CHAPTER 11
Viscoelastic Assays and Hypercoagulability

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Introduction

In 1910, Kottman reported the data from the first instrument used to measure blood coagulation. His “Koaguloviskosimeter” exploited the increasing viscosity of blood during coagulation, measuring the movement of a blade placed in the specimen while the specimen was rotated.¹ In the subsequent century, methodologies exploiting mechanical, turbidometric, spectrophotometric, and nephelometric processes have been developed that are directed at the diagnosis of bleeding disorders and the monitoring of anticoagulation.² Although the majority of studies have been directed at the prolongation of tests, there have been a number of studies directed at the acceleration of clotting times³ in an effort to detect hypercoagulability.³–⁵

More recently, there has been a remarkable increase in the understanding of the risk factors for thrombosis. Despite the strides that have been made, other than monitoring of anticoagulation, there are still few laboratory tests that actually guide the initiation of therapy of an individual patient, the therapeutic intervention that is still guided by the clinical findings in the patient.

Testing of hypercoagulability of blood has been focused on measuring activation of platelets, plasma hypercoagulation, and, to a lesser degree, inhibition of fibrinolysis. Activation of plasma coagulation has been reflected in the acceleration of initial coagulation tests,³–⁵ elevation of coagulation factors,⁶–⁹ detection of activation peptides in the plasma, and the presence of complexes of activated coagulation factors with their inhibitors. In the case of platelet activation, the measurements have focused on circulating platelet aggregates, analytes that are secreted by the platelet, and measurement of the activation of receptors on the surface of the platelet.¹⁰–¹⁵ In general, hypercoagulability related to fibrinolysis is a reflection of the concentration of the natural fibrinolysis inhibitors (Table 11-1).⁷,¹⁶ VET demonstrates evidence of hypercoagulability in obese patients and perioperatively, in the peripartum period and other clinical settings.

It is the intent of this chapter to review the findings and current use of VET in the evaluation of the hypercoagulable state, comparing those findings to those of other available assays.
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Table 11-1. Measurement of Coagulation Hypercoagulability.

<table>
<thead>
<tr>
<th>Platelet Hyperactivity Products Measured</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circulating platelet aggregates</td>
<td>12, 14, 15</td>
</tr>
<tr>
<td>Secretory products</td>
<td>10, 11</td>
</tr>
<tr>
<td>Activated platelet receptor</td>
<td>13</td>
</tr>
<tr>
<td>Accelerated PFA-100</td>
<td>17</td>
</tr>
<tr>
<td>Coagulation Hypercoagulability Products Measured</td>
<td></td>
</tr>
<tr>
<td>Acceleration of aPTT and/or PT</td>
<td>18, 3-5</td>
</tr>
<tr>
<td>Elevation and activation of coagulation factors</td>
<td>7, 8</td>
</tr>
<tr>
<td>Acute decrease in coagulation inhibitors</td>
<td>19</td>
</tr>
<tr>
<td>Presence of complexes of factor-inhibitor</td>
<td>7, 20-22</td>
</tr>
<tr>
<td>Inhibition of Fibrinolysis Products Measured</td>
<td></td>
</tr>
<tr>
<td>Increased plasmin inhibitors</td>
<td>7</td>
</tr>
</tbody>
</table>

*PFA-100, Platelet Function Analyzer 100; aPTT, activated partial thromboplastin time; PT, prothrombin time.

Case 1: Global Hemostasis Assessment in a Patient with Thrombosis but Negative Hypercoagulable Workup

History

A 52-year-old, Caucasian woman experiencing obesity (body mass index [BMI] = 40.1) presented to the emergency department with acutely worsened abdominal pain that began one week before her presentation. She also had left leg pain for the past two months. The patient suffered from a pulmonary embolism three years before her presentation. She has a 20-pack/year history of smoking, which she halted 12 months before presentation.

Clinical Course

The patient was diagnosed with descending aortic thrombus with bilateral lower extremity arterial emboli. She underwent thrombectomy. She also suffered from a splenic infarct.

