Coagulation Assays
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Case 1: Mr F, a 75-year-old man with diabetes, was admitted with non–ST-segment-elevation acute coronary syndrome. In addition to other medications, he is receiving 1 mg/kg enoxaparin every 12 hours. He is scheduled for cardic catheterization later in the afternoon. Earlier this morning, his activated partial thromboplastin time (aPTT) was 30 seconds. How should his anticoagulants be managed?

Case 2: Mrs G was admitted with chest pain and shortness of breath. A ventilation-perfusion lung scan was consistent with a large pulmonary embolism. Because of renal dysfunction, initial therapy consisted of unfractionated heparin. After 2 days of intravenous heparin and 5 mg/d warfarin, her aPTT is 48 seconds, and her international normalized ratio (INR) is 1.3. She is currently receiving 45 000 U heparin per day. How should her anticoagulants be managed?

Normal Coagulation
Coagulation in arteries or veins is triggered by tissue factor, a cellular receptor for activated factor VII (VIIa) that is exposed after vessel wall injury. Lipid-laden macrophages in the core of atherosclerotic plaques are particularly rich in tissue factor, which explains the propensity for arterial thrombosis at sites of plaque disruption. Although direct injury also can induce thrombosis in veins, more commonly, coagulation is triggered by tissue factor–bearing monocytes or microparticles that become tethered to activated endothelial cells or platelets.

Once bound to tissue factor to form extrinsic tenase, factor VIIa activates factors IX and X (Figure 1). Factor Xa converts small amounts of prothrombin to thrombin. This low concentration of thrombin amplifies coagulation by activating platelets and factors V and VIII, key cofactors for coagulation.

Coagulation is propagated when factor IXa binds to factor VIIIa on the surface of activated platelets or monocytes to form intrinsic tenase, a complex that efficiently activates factor X. Factor Xa then binds to factor Va to form prothrombinase, thereby increasing the rate of factor Xa–mediated conversion of prothrombin to thrombin >300 000-fold. The resultant burst of thrombin rapidly converts fibrinogen to fibrin, and fibrin monomers polymerize to form the fibrin mesh that is stabilized and cross-linked by factor XIIIa, a thrombin-activated transglutaminase.

Aside from factor VIII, all coagulation factors are synthesized in the liver. The hepatic synthesis of factors VII, IX, X, and prothrombin is vitamin K dependent. Vitamin K is required for a posttranslational modification of these factors that results in gamma-carboxylation of glutamic acid residues at their N termini. Gamma carboxylation endows these factors with the capacity to bind calcium and to interact with negatively charged phospholipid membranes. Without this step, vitamin K–dependent clotting factors are nonfunctional.

Abnormalities of Coagulation
In addition to hereditary deficiencies of coagulation factors, there are numerous acquired coagulation abnormalities. Hepatic dysfunction or vitamin K deficiency (resulting from inadequate dietary intake, malabsorption, or warfarin therapy) results in reduced production of functional coagulation factors. The coagulation disorder encountered in severe liver disease is compounded by enhanced fibrinolysis with resultant consumption of clotting factors. Vitamin K antagonists like warfarin block the gamma-carboxylation step, leading to synthesis of biologically inactive coagulation factors. Excess activation of coagulation can cause disseminated intravascular coagulation, which leads to con-
sumption of coagulation factors. Secondary fibrinolysis triggers breakdown of fibrin and generation of fibrin degradation products, which can impair hemostasis by inhibiting fibrin polymerization. Finally, factor deficiencies can be caused by autoantibodies against coagulation factors. These most often occur in hemophiliacs or in patients with autoimmune disorders.

Coagulation Assays
Several techniques, including clot-based tests, chromogenic or color assays, direct chemical measurements, and ELISAs, are used for coagulation testing. Most of these tests use citrated plasma, and the end point for all of them is fibrin clot formation. Some of the technical and analytic variables that can influence assay results are listed in Table 2.

