Monitoring of Hemostasis

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2.1 Introduction

Critically ill patients (CIP) are patients who, because of dysfunction or failure of one or more organs/systems depend on advanced instruments for monitoring and therapy for their survival. Coagulation abnormalities are common in CIP. The most common (35–44%) is thrombocytopenia (platelet count less than 150,000/μl), due to sepsis, disseminated intravascular coagulation (DIC), thrombotic microangiopathy, or induced by drugs, such as heparin. Prolonged global coagulation times, beyond antithrombotic therapy, are also found, and are a result of quantitative defects of coagulation factors, caused by synthesis defects (liver insufficiency), excessive loss (massive hemorrhage), or consumption (DIC), low levels of one or more coagulation factors due to antibodies, antiphospholipid antibodies, or the presence of inhibitors [1–3]. This chapter will provide information on the principles, clinical significance, technology and open problems in laboratory assessment of hemostasis in critically ill patients.

2.2 Principles and Limitations of Laboratory Assessment

Healing a macroscopic or microscopic injury represents a great biologic advantage for every living being. It requires a complex sequence of cellular and extracellular events, partly unknown, aiming at rebuilding the scaffold and, eventually, structure of an organ or tissue. Hemostasis, the earliest and quickest mechanism of tissue

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G. Berlot (ed.), *Hemocoagulative Problems in the Critically Ill Patient*,
DOI: 10.1007/978-88-470-2448-9_2, © Springer-Verlag Italia 2012

repair, prevents excessive loss of blood, a liquid connective tissue. This process has evolved during evolution to become highly efficient in mammals, in which high blood pressure poses an important challenge to hemostasis.

There is not an ideal model to study in vitro hemostasis, and vast majority of tests revolve around the concept of triggering a biochemical pathway to highlight a deficiency of the system rather than evaluating excessive in vivo efficiency of the system maybe leading to thrombosis. The tests designed to match specific requirements (drug monitoring, or detection of specific coagulation abnormalities) are mainly oriented to reproduce in vitro—rather than to assess in vivo—a specific condition or drug effect. This leaves almost completely unexplored the actual state of the patient, in favor of the analysis of very few reactions. Only recently, in vivo coagulation markers have been introduced in hemostasis laboratory equipment, but cost, sampling peculiarities, and lack of studies are still limiting their use.

Another limitation is in the method which is used for the common test. Armand James Quick developed the test which has his name with laboratory equipment worth around US $100, according to George Collentine, one of his students. With such a tiny budget, poor technology, and very limited knowledge of the complex coagulation enzyme network and kinetics, the observation of the coagulation phenomena relied on measuring the time to (visually) observe the formation of the first fibrin filaments. Such observation constituted the paradigm for all the coagulation tests developed thereafter. For decades, the method for the tests used in clinics to evaluate the global efficiency of hemostasis relied on the time to reach a certain clot size. Indeed, clot formation in vitro requires the generation of 50 nM thrombin, which represents only about 1% of the whole potential of thrombin generation in vivo, and is therefore poorly predictive of the in vivo process. Assessment of the whole potential of thrombin generation holds great promise in the study of hemostasis, but has been introduced only recently and, notably, describes the in vitro potential of blood, not the actual in vivo state. Therefore, for clinical purposes, such assessment of hemostasis based on coagulation times is widely accepted and actual evaluation of hemostasis defects or monitoring of some old drugs is based on such a technique.

As a consequence, the pathophysiological significance, i.e., correlation of an in vitro test with in vivo activity as well as interlaboratory standardization, has always been a critical point in hemostasis. The prothrombin time, activated partial thromboplastin time (aPTT), and specific assays of platelet function and fibrinolysis were invented in the 1930s and 1940s and led to more sophisticated laboratory tests based on the very same method and inspired by the same approach.

A peculiar problem in emergency medicine, perioperative, and critical care areas, and for CIP in general, is the turnaround time. The importance of a precise diagnosis is obvious for every patient, but in an emergency environment this has to be obtained in a short time, often within the turnaround time of a common laboratory test. Therefore, in recent years, micronization and the development of new technologies have allowed the development of a rapid hemostasis test which can be done at the bedside, by generic personnel, providing a test and diagnosis within minutes instead of some hours.

