

# Chapter 3

## Understanding Advanced Hematologic Testing



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### Introduction

Critically ill patients often have anemia, sepsis, multiorgan failure (MOF), coagulopathies, and/or infection. For the patient to receive the correct diagnosis and treatment, laboratory tests must be carefully ordered and interpreted. Many laboratory tests pertaining to anemia and coagulopathy evaluation and diagnosis will be discussed in this chapter. Test interferences as well as caveats that are important to recognize in test result interpretation will be addressed. Additionally, recent advances in hematologic testing for anemia and coagulopathies will be described.

One emerging area that is of importance in blood conservation is the frequency and volume of laboratory blood draws, but this topic is beyond the scope of this chapter. Laboratory tests are expensive with point-of-care (POC) tests typically costing more. Additionally, laboratory tests require blood draws that contribute to the development of iatrogenic anemia during hospitalization [1]. Patients in the intensive care units (ICU) are quite ill and require careful monitoring, and how frequently certain tests should be ordered is unknown. However, many hospitals have begun stressing to their staff the costs of unnecessary testing and the risks to the patients [2]. Frequent lab testing has not been linked to improved patient outcome [3]. Notably, it has been shown that when laboratory tests were reduced, patient outcomes remained the same, and blood component use was decreased [4].

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## Anemia Tests

Anemia is characterized by a decrease in red blood cells (RBCs) and/or hemoglobin (Hgb). There are several forms of anemia that are defined by the mean corpuscular volume (MCV). Typically, RBCs have a MCV of 80–100 fL. Microcytic anemia is characterized by a MCV below the normal range, normocytic anemia has a normal MCV, and macrocytic anemia is characterized by a MCV above the normal range. There are various causes of each of these forms of anemia.

The most common cause of microcytic anemia is iron deficiency. Iron-deficiency anemia (IDA) can be suspected by a variety of different laboratory values in the CBC. Typically, not every laboratory parameter will be an exact fit. Besides a low MCV, patients with IDA often have an increased red blood cell distribution width (RDW), which is indicative of a wide range of red blood cell sizes. The RDW is the coefficient of variation of the erythrocyte size and is calculated by dividing the standard deviation of the MCV by the MCV value and multiplying it by 100 [5]. Interestingly, an increased RDW has been shown to be prognostic and is associated with cardiovascular and cerebrovascular diseases [6]. The increased RDW of iron-deficiency anemia (IDA) is due to the concomitant presence of microcytic RBCs and reticulocytes and possibly nucleated RBCs in the patient's circulation. Patients usually respond to anemia by increasing their production of reticulocytes. This is reflected as an increase in either the absolute reticulocyte count or as an increase in the reticulocyte percentage. Notably, the reticulocyte percentage should be adjusted to the patient's anemia to yield the corrected reticulocyte percentage. This value can be used to correctly assess the bone marrow's response to anemia. Together, the absolute reticulocyte count and the corrected reticulocyte percentage can be used to determine if the patient's anemia is due to RBC loss or inadequate RBC production. Recently, the reticulocyte hemoglobin has become an important measure in evaluating anemia; this value is frequently reported as mean reticulocyte hemoglobin content (CHr) or reticulocyte hemoglobin equivalent (Ret-He) (Table 3.1). Most automated hematology analyzers have the capability to report one of these values,

**Table 3.1** Nonroutine laboratory tests to evaluate a patient with anemia

Test name	Comments
Mean reticulocyte hemoglobin content (CHr) or reticulocyte hemoglobin equivalent (Ret-He)	Useful to detect functional iron deficiency
CHr or Ret-He	Decreased in iron-deficiency anemia and thalassemias; improves fast with iron supplementation
Mean reticulocyte volume	Decreased in iron deficiency; reference range dependent on methodology
Hepcidin (serum, plasma, or urine)	Increased in anemia of chronic disease; variable results depending on method used
Plasma methylmalonic acid (MMA)	Increased in early or mild vitamin B <sub>12</sub> deficiency

which provide an assessment of functional iron availability for incorporation into RBC production over the previous 3–4 days [7, 8]. A decreased value indicates reduced cellular hemoglobin content as seen in functional iron deficiency. Reticulocyte hemoglobin is also the strongest predictor of IDA in children [9]. Reticulocyte hemoglobin is particularly helpful in diagnosing IDA in the critically ill as ferritin values may be falsely elevated due to inflammation and/or chronic renal disease. Additionally, serum iron values may also be affected by patient dietary intake and may not correctly reflect the patient's iron status. The CHr/Ret-He decreases with IDA and is one of the first laboratory values to recover with iron supplementation [10, 11]. Notably, CHr/Ret-He can be decreased in thalassemias, and these RBC disorders should be excluded prior to using CHr/Ret-He to assess a patient's iron status [12].

Mean reticulocyte volume is another parameter that is available; however, there are limited studies on its clinical usefulness. In patients with low iron stores, the mean reticulocyte volume increases quickly after iron therapy and decreases at a similar speed with the development of iron-deficient erythropoiesis. The mean reticulocyte volume also decreases quickly following treatment with vitamin B<sub>12</sub> and/or folate in macrocytic anemia [13]. One problem with using mean reticulocyte volume is the variation in values obtained from different analyzers.

Quite frequently, an iron panel consisting of serum iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin is ordered to assess microcytic anemias. As mentioned earlier, ferritin is an acute phase reactant and often elevated in critically ill patients. Transferrin saturation may also be affected by the presence of inflammation which can cause increased transferrin in the setting of a constant amount of iron which would appear as low transferrin saturation. The transferrin concentration may also be low due to decreased synthesis caused by liver impairment, malnutrition, or chronic disease. In these cases, the transferrin saturation could appear increased. Finally, transferrin saturation also experiences substantial fluctuations in values between 17% and 70% due to diurnal variation [14, 15].