Prothrombin Time
This test is performed by adding a thromboplastin reagent that contains tissue factor (which can be recombinant in origin or derived from an extract of brain, lung, or placenta) and calcium to plasma and measuring the clotting time (Figure 2A). The prothrombin time (PT) varies with reagent and coagulometer but typically ranges between 10 and 14 seconds.9 The PT is prolonged with deficiencies of factors VII, X, and V, prothrombin, or fibrinogen and by antibodies directed against these factors. This test also is abnormal in patients with inhibitors of the fibrinogen-to-fibrin conversion reaction, including high doses of heparin and the presence of fibrin degradation products. Typically, PT reagents contain excess phospholipid so that nonspecific inhibitors (ie, lupus anticoagulants), which react with anionic phospholipids, do not prolong the clotting time.10 The PT is most frequently used to monitor warfarin therapy.

Commercially available thromboplastins vary in their tissue factor source and method of preparation, leading to differing sensitivities to factor deficiencies11; therefore, PT results reported using different reagents are not interchangeable.12 The INR corrects for differences in thromboplastin potency. The World Health Organization has established a reference thromboplastin against which commercially available reagents are compared. The international sensitivity index (ISI) describes the responsiveness of each thromboplastin reagent to reductions in the vitamin K–dependent clotting factors compared with a sensitive standard, which is assigned an ISI of 1.0. Commercial thromboplastins derived from animal sources are less sensitive than the reference standard and commonly have ISI values of 1.2 to 2.8.13 Using the ISI, we can convert PT to an INR with this formula: INR = (patient PT/mean normal PT)ISI. Although the INR has helped to standardize anticoagulant monitoring, problems persist. The precision of INR determination varies, depending on reagent-coagulometer combinations. Unreliable reporting of the ISI by thromboplastin manufacturers also complicates INR determination.14 Finally, with new batches of thromboplastin reagent, each laboratory must establish a mean normal PT using blood from at least 20 healthy volunteers.14

aPTT
The aPTT is performed by first adding a surface activator (eg, kaolin, celite, ellagic acid, or silica) and diluted phospholipid (eg, cephalin) to citrated plasma (Figure 2B). The phospholipid in this assay is called partial thromboplastin because tissue factor is absent. Activated platelets or monocytes provide negatively charged phospholipid surfaces on which these clotting reactions occur.

Figure 1. Coagulation pathways. Coagulation is initiated by extrinsic tenase, which forms when factor VIIa binds to tissue factor. Extrinsic tenase activates factors IX and X. In the presence of calcium, factor Xa binds to negatively charged phospholipid surfaces where it interacts with factor VIIIa to form intrinsic tenase, a complex that efficiently activates factor X. Factor Xa binds to factor Va on negatively charged phospholipid surfaces to form prothrombinase, the complex that activates prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin. Activated platelets or monocytes provide negatively charged phospholipid surfaces on which these clotting reactions occur.

bleeding abnormalities and to monitor anticoagulant therapy (Table 1).9 Most of these tests use citrated plasma, and the end point for all of them is fibrin clot formation.
factor XI, prekallikrein, and high-molecular-weight kininogen), calcium is then added, and the clotting time is measured.9

Although the clotting time varies according to the reagent and coagulometer used, the aPTT typically ranges between 22 and 40 seconds. The aPTT may be prolonged with deficiencies of contact factors; factors IX, VIII, X, or V; prothrombin; or fibrinogen. Specific factor inhibitors, as well as nonspecific inhibitors, may also prolong the aPTT. Fibrin degradation products and anticoagulants (eg, heparin, direct thrombin inhibitors, or warfarin) also prolong the aPTT, although the aPTT is less sensitive to warfarin than is the PT.15

**Thrombin Clotting Time**

The thrombin clotting time (TCT) is performed by adding excess thrombin to plasma (Figure 2C). The TCT is prolonged in patients with low fibrinogen levels or dysfibrinogenemia and in those with elevated fibrin degradation product levels.9 These abnormalities are commonly seen with disseminated intravascular coagulation. The TCT is also prolonged by heparin and direct thrombin inhibitors.10