2.3 Blood Collection Guidelines for Monitoring Hemostasis

Blood sampling is the first, unavoidable, step in order to obtain a hemostasis test. Blood can provide different results according to the source (venous, capillary, or arterial), but also according to the quality of the preanalytical management, i.e., sampling. Surprisingly, the most common cause of an abnormal clotting test is not a pathology-related issue, but rather an improperly obtained sample.

The standard approach in hemostasis is to collect blood and stop coagulation reactions by chelating the plasma calcium with sodium citrate. This blocks the coagulation reactions, except for minimal calcium-independent thrombin activity. Samples can be kept at room temperature. When platelet in vivo activation markers (β-thromboglobulin, βTG, platelet factor 4, PF4) or coagulation activity (fibrinopeptide A, FPA) is being evaluated, calcium chelation is not sufficient, since serious ex vivo platelet aggregation and coagulation could produce false-positive results. For these markers, either blockers of platelet activities (adenosine, citrate, theophylline) or broad spectrum protease inhibitors (namely, aprotinin) for coagulation factors together with immediate cooling (4°C) have to be used.

The personnel conducting the sampling are responsible for adhering to collection and processing guidelines.

Specimen requirements for coagulation assays (described in Ref. [4]) are summarized below.

2.3.1 Before Collection

Patient Identification and Conditions

- Prior to a blood specimen being obtained from any patient, the personal or documental identification of the patient must be verified by checking the patient's wristband for the name and the hospital number.
- Hyperlipemia (and hyperbilirubinemia) can interfere with the test results and their accuracy. For the former condition, if possible, the test should be planned for fastening patients or far from meals.
- Check if the patient has a hematocrit between 20 and 55. If not, see below for special directions.

Identification of the Blood Collection Site

The best samples are obtained with a *peripheral stick* with evacuated tube system. Vascular access devices are not suitable for many routine assessments and definitely *not* for measurement of in vivo activity markers.

Identification and Preparation of Consumables for the Collection

Vacutainer collection tubes (light blue) should be used together with another vial to take out the first milliliters of blood and a 19G to 22G needle. Smaller needles

could cause hemolysis; larger are probably unnecessary, unless a platelet aggregation test is conducted. If Vacutainer collection tubes are not available or such a type of sampling is not possible, then a syringe draw is an alternative, using a small (less than 20 ml) syringe and adding blood to the anticoagulant in less than 1 min.

If a sample is drawn from pediatric patients, a pediatric size tube may be used (2.7 ml blood to 0.3 ml of citrate is necessary).

Special Case: Anemia and Polycythemia

To perform a functional test (aPTT, international normalized ratio, INR, and others), plasma samples are collected with calcium-chelating sodium citrate and are then recalcified with calcium chloride in the reaction cuvettes to restore the plasma calcium concentration and the coagulation potential. A sodium citrate concentration of 3.6% provides adequate calcium chelation when the volume of plasma (determined by the red blood cell count of the blood) is within physiological ranges.

In the usual mixing proportions, 10% of the final volume of the anticoagulant solution combines with 90% of blood, which is 60% plasma. In a 5-ml vial, 4.5 ml blood has 2.7 ml of plasma (4.5 × 0.6), which combines with 0.5 ml of 3.6% sodium citrate in a ratio of approximately 5:1, or 0.2 ml of anticoagulant solution for each milliliter of plasma.

The amount of plasma in blood samples varies as the result of changes in the hematocrit, like in anemia and polycythemia. If a standard volume of anticoagulant is used, as in Vacutainer tubes, large amounts of plasma, typical of anemia, can result in a higher amount of available calcium and insufficient neutralization; in contrast, in polycythemia, with less plasma calcium available in the vials, residual chelating activity could interfere with calcium chloride and falsely lengthen functional tests.

These conditions require either modification of the filling of the tube or a different amount of anticoagulant. Keeping in mind the ratio above, we can assume that a sample with a hematocrit of 20 (i.e., around 4 ml of plasma) requires 0.8 ml of sodium citrate (4 ml of blood × 0.2).

If the amount of blood is the target of the correction, then for a standard amount of anticoagulant (0.5 ml) the volume has to be (0.5/0.2) 2.5 ml of plasma. With a hematocrit of 20, the whole volume of blood is approximately 3.1 ml (2.5/0.8). The same principles apply to calculations for polycythemia.