Many critically ill patients have normocytic anemia, with anemia of chronic disease being its most common cause, affecting 6% of hospitalized adult patients [16]. There are several causes of normocytic anemia: decreased production, increased destruction, or an uncompensated increase in plasma volume such as that which occurs in fluid overload or pregnancy. Anemia of chronic disease (aka anemia of inflammation) is a diagnosis of exclusion. All iron studies are normal indicating adequate iron stores, but iron delivery to developing RBCs is impaired, and erythropoietin (EPO) production is typically suppressed. In addition to decreased EPO levels, anemia in critically ill adults also can have high hepcidin levels. Notably hepcidin is increased as a result of inflammation as part of a host defense mechanism to reduce iron availability to microbes and malignant cells [17–19]. Hepcidin is a liver-derived peptide hormone that is involved in iron homeostasis, and increased hepcidin decreases iron availability via internalization and destruction of ferroportin and decreased iron absorption in the gastrointestinal tract [20]. Decreased ferroportin levels prevent iron export from cells, essentially resulting in iron being locked in macrophages in the reticuloendothelial system [21]. Erythroferrone is a

hormone which mediates hepcidin suppression and subsequent increased iron absorption and mobilization [21, 22]. Hepcidin levels can be measured in critically ill adults to assess its role in normocytic anemia. This testing is likely to be sent out at most hospitals and have a turnaround time of several days. Hepcidin testing is performed either using mass spectrometry or immunoassays [23]. Importantly, hepcidin results can vary widely depending on the method used and testing center employed. This is due in part to a lack of reference material, no agreed-upon reference method, and no commutable calibrators [24]. Additionally, some studies have shown that there is considerable within-patient variability for hepcidin levels, with hepcidin levels increasing with fasting and showing circadian rhythm and daily variability [25, 26]. Currently, researchers are working on developing hepcidin antagonists to treat anemia of chronic disease [19]. With the advent of such new drugs, hepcidin measurement is likely to become more prominent in clinical practice.

The direct antiglobulin test (DAT), lactate dehydrogenase (LDH), haptoglobin, and bilirubin tests are useful in diagnosing anemia due to increased destruction (hemolysis). A positive DAT, increased LDH, decreased haptoglobin, and increased bilirubin are supportive of a hemolytic anemia diagnosis. The DAT assesses whether the patient's RBCs have been coated *in vivo* with IgG and/or complement. This test has a false-negative rate of 5–10% which can be potentially due to extensive hemolysis, hemolysis caused by IgA or IgM, low levels of IgG bound to the RBC (below detection limit), low-affinity antibodies, and technical error [27]. The DAT may also be falsely positive in a clotted specimen which can lead to *in vitro* complement binding, high serum immunoglobulin or protein levels, intravenous immunoglobulin, antiphospholipid syndrome, and infection such as HIV. Notably, the DAT can also help distinguish the type of autoimmune hemolytic anemia based upon the thermal amplitude of the antibody. In the absence of recent transfusion, a positive DAT for IgG only indicates warm autoimmune hemolytic anemia. A DAT negative for IgG and positive for C3 is consistent with cold autoimmune hemolytic anemia or cold agglutinin disease. Finally, a mixed-type autoimmune hemolytic anemia can be positive for IgG and C3 [28]. Additionally, the DAT may also be helpful in detecting drug-induced hemolytic anemia, as when there is a positive DAT for IgG but an eluate from the red cells that does not bind to any reagent cell. A thorough history of commonly implicated drugs such as some penicillins and cephalosporins is essential to seal the diagnosis.

LDH is an intracellular enzyme that is very prevalent in RBCs. Thus, although increased serum LDH is a sensitive marker of hemolysis, it is not specific, as increased LDH can be seen in many conditions such as cancer and liver disease. Haptoglobin is a protein that scavenges free hemoglobin and protects the patient from its toxic effects [29]. Decreased haptoglobin is also sensitive but not specific for hemolysis; liver disease, exercise, and blood transfusions can also decrease haptoglobin levels [10]. Interestingly, approximately 1:1000 Caucasians and 1:25 African-Americans have a complete absence of haptoglobin [30]. Lastly, bilirubin levels, particularly indirect bilirubin, increase with hemolysis due to the liver's inability to keep up with conjugation and excretion into the bile.

Two common causes of macrocytic anemia are B<sub>12</sub> and folate deficiencies, and serum levels are helpful for diagnosis. However, increased levels of transcobalamin I-bound B<sub>12</sub>, as can be seen in patients with chronic myelogenous leukemia, liver disease, and other myeloproliferative disorders, can cause normal or high serum B<sub>12</sub> even in the setting of B<sub>12</sub> deficiency [31]. Normal serum B<sub>12</sub> can also be observed in patients with prolonged exposure to nitrous oxide [31]. Decreased serum B<sub>12</sub> is not specific to B<sub>12</sub> deficiency and can be seen in folate deficiency, pregnancy, multiple myeloma, HIV infection, and in patients that are receiving anticonvulsants. Thus, folate and B<sub>12</sub> levels should be checked concomitantly. An alternative to detect B<sub>12</sub> deficiency is to measure serum or plasma methylmalonic acid (MMA) and plasma total homocysteine. Increased MMA is highly specific for B<sub>12</sub> deficiency, more than a low serum B<sub>12</sub> level. Notably, the MMA assay is quite expensive and is typically a sendout test at most hospitals. It is also important to remember that impaired renal function can alter the results of the MMA assay. Total homocysteine testing has much lower specificity and can be increased not only in B<sub>12</sub> deficiency but also folate deficiency, renal failure, vitamin B<sub>6</sub> deficiency, hypothyroidism, and alcohol abuse, in patients taking certain medications and in carriers of mutations in the homocysteine metabolism pathway [31].

Folate levels can be measured in serum or in RBCs. A serum folate level reflects the patient's current diet, while the RBC folate is a measure of the folate status over the previous 90 days. Since a decreased folate can be seen in a large number of B<sub>12</sub>-deficient patients, a diagnosis of folate deficiency is typically reserved for someone with decreased folate and a normal B<sub>12</sub> result.

In many patients with the laboratory tests discussed above, the etiology of the anemia remains unclear. For such patients, a bone marrow biopsy (BMBx) may be useful. BMBx may also be helpful in evaluating critically ill patients with immature cells such as blasts identified in the complete cell count (CBC), pancytopenia, extremely low reticulocyte count, serum or urinary monoclonal proteins, or unexplained severe anemia, particularly with a history of cancer or chemotherapy. Iron staining can be performed on the BMBx to evaluate bone marrow iron stores as long as the specimen contains enough spicules for adequate assessment. Some experts recommend a minimum of seven spicules to be able to accurately assess iron stores [32]. A reticulin stain may also be useful to detect fibrosis within the bone marrow, another cause of anemia. Cytogenetic studies and FISH may also be beneficial in evaluating the BMBx. Lastly, assessment of the BMBx aspirate and core for cellularity, dysplasia, increased blasts, increased plasma cells, and other abnormalities can be helpful when no other cause of anemia has been ascertained.