**Activated Clotting Time**

Activated clotting time (ACT) (Figure 2D) is a point-of-care whole-blood clotting test used to monitor high-dose heparin therapy or treatment with bivalirudin.10 The dose of heparin or bivalirudin required in these settings is beyond the range that can be measured with the aPTT.16 Typically, whole blood is collected into a tube or cartridge containing a coagulation activator (eg, celite, kaolin, or glass particles) and a magnetic stir bar, and the time taken for the blood to clot is then measured.10 The reference value for the ACT ranges between 70 and 180 seconds. The desirable range for anticoagulation depends on the indication and the test method used. During cardiopulmonary bypass surgery, the desired ACT range with heparin may exceed 400 to 500 seconds.17 In contrast, in patients undergoing percutaneous coronary interventions, a target ACT of 200 seconds is advocated when heparin is administered in conjunction with a glycoprotein IIb/IIIa antagonist, whereas an ACT between 250 and 350 seconds is targeted in the absence of such adjunctive therapy.18 The ACT does not correlate well with other coagulation tests.

**Ecarin Clotting Time**

For the ecarin clotting time (ECT), venom from the *Echis carinatus* snake is used to convert prothrombin to meizothrombin, a prothrombin intermediate that is sensitive to inhibition by direct thrombin inhibitors.19 The ECT cannot be used to detect states of disturbed coagulation and is useful only for therapeutic drug monitoring. This assay is insensitive to heparin because steric hindrance prevents the heparin-antithrombin complex from inhibiting meizothrombin.19 Because ecarin also activates the noncarboxylated prothrombin found in plasma of warfarin-treated patients, levels of direct thrombin inhibitors can be assayed even with concomitant warfarin treatment.19 Although the ECT has been used in preclinical research, the test has yet to be standardized and is not widely available. A chromogenic variant of this assay has also been developed in which ecarin is added to a plasma sample and meizothrombin generation is measured with a chromogenic substrate.20

### TABLE 1. Causes of Clot-Based Assay Prolongation10,55

<table>
<thead>
<tr>
<th>Scenario</th>
<th>aPTT</th>
<th>INR</th>
<th>TCT</th>
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<tbody>
<tr>
<td>Factor deficiency</td>
<td>Prolonged</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Prekallikrein</td>
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<td>Factor XII</td>
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<tr>
<td>Factor XI</td>
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<td>Factor IX</td>
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<tr>
<td>Factor VIII</td>
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<tr>
<td>Factor deficiency</td>
<td>Prolonged</td>
<td>Prolonged</td>
<td>Normal</td>
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<tr>
<td>Factor X</td>
<td></td>
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<tr>
<td>Factor V</td>
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<tr>
<td>Prothrombin</td>
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<tr>
<td>Factor VII</td>
<td>Normal</td>
<td>Prolonged</td>
<td>Normal</td>
</tr>
<tr>
<td>Factor deficiency</td>
<td>Prolonged</td>
<td>Prolonged</td>
<td>Prolonged</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Nonspecific inhibitor</td>
<td>May be prolonged (depends on reagent)</td>
<td>Usually normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Heparin (therapeutic doses)</td>
<td>Prolonged</td>
<td>Less affected than aPTT, may be normal</td>
<td>Prolonged</td>
</tr>
<tr>
<td>LMWH</td>
<td>Normal</td>
<td>Normal</td>
<td>Prolonged</td>
</tr>
<tr>
<td>Hirudin, bivalirudin, argatroban</td>
<td>Prolonged</td>
<td>Variably prolonged</td>
<td>Prolonged</td>
</tr>
<tr>
<td>Warfarin (therapeutic doses)</td>
<td>Less affected than INR, may be normal</td>
<td>Prolonged</td>
<td>Normal</td>
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<tr>
<td>Vitamin K deficiency</td>
<td>Less affected than INR, may be normal</td>
<td>Prolonged</td>
<td>Normal</td>
</tr>
<tr>
<td>Liver dysfunction</td>
<td>Less affected than INR, may be normal</td>
<td>Usually prolonged</td>
<td>Prolonged</td>
</tr>
<tr>
<td>DIC</td>
<td>Less affected than INR</td>
<td>Usually prolonged</td>
<td>Usually prolonged</td>
</tr>
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HMWK indicates high-molecular-weight kininogen; DIC, disseminated intravascular coagulation.
Chromogenic Assays

