still unsatisfactory harmonization of many preanalytical, analytical and postanalytical procedures.

Keywords: Blood coagulation; hemostasis; diagnosis; laboratory; bleeding; thrombosis

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Introduction

Physiological hemostasis is conventionally defined as a complex biological pathway aimed at arresting blood leakage from injured venous and arterial vessels, and concomitantly preventing excessive or unwarranted blood clotting when these structures are undamaged (1). Albeit a thorough description of physiological hemostasis shall be omitted here due to space constraints, some important elements need to be described. Briefly, hemostasis is typically divided into primary and secondary, the former mainly encompassing vasoconstriction and generation of a preliminary and labile platelet plug, the latter being represented by 'blood coagulation' (2). A third biological mechanism, conventionally called fibrinolysis, integrates into hemostasis by dissolving blood clots once these are no longer necessary (i.e., once the haemorrhage has been definitely arrested) (1) (*Figure 1*).

Physiological hemostasis

A blood vessel injury is suddenly accompanied by the onset of an arterial vasoconstriction, aimed at reducing the size of the vessels and thereby the potential amount of blood that may leak outside of the vessel itself, a process then followed by activation of blood platelets neighbouring the injury. Platelets can be activated by either direct contact with subendothelial surfaces (i.e., collagen) that become exposed after endothelial injury, or by thrombin generated by blood coagulation, as described elsewhere in this article. Once activated, platelets undergo a series of biochemical and structural changes, mainly entailing shape changes

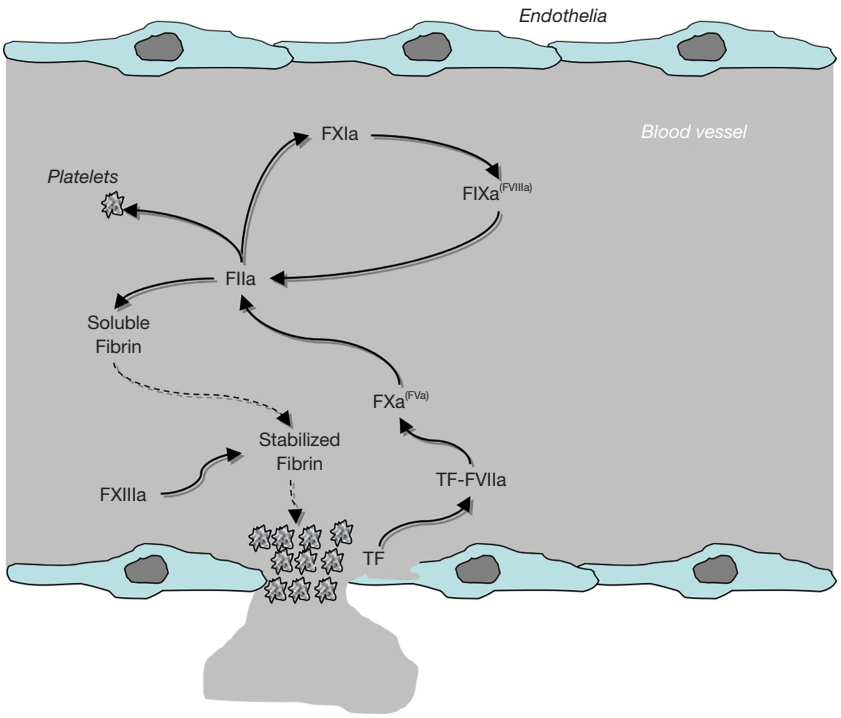

Figure 1 Schematic representation of physiological hemostasis. a, activated; F, factor; TF, tissue factor.

(to facilitate platelet-to-platelet interaction), adhesion to subendothelial structures, and aggregation (i.e., platelet-to-platelet binding) (1) (*Figure 1*). Provided that platelet number and function are preserved, this former mechanism enables the generation of a preliminary plug, which contributes to slow down or completely arrest the leakage of blood outside the vessel. Nevertheless, the stability of this plug is strongly challenged by blood flow and shear stress, which may both contribute to gradually dissolve the clot, thus exposing the patient to the risk of subsequent hemorrhage (3).