She had a comprehensive thrombophilia evaluation (lupus anticoagulant, anti-cardiolipin antibody, anti–β-2 glycoprotein-1 [anti-β2GPI] antibodies, antithrombin, protein C function, protein S function, homocysteine, prothrombin G20210A, and FVL gene mutation). All of these tests were negative, failing to support the presence of a hypercoagulable state.

Laboratory Tests

Results of conventional laboratory tests are shown in Table 11-2. LTA demonstrates spontaneous aggregation.
The data in Figure 11-1 show a normal clotting time (R = 5.8 minutes), indicating a normal rate of thrombin generation. The α angle is slightly elevated at 75 degrees, which may indicate a slight acceleration of clot development. The MA is elevated to 81 mm, which is indicative of platelet hyperactivity.

Case Follow-Up
A decision was made to treat the patient with anticoagulant (Coumadin) and dual antiplatelet therapy (aspirin and Plavix).

Discussion of Case 1
This case reveals platelet hyperactivity with no evidence of enzymatic hypercoagulability. The patient has a history of venous thromboembolism (VTE), but the immediate problem is arterial thrombosis. The R time is within normal limits, as are the standard plasma coagulation tests (PT and aPTT). The only (nonviscoelastic) indication of hypercoagulability comes from platelet aggregometry that reveals spontaneous platelet aggregation. Platelet aggregometry (especially LTA) is not readily available in the short term as a rapid test and is labor intensive. Rapid VET in the form of TEG shows a markedly enhanced clot strength (MA), which is a consequence of platelet hyperactivity. The latter has been associated with VTE and peripheral arterial disease. Although laboratory testing reveals no obvious cause for thrombosis, the patient is experiencing obesity, which is a risk factor for VTE.
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Case 2: Global Hemostasis Assessment in a Patient with Thrombosis Who Desires to Have Children

History

A 39-year-old Caucasian woman with history of deep venous thrombosis (DVT) developed a pulmonary embolism while on oral contraceptive therapy for the DVT. She is a nonsmoker and eats a vegan diet. Her parents are both smokers. Her father suffers from lung cancer, diabetes mellitus, and stroke. There is no family history of hypercoagulability or thrombosis. Findings: Height 5 ft. 7 in.; Weight 143 lbs. (BMI = 22.4)

Laboratory Tests

Results of conventional laboratory tests are shown in Table 11-3.

Thrombophilia Evaluation

Lupus anticoagulant, anti-cardiolipin and anti-β2GPI antibodies, antithrombin, protein C function, protein S function, homocysteine, and prothrombin G20210A gene mutation were all within the reference interval (RI). She was found to be heterozygous for the FVL gene mutation.

TEG 5000 Interpretation

TEG showed shortened R, indicating accelerated thrombin generation (Figure 11-2). Other parameters are within the RI.

Treatment

The patient was treated with Coumadin therapy for six months in Europe. Lifelong prophylactic aspirin therapy was recommended by a hematologist at that time. The patient followed this regimen for a few years but discontinued the aspirin herself. The patient uses short-term, low-molecular-weight heparin prophylaxis before long airline flights.

Current issue: Patient desires to have children. She has had no prior pregnancies.

Discussion of Case 2

The history of a young woman with the FVL mutation and venous thrombosis is a common one. The treatment and recovery were uncomplicated, and she has no evidence of post-thrombotic syndrome. The issue of the presence of a thrombophilic risk factor (FVL) and anticipated pregnancy raises several issues. As it relates to hemostasis, pregnancy has been described as the acute phase reaction in slow motion, leading to a hypercoagulable state peripartum. Coagulation factors (factor VIII, vWF, and fibrinogen) all rise significantly during a normal pregnancy, and coagulation inhibitor protein S falls at the same time.¹⁹ This may be thought of as preparing the mother for the bleeding event of delivery. Addition of a thrombophilic risk factor

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Table 11-3. Case 2: Results of Conventional Laboratory Assays.