Anti–factor Xa assays are used to measure levels of heparin and low-molecular-weight heparin (LMWH). These are chromogenic assays that use a factor Xa substrate onto which a chromophore has been linked (Figure 3). Factor Xa cleaves the chromogenic substrate, releasing a colored compound that can be detected with a spectrophotometer and is directly proportional to the amount of factor Xa present. When a known amount of factor Xa is added to plasma containing heparin (or LMWH), the heparin enhances factor Xa inhibition by antithrombin rendering less factor Xa available to cleave the substrate. By
correlating this result with a standard curve produced with known amounts of heparin, we can calculate the heparin concentration in the plasma.

**Use of Anticoagulant Assays to Monitor Therapy**

Anticoagulant drugs in clinical use include warfarin, heparins (unfractionated heparin and LMWH), and direct thrombin inhibitors (bivalirudin, hirudin, and argatroban).

**Warfarin**

Warfarin is effective for primary and secondary prevention of venous thromboembolism; for prevention of cardioembolic events in patients with atrial fibrillation or prosthetic heart valves; for prevention of stroke, recurrent infarction, or cardiovascular death in patients with acute myocardial infarction; and for the primary prevention of acute myocardial infarction in high-risk men. Warfarin dosage is usually adjusted to attain a desired INR (Table 3). Because of the variability in the anticoagulant response to warfarin, which reflects genetic variations in metabolism and environmental factors such as medications, diet, and concomitant illness, regular coagulation monitoring and dosage adjustment are required to maintain the INR within the therapeutic range.

**Heparins**

Heparins are indirect anticoagulants that activate antithrombin and promote its capacity to inactivate thrombin and factor Xa. To catalyze thrombin inhibition, heparin binds both to antithrombin via a high-affinity pentasaccharide sequence and to thrombin. In contrast, to promote factor Xa inhibition, heparin needs only to bind to antithrombin via its pentasaccharide sequence. Heparin molecules containing <18 saccharide units are too short to bind to both thrombin and antithrombin and therefore cannot catalyze thrombin inhibition. However, these shorter heparin fragments can catalyze factor Xa inhibition, provided that they contain the pentasaccharide sequence. Because almost all of the chains of unfractionated heparin are of sufficient length to bridge antithrombin to thrombin, heparin promotes thrombin and factor Xa inhibition equally well and is assigned a ratio of anti-Xa to anti-IIa of 1.

The anticoagulant response to heparin is unpredictable because of variable nonspecific binding to endothelial cells, monocytes, and plasma proteins. Because of this variable anticoagulant response, coagulation monitoring is routinely performed when heparin is given in greater than prophylactic doses. The aPTT is the test most often used to monitor heparin. Unfortunately, aPTT reagents vary in their responsiveness to heparin, and the aPTT therapeutic range differs, depending on the sensitivity of the reagent and the coagulometer used for the test. The aPTT has proved more difficult to standardize than the PT, and the commonly quoted therapeutic range of 1.5 to 2.5 times the control value often leads to systematic administration of subtherapeutic heparin doses. Consequently, it is recommended that the therapeutic aPTT for heparin correspond to that which results in a heparin concentration of 0.35 to 0.7 anti–factor Xa heparin units per 1 mL. Evidence supporting the concept of an aPTT therapeutic range that predicts efficacy and safety (with respect to bleeding) is somewhat tenuous.