## Tests to Evaluate Thrombocytopenia

Thrombocytopenia is typically defined as a platelet count  $<150 \times 10^6/\mu\text{L}$ . Thrombocytopenia can be due to decreased production (hypoproliferative), increased breakdown or clearance, or splenic sequestration. Hypoproliferative

**Table 3.2** Components of the PLASMIC score – each parameter present contributes one point to the score [34]

P: Platelet count less than $30 \times 10^6/\mu\text{L}$
L: Laboratory evidence of hemolysis (any)
A: Absence of active cancer
S: No history of stem cell or solid organ transplant
M: Mean corpuscular volume (MCV) less than 90 fL
I: International normalized ratio (INR) less than 1.5
C: Creatinine less than 2 mg/dL

thrombocytopenia may be caused by leukemia or metastatic cancer to the bone marrow, chemotherapy, a primary bone marrow diagnosis such as myelodysplastic syndrome, viral infections such as hepatitis C or HIV, or alcoholism. It is often accompanied by other cytopenias, such as anemia and leukopenia. Causes of increased platelet destruction/consumption should be divided into immune and nonimmune. The latter includes immune thrombocytopenia (ITP), drug-induced ITP, and heparin-induced thrombocytopenia (HIT). Nonimmune causes are more common in the critical care setting, such as sepsis/disseminated intravascular coagulation (DIC), malignant hypertension, large intravascular devices, thrombotic thrombocytopenic purpura (TTP), and atypical hemolytic uremic syndrome (aHUS), among others [33]. In addition to a complete history, review of the peripheral blood smear aids in the differential diagnosis. A BMBx is rarely needed but may be helpful in selected cases. A newly developed index to detect ADAMTS13 deficiency, the PLASMIC score, should be utilized in patients with thrombocytopenia and microangiopathic hemolytic anemia in whom TTP is being considered (Table 3.2) [34]. If the PLASMIC score is intermediate or high, the patient must be treated with therapeutic plasma exchange emergently until the result of the ADAMTS13 activity is available. TTP is a medical emergency, as 90% of patients die without therapeutic apheresis. To diagnose or rule out HIT, another potentially serious complication, there are two indices that can be used to determine the likelihood of the diagnosis prior to ordering a laboratory test [35, 36]. When indicated, the initial test should be heparin-platelet factor 4 (PF4) antibodies by ELISA, which measures heparin-PF4 antibodies of all immunoglobulin classes. This test has a high incidence of false positives which has been reported to occur in 1–3% of dialysis patients, 10–15% of medical patients, and >20% of patients receiving heparin for peripheral vascular surgery [33]. Thus, false-positive HIT results can be a problem in critically ill patients. A positive ELISA result for HIT should be followed by a serotonin release assay, which determines the functional activity of the heparin-platelet factor 4 antibodies [37, 38].

## Interpretation and Testing for Coagulation Abnormalities

Coagulopathy is a common problem in the critically ill. Many different conditions can result in coagulopathies which make careful assessment important in correctly diagnosing the etiology and administering the most efficacious

treatment. The most common coagulation tests ordered are the prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer. Some of these tests are currently ordered for uses they were not designed for. This is most true for the PT and aPTT tests. The PT was intended to monitor patients receiving warfarin and the aPTT to monitor heparin therapy. However, they are often utilized to assess a patient's bleeding risk or tendency. It is important to note that neither of them has predictive value for bleeding [39]; however, prolongation of either the PT or aPTT has been shown to be a strong predictor of mortality in trauma patients [40]. Furthermore, their use to guide transfusions has resulted in substantial variation in plasma administration practices to the critically ill [41, 42].

### ***Routine Coagulation Assays***

As discussed earlier, PT, aPTT, fibrinogen, and D-dimer are among the most frequently ordered coagulation tests on critically ill adults. Fibrinogen is also an acute phase reactant and can be elevated in such patients. Additionally, fibrinogen can be decreased due to liver failure or increased consumptions such as in DIC. Thus, the fibrinogen result must be interpreted with caution and measurements compared with each other over time to detect significant changes. The D-dimer assay is useful in detecting fibrinolysis and can be indicative of excessive clotting or sepsis. D-dimers are generated by the breakdown of cross-linked fibrin by plasmin. All D-dimer assays use antibodies against epitopes only exposed once cross-linked fibrin is broken down. While D-dimers are increased in a variety of settings including pregnancy, burns, sickle cell crisis, surgery, malignancy, infection, liver disease, atrial fibrillation, renal failure, cardiac failure, aortic dissection, and VTE, the D-dimer concentration in DIC is expected to be at least 20 times higher than the reference range for the specific assay. A low D-dimer in a patient with a low pretest probability of VTE has a very high negative predictive value and can be useful to guide management [56, 57].

Many critically ill patients have prolonged PT and aPTT values. The PT measures the extrinsic and common pathways of coagulation and is most sensitive to deficiencies of factors VII, X, and V and prothrombin. Frequently, the PT is prolonged due to sepsis/DIC and coagulation factor consumption or poor factor synthesis due to liver dysfunction, malnutrition, and GI malabsorption, especially of vitamin K. The aPTT measures the intrinsic and common pathways of coagulation and is most sensitive to deficiencies of factors XII, XI, IX, and VIII and prekallikrein. In the critically ill, the aPTT is commonly prolonged due to heparin administration or blood sample contamination with heparin, severe DIC/sepsis, and liver failure. The PT and aPTT tests are also affected by anticoagulants that the patients may be receiving for thrombosis prophylaxis or treatment. Critically ill patients with prolonged PT and/or aPTT values with no clear etiology can be evaluated further using mixing studies.