2.3.2 During Collection

From a Peripheral Vein

Clean venipuncture is essential; therefore, trauma should be avoided. Prolonged tourniquet use should be avoided as should “digging” to find the vein since it can cause activation of clotting factors, which can result in a clotted sample. Contamination with tissue factor, activation of clotting factors and platelets, and hemolysis can occur from use of multiple sticks, slapping the vein, excessive pumping of the

hand, air bubbles in the syringes, or placing the tourniquet on too tightly or for too long a period of time.

From a peripheral vein, draw 2–3 ml of blood (which can be rich in cell debris and tissue factor) into a vial and discard it or use it for other purposes. Then use a 5-ml blue-top tube containing the anticoagulant.

The tubes must be properly filled to achieve the proper ratio of blood to citrate (9:1, i.e., nine parts blood to one part anticoagulant). Vials less than 90% full are unacceptable for many routine laboratories, but never combine the contents of two underfilled tubes to make one filled tube.

Mix the anticoagulant with whole blood promptly and thoroughly by gently inverting (do not shake) the vial.

From a Vascular Access Device

If one is drawing from central line, flush the line with 5 ml saline and discard the first 5 ml or six dead space volumes. If one is drawing from a saline lock, discard two dead space volumes.

Use a discard tube (around 3–5 ml) before using light-blue-stoppered tubes (the discard will fill the "dead space" of winged collection set tubing). If this is not done, an underfilled tube or diluted sample/analyte could result.

2.3.3 Afterward

Mix the contents thoroughly by gentle inversion. Do not shake the vials. The samples should be labeled in the patient's presence. Deliver the samples by hand (never by a pneumatic tube) as soon as possible to the laboratory, or conduce the test in a point-of-care setting within the appropriate time. Usually, samples should be stored at *room temperature* (ice will activate leukocytes in the sample, resulting in shortened clotting times), unless special tests are to be run (β-TG, PF4, FPA).

2.4 The Particular Approach for CIP

Some critical aspects of the treatment of CIP need to be considered in the choice, performance, and interpretation of the hemostasis test. The extension of the time of care, the turnaround time for the laboratory test, and deployment are the main issues in treating CIP.

Time extension. CIP require special monitoring as long as they remain in such state. This means that the hemostasis test should be repeated every 24 h using, for comparison purposes, the same instrument and assuming the same (possibly high) grade of reliability.

Turnaround time. The time needed to conduct a test is critical in those patients with intense anticoagulation such as in cardiothoracic surgery, bleeding, or simply who are at risk of bleeding. Sometimes, even the analytic phase of standard coagulation tests (30–60 min) is often too long an interval to wait for reliable

guidance on the use of blood products for a massively bleeding patient. As a result, many clinicians have turned their attention toward point-of-care coagulation testing, which is able to provide a less precise result in 15 min, rather than a perfect result in 30–60 min, as long as most of the time the result they obtain is close to the "true" value or at least is sufficiently accurate to prevent the inappropriate transfusion of blood products.

Settings. CIP may be in different settings (some within and some beyond the reach of a hemostasis laboratory). Among the many are: surgery rooms and emergency areas, but also in war settings, tragedy settings (in mobile clinics), and also the patient's house. Different locations also drive different prognoses: military patients with trauma have a 31–38% chance of coagulopathy, associated with a fivefold increase in mortality when compared with civilian trauma patients [5]. Prompt diagnostic support is essential for these patients. Two strategies have been developed to manage this problem. The first, which can be offered only to patients with reach of a laboratory, is to create an array of tests which can be conducted in the routine central laboratory and in a short time. In a study omitting some steps of the laboratory workbench and speeding up others, some hemostasis tests maintained sufficient/good quality and the results were ready after around 20 min [6]. Such a strategy is not suitable when a patient is far from a laboratory facility.

The most relevant advance in this context is the development of point-of-care testing (POCT), a bedside set of coagulation tests. Even considering the many limitations in reproducibility and comparability, POCT can provide information on hemostasis in real time and drive diagnosis and therapy of some CIP.