Approximately 25% of patients require doses of heparin of >35 000 U/d to obtain a therapeutic aPTT and are called heparin resistant. Most of these patients have therapeutic heparin levels when measured with the anti-Xa assay, and the discrepancy between the 2 tests is the result of high concentrations of procoagulants such as fibrino-
gen and factor VIII, which shorten the aPTT. Heparin therapy in these patients can be managed safely with heparin levels. Less often, patients with a subtherapeutic aPTT also have a subtherapeutic heparin level despite large doses of heparin. This scenario usually reflects a combination of increased levels of heparin-binding proteins and increased heparin clearance. Rarely, this form of heparin resistance is caused by low levels of antithrombin.

Although the aPTT response is linear with heparin levels within the therapeutic range, the aPTT becomes immeasurable with higher heparin doses. Thus, a less sensitive test of global anticoagulation such as the ACT is used to monitor the level of anticoagulation in patients undergoing percutaneous coronary interventions or aorticcoronary bypass surgery. Although several retrospective studies defined an inverse relationship between the likelihood of a thrombotic event and the ACT after heparin administration for percutaneous coronary intervention (PCI), more recent data suggest that ischemic end points do not increase with decreasing ACT values, provided that the ACT is ≥200 seconds.

LMWH is derived from unfractionated heparin by chemical or enzymatic depolymerization. With a mean molecular weight about one third that of unfractionated heparin, only 25% to 50% of LMWH molecules contain ≥18 saccharides. Consequently, these agents have ratios of anti-Xa to anti-IIa that range from 2:1 to 4:1.

LMWH has gradually replaced heparin for most indications. LMWH is typically administered in fixed doses when given for prophylactic purposes or in weight-adjusted doses when given for treatment. LMWH has advantages over heparin that enable once- or twice-daily subcutaneous administration without coagulation monitoring (Table 4). Exceptions include patients with renal dysfunction (shorter LMWH chains are cleared via the kidneys), those at extremes of weight, infants, and perhaps pregnant women who are receiving full treatment doses. LMWH has little effect on the aPTT. Consequently, when monitoring is required, anti-Xa levels are measured with an LMWH standard.

Pitfalls in the monitoring of LMWH by anti–factor Xa levels include poor comparability between commercially available anti–factor Xa chromogenic assays, differences in ratios of anti-Xa to anti-IIa among the various LMWH preparations, and the importance of timing of blood sampling in relation to dosing. In general, it is recommended that blood samples for LMWH monitoring be obtained 4 hours after a subcutaneous injection. Although the relationship between anti-Xa levels and clinical outcomes is unclear, typically recommended therapeutic anti-Xa levels for twice-daily LMWH therapy range from 0.5 to 1.0 U/mL and for once-daily treatment between 1.0 and 2.0 U/mL.

Although the aPTT may be prolonged with high doses of LMWH, this assay is not used for monitoring. Because LMWH has less effect on the ACT than heparin, empiric LMWH dosing algorithms have been developed in the PCI setting.

### Direct Thrombin Inhibitors

Direct thrombin inhibitors bind directly to thrombin and block the interaction of thrombin with its substrates. Three parenteral direct thrombin inhibitors have been licensed for limited indications in North America. Hirudin and argatroban are approved for treatment of patients with heparin-induced thrombocytopenia, whereas bivalirudin is licensed as an alternative to heparin in patients undergoing PCI.

Hirudin and argatroban require routine monitoring. The TCT is too sensitive to small amounts of hirudin and argatroban to be used for this purpose. Although the ACT has been used to monitor the higher doses of direct thrombin inhibitors required in interventional settings, it does not provide an optimal linear response at high concentrations. The aPTT is recommended for therapeutic monitoring; however, each direct thrombin inhibitor has its own dose response, and the sensitivity of the test to drug levels varies between aPTT reagents. When hirudin therapy is monitored with the aPTT, the dose is adjusted to maintain an aPTT that is 1.5 to 2.5 times the control, whereas for argatran, the target aPTT is 1.5 to 3 times control (but not to exceed 100 seconds). The aPTT appears less useful in patients requiring higher doses of direct thrombin inhibitor in cardiopulmonary bypass procedures because this test becomes less responsive at increasing drug concentrations. The ECT appears to be useful for both low and high concentrations of direct thrombin inhibitors and is less affected by interfering substances than the aPTT. However, as stated above, it is not routinely available.