In mixing studies, a fixed volume of the patient's plasma is incubated at 37 °C with an equal volume of normal plasma, and then the PT and/or aPTT are repeated immediately after 1–2 h (“incubated mixing study”). If the prolonged screening test is due to a factor deficiency, the PT or aPTT in the mix will normalize or “correct,” which is defined as a result in the reference range of the test or within 10% of the result of the normal plasma alone incubated under the same circumstances. If the result of the mixing study does not show correction, it suggests the presence of an inhibitor in the patient's plasma. The most common inhibitor is heparin, which is the reason why some laboratories perform a thrombin time and an anti-Xa test to rule out the presence of unfractionated heparin and/or a direct factor Xa inhibitor before performing the mixing study. In the setting of multiple factor deficiencies, such as in DIC or severe liver failure, the mixing studies may also not fully correct as per the definition above, but the result of the PT or aPTT in the mix should be shorter than the original PT or aPTT in the patient's plasma. Thus, interpretation of mixing studies must take into account the most likely cause of the coagulopathy. A less common cause of lack of correction in the aPTT mixing study in a critically ill patient is a lupus anticoagulant, which is often an incidental finding.

In the setting of mixing studies that correct, individual factor levels can be performed to evaluate which particular factors are deficient. Vitamin K deficiency will result in decreased levels of prothrombin and factors VII, IX, and X. In liver failure, all coagulation factors except factor VIII should be decreased because factor VIII is made by both the liver and endothelial cells. Liver failure can be distinguished from vitamin K deficiency by measuring factors V and VIII, which are not vitamin K-dependent. Factor V will also be decreased in liver failure, but factor VIII will be normal or even high due to the acute phase reaction [49]. Isolated deficiency of factor X is rare as a congenital defect, but it can be seen in patients with amyloidosis and unexplained bleeding [58, 59].

### ***Testing for Lupus Anticoagulant***

Current International Society for Thrombosis and Haemostasis (ISTH) guidelines recommend screening for lupus anticoagulants using two different tests such as a dilute aPTT or silica clotting time and a dilute Russell viper venom time (DRVVT). Dilute refers to the use of limited amount of phospholipid to increase the sensitivity of the assay to antiphospholipid antibodies. If one of these screening tests is prolonged, a confirmatory test must be done, in which a large amount of phospholipid is added to the patient's plasma and a decrease in the clotting time is observed if a lupus anticoagulant is present. A positive test for lupus anticoagulant should be repeated 6–12 weeks later to confirm its persistence and to make the diagnosis of antiphospholipid syndrome if the patient has clinical manifestations such as VTE or pregnancy complications. Patients receiving direct thrombin and direct factor Xa inhibitors can have false-positive lupus anticoagulant results and should not be tested [60, 61]. Also, if a lupus anticoagulant is present and is causing a



prolongation of the aPTT, the latter is unreliable to monitor heparin, and the anti-Xa assay must be employed. In rare patients with a lupus anticoagulant that prolongs the PT, an alternative test must be used to monitor warfarin. Several studies have shown that under these circumstances, INR values vary significantly depending on the thromboplastin used. Consequently, the extent of anticoagulation was frequently overestimated [62, 63]. Instead, the chromogenic factor X assay should be used to guide therapy.

### ***Chromogenic Factor X Assay***

The chromogenic factor X assay (not commonly available in hospital laboratories) is a test of factor X activity that it is not clot-based and contains no phospholipid; therefore it is not affected by lupus anticoagulant [64]. The measurable factor X activity decreases as the patient's INR increases. A factor X activity of 20–40% corresponds to an INR of 2.0–3.0 [65]. The chromogenic factor X assay is also useful in patients receiving the thrombin inhibitor, argatroban, while being transitioned to warfarin [66]. However, patients with liver disease and vitamin K deficiency may have decreased factor X activity levels.

### ***Factor VIII Inhibitor***

If the aPTT mixing study did not correct and lupus anticoagulant testing was negative, the patient may have a specific factor inhibitor, such as a factor VIII inhibitor. Patients with factor VIII inhibitors typically have hematomas and bleeding at various sites, which characterize acquired hemophilia [67]. If a factor VIII inhibitor is suspected, close consultation with the coagulation laboratory may be particularly helpful, and treatment with appropriate activated factors must be initiated promptly in the setting of bleeding. Plasma transfusion is not effective. Furthermore, determination of the inhibitor titer using either the Bethesda or Nijmegen-Bethesda assay will help guide patient management.

### ***The Anti-Xa Assay***

The anti-Xa assay is the most accurate test to monitor unfractionated heparin (UFH) and the only test to monitor low-molecular-weight heparin (LMWH), when needed. As mentioned above, patients with a lupus anticoagulant who are receiving UFH should be monitored with the anti-Xa, a non-clot-based method to measure the ability of the heparin-antithrombin (AT) complex to inhibit factor Xa. The residual amount of factor Xa activity is inversely proportional to the amount of UFH or

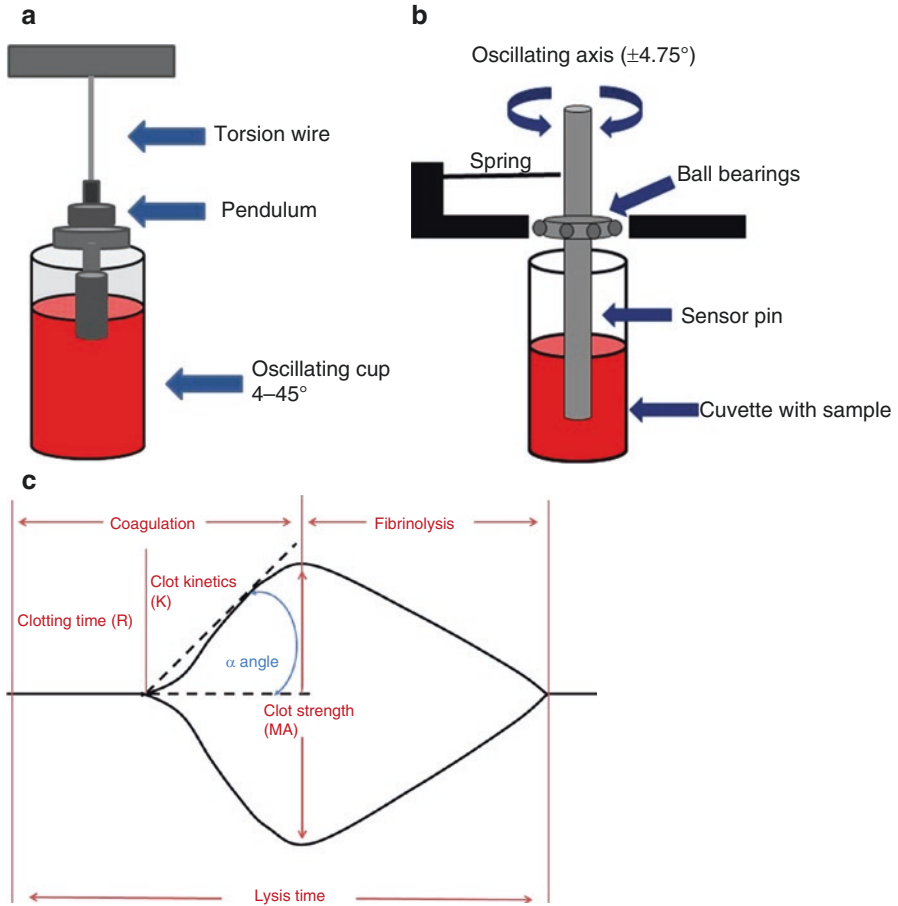
LMWH in the patient's plasma and is determined using a calibration curve [68, 69]. The reference range of the anti-Xa assay depends on the type of heparin the patient is receiving, dose schedule, and the indication (prophylactic or therapeutic). For LMWH, the anti-Xa level should be drawn 3–4 h after a subcutaneous dose. The anti-Xa assay can also be used to monitor fondaparinux; however, a separate calibration curve using fondaparinux is needed [70].