2.5 Evaluation and Use of Coagulometers

Automation in coagulation laboratories has greatly improved accuracy, precision, and standardization, and has facilitated tests requiring specific training and special conditions. In the 1990s, a number of manufacturers successfully included multiple detection methods, which now give a single laboratory the possibility of applying different methods using the same equipment, and fully automated instruments are today quite common in hemostasis laboratories. They provide efficiency, comparability, repeatability, and reproducibility. Today, instruments can perform clotting, chromogenic, and immunological tests.

2.5.1 Principles of Operation

Mechanical tests. These rely on the increased viscosity of the plasma when fibrin is formed. A steel ball within the plasma sample is subjected to a magnetic field, resulting in a swinging movement of the ball. The increased viscosity from coagulation hinders movement and when a predetermined level of oscillation is not reached, the chronometer stops. The time relates to the speed of fibrin formation.

Photo-optical test. Fibrin clot formation induces a change in the optical density of the specimen: therefore, the time to reach certain a degree of change, assessed with a light beam, is related to the coagulation efficacy.

Nephelometric principle. Laser dispersion can be an alternative way to assess fibrin formation. Light is scattered when it encounters a fibrin clot and measurements are related to a certain level of dispersion (90° or 180°) lateral or backward with respect to the vial.

Chromogenic principle. A color-specific generating substance known as a chromophore (*para*-nitroaniline is the most common) can be linked to synthetic substrates (chromogenic substrate) of proteins involved in coagulation. The analyte protease cleaves the chromogenic substrate, generating a yellow color (*para*-nitroaniline is measured at 405 nm) proportional to the amount/activity.

Immunological principle. Specific antibodies can be bound to latex microparticles, and reaction with the specific antigen will cause agglutination of many particles into a larger agglomerate of particles. This method assesses the amount of an analyte/antigen using the property of microparticles absorbing light when their size approaches the wavelength of (monochromatic) light. Such an increase in light absorbance is proportional to the agglutination, which, in turn, is proportional to the amount of the antigen present in the sample.

2.6 Primary Hemostasis Tests

A test able to screen primary hemostasis is not available in clinical settings. So far the only test, the Ivy skin test, is expressed as the time required to observe the primary hemostasis after a standardized incision in the volar surface of the forearm. Platelet adhesion and aggregation (intrinsic defects, von Willebrand disease, drugs), vascular tone, and integrity, as well as skin resistance, determine the bleeding time, but the thickness and vascularization of the skin, the site and depth of the incision, the skin temperature, and patient anxiety can affect the results, making the Ivy test imprecise, inaccurate, and irreproducible. These limitations make the bleeding time not suitable for any clinical use.

2.6.1 Platelet Function

Platelet Count

A low platelet count is the most common hemostasis defect in CIP. The accuracy of hematology analyzers, based on electrical impedance or light scattering techniques, is ±5% in the range between 1,000 and 3,000,000 platelets per microliter. The mean platelet volume is also provided by the same instruments, together with a platelet size distribution curve.

Spontaneous platelet aggregates, cold agglutinins, or use of EDTA as an anticoagulant can spuriously reduce the platelet count, inducing so-called pseudothrombocytopenia. In contrast, particulate debris, i.e., red or white cell fragments, can account for spurious thrombocytosis.

Since thrombocytopenia can induce an overestimate of the platelet count, low platelet counts need manual inspection of a peripheral smear [7].

Platelet Aggregation

Platelet aggregation is measured by either conventional or whole blood platelet aggregometry. The former uses a modified spectrophotometer using light transmission variation to measure formation of the platelet, whereas the latter is based on changes of electrical impedance due to the cell–cell (platelets and leukocytes) contact in whole blood. Platelets in platelet-rich plasma are triggered in vitro by an agonist to diagnose inherited and acquired platelet disorders, for the assay for von Willebrand factor activity (ristocetin cofactor assay), and for the diagnosis of heparin-induced thrombocytopenia.

The type of agonist is chosen to diagnose specific platelet defects. Whole-blood aggregometers have a faster turnaround time. Also, lumiaggregometers measure aggregation and ATP release at once, to provide a more accurate diagnosis of platelet function defects. Routinely, the platelet agonists used to differentiate various platelet function defects are ADP, adrenaline, collagen, ristocetin, and arachidonic acid, whereas thrombin, vasopressin, serotonin, thromboxane A_2, platelet-activating factor, and other agents are mainly used for research.