The responsiveness of the INR to different drug concentrations differs with assay reagent and with the type of direct thrombin inhibitor. Although all direct thrombin inhibitors prolong the INR, argatroban has the greatest effect on this test. This feature complicates the transitioning of patients with heparin-induced thrombocytopenia from argatroban to vita-

<table>
<thead>
<tr>
<th>TABLE 4. Advantages of LMWH Over Heparin and Their Consequences</th>
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<tbody>
<tr>
<td>Advantage</td>
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<tr>
<td>Better bioavailability after subcutaneous injection</td>
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<tr>
<td>Longer half-life</td>
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<tr>
<td>More predictable anticoagulant response</td>
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<tr>
<td>Less platelet activation and binding to platelet factor 4</td>
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</tbody>
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Point-of-Care Monitoring

Most coagulation assays are performed in centralized laboratories using blood collected from indwelling lines or via venipuncture. This approach introduces problems with respect to turnaround time, venous access requirements, and difficulties associated with sample transport and processing. To circumvent these problems, several point-of-care coagulation tests have been introduced. Of these, the ACT remains the most commonly used, reflecting, at least in part, the lack of rapid, readily available, inexpensive alternatives. Point-of-care INR monitoring is both feasible and practical and is used by many specialized coagulation clinics to streamline care. Although there are concerns about discrepancies between INR results obtained by near-patient testing and those measured in hospital laboratories, several investigators have reported that self-management with point-of-care INR devices is safe for selected patients and results in the same quality of care provided by specialized anticoagulation clinics. Although point-of-care aPTT results appear to be clinically reliable and reproducible, there is less experience with these techniques. The varying responsiveness of aPTT reagents and the need for calibration with heparin levels to establish an appropriate aPTT range limit the utility of these tests. There is a point-of-care device that can be used to monitor anti-Xa levels. This clot-based test detects LMWH levels above or below 1.0 U/mL. Warfarin, liver disease, and coagulation factor deficiencies can produce falsely high readings with this system. A point-of-care test based on the ECT also has been developed for monitoring direct thrombin inhibitors, but the test has yet to be fully validated.

Point-of-care tests are more expensive than centralized assays. Therefore, cost-effectiveness analyses are needed to justify their widespread use.

Review of Cases

Case 1

The attending physician was not surprised that Mr F’s aPTT was unaffected by LMWH. The physician debated performing an anti-Xa level but, on the basis of the results of the SYNERGY trial, which used a fixed-dosing strategy, decided that the result would not change management. Mr F proceeded to catheterization as scheduled. Because Mr F’s last dose of enoxaparin was given <8 hours before balloon inflation, no additional enoxaparin was given during the procedure. Mr F’s sheath was removed 8 hours after his last enoxaparin dose, and there were no complications.

Case 2

Mrs G’s physician drew blood for an anti-Xa level at the same time as her next aPTT. Although Mrs G’s aPTT remained subtherapeutic at 50 seconds, her anti-Xa level was 0.40 U/mL. Because the heparin level was well within the therapeutic range, heparin was dosed according to daily anti-Xa levels. Warfarin therapy was continued. By the seventh day of warfarin therapy, Mrs G’s INR was >2.0 for 2 consecutive days, and the heparin was discontinued. Mrs G was discharged home on warfarin, and a repeated INR was ordered 3 days later.

References

17. Hirsh J, Ratschke R. Heparin and low-molecular-weight heparin: the Seventh ACCP Conference on Antithrombotic and