The anti-Xa assay is also affected by the presence of a direct factor Xa inhibitor in plasma and may be useful to monitor rivaroxaban, apixaban, or edoxaban. However, most laboratories do not have a reference range for any of these drugs due to lack of commercially available controls to generate a calibration curve for each direct factor Xa inhibitor [71, 72]. Importantly, the anti-Xa calibration curves for UFH or LMWH cannot be used to monitor the direct factor Xa inhibitors. The “gold standard” for measurement of the plasma concentration of these agents is high-performance liquid chromatography coupled to mass spectrometry (HPLC-MSMS). A recent study by Bardy et al. showed that the anti-Xa assay was effective in evaluating plasma concentrations of rivaroxaban >52 ng/mL; however, at lower concentrations, it was not as accurate in comparison to HPLC-MSMS [73].

### *Viscoelastic Testing*

While the PT and aPTT only measure specific steps of the coagulation cascade in plasma, thromboelastography (TEG) and rotational thromboelastometry (ROTEM) offer a more holistic overview of clot development, stabilization, and dissolution in whole blood, more closely reflecting in vivo hemostasis. Thus, TEG and ROTEM detect platelet dysfunction and fibrinolysis, which are important aspects of coagulopathies in the critically ill. Numerous studies have described the usefulness of TEG and ROTEM in assessing trauma patients, guiding blood component use and administration, assessing coagulopathy, monitoring liver transplant surgeries, and assessing heparin reversal following cardiac bypass surgery, among many things [43–48].

In TEG and ROTEM, testing of citrated blood must be performed within 2 h of collection. Fig. 3.1a shows a schematic representation of TEG, where the patient's blood is added to a preheated cuvette, a pin is suspended from a torsion wire into the cup, and the cup moves with each rotation cycle. As the clot forms, the cup's impeded movement is transmitted to the pin, and a tracing is generated. In ROTEM (Fig. 3.1b), the pin transmits a signal via an optical detector system and not a torsion wire as in TEG [45]. A diagram of a TEG tracing is shown in Fig. 3.1c. The time until initial fibrin formation is measured by the reaction time (R). The R value is



**Fig. 3.1** TEG. (a). In TEG, the pin is suspended from a torsion wire and is inserted into the cuvette or cup which rotates through an angle of 4–45°. As fibrin strand forms in the cuvette/cup, the impeded movement is transmitted via the pin and torsion wire to a tracing. (b). In ROTEM, the cup is stationary and a ball bearing pin rotates back and forth 4.75°. The movement of the pin is driven by an elastic spring. As long as the blood is liquid, the rotation of the pin is unrestricted. When the blood starts to clot, the rotation of the pin is restricted as the firmness of the clot increases. (c). The clot time or reaction time (*R* value) reflects the time to initiate fibrin formation. The kinetics of clot formation (*K* value) is the time at which the amplitude reflecting clot strength reaches 20 mm. The *R* and *K* values are measurements of coagulation. The  $\alpha$  angle is the angle between the midline and a line tangential to the developing TEG® trace. The  $\alpha$  angle represents the clot kinetics of clot buildup and fibrin cross-linking. The maximum amplitude (*MA*) is the maximum width of the TEG® trace. It is a measurement of the ultimate clot strength and reflects platelet number and function

prolonged in the setting of single hereditary (e.g., hemophilia A or B) or multiple and acquired (e.g., warfarin) coagulation factor deficiencies, when the coagulation cascade is inhibited by either unfractionated heparin, direct thrombin inhibitors (e.g., argatroban, bivalirudin, dabigatran), or direct factor Xa inhibitors (e.g., rivaroxaban, apixaban, edoxaban, etc.). The cutoff R value varies depending on which activator is used in the TEG assay. The kinetics of clot formation (K value) is the time at which the amplitude reflecting clot strength reaches 20 mm. The K value can be prolonged in coagulation factor deficiencies, hypofibrinogenemia, and thrombocytopenia. Similar to the R value, the K value is dependent on the activator used. The  $\alpha$  angle is the angle between the midline and a line tangential to the developing TEG trace, and it represents the kinetics of clot buildup and fibrin cross-linking. The  $\alpha$  angle is increased in hypercoagulable states and decreased in thrombocytopenia or hypofibrinogenemia. The maximum amplitude (MA) predominantly reflects the strength of the clot and is affected by the fibrinogen concentration and platelet count and function. The MA is increased in hypercoagulable states and decreased in thrombocytopenia, platelet dysfunction, and hypofibrinogenemia. Decreased MA values are frequently seen in TEG tracings from patients who have been on cardiopulmonary bypass or extracorporeal membrane oxygenation (ECMO) [49, 50]. The use of the MA to predict thrombotic states in patients undergoing coronary artery bypass and percutaneous intervention has also been suggested [51]. An MA above 68–70 mm has been evaluated in the literature to assess risk of thrombosis. Although there was only a trend toward worse 30-day outcomes for either myocardial infarction, stroke, or mortality in patients who had MA values >68–70 mm, this study may have been underpowered to examine these issues since a composite of the three outcomes was higher ( $p = 0.014$ ) in patients with this MA cutoff [52]. Another study evaluated development of venous thromboembolism (VTE) in trauma patients. The rate of deep vein thrombosis (DVT) was double in trauma patients with hypercoagulable TEG indices despite prophylaxis, as compared to trauma patients with normal TEG results (odds ratio 2.41,  $p = 0.026$ ) [43]. Notably, none of the individual TEG indices alone was associated with DVT, but the combination of  $R < 5$  min,  $\alpha$  angle  $> 72$  degrees, and MA  $> 74$  mm was associated with DVT development [43].