The main utilization is in the diagnosis of platelet defects. The most relevant is *Glantzman* thromboasthenia, consisting in the aberration of the activatable fibrinogen-binding glycoprotein (GP) IIb/IIIa. In this condition, all fibrinogen-dependent aggregation is defective, i.e., that to ADP, epinephrine, arachidonic acid, and collagen, whereas passive aggregation ristocetin-mediated aggregation is maintained. *Bernard–Soulier* disease is characterized by lack of GPIb/IX, thrombocytopenia, and large platelets. In these patients, fibrinogen-mediated aggregation is efficient, whereas binding to thrombin, ristocetin, and von Willebrand factor (i.e., GPIb/IX-mediated aggregation) is poor.

A more sophisticated use of aggregometry relies on mixing studies, i.e., tests where either the patient's platelets or plasma is mixed with control components and triggered with specific agonists. In *heparin-induced thrombocytopenia*, plasma (containing antibodies against the PF4–heparin complex) can induce control platelet aggregation in the presence of heparin as an agonist. The complex binds to CD32 (FcyRIIA), resulting in platelet aggregation. The relatively low sensitivity and turnaround time make enzyme immunoassay and flow cytometry preferable in the initial diagnosis of heparin-induced thrombocytopenia, and the platelet aggregation test should only be used as a confirmatory test in antibody-positive patients.

Von Willebrand disease is another condition requiring platelet aggregation. Ristocetin aggregability is impaired when plasma from these patients, lacking von

Willebrand factor, is used, but not when the patient's platelets are matched with control plasma.

In conclusion, although very precise, platelet aggregometry is a complex, time-consuming, expensive test, the utility of which, in CIP, is limited to confirmation of inherited diseases, but with few consequences for acute treatment of the patient.

2.7 Clot-Based Assays

Although some guidelines state that the INR can be conducted even after 24 h, no matter whether the sample is centrifuged or not, or is taken from thawed plasma stored at −70°C for 6 months, fresh clean plasma should be the only option for CIP.

2.7.1 Activated Partial Thromboplastin Time

The aPTT is a functional evaluation of the intrinsic pathway of coagulation (involving fibrinogen, prothrombin, factors V, VIII, IX, XI and XII, prekallikrein, high molecular weight kininogen). In the aPTT, an aliquot of undiluted, platelet-poor plasma is incubated with a particulate factor XII activator (e.g., silica, Celite, kaolin, micronized silica, ellagic acid, etc.) and a reagent containing phospholipid (partial thromboplastin) at 37°C. After 5 min, i.e., after generation of a sufficient amount of activated factor XI, the sample is recalcified with $CaCl_2$ and the chronometer starts. A cascade reaction takes place and results in fibrin production, which stops the chronometer. The normal value is between 24 and 37 s [3]. Statistically, the aPTT is slightly lengthened in young individuals and slightly shortened in older populations. Premature infants have prolonged aPTT values, which return to normal by 6 months of age. However, age-specific normal ranges are not utilized in patient care at this time.

The sensitivity of the assay to factor deficiencies, inhibitors, and heparin varies with the reagents used in the assay. Lipemia and hyperbilirubinemia interfere with the detection of clot formation by photo-optical methods.

Clinical Use

The aPTT is a fundamental assay of the intrinsic pathway of the coagulation system. The principal clinical uses of the aPTT include:

1. The detection of hereditary or acquired deficiencies or defects of the intrinsic and common pathway coagulation factors and monitoring their eventual correction, such as coagulation factor replacement therapy in patients with hemophilia. The time increases beyond the upper limit when a single factor is below 40% of activity.
2. Monitoring intravenous unfractionated heparin anticoagulant therapy.

3. Detection of coagulation inhibitors (i.e., lupus anticoagulant, antibodies against coagulation factors), and of resistance to activated protein C (i.e., genetic defects of factor V).

Liver disease, disseminated intravascular coagulation (DIC), intense oral anticoagulant therapy, and improper specimen collection (i.e., traumatic phlebetomy or hemolyzed specimen) are other causes of inaccurate aPTT.

For some uses, variants of the test have been developed. These include reptilase time, protein C and protein S activity, tests for lupus anticoagulant diagnosis (dilute Russell's viper venom time, dRVVT), and activated C protein resistance test.