The stabilization of the platelet plug, which is hence necessary to efficiently counteract bleeding, is mediated by the activation of blood coagulation, whose ultimate scope is generating a sufficient amount of fibrin strands to act like mortar for the bricks (i.e., the platelets). Unlike former theories, the modern and "unified" vision of blood coagulation no longer encompasses three different pathways (i.e., the so-called intrinsic, extrinsic and common pathways), but considers blood coagulation as an integrated biological system, primarily assembled on cellular structures, starting from release of tissue factor (TF) from damaged endothelia, and proceeding further with sequential activation of many inactive clotting proteins, up to fibrin generation1) (*Figure 1*). Rapidly upon release into the blood stream, TF associates with factor (F) VII, thus generating a dimeric protein complex which is auto-catalytic, but is also capable of activating FX, the ensuing factor in the coagulation cascade. Combined with activated (a) FV to form the so-called prothrombinase complex, FXa catalyzes the conversion of prothrombin (FII) to thrombin (FIIa), which consequently catalyzes the conversion of fibrinogen to fibrin. Whilst this straightforward biological pathway is actually effective to generate fibrin, the overall amount of fibrin produced here is largely ineffective to ensure clot stability (i.e., between 3–5% of the total amount needed). It is at this point that the clotting factors of the formerly known "intrinsic" pathway come into play (1). Thrombin not only is capable of activating platelets and transforming fibrinogen into fibrin (*Figure 1*), but also catalyzes the conversion of FXI into FXIa. This latter protein in turns activates FIX to FIXa which, in conjunction with its cofactor FVIIIa, promotes the conversion of FX into FXa, thus closing the "circle" and bringing back the pathway to the prothrombinase complex. This specific mechanism, which has been for long identified as the "intrinsic" pathway, is now more conveniently known as "thrombin burst", which enables (provided that concentration and activity

of all clotting factors are preserved) the generation of the necessary amount of fibrin for more efficiently stabilizing the platelet plug after FXIIIa-mediated polymerization of soluble fibrin (*Figure 1*) (1). Under physiological conditions, the blood clot is then gradually dissolved by the activity of the fibrinolytic system (i.e., by plasmin, converted from plasminogen by tissue plasminogen activator), with release into the circulation of the co-called fibrin/fibrinogen degradation products (FDPs), including D-dimer (1).

The hemostatic balance is then finely modulated by a number of endogenous inhibitors, which are capable to neutralize one or more clotting factors, and are mostly represented by antithrombin (whose function is enormously magnified by heparin and other glycosaminoglycans), the protein C-protein S pathway, TF pathway inhibitor (TFPI, formerly known as extrinsic pathway inhibitor), thrombin activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor-1 (PAI-1) (*Table 1*) (4).

Table 1 Main endogenous inhibitors of blood coagulation and fibrinolysis

Inhibitor	Target protein
Tissue factor pathway inhibitor (TFPI)	Tissue factor
Antithrombin	Factor II and X
Protein C-protein S	Factor V and VIII
Thrombin activatable fibrinolysis inhibitor (TAFI)	Fibrin
Plasminogen activator inhibitor-1 (PAI-1)	Tissue plasminogen activator (t-PA)

Integrated approach to hemostasis disturbances

In accordance with the earlier definition of hemostasis, disorders of this biological system are typically classified as being either hemorrhagic (i.e., relatively insufficient clotting) or thrombotic (i.e., relatively disproportionate clotting). Regardless of the nature and the underlying causes, the diagnostic approach to patients with hemostasis disorders must inevitably involve an accurate collection of clinical history, physical examination, and results of an appropriate number and type of laboratory investigations (*Figure 2*) (1,4-7). Clinical history is indeed the mainstay for an accurate diagnosis, since in many cases it provides essential information to distinguish between the inherited or acquired nature of bleeding or thrombosis. Signs and symptoms are also crucial. Albeit the clinical distinction between disorders of primary and secondary hemostasis is not always so straightforward, especially for certain conditions such as von Willebrand disease (VWD), the defects of primary hemostasis are more often accompanied by

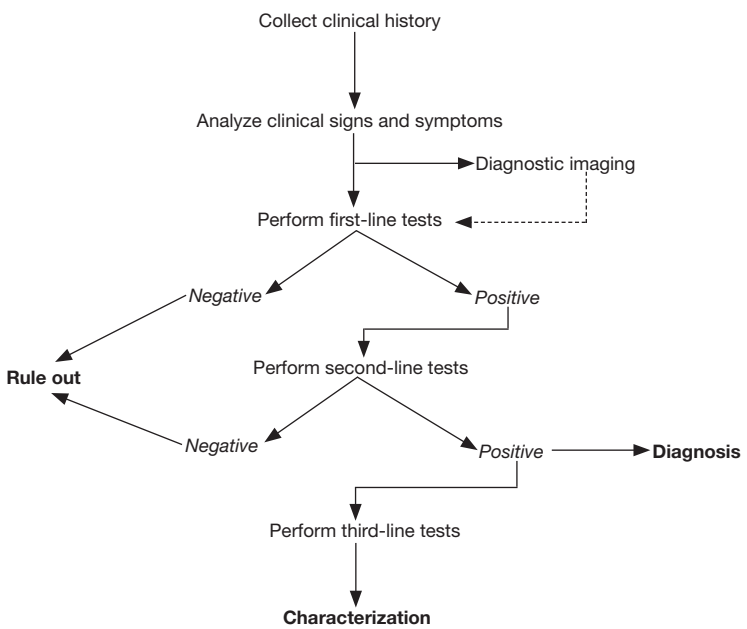

Figure 2 A practical algorithm to assist the diagnosis of hemostasis disorders.