The TEG and ROTEM measure the viscoelastic properties of blood under static conditions in a cuvette and not under flow that exist in an endothelialized vessel. Thus, they are not wholly reflective of what is occurring in vivo; in addition, they are very difficult to standardize as results are affected by age and gender as well as blood collection site and time delay in processing [53]. Patients receiving heparin should have a heparinase TEG which utilizes the enzyme heparinase to degrade the heparin followed by the addition of kaolin as an activator to test the patient's viscoelastic coagulation properties. In the absence of heparinase use, patients receiving heparin anticoagulation may have a falsely elevated R value.

As mentioned above, TEG and ROTEM can be used to guide blood component administration; this practice is particularly well-studied for trauma victims. A shortened R value is frequently seen in the acute setting of trauma, and no action is typically needed. A prolonged R value can indicate the need for plasma to replenish coagulation factors. A decreased  $\alpha$  angle may be an indication for plasma or

cryoprecipitate, and a decreased MA supports the use of cryoprecipitate and/or platelet transfusions. Patients with increased MA values and/or an overall increase in the coagulative index (CI) may benefit from anticoagulation. Increased LY30 values are indicative of increased fibrinolysis, and tranexamic acid may be useful under the right circumstances [54].

Despite trauma and injury, many patients with coagulopathy still have normal TEG values at presentation. Savage et al. have described a new TEG value, the MA-R ratio, which they believe reflects a patient's hyper- and hypocoagulability [55]. Patients with the lowest MA-R ratios had higher mortality [55]. Further, they hypothesized that the lower MA-R ratio is due to decreased fibrinogen and earlier cryoprecipitate transfusion may be beneficial [55].

TEG and ROTEM are point-of-care (POC) tests and are frequently kept in the operating room and not in a centralized laboratory. Hence, as with many POC test devices, special attention is needed to comply with standards with respect to QC/QA and monitoring of user proficiency as is routine in clinical laboratories. Maintenance of these standards is important, non-laboratory-trained individuals often perform these tests, and TEG is classified as a moderately complex test by CLIA. Due to these concerns, many institutions have moved the TEG and ROTEM to either a central or a satellite laboratory where laboratory-trained personnel maintain the devices and perform the tests. Another advantage of these tests is that the tracings can be transmitted in real time to where the patient is, in order to facilitate semi-real-time decision-making with reliable results.

### ***von Willebrand Disease***

von Willebrand disease (vWD) is reported to affect ~1% of people [74, 75]; however, only approximately 0.1% are symptomatic [76]. Patients with vWD have a tendency to bleed at mucosal surfaces such as the nose, oropharynx, and urogenital system. Most people have type 1 vWD, which is a partial quantitative deficiency. Several factors influence the plasma concentration of von Willebrand factor (vWF), such as the blood type, stress, and inflammation [77]. Individuals of blood type O have vWF levels that are 10–30% lower than those of other blood types [78]. This difference is likely due to increased clearance of vWF in the circulation [79]. Several studies have shown that patients that have the blood type O comprise a much larger percentage of individuals diagnosed with vWD than would be expected, suspicious for their misclassification due to constitutively lower vWF [80, 81]. Thus, when evaluating a patient for vWD, it is important to know the blood type; prior surgical, dental, and bleeding history; and test for vWF antigen, vWF activity, and factor VIII activity. Conversely, patients with vWD may have normal test results because they are acutely ill (stress and acute phase reaction).

A smaller percentage of patients with vWD have a qualitative defect of vWF that can be detected by calculating the vWF activity/vWF antigen ratio. Typically, the vWF activity and antigen are present in an essentially 1:1 ratio. When the ratio is

<0.7, a qualitative defect is indicated, and a diagnosis of type 2 vWD should be investigated further. In rare patients, vWF activity, vWF antigen, and factor VIII activity are severely depressed, and type 3 vWD is likely. For such patients, vWF replacement with concentrates is the appropriate therapy for bleeding. While patients with type 1 vWD whose vWF levels have normalized as part of an acute phase reactant most often do not require treatment, DDAVP and vWF replacement should be available.

Another cause of bleeding in critically ill patients is acquired vWD (AvWD). These patients do not have a personal or family history of abnormal vWF testing or bleeding. Patients with AvWD frequently have diseases such as lymphoproliferative or myeloproliferative neoplasms, cardiovascular disease, solid tumors, and autoimmune disorders [82–84]. AvWD should be suspected in patients with mechanical heart valves, ventricular assist devices (VADs), artificial hearts, status post-cardiac bypass, or on extracorporeal membrane oxygenation (ECMO) [85–88]. While the prevalence of AvWD in the general population is estimated at 0.04–0.2% [84], it is likely much higher (although unknown and probably underrecognized) in critically ill patients with one or more of these intravascular devices. AvWD is thought to occur via three mechanisms: (1) loss of high-molecular-weight vWF multimers due to high shear stress and proteolysis, (2) vWF adsorption onto cancer cells resulting in increased clearance, and (3) autoantibodies that impair vWF function and increase its clearance [83, 89]. In mechanism 1, vWF activity is much lower than the vWF antigen; in mechanism 2, both vWF activity and antigen are decreased, or vWF activity is decreased proportionally with the vWF antigen; in mechanism 3, both the vWF activity and antigen are decreased. vWF multimer analysis is helpful in distinguishing between AvWD and vWD. Typically, AvWD is associated with decreased or absent high-molecular-weight vWF multimers [84, 91–93]. However, this pattern is also seen in congenital type 2 vWD. AvWD can also be drug-induced. Several drugs have been implicated, including valproic acid, ciprofloxacin, griseofulvin, tetracycline, pesticide, thrombolytic agents, and hydroxyethyl starch. Discontinuation of the offending agent results in resolution of the AvWD [84, 89]. Although less common, AvWD has also been observed with hypothyroidism and uremia and without an underlying predisposing condition [82, 90]. When the diagnosis of AvWD is suspected, especially in critically ill patients with intravascular devices, the proper levels should be promptly checked in order to replace vWF as necessary.