2.7.2 Prothrombin Time and INR

Test methodology. In the prothrombin time, an aliquot of plasma is incubated at 37°C with a reagent containing a phospholipid–protein tissue extract mix (complete thromboplastin). $CaCl_2$ is then added and the time required for clot formation is measured by one of a variety of techniques (photo-optical, electromechanical, etc.). The result is reported in seconds (prothrombin time), or as a ratio compared with the laboratory mean normal control (prothrombin ratio). Indeed, the prothrombin time is critically dependent on the characteristics of the thromboplastin used in the assay and in particular on tissue factor content. This receptor (CD142), a transmembrane protein widely expressed on cells of nonvascular origin, activates factor VII during the initiation of the extrinsic coagulation pathway. It is commercially taken from the uterus and brain of animals (and only recently has been obtained by recombinant technology) and its amount differs critically according to the type of organ and the age and strain of the animal. Thus, potentially, different laboratories might provide different and sometimes opposite results, leading to different clinical decisions. For this reason, the WHO requires that the activity of each batch of thromboplastin from manufacturers of INR kits is matched with a reference sample from the WHO. The result of such a comparison is the international sensitivity index (ISI), a power factor to be assigned to the ratio of the times (patient/reference) to obtain the INR. Nowadays, only the INR should be considered for monitoring the extrinsic pathway.

What the prothrombin time measures. The prothrombin time is a functional determination of the extrinsic (tissue factor) pathway of coagulation (prothrombin, factors V, VII, and X, fibrinogen) and is extremely sensitive to the vitamin K dependent clotting factors (prothrombin, factor VII and X). The prothrombin time is used for the detection of inherited or acquired coagulation defects related to the extrinsic pathway of coagulation and for monitoring oral anticoagulation maintained with vitamin K antagonists. Its normal values are in the range 8.8–11.6 s and the INR should be between 0.8–1.2 [7].

2.7.3 Fibrinogen Assay

Fibrinogen is a large asymmetrical clotting protein in the plasma, with a normal level ranging from 200 to 350 mg/dl and a half-life of 4 days. Its plasma concentration is essential in the diagnosis and management of many coagulopathies. In addition, it is also an acute phase reactant, and high levels represent a risk index in some patients who will develop myocardial infarction and stroke.

The methods for its measurement are numerous and many of them provide significantly different results. The washed clot assay (dry weight of the total clottable fibrinogen, or the protein content in a washed and dissolved clot), proposed by the WHO, is a time-consuming reference technique but is definitely not suitable for CIP and emergency settings.

The von Clauss technique is the standard method used in clinical laboratories, and provides the best results in terms of interlaboratory and intralaboratory accuracy and precision [8]. In this technique, a high concentration of thrombin is added to buffer-diluted (1:5 or 1:10) plasma. Dilution reduces the effect of clotting inhibitors and the clotting time is, therefore, directly proportional to the level of clottable fibrinogen. The times are then converted into fibrinogen concentration on a standard curve made with purified fibrinogen of known concentration. Accuracy is good in the range of approximately 50–800 mg/dl.

Interference in such a measurement can come from high (beyond therapeutic) levels of heparin or hirudin, hyperfibrinolytic activity (which will require a special tube containing aprotinin or another plasmin inhibitor), and fibrin degradation products (FDPs). All these can result in underestimation by the Clauss or coagulation-based technique.

2.8 Plasma Mixing Studies

A high INR value or a prolonged aPTT indicate either a factor deficiency or the presence of an inhibitor of coagulation. The plasma mixing study is the initial step in the evaluation of a prolonged clotting time, provided that 50% of any coagulation factor is sufficient to restore a normal value of the test, but the presence of an inhibitor requires a more important dilution and is able to prolong coagulation times of normal pooled plasma. So, even the 1% factor VIII level encountered in severe hemophilia can be corrected to the normal range by mixing with normal pooled plasma, whereas an inhibitor requires a higher plasma dilution and 1:1 mixing will not completely restore the coagulation time. The single deficient clotting factor will be then identified by “factor assays”: in principle, mixing the plasma of the patient with plasmas known to be deficient in a different coagulation factor will identify the defective protein (the level in the mix in one of the vials will not be above 50%, resulting in a prolonged time). The failure of a mixing study to correct a prolonged clotting test indicates an “inhibitory” substance retarding coagulation.