<table>
<thead>
<tr>
<th>Laboratory Assay</th>
<th>Patient's Results</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (seconds)</td>
<td>13.3</td>
<td>11.0–14.4</td>
</tr>
<tr>
<td>aPTT (seconds)</td>
<td>25</td>
<td>22–37</td>
</tr>
<tr>
<td>Platelets (x10³/µL)</td>
<td>261</td>
<td>150–450</td>
</tr>
<tr>
<td>D-dimer (µg/mL)</td>
<td>0.39</td>
<td>&lt;0.50</td>
</tr>
</tbody>
</table>

¹PT, prothrombin time; aPTT, activated partial thromboplastin time.
to this physiologic hypercoagulability significantly increases the risk of peripartum thrombosis in the mother and poses increased risk to the pregnancy itself. In the antiphospholipid syndrome, recurrent spontaneous abortion and pregnancy complications along with antiphospholipid antibody and/or lupus anticoagulant are among the diagnostic criteria. The presence of other thrombophilic risk factors have also been associated with pregnancy loss and complications, as well as with thrombosis in the mother. Examination of global hemostasis with VET has also demonstrated that shortened R time (accelerated thrombin generation), increased α angle (more rapid incorporation of fibrinogen in the clot), and increased MA (activation of platelets) during pregnancy have also been associated with pregnancy complications and loss. It is interesting that this patient has evidence of hypercoagulability in her VET at a time when she is not pregnant and has no other identified medical problems other than her FVL. FVL alone has not been reported to accelerate coagulation as measured with VET unless there is other complicating thrombosis or pregnancy. There may be additional issues in this patient that have not been defined.

**Case 3: Postoperative Thromboembolic Complication**

**History**

The patient was a 71-year-old Caucasian woman with a thoracoabdominal, fusiform aortic aneurysm who was admitted for an open surgical repair. She had a complex history, including polymyalgia rheumatica, hypertension, hypertrophic cardiomyopathy, and multiple myeloma (in remission). She had an uneventful surgery and, on postoperative day 12, the patient was transferred to a skilled nursing facility for rehabilitation.

Medications: Aspirin, 81 mg po daily

**Clinical Course**

She was recovering unremarkably at the facility until she was found unresponsive. She
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expired on postoperative day 15. An autopsy was performed. Postmortem examination revealed pulmonary thromboembolism involving complete occlusion of the right pulmonary artery, with smaller emboli also present. The right popliteal vein contained a loosely adherent thrombosis, the likely source of the pulmonary embolus.

Preoperative Evaluation

Hypercoagulable workup was not performed; she had no prior personal or family history of thrombosis.

Preoperative TEG 5000 Interpretation

TEG showed combined enzymatic hypercoagulability and platelet activation: shortened clotting time (R = 4.2 min), indicating increased rate of thrombin generation; elevated angle (α angle = 74.8 degrees), indicating increased contribution of fibrinogen to the clot stability; and elevated MA (MA = 72.5 mm), indicating platelet activation (Figure 11-3).

Discussion of Case 3

This patient had a number of risk factors for VTE, including recent major surgery (and its attendant inflammation), as well as, most likely, prolonged immobilization. In addition, she had a known history of polymyalgia rheumatica, which has a known association with VTE, as do other systemic autoimmune diseases. These factors can conspire to create an environment conducive to hypercoagulability and increased platelet activation. Underlying mechanisms include elevations in plasma factor VIII, vWF, and fibrinogen. In addition, there is increased TF expression, as well as the release of microparticles from platelets, lymphocytes, and monocytes. Platelet microparticles contain surface TF as well as phosphatidyl serine on the outer

Figure 11-3. TEG 5000 graph from Case 3, demonstrating a slight increase in the α angle and increase in the maximal amplitude (MA). Reference tracings are superimposed in the background (red dashed line).
leaflet of the microparticle membrane. Coagulation factors bind best to cell membranes rich in phosphatidylserine. Inflammation is also associated with reduced thrombomodulin expression and increased PAI-1. Thrombomodulin, together with thrombin, activates the protein C system, while PAI-1 blocks plasminogen activator and hence fibrinolysis. A meta-analysis by Zöller, et al., demonstrated an increased risk of VTE in those with polymyalgia rheumatica. The standardized incidence ratio for subsequent pulmonary embolism was 7.86 within the first year of follow-up in those with polymyalgia rheumatica.