Table 2 Hierarchic classification of tests for the screening, diagnosis and therapeutic monitoring of hemorrhagic and thrombotic disorders

Classification	Setting	Hemorrhagic disorders	Thrombotic disorders
First-line	All clinical laboratories	• Prothrombin time (PT) • Activated partial thromboplastin time (APTT) • Fibrinogen • Platelet count • Platelet function screening	• D-dimer • Prothrombin time (PT)
Second-line	"Hub" laboratories	• Mixing test • Clotting factors deficiency (clotting activity) • Titration of inhibitors • von Willebrand disease testing • Platelet aggregation studies • Molecular testing (SNPs identification)	• Inhibitors deficiency (clotting or chromogenic activity, concentration) • Activated protein C resistance (APCr) • Lupus anticoagulants (LAC) • Anticardiolipin antibodies • Molecular testing (SNPs identification) • Activity of direct oral anticoagulants (DOACs)
Third-line	Reference hemostasis laboratories	• Clotting factors deficiency (chromogenic activity or concentration) • Cytofluorometry • Molecular testing (gene sequencing) • Thrombin generation	• Concentration of direct oral anticoagulants (DOACs) • Molecular testing (gene sequencing)

SNP, single nucleotide polymorphism

instantaneous and more superficial bleeding, whilst those of the secondary hemostasis are more typically delayed and encompass profound haemorrhages (i.e., in muscles, joints, brain, internal organs) (5). Regarding venous thromboembolism (VTE), even in such cases the clinics is fundamental, since both deep vein thrombosis (DVT) and pulmonary embolism (PE) may be characterized by a kaleidoscope of suggestive symptoms, which may also lead to highly unfavourable consequences (up to death) when left untreated (8). Diagnostic imaging may then be useful, especially for revealing the presence of clots in peripheral veins or pulmonary arteries, but also for identifying the site and severity of haemorrhages, especially when these involve internal organs. Last but not least, the contribution of laboratory hemostasis is now unavoidable for the screening (i.e., pre-operative testing), diagnosis and therapeutic monitoring of both hemorrhagic and thrombotic disorders (9).

A hierarchic classification of laboratory investigations

Squeezed between a continuously expanding volume of tests and an even more often concerning shortage of public funding (10,11), clinical laboratories are now fully committed to quality and appropriateness, which not only would contribute to the sustainability of the healthcare systems, but will also prevent the generation of potentially avoidable harms to the patients (i.e., false positive test results generated by unnecessary or unwarranted testing) (12). Since the list of potentially useful hemostasis tests is continuously expanding, the most reliable strategy to contain inappropriateness entails developing a reasonable priority of eligible tests, which can hence be arbitrarily classified as first-line (i.e., screening), second-line (i.e., for the etiological diagnosis) and third-line (i.e., for biochemical or even molecular characterization) analyses. A reliable example of how hemostasis tests could be classified for optimizing resources and improving the diagnostic and therapeutic reasoning is summarized in *Table 2*. This possible classification actually mirrors that of laboratory services, which are now increasingly organized in "spoke" (i.e., peripheral), "hub" (i.e., core) and reference facilities within the same geographical area (13), and takes also great advantage from a kaleidoscope of technological innovations occurred in this specific branch of laboratory testing (14-16).

Table 3 Major unresolved issues in hemostasis testing

Poor knowledge of hemostasis in health and disease
Heterogeneity of available guidelines for diagnosis and therapeutic management
Inaccurate definition of reference ranges
Identification and communication of critical values
Harmonization of preanalytical, analytical and postanalytical procedures

Unlike other areas of laboratory medicine (17), standardization, and even harmonization, are not always met for hemostasis testing (18). More specifically, albeit an acceptable degree of harmonization has now been achieved for certain tests such as PT and APTT, there is still evidence that other tests such as D-dimer and von Willebrand factor (VWF) testing are still plagued by major inconsistency. Many ongoing supranational initiatives are underway, with the obvious expectation that a better degree of harmonization shall be soon achieved (19,20).

Conclusions

There is now incontrovertible evidence that laboratory tests are integral to the diagnostic reasoning and to the managed care of patients with hemostasis disturbances, both haemorrhagic and thrombotic. Nevertheless, several important issues still plague this important branch of laboratory medicine (*Table 3*), including the relatively modest knowledge that many laboratory professionals have of hemostasis in health and disease, the unacceptable heterogeneity of available diagnostic algorithms for both diagnosis (21) and therapeutic management of hemostatic diseases (22), the accurate definition of reference ranges (23), the identification and communication of critical values (24), as well as the still unsatisfactory harmonization of some preanalytical (25), analytical (18) and postanalytical (26) procedures.

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