Precise identification of the inhibitor requires cooperation with the clinicians and further laboratory tests. Inhibitors can be considered "specific," such as antibodies directed against phospholipids (like in lupus anticoagulant) or a specific coagulation factor (factor inhibitor), or "global", i.e., FDPs, monoclonal paraproteins, and drugs such as heparin. The former group is not corrected by the mixing studies, and the latter is corrected only in part, but not completely. Clinical and other laboratory clues are necessary to identify the inhibitor. For example, lupus anticoagulant is not associated with clinical bleeding, whereas specific factor inhibitors frequently induce bleeding. The diagnosis can be confirmed by a phospholipid-sensitive test such as the dRVVT.

Heparin and other global inhibitors (paraproteins and FDPs) can be confirmed by other coagulation tests, such as the thrombin clotting time and the reptilase time, which are insensitive to these inhibitors.

The incubation time can provide further information on the activity of the inhibitor. Fast-reacting inhibitors prolong the coagulation time immediately after the mixing (most factor inhibitors, except factor VIII, and most lupus anticoagulants), whereas slow-reacting inhibitors (most factor VIII inhibiting factors and 15% of lupus anticoagulants) require 1–2 h of incubation at 37°C to inhibit the test.

In the case of hereditary prekallikrein deficiency, inducing a markedly prolonged aPTT, a prolonged preincubation (i.e., 10 min) of the plasma with aPTT reagent before the assay is performed normalizes the time.

2.8.1 Reptilase Time

Reptilase replaces thrombin and is not affected by heparin, hirudin, and antithrombin antibodies, but is still sensitive to the presence of myeloma proteins and FDPs. Prolongation of both the thrombin time and the reptilase time suggests myeloma, FDPs, hypofibrinogenemia, or dysfibrinogenemia. A prolonged aPTT and a normal reptilase time indicates that, beyond the aforementioned causes, heparin, hirudin, or other antithrombins are the cause of the prolonged aPTT. If these drugs, FDPs or myeloma can be ruled out, then the cause of the prolonged reptilase time may be hypodysfibrinogenemia.

2.8.2 Dilute Russell's Viper Venom Assay

Lupus anticoagulants are IgG and IgM autoantibodies that interfere with the function of anionic phospholipids found in patients with antiphospholipid antibody syndrome. The effect on coagulation is to prolong phospholipid-dependent clotting tests such as the aPTT and particularly the dRVVT. Russell's viper venom contains a serine protease that directly activates factor X. The activated factor X then activates prothrombin (factor II) in the presence of factor V and phospholipid. In the dRVVT test, the phospholipid content (of plant origin) is lowered to make

the test more sensitive to the presence of substances blocking the availability of the phospholipid surface. Starting by activation of factor X, moreover, the test is not influenced by deficiencies of the contact or intrinsic pathway factors or antibodies against factors VIII, IX, or XI, as can be the case for aPTT.

2.9 POCT Technologies

POCT refers to testing that is performed near or at the site of the patient, with the result leading to a possible change in the care of patient. The development of devices incorporating microchemistry, miniaturization, and microcomputerization has led us to a point where many of the hemostasis tests can be performed in real time at the bedside or at the point of patient interaction.

Hemostasis tests currently available in POCT are:

- International normalized ratio (INR)
- Activated partial thromboplastin time (aPTT)
- Activated clotting time (ACT)
- Modifications of the thrombin time
- Thromboelastography
- Platelet function
- D-dimer

They can be performed in venous (whole or centrifuged) as well as in capillary blood. Some further difficulties can be encountered with POCT devices compared with standard devices: insufficient volume of blood sample (usually 3–50 μl), coordination between warm up of the strip and capillary blood sampling, access to the test strip (when inserted in the device), unavailable heparin neutralizing reagent in some kits, high INR (above 4.5), high viscosity, hematocrit extremes (below 25 or above 55), hyperbilirubinemia, and hypertriglyceridemia.

The technologies used in POCT are quite different and the main problems are standardization of the results and comparability among different POCT devices and coagulometers. Conflicting reports exist on comparability with regular coagulometers. External quality assessment programs do exist but, at the moment, they are very few. The tests described below are those peculiar to or better performed with POCT technologies [9].