**Discussion**

Remarkable progress has been made in the past few decades regarding the identification of thrombophilic risk factors. Thus, the risks of FVL, prothrombin G20210A, and deficiencies of proteins C and S and others have added much to the understanding of the control of hemostasis and the possible etiologies of thrombosis. However, these analytes do not answer the question of the risk of thrombosis at a point in time. As summarized in Table 11-1, there are a number of markers that imply immediate risk—such analytes as circulating platelet aggregates, elevated β thrombomodulin, elevated factor VIII, elevated fibrinogen, and others. The finding of any of these markers raises the concern for immediate risk. The shortcomings are the variability of the assays, the variability among the analytes among individuals, and the lack of close correlation of the findings to the clinical outcomes. In addition, the assays are not easy to perform in real time.

VET offers the possibility to assay the blood in a given clinical setting and, via the findings of shortened R time, shortened K time, increased α angle, and/or increased MA can lead to the possible diagnosis of hypercoagulability. As outlined above, these findings have been defined in a number of clinical settings; however, the correlation with clinical thrombosis is also highly variable. In a meta-analysis of VET and hypercoagulability, Brown, et al. have shown that VET consistently demonstrated perioperative hypercoagulability beginning on postoperative day 1. In their analysis, only MA was consistently used to both define hypercoagulability and be predictive of VET after traumatic injury and surgical intervention; however, there remains a broad variability in the definition of hypercoagulability as determined by MA and this limits its predictive ability.

In what may be the earliest publication on this subject, Hewison, in 1780, reported on the rapid clotting of the blood in a patient with infection. Scattered studies occurred over the subsequent two centuries. More recently, prothrombotic factors (fibrinogen, vWF, and factor VIII) have shown to be elevated in patients with obesity when compared with non-obese patient controls, with a positive association with central fat. Likewise, plasma concentrations of PAI-1 have shown to be higher in patients with obesity and to be directly correlated with visceral fat. In addition, obesity is characterized by higher plasma concentrations of coagulation inhibitors, such as tPA and protein C, when compared with non-obese patient controls. Lifestyle interventions have had some success in reducing apparent hypercoagulable risk. Accelerated closure time
in the PFA-100 platelet function assay also has been associated with risk of VTE.\textsuperscript{17} Although relationships of accelerated coagulation and thrombosis could be demonstrated, the variability of the clinical setting, the patients, and the tests greatly limited the value of these data in individual patient management.

Platelet hyperactivity has been implicated as one of the factors contributing to venous thrombosis. This has not always been easy to demonstrate. In the Framingham study, there was no significant association between platelet aggregation data and venous thromboembolic events.\textsuperscript{31} Any trends in this study did not remain significant after adjusting for BMI. Another study by Vázquez-Santiago, et al., did not find an association between VTE risk and whole blood platelet aggregation.\textsuperscript{17} However, the PFA-100 assay, which measures platelet adhesion, showed a significant association between a short closure time (that is, increased adhesion) and VTE risk. In a focused study on platelet hyperreactivity in the setting of total knee arthroplasty, Kim, et al., demonstrated that enhanced platelet aggregation was apparent in those with DVT; the best combination of sensitivity and specificity was seen when the platelets were stimulated by low epinephrine concentrations.\textsuperscript{32} Peripheral arterial disease has also been associated with increased platelet aggregation and activation, including spontaneous aggregation in the absence of an added agonist.\textsuperscript{33,34}