### *Hypercoagulability Tests*

During the course of a hospital stay, critically ill patients frequently develop blood clots. In many cases, this is despite prophylactic measures. A hypercoagulable work-up may be warranted. A hypercoagulable panel, comprised of antithrombin (AT), protein S, and protein C activity, activated protein C resistance (APCR), prothrombin

G20210A gene mutation analysis, and antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibodies, and anti- $\beta_2$ -glycoprotein I antibodies), is rarely indicated in the intensive care unit. In the setting of a recent clot, AT, protein S, and protein C levels are likely to be decreased and not reflective of baseline values. Although normal results rule out a deficiency, misinterpretation of abnormally low results is likely and has significant long-term implications for the patient. Furthermore, proteins C and S are vitamin K-dependent and may be decreased because of liver disease, malnutrition, malabsorption, vitamin K deficiency, or current or recent warfarin. Furthermore, protein S activity measures free protein S, which decreases during an acute phase reaction. It is not surprising, therefore, that decreased levels of protein C and AT have been observed in 40–60% of trauma patients and 90% of sepsis patients [94, 95]. In addition, direct thrombin and direct factor Xa inhibitors can interfere with functional protein S assays [96]. Patients receiving UFH or LMWH may also have decreased levels of AT due to increased clearance. The only indication for testing for AT during an acute VTE episode is when there appears to be heparin resistance, i.e., difficulty reaching a therapeutic anti-Xa level despite increased intravenous infusion of UFH or subcutaneous doses of LMWH. In such circumstances, a low AT activity may explain lack of response to heparin, and AT replacement is indicated in order to reach the desired target anti-Xa.

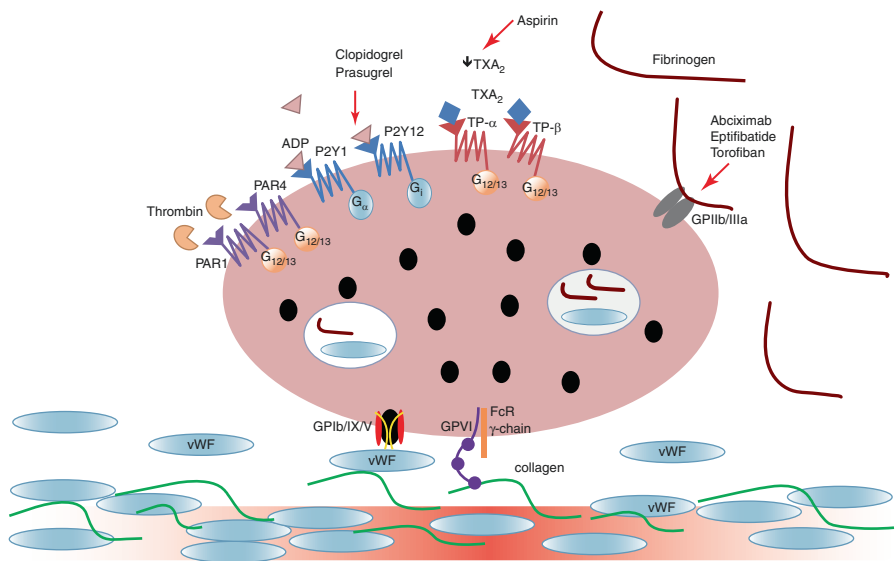
Testing a patient with unexplained VTE for factor V Leiden mutation with the APCR assay and for the prothrombin G20210A mutation is not affected by acute illness, but the results of these tests do not affect anticoagulation in the hospital. The presence of these hypercoagulable risk factors plays a role in the duration of treatment, which is the basis for the recommendation to perform them after discharge. The APCR evaluates the ability of activated protein C (APC) to cleave and inactivate factors Va and VIIIa. In the presence of factor V Leiden mutation, APC is unable to cleave and inactivate factor Va which leads to increased thrombin generation. Although factor V Leiden testing can be done via DNA analysis in a molecular diagnostic laboratory, the APCR is quicker and much less expensive. The APCR is not accurate when a lupus anticoagulant is present and direct oral anticoagulants also interfere with the APCR assay [97]. Except for testing for lupus anticoagulants which is affected by anticoagulation, assessing for the other antibodies that may cause antiphospholipid syndrome may be performed in the intensive care unit. However, once again, the acute management of the patient with VTE does not vary if such a diagnosis is made.

### ***Platelet Function Tests***

With the adoption of TEG and ROTEM by many hospitals, interest in critically ill patients' platelet function has increased. Prior to TEG and ROTEM availability, the methods available to assess platelet function were whole blood platelet aggregometry, platelet-rich plasma (PRP) platelet aggregometry, and the PFA-100. The first

two may be considered the gold standard in platelet function but are not widely available because of their high cost and complexity. The PFA-100 is widely available and a POC test. It assesses the shear rates as blood is aspirated through a cartridge that has an aperture coated with either collagen and epinephrine or collagen and ADP. The closure time (CT) is determined once the blood ceases to flow through the aperture. PFA-100 testing is affected by the hematocrit (decreased hematocrit is associated with increased CT), platelet count (increased CT with platelet count below  $100 \times 10^6/\mu\text{L}$ ), vWF (decreased levels associated with increased CT), platelet inhibitor drugs, uremia, and cardiopulmonary bypass.