2.9.1 Thromboelastography

Native blood is put in the cuvette and left to coagulate with a 45° swinging movement. A pin is immersed in the vial and suspended by a torsion wire—a transductor attached to the wire records the strength of the forming clot. This allows one to record the formation/dissolution speed, and the amplitude of the clot. Thromboelastography is mainly used in monitoring blood replacement products in perioperative patients.

Thromboelastography has been adopted into the resuscitation protocols of some centers. However, although the turnaround time for rapid thromboelastography (19 + 3.1 min) may seem advantageous compared with that of routine laboratory tests, in most centers this time is spent by the clinician performing laboratory testing, instead of direct treatment of the patient, which could negatively affect the delivery of health care.

2.9.2 Platelet Test

Recently, POCT platelet tests have been used in CIP treatment. One type of assessment studies aggregability and is based on impedance platelet counting. The principle is that a thrombocytopenia (low platelet count) is expected in a blood sample aggregated by an agonist and such a reduction in platelet count is directly proportional to the aggregation potential. Two separate samples are taken, one containing a prefixed dose of angonist (ADP and collagen), and the difference in the platelet counts between the two samples expressed as a percentage reflects the effect of antiplatelet drugs (clopidogrel, NSAIDs, or GPIIb/IIIa antagonists) in patients with different cardiovascular disease undergoing procedures.

Another method (PFA-100) is to study aggregation under flow conditions. Blood mixed with a platelet agonist is forced through a narrow pore (150 μm) at a high shear rate corresponding to that of a small artery. Platelets undergo platelet adherence, activation aggregation, and finally occlusion of the pore. The time to occlusion (closure time) is the final result of the assay. This test is versatile and can provide information also on inherited platelet dysfunction, aspirin resistance or antiplatelet compliance, monitoring desmopressin acetate in von Willebrand disease patients (types 1 and 2).

The limitations of this test are the insensitivity to low levels of fibrinogen or other coagulation factors and some platelet defects, requiring a strict clinical cooperation in order to evaluate the results. Moreover, the test is affected by preanalytical conditions such as hematocrit, platelet count, and the collection technique.

Alternatively, in the platelet test, blood is forced into a slit at constant pressure. The bleeding time is the time that elapses between the start of the flow and the blood clotting in the slit. Phase I studies suggest that such a test is able to diagnose dysfunctional platelets and thrombotic thrombocytopenic purpura and to monitor the effects of plasma exchange.

Recently, microbead agglutination technology has been used in the evaluation of platelet function (VerifyNow system). Anticoagulated blood is put into contact with fibrinogen-coated microbeads, which, after agglutination, change the optical absorbance of the sample.

All these POCT devices/techniques have a shorter turnaround time but none are comparable in terms of sensibility or sensitivity with classic platelet aggregometry.

2.9.3 Activated Clotting Time

The ACT is useful in monitoring coagulation status and high-level heparinization in immediate-need situations such as cardiac surgery. The ACT uses tubes containing a negatively charged particulate activator of coagulation, such as kaolin, Celite, or diatomaceous earth. When whole blood is drawn into the tube, the contact system is activated and clotting occurs. The manual ACT device has been replaced in recent years by an increasingly sophisticated variety of microprocessor-controlled instruments. The assay is useful at high levels of heparin, when the aPTT assay would provide an unclottable sample, but is also affected by platelets. For this reason, some manufacturers also provide ACT reagents containing heparinase so that a patient's baseline value can be established in the presence of heparin. These instruments are increasingly being applied to the near-patient monitoring of direct thrombin inhibitors and low molecular weight heparins in critical situations.

2.9.4 D-dimer

D-dimer is a marker of fibrin turnover, its level being increased as a result of both increased formation and increased degradation of fibrin. Although this marker is hardly used in emergency situations, it still retains its utility in the treatments of CIP to rule out deep vein thrombosis. This semiquantitative test has reached sufficient reliability for clinical settings and is considered negative when values are below 500 ng/ml. Different antibody-related techniques are employed in the analysis of this marker.

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http://www.springer.com/978-88-470-2447-2

Hemocoagulative Problems in the Critically Ill Patient
Berlot, G. (Ed.)
2012, XIII, 238 p., Hardcover
ISBN: 978-88-470-2447-2