Intraoperative viscoelastic hemostatic testing has found a use for several decades in liver transplantation.\textsuperscript{35} More recently, there have been many examples demonstrating the use of viscoelastic hemostatic studies in identifying hypercoagulable states. With respect to platelet hyperactivity, a TEG tracing will yield the MA, which is the maximum distance in millimeters between the upper and lower lines of the TEG tracing.\textsuperscript{36,37} With ROTEM, the equivalent of the MA is the MCF.\textsuperscript{38} Whereas MA or MCF is an approximation of the clot strength, the true strength is represented by G, which is the shear elastic modulus strength of the clot and is measured in dynes or kilodynes (1,000 dynes) per square centimeter. G is a function of MA, such that \( G = (5,000 \times MA)/(100 – MA) \). Both MA and G are determined by platelet function and also by the formation of the insoluble fibrin strands produced through the action of thrombin. For MA values of 40, 60, and 80 mm, the corresponding G is 3.3, 7.5, and 20 kdynes/cm\(^2\), respectively. G is more sensitive than MA or MCF to small changes in the clot strength. On the basis of the authors’ experience with platelet mapping, approximately 80 to 85 percent of the clot strength is a consequence of platelet function.\textsuperscript{39} Oshita and co-workers demonstrated a linear relationship between the MA and \( \log_{10} \) platelet count with \( R^2 = 0.739 \).\textsuperscript{40}

Increasing evidence from ongoing investigations confirms that trauma patients show hypercoagulability when the specimen is analyzed by viscoelastic hemostatic analysis.\textsuperscript{41} Although some studies emphasize the effect upon the coagulation reaction time (R time), the role of platelet hyperactivity is nevertheless believed to be very important.\textsuperscript{42} However, hypercoagulability, especially platelet hyperactivity, has not been shown to be consistently true in all clinical investigations. Van Haren, et al., tested the hypothesis that TEG data on ICU admission can help to
stratify risk assessment for VTE.\textsuperscript{43} The results of this study indicated that this approach was not feasible. The MA was least affected by trauma in this study, and there was no significant difference in VTE rates between patients who did or did not exhibit hypercoagulability on admission. However, many other studies have found evidence for platelet hyperactivity in trauma. Park, et al., studied critically injured burned versus nonburned trauma patients.\textsuperscript{41,44} Trauma patients showed higher TEG $\alpha$ angle (which reflects the degree of fibrin cross-linking) and a greater MA (clot strength). In this population, results of conventional clotting studies (PT and aPTT) were not significantly different from those of controls. Watters, et al., evaluated coagulation parameters in trauma patients who had suffered splenic injury.\textsuperscript{41,45} In this study, the baseline TEG showed a higher mean $\alpha$ angle and enhanced fibrinolysis after splenectomy compared with controls. Clot strength (MA) was elevated above the baseline in the splenectomy patients, but this was not significant compared with the controls. At the six-week follow-up, the MA was significantly greater in the splenectomy group compared with the MA in controls. Significant differences were also detected between splenectomy and control patients with respect to the incidence of DVT (6.7 percent in splenectomy patients versus 0 percent in controls). In another study using rapid TEG on critically ill patients in the surgical ICU, the data showed that increased G was associated with thromboembolic complications after controlling for thromboprophylaxis.\textsuperscript{46} For every 1 dyne/cm$^2$ increase in G, the odds of thromboembolism increased by 25 percent. Differing, et al., conducted a TEG-based prospective study that showed that trauma patients blood were more hypercoagulable compared with healthy volunteers.\textsuperscript{47} This conclusion was reached by assessing time to clot formation, clot strength, and fibrin cross-linking at a range of temperatures. A cohort study suggested an association between admission MA and pulmonary embolism. Rapid TEG was obtained on 2,070 consecutive trauma patients.\textsuperscript{48} When controlling for gender, race, age, and Injury Severity Score, elevated MA at admission was an independent predictor of pulmonary embolism. A large meta-analysis investigated and reviewed the use of TEG and ROTEM in trauma and postsurgical cases in a variety of surgical fields.\textsuperscript{30} Seventeen studies consisting of 6,348 patients reported elevated MA values. Five studies, looking at 2,284 patients, described an elevated G. However, eight studies reported no evidence of hypercoagulability. Two studies identified an increased odds ratio based upon the MA for development of VTE. However, in the meta-analysis of all the studies, the odds ratio for developing a VTE in those with an elevated MA was 1.31 and was deemed not significant.