To determine if a patient’s platelets are adequately “blocked” by ADP inhibitors such as clopidogrel or prasugrel, GPIIb/IIIa receptor inhibitors, or aspirin (ASA), VerifyNow can be used (Fig. 3.2). The VerifyNow is a POC device that uses whole blood in a turbidimetric assay. It employs fibrinogen-coated beads and measures



**Fig. 3.2** Schematic representation of platelet receptors and ligands. The GP IIb/IIIa receptor is shown in gray, and its ligand, fibrinogen, is shown in maroon strands. The GP Ib/IX/V receptor is shown in red, black, and yellow, and its ligand, vWF, is shown in blue ovals. The GP VI receptor is shown in purple, and its ligand, collagen, is shown as green strands. The protease-activated receptors (PARs) PAR1 and PAR4 are shown in purple, and their activating serine protease, thrombin, is shown in orange. The ADP receptors, P2Y1 and P2Y12, are shown in blue, and their ligand, ADP, is shown in pink triangles. The  $\text{TXA}_2$  receptors, TP- $\alpha$  and TP- $\beta$ , are shown in red with their ligand,  $\text{TXA}_2$ , shown in blue diamonds. The G-protein-coupled receptors, PAR1, PAR4, P2Y1, P2Y12, TP- $\alpha$ , and TP- $\beta$ , are shown associated with representative G-proteins in the interior of the platelet. Dense granules are shown as black circles, and  $\alpha$ -granules are shown as white ovals. The  $\alpha$ -granules are shown carrying fibrinogen and vWF. Prasugrel and clopidogrel act by irreversibly inhibiting the P2Y12 receptor. Aspirin works by irreversibly inhibiting the COX-1 enzyme and preventing arachidonic acid conversion to  $\text{TXA}_2$ . Lastly, abciximab, eptifibatid, and tirofiban work by inhibiting the GP IIb/IIIa receptor



differences in platelet aggregation in response to various agonists [98]. To test for platelet response to clopidogrel, the VerifyNow P2Y12 assay can be used. Using this specific cartridge, whole blood from the patient is exposed to 20  $\mu\text{mol}$  ADP and 22 nmol prostaglandin E1 (PGE1). The PGE1 acts to increase the assay specificity by suppressing ADP-mediated P2Y1 intraplatelet signaling. The patient's platelets will agglutinate around the fibrinogen-coated beads and increase light transmittance. The results are reported as P2Y12 reaction units (PRU): the lower the PRU, the higher the clopidogrel inhibition. High platelet reactivity (HPR) after clopidogrel treatment was defined as a PRU  $\geq 240$  [99]. The VerifyNow® P2Y12 assay also calculates the percentage of P2Y12 inhibition based upon thrombin receptor-activating peptide (TRAP) activation of platelets via PAR1, which is expressed as *base* value. Besides the P2Y12 assay, VerifyNow® can perform an ASA assay to examine response to ASA therapy. Using a separate cartridge, whole blood is exposed to 1.0 mM arachidonic acid (AA). The patient's platelets will agglutinate around the fibrinogen-coated beads and increase light transmittance, and the results are reported as aspirin reaction units (ARU): the lower the ARU, the higher the ASA inhibition of platelet aggregation. In contrast, high platelet reactivity (HPR) after ASA treatment was defined as an ARU  $\geq 550$  [100]. The VerifyNow system can also be used to assay for GPIIb/IIIa inhibitor therapy, although it is not as widely utilized. Limitations to this testing include non-steroidal anti-inflammatory drugs (NSAIDs), GPIIb/IIIa inhibitors, and other antiplatelet agents which can interfere with the aspirin assay; and GPIIb/IIIa inhibitors, anemia, and thrombocytopenia which can interfere in the P2Y12 assay.

TEG-based platelet mapping is also available to assess a patient's platelet response to AA and ADP agonists. Platelet mapping is frequently ordered for patients requiring emergent neurosurgery, for some trauma patients, and for patients in the cardiac ICU. Patients with VADs may also benefit from platelet mapping to assess platelet inhibition in order to prevent clotting of extracorporeal circuits. If a patient is on UFH, HTEG (with heparinase) platelet mapping should be performed. In the platelet mapping assay, a kaolin-activated sample is used to generate a strong thrombin response to maximally activate all platelets and cleave fibrinogen demonstrating the underlying potential for maximum clot strength ( $\text{MA}_{\text{thrombin}}$ ). A second assay follows, where all thrombin is blocked, and is used to demonstrate the clot strength from the fibrin component ( $\text{MA}_A$ ). Thrombin may also be blocked and platelets activated via the ADP receptor or thromboxane A2 receptor, thus assessing clot strength when platelets are activated through those specific receptors ( $\text{MA}_{\text{ADP}}$  or  $\text{MA}_{\text{AA}}$ ). The degree of inhibition in platelet mapping is calculated by using the patient's full hemostatic potential  $\text{MA}_{\text{thrombin}}$  as the baseline and the contribution of platelets activated through specific receptors. For patients on ADP inhibitors such as clopidogrel or prasugrel, the goal  $\text{MA}_{\text{ADP}}$  is 35–50 mm. For patients on ASA, the goal  $\text{MA}_{\text{AA}}$  is 35–50 mm. A  $\text{MA}_{\text{AA}}$  or  $\text{MA}_{\text{ADP}} < 30$  mm is associated with an increased risk of bleeding, and a  $\text{MA}_{\text{AA}}$  or  $\text{MA}_{\text{ADP}} > 50$  mm is indicative of little to no inhibition. If the  $\text{MA}_A$  is expanding over time, a functional fibrinogen needs to be done for the platelet mapping assay to be accurate. Functional fibrinogen is measured using the GPIIb/IIIa inhibitor, abciximab [101].

## ***Fibrinolysis Tests***

Increased fibrinolysis is seen in critically ill patients with sepsis/DIC and multiorgan failure. It can also be due to malignancies such as acute promyelocytic leukemia and prostate cancer [102]. The most commonly available and used assay for fibrinolysis is the D-dimer assay. In addition, TEG can detect increased fibrinolysis with the LY30 value. Measurement of  $\alpha_2$ -antiplasmin, a *serpin* and the main inhibitor of plasmin, may be useful in patients with hyperfibrinolysis, but it is rarely available in a hospital-based laboratory. Decreased levels of  $\alpha_2$ -antiplasmin can lead to increased fibrinolysis such as in patients with systemic amyloidosis. Measurement of fibrin degradation products (FDPs) has been mostly replaced by the D-dimer assay, which is much more accurate compared with the semiquantitative tests for FDPs.

## **Summary**

Hematologic testing, including assessment of primary (platelet) and secondary (coagulation cascade) hemostasis, takes various forms in critically ill patients. In addition to understanding the role and the utility of the various assays, it is essential to understand how they perform in the setting of acute illness and multiple comorbidities and drugs, such as anticoagulants. Furthermore, patients often have more than one laboratory abnormality and the interpretation of their clinical significance may not be straightforward.

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