Ischemic stroke has naturally been an important focus of hypercoagulability studies. A 1974 study proved to be an early analysis of hypercoagulability.\textsuperscript{49} In this analysis, the ratio MA/(R + K) was calculated. The rationale was the observation (that still holds) that hypercoagulability is associated with smaller values for R (shorter reaction time) as well as K (rapid kinetics) together with an increase in MA. Hypercoagulability was defined as a ratio of greater than 4.0 and was observed after cerebral infarction in 29 to 38 percent of the patients studied. In 1999, Handa, et al., showed that TEG can be
used to differentiate ischemic from hemorrhagic stroke. Ischemic patients produced hypercoagulable TEG tracings. However, the data did not differentiate platelet versus enzymatic hypercoagulability and instead calculated the overall CI. In a TEG-based study by Elliott, et al., of patients who experienced acute ischemic stroke, enrolled individuals had shorter R, greater α angle, and shorter K when compared with the control population; 24 percent had a clot strength or G that exceeded the highest control (mean + one SD) and probably represented a subpopulation with the ability to form strong, fibrin-platelet–rich clots.

Hypercoagulability with a tendency to VTE is a well-recognized paraneoplastic effect of tumors. In a study by Toukh, et al., 32 patients with prostate cancer were compared to a control group composed of eight men with negative prostate biopsy. Hypercoagulability in this study was defined by having at least three abnormal TEG values. Hypercoagulability was most evident in those patients with metastatic disease who were on androgen-deprivation therapy. There was a significant reduction of R, an elevation of α angle, and an increase in MA in this latter group. In a retrospective study of adult patients with a confirmed diagnosis of gynecological malignancy, 10% percent developed VTE. Univariate analysis showed significant risk factors for VTE with respect to the R value, K value, α angle, MA, and CI. Multivariate analysis demonstrated that TEG CI is one of the independent risk factors for VTE. The CI is derived from all the TEG measurements, including R, K, α angle, and MA. Receiver operating characteristic curve analysis demonstrated that the TEG CI cutoff value for VTE was 2.55. In a study of newly diagnosed primary lung cancer by Davies, ROTEM parameters of patients were significantly different when compared with age-matched controls, including increased MCF and α angle; however, no differences were observed between patients who developed a VTE and those who did not.

A number of viscoelastic hemostatic studies have supported the hypothesis that obesity predisposes to a hypercoagulable state. Coelho, et al., found an increase in clot strength (as indicated by the ROTEM MCF) as well as an increase in clot formation speed in obese versus nonobese patients with Cushing syndrome. ROTEM studies by Campello, et al., demonstrated significant platelet hyperactivity with a higher clot strength in those with severe or class 3 obesity (BMI ≥ 40 Kg/m²).

Evaluation of hypercoagulability using VET requires some discussion of the quality parameters of the assay itself. There are examples described above that demonstrate, in the hands of the authors, a threshold level of a VET parameter predicting an outcome. For example, a pre-pregnancy MA value of greater than or equal to 64 mm was found to predict an increased risk for pregnancy loss. Those with pregnancy loss also had a reduced LY30. The threshold values were within the RI of the laboratory used in the study. In addition, there was significant overlap of the ranges for these tests when comparing the patients with pregnancy loss (MA range: 48 to 76 mm) with matched controls (MA range: 50 to 67 mm). VET has considerable within-run and between-run imprecision, and there is also variability among patients. The message from these
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data is twofold: women with pre-pregnancy increased MA and/or reduced LY30 did have an increased risk of pregnancy loss, and application of this information in another clinical setting requires that the local RI is well defined. The RI published by the manufacturer and that determined locally can differ significantly. In addition, any levels defining clinical risk must be locally validated.

References

15. Salem HH, Koutts J, Firkin BG. Circulating platelet aggregates in ischaemic heart
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